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DEPARTMENT OF BIOTECHNOLOGY

THE ROLE OF MODERN PLANT BREEDING TO CONTROL PLANT DISEASE

A REVIEW: SUBMITTED TO DEPARTMENT OF BIOTECHNOLOGY FOR PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE BACHELOR OF SCIENCE DEGREE IN BIOTECHNOLOGY

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TABLE OF CONTENTS

ACKNOWLEDGEMENT ii
LIST OF TABLES iii
LIST OF ABbREViATION iv
ABSTRACT v
1. INTRODUCTION 1
1.1. Background 1
2. LITERATURE REVIEW 2
2.1. Plant breeding for disease control 2
2.2. Plant disease 3
2.3 Objectives of plant breeding 4
2.3.1 Higher yield 4
2.3.2. Improved quality 4
2.3.3. Abiotic resistance 5
2.3.4. Change in maturity duration 5
2.3.5. Desirable Agronomic Characteristics 5
2.3.6. Elimination of toxic Substances 6
2.3.7. Wider adaptability 6
2.3.8. Biotic resistance 6
2.4. Conventional plant breeding for disease control 7
2.5. Conventional plant breeding limitation for disease control 7
2.6. Modern plant breeding methods for disease control 8
2.6.1. Molecular marker -assisted breeding for disease control 8
2.6.2 Transgenic approach for disease control 10
2.6.3. RNAi mediated gene silencing technology for plant disease control 11
2.6.4. Tissue culture Approach 12
2.6.5. CRISPR/Cas9 and CRISPR/Cpf1 technology used in modern plant breeding 13
3. CONCLUSION 14
4. RECOMMENDATION 15
5. REFERENCEs 16

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LIST OF TABLES

Table page

Table 1: Crops disease resistant varieties developed through Marker assisted breeding 10

Table 2: disease resistant cultivars released by RNAi gene silencing technology 12

LIST OF ABBREVIATION

FAO	Food and agricultural organization
GMO	Genetically modified organisms
MAS	Marker assisted selection
MAB	Marker assisted breeding
RFLP	Restriction fragment length polymorphism
SSR	Simple sequence repeats
RAPD	Random amplified polymorphic DNA
AFLP	Amplified fragment length polymorphism
SNP	Single nucleotide polymorphism
DNA	Deoxyribonucleic acid
ROS	Reactive oxygen species;
RNAi	Ribonucleic acid interference
mRNA	Messenger ribonucleic acid
CRISPR	Clustered Regularly Interspaced Short Palindromic Repeats
RNA	Ribonucleic acid

ABSTRACT

Plant breeding is a technology which deals with genetic improvement of crop plants in relation to their economic use, to improve crops selected for desirable characteristics that can be reproduced to satisfy a variety of needs and overcome a multitude of challenges. The main aim of plant breeding is to improve yields, quality, disease resistance, drought resistance, and other desired agronomic characteristics. Agricultural production is affected by many factors such as pests, disease, and environmental changes. Crop plants are attacked by various diseases and insects resulting in considerable yield loss. Thus, plant breeding for disease resistance is a central focus of plant to produce disease-free plants and improve plants quality, quantity and have been made more productive and safer to grow free from disease. Conventional plant breeding for disease resistance has an important role to produce disease resistance crops up to now. However, the emphasizes is for disease resistance traits which preexist in the genetic potential of the species. Modern breeding for disease resistance enables us to introduce fungicidal, insecticidal, nematocidal or bactericidal coding genes from outside the plant species. Modern plant breeders, in along with plant pathologists, have had notable successes in breeding disease-resistant varieties, thus ensuring the economic production of crops. Recently modern plant breeding methods includes marker assisted breeding, transgenic approach, tissue culture, ribonucleic acid interference, and Clustered Regularly Interspaced Short Palindromic Repeats gene editing technology plays a central role to produce disease resistance plants. Therefore, applying modern plant breeding technology will help to develop disease resistant varieties that can minimize crop losses, and it will play a fundamental role in ensuring food security to the ever-increasing human population.

