

## Full Length Research

# Plant Diseases Management through Gene Pyramiding

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Plant diseases have caused severe losses to humans in several ways. The goal of plant disease management is to reduce the economic and aesthetic damage caused by plant diseases. The main objective of this review was to understand about a gene pyramiding concepts with principles & application in disease management. Disease management procedures are frequently determined by disease forecasting or disease modeling rather than on either a calendar or prescription basis. Correct diagnosis of a disease is necessary to identify the pathogen, which is the real target of any disease management program. Improving disease resistance in crops is crucial for stable food production. Quantitative trait loci (QTLs), which usually have smaller individual effects than R-genes but confer broad-spectrum or non-race-specific resistance, can contribute to durable disease resistance (DR). Gene pyramiding holds greater prospects to attain durable resistance against biotic and abiotic stresses in crop. Agene pyramiding involves the use of several genes in a single cultivar to provide a wider base of disease resistance. Marker assisted breeding and functional genomics tools are effective strategies to develop resistant cultivars against fungal diseases in wheat for achieving estimated production paradigm. In future, functional genomics approaches such as TILLING; RNAi and epigenetics are needed to strengthen the development of resistant varieties.

**Keywords:** Disease Management, Disease Resistance, Genetically Modified Organism, Gene Pyramiding, Marker Assisted Selection, Molecular Marker

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## INTRODUCTION

Plant diseases have caused severe losses to humans in several ways. Starvation and uprooting of families resulted from the Irish famine caused by potato late blight (caused by *Phytophthora infestans*) (Coca-Morante, M. and Tolín-Tordoya, I., 2013). A valued resource was lost with the virtual elimination of the American chestnut by chestnut blight (caused by *Cryphonectria parasitica*). And direct economic loss such as the estimated one billion dollars lost in one year to American corn growers from southern corn leaf blight (caused by *Cochliobolus maydis*,

anamorph *Bipolaris maydis*) (Wisser, R. J., *et al.*, 2011). Many plant diseases cause less dramatic losses annually throughout the world but collectively constitute sizable losses to farmers and can reduce the aesthetic values of landscape plants and home gardens.

The goal of plant disease management is to reduce the economic and aesthetic damage caused by plant diseases. More multifaceted approaches to disease management, and integrated disease management, have resulted from this shift in attitude, however. Single, often severe, measures, such as pesticide applications, soil fumigation or burning are no longer in common use

(Mazzola, M. and Freilich, S., 2017). Further, disease management procedures are frequently determined by disease forecasting or disease modeling rather than on either a calendar or prescription basis. Disease management might be viewed as proactive whereas disease control is reactive, although it is often difficult to distinguish between the two concepts, especially in the application of specific measures (Fukuoka, S., *et al.*, 2015).

Plant disease management practices rely on anticipating occurrence of disease and attacking vulnerable points in the disease cycle (i.e., weak links in the infection chain) (Chaube, H., 2017). Therefore, correct diagnosis of a disease is necessary to identify the pathogen, which is the real target of any disease management program. A thorough understanding of the disease cycle, including climatic and other environmental factors that influence the cycle, and cultural requirements of the host plant, are essential to effective management of any disease (Servin, A. D. and White, J. C., 2016).

The many strategies, tactics and techniques used in disease management can be grouped under one or more very broad principles of action. Differences between these principles often are not clear. The simplest system consists of two principles, prevention and therapy (treatment or cure). There are a methods used to manage plant disease, such as exclusion, eradication, protection, resistance and application of fungicides (Villa, F., *et al.*, 2017). Development of disease-resistant plants has been relatively successful with annual and biennial plants, but less so with perennials, primarily because of the longer time required to develop and test the progeny. Woody perennials, such as ornamental, forest, and orchard trees, has been especially difficult for plant breeders to develop useful disease resistance (Rawal, G., *et al.*, 2017). Improving disease resistance in crops is crucial for stable food production. Although the use of race-specific resistance genes (R-genes) is a major strategy for disease control, these genes are vulnerable to counter evolution of pathogens. New resistance genes are then needed, thus continuing a cycle referred to as an evolutionary “arms race” between crops and pathogens. Quantitative trait loci (QTLs), which usually have smaller individual effects than R-genes but confer broad-spectrum or non-race-specific resistance, can contribute to durable disease resistance (DR) (Singh, S., *et al.*, 2013).