***Key words**: plant breeding, plant disease, disease resistance, modern plant breeding*

1. INTRODUCTION

1.1. Background

Modern agriculture is expected to provide sufficient nutrients to feed the world's growing population, which is projected to increase from 7.3 billion in 2015 to at least 9.8 billion by 2050. To sufficiently feed all these people, the total food production will have to increase 60% to 70% to meet a net demand of 1 billion tons of cereal for food and to feed by 2050 (Tillman *et al*., 2011). This goal is facing more challenges because of many factors like climate change, pests, disease. Diseases are one of the most dangerous enemies of crop which leads to entire failure of crop plant. From ancient times it occurs in minute form to most severe form leading to complete failure of crop. Some of them become epidemic also like Bengal famine, late blight of potato, powdery mildew of grapes and coffee rust (Mishra *et al.*, 2016). Bacterial and fungal pathogens reduce crop yields by about 15% and viruses reduce yields by 3%, For some crops, such as potatoes, the loss caused by microbial infection is estimated to be as high as 30% (Oerke and Dehne, 2004). To meet these challenges, breeding programs have largely reoriented their objectives towards stress tolerance, both biotic and abiotic (Borém *et al.*, 2012).

Plant breeding is a deliberate effort by humans to improve certain aspects of plants to perform new roles or enhance existing ones (Acquaah, 2012). The breeding strategies can be divided into two conventional and modern methods. Conventional breeding (classical breeding), is the development of new varieties (cultivars) of plants by using older tools and natural processes, as opposed to the newer, more sophisticated and sometimes radical tools of molecular plant breeding (Jameel *et al* ,2015). In conventional breeding, breeders assemble desirable traits from different but usually closely related plants into the new cultivar using the techniques of crossing (hybridization) (Acquaah, 2012). Consequently, the product of conventional breeding only emphasizes target traits which preexist in the genetic potential of the species, without introducing new genes (Jakowitsch *et al.*, 1999). However, its chief limitation is the fact that it depends on variability that can be exchanged within natural boundaries, Time-consuming, laborious, and untargeted breeding programs can not satisfy the increasing global food demand.

Modern Plant breeding is the art and the science of changing the trait of plant in order to produce desired characteristics or improving the heredity of plants. Modern plant breeding is based on

understanding and utilization of genetic principles. It presupposes knowledge of the botanical characteristics of the species, plant diseases and their epidemiology, insect pests that feed upon the different plant species, of physiological factors related to adaptation of plants, and of biochemical characteristic affecting utilization and nutritive value (Poehlman, 2013). Knowledge about the extent of genetic variability and patterns of agro-morphological variation in local germplasm from a target region is an important prerequisite for efficient crop improvement (Bashir *et at.*, 2014). Modern plant breeding has significant advantages over the conventional breeding such as it allows selection specific traits at seedling stage, reduces the time required before the phenotype of an individual plant is known, the selection to be performed under any environmental conditions, there is also higher effectiveness and efficiency in terms of time and resources (Onasanya *et al.*, 2010). Breeding for disease resistance is a central focus of plant breeding programs, as any successful variety must have the complete package of high yield, disease resistance, agronomic performance, and end-use quality. Modern plant breeding tools such as marker assisted selection, transgenic approaches, RNAi interference technology, plant tissue culture and CRISPR/cas9 genome editing approaches provide resistance against disease like fungal, viral and bacterial diseases has been controlled (Varshney *et al*., 2005). The impact of modern plant breeding in disease control was cited throughout these reviews to illustrate the ways in which the important crop plants have been made more productive and safer to grow free from disease. Developing sustainable societies is the grand challenge of the coming century. More food, feed, fiber, fuel, and forest products necessary to meet basic human needs must be produced from less land, water, and nutrients. (Brummer *et al.*, 2011). The objective of this paper is to review the role of modern plant breeding in agriculture to produce disease free plants and actually observed impacts of modern plant breeding with a particular emphasis on their role for food security and sustainable agricultural development.