## OBJECTIVE

The main objective of this paper is to understand about a gene pyramiding concepts with principles& application in disease management

## LITERATURE REVIEW

Development of resistance has been most successful against the most specialized pathogens such as rust fungi, smut fungi, powdery mildew fungi, and viruses, but less so against general pathogens such as much blight, canker, roots rot and leaf spotting pathogens. A major problem with genetically resistant plants is that host-differentiated pathogenic races can be selected, so that many breeding programs become continuous processes to develop disease resistant plant lines. Disease resistance conferred by a single major gene is sometimes called specific or qualitative resistance and is race-specific. This type of resistance is often unstable, and emergence of a pathogenic race that can attack that genotype can completely overcome this type of resistance. Quantitative resistance or general resistance derives from many different genes for resistance with additive effects to provide more stable (or durable) resistance to pathogens (Singh, S., *et al.*, 2013). There are several strategies to minimize this race development and resistance failure. These include methods of gene deployment, where different genetic plant types are interspersed on a regional basis to avoid a genetic monoculture, or planting mixtures of cultivars having different genetic compositions to ensure that some component of the crop will be resistant to the disease (Singh, R. P., *et al.*, 2015; Goutam, U., *et al.*, 2015). Molecular techniques involve the use of newer molecular biology methods, instruments, reagents and analytical techniques to understand and/or provide solutions to crop pest management problems (Hartung, F. and Schiemann, J., 2014). A recent and controversial technique in developing disease resistant plants is the insertion of genes from other organisms into plants to impart some characteristic. For example, genes from the bacterium *Bacillus thuringiensis* have been inserted into plants to protect against insect attacks. Plants with these inserted genes are called genetically-modified organisms (GMOs), and have caused concern that unanticipated, and perhaps detrimental, characteristics, such as unforeseen allergies, may also be transferred to the new plants (Tosun, J. and Hartung, U., 2018). However, unforeseen and undesirable qualities also can be transmitted by conventional plant breeding techniques. The potato cultivar Lenape was developed in part because of its resistance to Potato virus A and resistance to late blight tuber infection. After it was released it was discovered that the tubers contained very high levels of solanine, a toxic alkaloid. The wheat cultivar Paha had resistance to stripe rust (caused by *Puccinia striiformis*) but also was very susceptible to flag smut (caused by *Urocystis*) (Ashkani, S., *et al.*, 2015).

### **Recent Advances on Mapping Genes**

Recently there have been advances in the mapping of genes involved in the variation of quantitative traits; through quantitative trait loci (QTL) mapping experiments and analysis of genomic data. QTL (quantitative trait locus) is a section of DNA (the locus) that correlates with variation in a phenotype (the quantitative trait). Usually the QTL is linked to, or contains, the genes that control that phenotype. Such studies on complex traits should lead to the identification of a great number of genetic factors responsible for the heritable variation of these traits. Furthermore, once these genetic factors are mapped, they can be controlled by molecular markers and the corresponding genotypes of individuals can be assessed easily (Yue, G. H., 2014; Gupte, R., Liu, Z. and Kraus, W. L., 2017). Gene pyramiding holds greater prospects to attain durable resistance against biotic and abiotic stresses in crop. Especially for widely grown crops such as wheat, corn, soybeans, rice, and the like, as social, legal, and economic obstacles is overcome. In a gene pyramiding scheme, strategy is to cumulate into a single genotype, genes that have been identified in multiple parents. The use of DNA markers, which permits complete gene identification of the progeny at each generation, increases the speed of pyramiding process (Poland, J. A. and Rife, T. W., 2012).

### **Some Common Crop Diseases and the Application of Gene Pyramiding History to Overcome the Pathogen**

#### **Wheat Rusts**

A number of different host genes or their combinations confer resistance to rusts. These genes are not expressed if virulence or virulence combinations occur in the rust population evaluated. Furthermore, a rust race can possess virulence to several resistance genes. Hence, it is extremely important to use races of known virulence combinations in genetic studies. Genetic studies should be conducted with pure races to avoid confusion in scoring infection types. Genetic studies in the field should use single races if possible. A gene pyramiding involves the use of several genes in a single cultivar to provide a wider base of disease resistance. Most breeders worldwide use this approach for the three rusts. Many gene pyramids have been successful, although some have quickly been rendered ineffective. At least in a few cases, Lr13 and 16 (332), Lr2a and 16 (117), Lr13 and 34 (101), Lr27 and 31 (351), and undesignated genes for stripe rust resistance (118, 344) seem to have an additive effect in combination (Aktar-Uz-Zaman, M., *et al.*, 2017).