2. LITERATURE REVIEW

2.1. Plant breeding for disease control

Plant breeding is the process of selecting plants with the most desirable qualities to produce offspring that inherit the desired traits. The practice of plant breeding has advanced from the cynical view of “crossing the best with the best and hoping for the best,” to the now carefully

planned and thought- out strategies for developing high performing cultivars with high predictability. Plant breeding has played an important role in the development of agriculture and the improvement of human life. Breeding programs aided by well-developed genetic principles and modern techniques, the breeder's experience and unique insight are an important factor for success in cultivar development (Acquaah, 2012). Both conventional and modern plant breeding methods currently have played a fundamental role in ensuring food security and safety and have had a profound impact on food production all over the world (Tester and Langridge, 2010). Plant breeders and geneticists are under constant pressure to sustain and expand food production by using innovative breeding strategies that provide a source of nutrition as well as crops having abiotic and biotic stress tolerances. Plant breeding has been a key science in improving crop production. Disease resistance is the most dynamic component of the crop breeding process. Many Researchers continues to unveil details mechanisms how plants defend themselves against plant disease, and implemented different breeding strategies to enhance resistance to pathogens in crop species and to expedite resistance breeding in the field. and generating novel resistances with components of the defense system. (Sharma *et al*, 2019).

2.2. Plant disease

The majority of plant diseases cause dramatic crop losses and reduce the artistic and practical values of landscape plants and home gardens. Plant diseases caused by bacteria, virus, fungi, and nematodes create several constraints to the production process. Plant disease is very difficult to control and their molecular basis for disease development is still under investigation (Jagtap *et al*, 2011). Tomato leaf curl virus, potato virus, barley yellow dwarf virus, bean golden mosaic virus, African cassava mosaic virus, rice dwarf virus, barley stripe mosaic virus tobacco rattle virus, citrus, mildew, blight, rusts, smuts, mildew, wilt, Bacterial pustule and other plant disease lost high yields annually (Edwards, 2001; Legg *et al.*, 2001). Recently Plant-parasitic nematodes are major biotic stress in successful crop cultivation (Karanastasi, 2003). Major issues at recently Rice root-knot nematode, Meloidogyne graminicola, was economically most serious causing yield loss of in rice, potato, carrot, onion, Citrus , banana, and tomato among the vegetable crops suffered comparatively more losses (Strange, 2005). Diseases of horticultural crops have always been a concern of crop producers, processors, merchandisers, and consumers because of reductions in yield and product quality. Some diseases pose a major constraint, or even threat,

which affects the economy of the nation those growth demotic product depends on it and its failure leads to breakdown of economy which is backbone of any nation for prosperity of its citizens. (Mishra *et al*, 2016). It is estimated that plant diseases crop yield loses is above 15% annually (Chakraborty and Newton, 2011). For cereals alone, a 10% loss amounts to a total of 300 million tons each year (the statistics division of the Food and Agricultural Organization of the United Nations (FAO, 2019). If all cereal diseases could be eliminated, this would result in additional food for 1.7 billion people. Plant disease management aims to reduce economically and aesthetically damages caused by plant diseases in a term called plant disease control. Plant breeders, in association with plant pathologists, have had notable successes in breeding disease-resistant varieties, thus ensuring the economic production of crop (Fisher *et al*., 2012).

2.3 Objectives of plant breeding

The breeder should first define a clear objective (or set of objectives) for initiating the breeding program, taking the needs of end-users into account (Acquaah, 2012).The primary objective of plant breeding is to increase crop yield and the secondary objectives are quality improvement, tolerance to biotic and abiotic stresses, Elimination of Toxic Substances, water and nutrient use efficiency, elimination of toxic substances, and different crop maturity groups Breeding objectives depend on the species and the intended use of the cultivar to be developed(Bhargava *et al.,* 2019).

2.3.1 Higher yield

Majority of breeding program aims to increase yields. This is achieved by developing more efficient genotypes. Conventional and modern plant breeding plays crucial role to improve the yield of plant products it may be grain yield, fodder yield, fibre yield, tuber yield, cane yield or oil yield depending upon the crop species. Improvement in yield can be achieved either by evolving high yielding varieties or hybrids. Recent advances in plant breeding techniques high yield product in different crops such as maize, wheat, rice, and potato achieved (Jameel, 2015).

2.3.2. Improved quality

Quality of produce is another important objective in plant breeding. The quality characters vary from crop to crop. Over all plant breeding used to improve the quality of grain size, color, milling and baking quality in wheat, Cooking quality in rice, malting quality in barley, color and

size of fruit, nutritive and keeping quality in vegetables fiber length in, strength and fineness in cotton, oil quality in oil seeds (Jameel, 2015). The amino acid content of food and feed is considered one of the most important agronomical traits (Collard, 2008). Plant breeding and transgenes is programs are racing to provide crops with enhanced levels of essential amino acids and storage proteins. Increasing the content of essential amino acids requires detailed study of its biosynthetic pathways, which have already been successful, used to engineer model plants (Ufaz and Galili, 2008).

2.3.3. Abiotic resistance

Crop plants also suffer from abiotic factors such as drought, soil salinity, extreme temperatures, heat, wind, and cold (Barkla *et al*. 2013b). Abiotic stress tolerance in plants is developed as a responsive behavior to overcome sudden changes in the environment, complex physiological and biochemical adaptation mechanisms towards temperature, water availability, and soil nutrients. Molecular biology tools have revealed that abiotic stress tolerance involves a network of biochemical events and many genes which operate under stress conditions (Senthil and Udayakumar, 2010).

2.3.4. Change in maturity duration

Earliness is the most desirable character which has several advantages. It requires less crop management period, less insecticidal sprays, permits new crop rotations and often extends the crop area. Development of wheat varieties suitable for late planting has permitted rice-wheat rotation. Thus, breeding for early maturing crop varieties or varieties suitable for different dates of planting may be an important to get high quality and quantity traits at a right time (Collard, 2008).

2.3.5. Desirable Agronomic Characteristics

It includes plant height, branching, growth habit, erect or trailing habit and other desirable characteristics to growth are often desirable. For example, dwarfness in cereals is generally associated with lodging resistance and better fertilizer response. Plants growth such types of desired characteristics important to get nullity plant products and prevent from different. Tallness, high tillering and profuse branching are desirable characters in fodder crops. Developing desired agronomic characteristic depend on the breeder objectives. The breeder

develops plants which used as frank furniture product growth erect and unbranched plant (Jameel *et al*, 2015).

2.3.6. Elimination of toxic Substances

It is essential to develop varieties free from toxic compounds in some crops to make them safe for human consumption Removal of different toxic substances would increase the nutritional value of different plant products. Some example Food allergy is a serious health problem affecting children and adults; it is a form of immune response against certain food proteins that are identified as harmful or toxic to the body. Allergens and toxins are found in various plant species, especially wild-types (Morandini, 2010). The breeding process focuses to produce Allergens and toxins free plants (Herman *et al.*, 2003).

2.3.7. Wider adaptability

Adaptability refers to suitability of a variety for general cultivation over a wide range of environmental conditions, different temperature conditions, different pH conditions, and different nutrient etc. Adaptability is an important objective in plant breeding because it helps in stabilizing the crop production over regions and seasons (Jameel, 2015).

2.3.8. Biotic resistance

Biotic stresses are the damage caused by other living organisms like viruses, fungi, bacteria, nematodes, insects, and weeds to plants, resulting in considerable yield losses (Rogers, 2004). Genetic resistance is the cheapest and the best method of minimizing such losses. Resistant varieties are developed through the use of resistant donor parents available in the gene pool. Biotic stresses resistance is often the most dynamic component of the crop breeding process, requiring continual updating owing to pathogen adaptation to plant genotypes. Conventional and modern plant breeding methods produce different disease resistance crops throughout the world and insect resistance. Examples illustrate the achievement insect resistance Transfer of insecticidal protein coding cry1Ac genes present in *Bacillus thuringiensis*. To crop plants has conferred resistance against chewing type insects termed Bt crops and Bt cotton, maize, and eggplant are the most noteworthy examples of such transgenic crops. These transgenic plants produce toxic proteins that damage the insect gut region, resulting in insect death (Kumar $et\ al.$, 2008).