According to Roelf's finding, some resistance gene combinations, such as the 'Sr2 complex' for stem rust

resistance (Singh, R. P., *et al.*, 2015; Shamanin, V., *et al.*, 2016), the 'Frontana complex' for leaf rust resistance (Li, Z., *et al.*, 2014), and the resistance of Anza and Little Joss for stripe rust (Ali, Y., *et al.*, 2018; Javaid, M. M., *et al.*, 2018), have shown long-term durability. These complexes provide the basic resistance in the emerging bread wheat germplasm at CIMMYT. Such durable resistance can be combined with other genes to provide some diversity. The breeding methodology for developing gene pyramids involves the identification of genetically different sources of resistance, followed by the incorporation of these resistances into a high yielding and adapted background (Fuchs, M., 2017). This can be accomplished by any selection methodology (pedigree or bulk breeding) following simple, top (three-way), or double crosses. CIMMYT breeders use the modified bulk method of selection (Singh, S., *et al.*, 2013). Similar methodology has been followed and worked well in India. The minor stem rust resistance gene Sr2 was pyramided with two major stem rust resistance genes Sr24 and Sr36 in the background of two important wheat varieties 'Lok-1' and 'Sonalika' (Singh, D. P., 2017; Goutam, U., *et al.*, 2015). Marker assisted selection using microsatellite markers gwm533, Sr24#12 and stm773-2 linked to Sr2, Sr24 and Sr36 were performed in the BC1F1, BC1F2, BC1F3 and BC1F4 generations for the successful pyramiding of these genes. As the minor gene Sr2 alone cannot provide adequate resistance to stem rusts, the two major genes Sr24 and Sr36 were pyramided along with it. The microsatellite markers eased the process of identification of lines carrying the pyramided genes. Although, these genes are ineffective individually to the upcoming pathotypes of stem rust in India and elsewhere in the world, pyramided lines are expected to provide durable resistance against all the races. Agronomic performance of the improved lines was compared with the recipient parent (Savadi, S., *et al.*, 2018).

#### **BLB and Blast Disease of Rice**

Marker aided pyramiding of rice genes for BLB and blast disease, the successful effort on gene pyramiding in rice includes resistance to blight, blast, gall midge etc. Bacterial blight (BB) caused by *Xanthomonas oryzae* pv. *Oryzae* (Xoo) is one of the most destructive diseases of rice throughout the world and in some areas of Asia it is responsible for yield loss of more than 60%. The most efficient approach to overcome bacterial blight caused by *X. oryzae* is to produce resistant varieties; more than 25 BLB resistant genes have been identified and subsequently transferred into modern rice cultivars by cross breeding. However, the recent breakdowns of many resistant genes to BLB pathogens have significantly affected the rice production in many countries. One way to delay such a breakdown is to pyramid multiple

resistance genes in to rice varieties. It is practically difficult to transfer genes through conventionally gene transfer process due to verti-folia effect. International Rice Research Institute (IRRI) have successfully used the MAS based gene pyramiding to transfer four genes Xa21, xa5, xa4 and xa13 in elite rice cultivars. The pyramided lines showed a wider spectrum and a higher level of resistance than lines with only a single gene (Song, S., *et al.*, 2019). Similarly, (Das G., *et al.*, 2018) successfully transferred three bacterial blight resistance genes into three susceptible rice lines possessing desirable agronomic characteristics via a marker-aided backcrossing procedure.

In India, at Punjab Agricultural University (PAU), three BB resistance genes xa5, xa13 and Xa 21 were pyramided in PR106 and Pusa 44 background and two of the PR1106 have been included in all India Coordinated testing during 2002. A similar work has also been successfully carried out in Central Rice Research Institute to pyramid three genes xa5, xa13 and Xa21 in to elite rice cultivars Lalat and Tapaswini. All combinations of the three resistance genes were pyramided using STS markers. Narayanan *et al.*, (2002) improved an elite indica rice line IR50 by pyramiding blast resistance gene Piz5 and bacterial blight resistance gene Xa21 through marker assisted selection and genetic transformation. Khare, M., (2015) made four cross combinations of IRBB21 and successfully obtained improved lines pyramided with Xa21 and Wx (waxy) gene showing durable resistance to bacterial leaf blight and high amylose content. Rice blast caused by the fungal pathogen *Magnaporthe grisea* is another devastating disease that provides constant challenge to rice production (Brar, D. S. and Khush, G. S., 2018).