2.4. Conventional plant breeding for disease control

Conventional breeding is the development of new varieties (cultivars) of plants by using older tools and natural processes; it involves identifying parent plants with desirable characteristics to create favorable combinations in the next generation. The process of selecting superior performing plants for food, feed and fiber products dates back more than10,000 years and has been substantially refined up to now. Hundreds of new crop varieties are released every year by commercial conventional breeding to improve crop productivity, bolster food security, enhance nutrition, and expand consumer choice (Jameel *et al*., 2015). Conventional approach includes pure line, pedigree, backcross, recurrent selection and interspecific hybrids through which various resistant varieties have been developed Possibilities for isolation of resistance factors are still restricted but nevertheless steadily improving (Smith, 2001). Conventional plant breeding methods played an important role in the development of disease resistant cultivars. Hybridization of less disease resistance plant with wild relative of high resistance plant Several examples illustrate the achievement for disease resistance by conventional breeding Laxmi of cotton resistance for leaf blight wheat is resistant against rust, cotton resistant for wilt, Wheat stripe rust resistance in USA, Wheat stripe and leaf rust resistance, Rice bacterial leaf blight resistance, Wheat stem rust resistance and many disease resistance plants released (Miah *et al.*, 2013).

2.5. Conventional plant breeding limitation for disease control

Conventional plant breeding techniques are inadequate for plant genome enhancement to develop new disease resistance verities if resistance sources is not available in the genetic boundaries of in the species, transfer of undesired genes alone with resistance genes, requires lot of time for testing and intensive recording disease resistance variety, it is costly in terms of land and labor requirement to develop disease resistance verities, the resistance mechanism is not understand well, it requires a lot of effort to develop disease resistance verities (Sharma, 2019). Therefore, plant breeders and researchers develop new and efficient modern methods plant breeding tools to overcome the limitation of conventional plant breeding. With the advancement of molecular genetics knowledge, many modern plant breeding methods have been developed for this purpose. The modern breeding procedures to overcome the problems associated with traditional breeding strategies (Khush, 2005).

2.6. Modern plant breeding methods for disease control

Modern plant breeding was revolutionized in the 21st century, leading to crop improvement based on genomics, molecular marker selection (Krimsky, 2019). Modern plant breeding can use techniques of molecular biology to select, or in the case of genetic modification, to insert desired traits into plants. Molecular breeding for resistance enables us to introduce fungicidal, insecticidal, nematocidal or bactericidal characters from outside the plant family or even outside the plant kingdom. One crucial task that can help crop modern plant breeding programs is identicaction of the novel genes that are responsible for desirable traits with a potential for utilization in commercial productions of field crops such as rice, wheat, maize, and other staple crop. Various molecular markers linked to disease resistance have been utilized by plant breeders to select disease resistance cultivars (Arens *et al*., 2010). Modern plant breeding techniques based on understanding how basic endogenous defense components work together and in generating novel resistances with components of the defense system such as pattern recognition receptors, which recognize conserved molecules in pathogens or the products of pathogen-mediated degradation of host molecules and trigger immune responses to a wide range of pathogens (Tang, 2017). The introduction of non-host resistance may thereby become possible. New perspectives might also arise from the introduction of an immune system that would enable the plant to produce antibodies against pathogens (Hiatt *et al.,* 1989). Numerous completed genome sequences e.g. Arabidopsis, rice, soybeans provide access to a greater number of genes to plant breeders (Eldakak *et al.,* 2013; Kim *et al.,* 2014). At recently many researchers and plant breeders successfully produce disease resistance plant by modern plant breeding techniques such as wheat, barley, coffee, rice, potato, tomato and other staple crops throughout the world. However, modern plant breeding is not yet widely accepted. Transgenic GMOs in particular are often seen very critically (Greenpeace, 2015). Even though 30 years of research and commercial applications suggest that modern plant breeding is not riskier than conventionally bred crops (EASAC, 2013).

2.6.1. Molecular marker -assisted breeding for disease control

Molecular marker -assisted breeding (MAB), is the application of molecular biotechnologies, specifically DNA markers, in combination with linkage maps and genomics, to alter and improve plant traits on the basis of genotypic assays (Jiang, 2013a). DNA marker can be defined as any

genetic marker which inherits together with the trait of interest throughout generation so it used to select the desired trait (He, 2014). DNA based markers are used extensively in MAB for various traits by the plant breeders such as disease resistance, drought tolerance, past resistance (Medina, 2013). The most commonly used markers in plant breeding are RFLP, SSR, RAPD, AFLP, SCAR, STS, and SNP (Agarwal, 2008). There are different types of marker assisted breeding methods available such as marker-assisted backcrossing, gene pyramiding, marker-assisted recurrent selection and genomic selection. Marker assisted selection can be affected by many factors such of reliability of DNA markers, quality of DNA extraction method, genetic maps, quick and efficient data processing, and availability of high throughput marker detection system marker analysis procedures, level of polymorphism (Dar, 2019). Various molecular markers linked to disease resistance have been utilized by plant breeders to select disease resistance cultivars. (Arens *et al*., 2010). At the last 20 years different researchers investigated markers linked to disease resistance have been utilized by plant breeders to select disease resistance cultivars (Chen and Zhang, 2002). Now a day's plant breeders produce many plants which are resistance to different bacteria, virus, fungi and nematode disease-free crops and plant produced. Several examples illustrate the achievement; form those achievements in this review some staple crop such as wheat, maize, and rice are described. Lagudah *et al*., (2006) reported that In wheat csLVLr34 DNA marker used to identify LR34 gens conferring resistance to leaf rust disease, Hiebert *et al*.,(2011) reported that FSD-RSA marker used to identify SCAR gens conferring resistance to Stem rust resistant, Galiano and Miedaner,(2017) reported that in maize SSR marker used to identify qMrdd gene conferring resistance to rough dwarf disease, and other marker such as RFLP markers helped to identify two genes i.e. Ht1, Ht2, conferring resistance to leaf blight, SSR used to identify Pi9, Pi2 gens conferring resistance to blast resistance disease, SSR used to identify Pi9, Xa23 genes conferring resistance to blight, Blast disease in rice RFLP used to identify Xa21 conferring resistance to Bacterial blight , in tomato AFLP used to identify genes Ve1 and Ve2 conferring resistance to mosaic virus.

Table1: Some major crops disease resistant varieties developed through marker assisted breeding

Crop	Gene	Marker	Disease	Reference
Wheat	LR34	csLVLr34	Leaf rust	Lagudah *et al., (*2006).
Wheat	SrCad	FSD-RSA	Stem rust	Hiebert *et al*., (2011).
Maize	qMrdd	SSR	rough dwarf disease	Galiano and Miedaner, (2017).
Maize	Ht1, Ht2	RFLP	leaf blight	Xu, (2020).
Rice	Xa21	RFLP	Bacterial blight	Chen, (2002).
Rice	Pi9, Pi2	SSR	blast resistance	Tian, (2019).
Rice	Pi9, Xa23	SSR	Blight, Blast	Nishizawa *et al*., (2015).
Tomato	Ve1andVe2	AFLP	mosaic virus	Arens *et al.,* (2010).

2.6.2 Transgenic approach for disease control

It is an advanced form of genetic engineering in which foreign genes are introduced into the genome of plant species for achieving desired goal. It a novel strategy through which diseases can be effectively managed by introducing and overexpressing of genes that encode proteins which are involved in the synthesis of compounds toxic to Bactria, viruses, fungi and nematodes with direct inhibitory effect on the growth (Cornelissen and Melchers,1993). The genes being inserted for disease resistance include chitinase, glucanases, thaumatin-like proteins, pathogen cell wall degrading enzymes and toxins. Such genes are introduced to plants that enhance the production of plant defense molecules like saponins, ROS, phytoalexin, and antimicrobial peptides. These are antimicrobial proteins that confer resistance to pathogens by attacking their virulence factors. Such gene introductions by transgenic approach confer resistance to plants against different diseases (Strange, 2005; Oldach *et al*., 2001). At recent years researchers introduced different disease resistance genes many crops successfully to prevent crop lost by transgenic approaches. Eastwood *et al*., (1994), reported that Cre3 gene that confers a high level of resistance to the cereal cyst nematode. Lagudah *et al*., (1997) introduce Cre3 gene into wheat and produce cyst nematode resistance wheat cultivator. Cre3 gene encode a nucleotide binding site and leucine-rich repeat (NBS-LRR), Stein *et al*., (2000) reported the leaf rust resistance gene Lr10 isolated from CIMMYT germplasm and introduce other wheat cultivator to produce leaf rust resistance wheat. Rice is affected by Magnaporthe grisea fungus, which caused rice blast disease. Coca *et al*., (2004), reported that afp gene was isolated from Aspergillus giganteus and introduced in to rice. afp gene expressed in rice produce AFP protein which is an antifungal