### **Gene Pyramiding for Powdery Mildew Resistance in Wheat**

The fungal pathogen *Blumeria graminis f. sp. tritici* is the causal agent of the powdery mildew disease in wheat (*Triticum aestivum* L.). Resistance to this pathogen is mediated by the Pm genes. Since race specific resistance is restricted to pathogens that carry the matching virulence (avr)-gene, this type of resistance can be overcome in the field. For breeders, it is therefore desirable to create plants with broader spectrum and long-lasting resistance features. One strategy to achieve this goal is to combine different resistance genes by classical breeding (Parlange, F., *et al.*, 2015). However, this is a time-consuming approach. MAS based gene pyramiding provides a more rapid tool to introduce new disease resistance specificities into crop plants. (Gupte, R., Liu, Z. and Kraus, W. L., 2017), have undertaken a gene pyramiding approach in which three powdery mildew resistance gene combinations, Pm2 + Pm4a, Pm2 + Pm21, Pm4a + Pm21 were successfully

integrated into an elite wheat cultivar 'Yang158' (Lu, Q., 2011). Double homozygotes were selected from a small F2 population with the help of molecular markers. As the parents were near-isogenic lines (NILs) of Yang158', the progenies showed good uniformity in morphological and other non-resistance agronomic traits. The present work illustrates the bright prospects for the utilization of molecular markers in breeding for host resistance (Ma, P., *et al.*, 2016).

### **Gene Pyramiding as a Bt Resistance Management Strategy in Cotton**

Reports on the emergence of insect resistance to *Bacillus thuringiensis* delta endotoxins have raised doubts on the sustainability of Bt-toxin based pest management technologies. Corporate industry has responded to this challenge with innovations that include gene pyramiding among others (Manyangariwa, W., *et al.*, 2006). Recently gene pyramiding has been hailed as a lasting Bt resistance management strategy. The strategy of Bt gene pyramiding rests on three core assumptions. The first assumption is that insects resistant to only one toxin can be effectively controlled by a second toxin produced in the same plant. This assumption forms the basis for the Bollgard® II cotton variety which has two toxins namely, Cry 1Ac and Cry 2Ac. The Cry 1Ac toxin controls tobacco budworm and pink bollworm while the Cry 2Ac toxin controls corn earworm. The second assumption is that strains resistant to two toxins with independent actions cannot emerge through selection pressure with one toxin alone. The third assumption underlying the strategy of Bt gene pyramiding is that a single gene will not confer resistance to two toxins that are immunologically distinct and that have different binding targets. Second generation pyramided dual- Bt gene cottons Bollgard II® (Cry 1Ac + Cry 2Ab) and Wide Strike™ (Cry1Ac + Cry 1F) express two Bt endotoxins and were introduced successfully by Monsanto in USA and India in order to raise the level of control for *H. zea*, which was not satisfactorily controlled by the Cry 1Ac toxin alone. The Cry 1Ac and 2Ab toxins have different binding sites in the larval mid gut and are considered to be a good combination to deploy in delaying resistance evolution. This is due to the fact that a species cannot easily evolve resistance to both toxins because that would require two simultaneous, independent mutations in genes encoding the receptors (Tangtra kulwanich, K. and Reddy, G. V., 2014). Future pest management practices will have to rely on the introduction of transgenic cottons that express other insecticidal toxins in addition to the Cry toxins. Biological pest control using parasitoids and predators, cultural practices and other pest management tactics are all essential tactics in

preserving the efficacy of Bt based products. But gene pyramiding approaches have definitely proven as effective method in broadening the scope and mode of action of toxins thereby providing growers with more options in their overall resistance management efforts (Carrière, Y., *et al.*, 2018).

### **Pyramiding Resistance Genes against the Barley Yellow Mosaic Virus Complex (BaMMV, BaYMV, BaYMV-2)**

Barley Yellow Mosaic Virus disease caused by different strains of BaYMV and BaMMV is a major threat to winter barley cultivation in Europe. Pyramiding of resistance genes has been effectively used as a promising strategy to avoid the selection of new virus strains and to create more durable resistances (Lüpken, T., *et al.*, 2013). For pyramiding of resistance genes *rym4*, *rym5*, *rym9* and *rym11*, located on chromosomes 3H and 4H of barley, two different strategies have been developed. These strategies are based on doubled haploid lines (DHs) and marker assisted selection procedures. On the one hand F1 derived DH-plants of single crosses were screened by molecular markers for genotypes being homozygous recessive for both resistance genes. These genotypes were crossed to lines carrying one resistance gene in common and an additional third gene, leading to a DH population of which 25% carry three resistance genes, 50% have two resistance genes and 25% possess a single resistance gene homozygous recessively. Alternatively, F1 plants having one resistance gene in common were directly inter-crossed [e.g. (*rym4* · *rym9*) · (*rym4* · *rym11*)] and about 100 seeds were produced per combination. Within these complex cross progenies plants were identified by markers being homozygous at the common resistance locus and heterozygous at the others. From such plants, theoretically present at a frequency of 6.25%, DH-lines were produced, which were screened for the presence of genotypes carrying three or two recessive resistance genes in a homozygous state (Ordon, F. and Kühne, T., 2014).

### **Gene Pyramiding for Soybean Mosaic Virus Resistance Using Microsatellite Markers**

Gene pyramiding has been used as an effective approach to achieve multiple and durable resistance to various strains of Soybean Mosaic Virus (SMV) in soybean [*Glycine max* (L.)] have successfully pyramided three genes *Rsv1*, *Rsv3*, and *Rsv4* for SMV resistance with the aid of microsatellite markers in order to develop new soybean lines containing multiple resistance genes (Shi, A., *et al.*, 2006). A population of 84 lines derived from J05 (*Rsv1*, *Rsv3*) x V94-5152 (*Rsv4*) were

developed, and six specific SSR markers were identified for SMV resistance genes (Liu, J. Z., *et al.*, 2016). Two SSR markers *Sat154* and *Satt510* were used for selecting lines having the *Rsv1* gene, *Satt560* and *Satt726* for *Rsv3*, and *Sat\_254* and *Satt542* for *Rsv4*. These SSR markers allowed for identification and selection of specific lines and individual plants containing different genes and for distinction of the homozygous and heterozygous lines or individual plants for all three resistance loci. Individual plants with homozygous alleles at three genetic loci (*Rsv1Rsv1*, *Rsv3*, and *Rsv4Rsv4*) have been identified and new soybean germplasm is expected to be released with three genes combined for SMV resistance (Ramteke, R., *et al.*, 2015).

### **Discussion on Recent Trend and Future Prospects of Gene Pyramiding for Disease Management**

Recently the new technologies are being used for sequencing of cereal crops, but the storage of data and analyses are difficult due to its vast size (Singh, R. P., *et al.*, 2015). Single nucleotide polymorphism (SNP) genotyping offers a solution to this problem and accelerates the crop improvement by providing insights into their genetic constitution. It has number of advantages over conventional marker system such as rapid processing of large populations, abundance of markers and varieties of genotyping system (Chawla, H., 2018). In quantitative trait locus (QTL) mapping experiments and genome-wide association studies (GWAS); SNP data is frequently used to detect marker-trait associations (Yang, J., *et al.*, 2015). Discovery of SNPs using complete genome is facilitated by recent advances in next-generation sequencing (Visscher, P. M., *et al.*, 2017).

### **CONCLUSION AND RECOMMENDATION**

Genetic studies of number of economically important crops have been successfully done by the application of high-density SNP arrays. Due to global food security and consistent increase in world population, there is an immediate need to increase crop yield considerably. Plant diseases continue to cause huge losses and pose a great challenge for crop production. Novel genetic tools based on molecular marker technologies provide a good alternative for developing improved resistant cultivars. Development of molecular markers such as RFLPs, SSRs, AFLPs, SNPs, and DArT in last more than two decades has revolutionized wheat genomics. Marker assisted breeding and functional genomics tools are effective strategies to develop resistant cultivars against fungal diseases in wheat for achieving estimated production paradigm.

In future, functional genomics approaches such as TILLING; RNAi and epigenetics etc. are needed to strengthen the development of resistant varieties. Mutagenesis-derived broad-spectrum disease resistance may lead to a better understanding of the regulation of defense response networks in crop. Large-scale genome sequencing and associated bioinformatics are becoming widely accepted research tools for accelerating the analysis of some crop genome structure and function. Currently, functional markers are being increasingly adopted in some crops breeding. These markers are needed for important traits such as disease and stress resistance in order to strengthen the application of molecular markers in breeding programs.

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