compound, so rice which résistance to rice blast disease produced. (Ahmed, 2019), reported Natural resistance genes R-genes are responsible for inducing resistance against nematodes in tomato. The genes Mi-1, Mi-2, Mi-3, Mi-4, Mi-5, Mi-6, Mi-7, Mi-8, Mi-9, and Mi-HT confer resistance to the root Knot nematode. Also, researchers produce synthetic peptide like D4E1 and introduce cotton providing resistance against Thielaviopsis basicola fungus in cotton (Rajasekaran *et al,* 2007).

2.6.3. RNAi mediated gene silencing technology for plant disease control

RNAi as a tool for plant gene therapy against, viral, bacterial and fungal infections lies in the fact that it regulates gene expression via mRNA degradation (Varshney *et al,* 2005). The mechanism of RNAi starts with degradation of dsRNA by Dicer enzyme into miRNA or siRNA of 21–24 nucleotides. These smaller RNAs are recruited by RISC complex (an effecter complex) to their target sequence of mRNAs, which results in its degradation (Ding, 2010). When a plant pathogen attacks the plant, plant machinery targets the pathogen dsRNA formed during host machinery-based pathogen replication and convert it into pathogen siRNA. Host RISC complex now recruits these siRNA to the photogene genome, resulting in inhibition of pathogen protein translation then the plant pathogen does not survive in the plant (Karthikeyan *et al.,* 2013). Host Induced Gene Silencing, has emerged in recent years that use the host plant as a delivery system to cause gene silencing in the pathogen (Mahmoud *et al*., 2008). The transgenic plants target the pathogen genome by using artificial miRNA, hairpin RNA, sense/antisense RNA, and miRNA construct targeting a pathogen gene. Once the transgenic plant is infected and the pathogen starts feeding from the host, the small interfering RNA (siRNA) molecules are transferred to the pathogen cells, ultimately inducing an RNAi response in the pathogen and the silencing of the targeted gene (Simon and Garcia, 2011). Escobar *et al*., (2001) reported RNAi induced technology are effective for control crown gall disease by targeting iaaM and ipt the transformed plants Arabidopsis thaliana showed resistance to crown gall disease. Different plant such as tobacco, cassava, cotton, rice, cabbage, tomato and, pea produce different disease resistance by RNAi silencing technology by targeting the pathogen proliferate mechanisms in the plants demonstrated in Table 2. In the next page

Table2: Different disease resistant cultivars released by RNAi mediated gene silencing technology across the globe.

Crop	Disease	Target region	Reference
Tobacco	rattle virus	Rar1, EDS	Di Stilio *et al*., (2010).
Cotton	crumple virus	ChlI, PDS	Tuttle *et al*., (2008).
Cassava	cassava virus	CYP79D1, CYP79D2	Fofana *et al*., (2004).
Rice	Bacilliform virus	bacilliform virus	Purkayastha *et al*., (2010).
Cabbage	curl virus	GUS, NtEDS1	Huang *et al*., (2011).
Tomato	vein virus	Comt	Del Rosario *et al*., (2008).
Pepper	mosaic virus	Su and luc	Carrillo *et al*., (2006).
Pea	browning virus	PDS	Constantin *et al*., (2008).

2.6.4. Tissue culture Approach

Tissue culture has been significant role in producing disease free planting materials of vegetative propagated crops in horticulture industry of many countries (Bachraz, 1998). Meristem culture is the propagation from meristematic tissue generally provides a method of cleaning up material from viruses and other systemic pathogen infections. Micro-propagation is a tissue culture (in vitro) method used for rapid and true to type multiplication of plants on artificial nutrient media under controlled environment Meristem culture the propagation from meristematic tissue generally provides a method of cleaning up material from viruses and other systemic pathogen infections (Mtui, 2011.). Currently many researchers reported meristem tissue culture is recognized as a useful tool for control and breeding for disease free plants and increase. Tiwari *et al*., (2008) investigate sugarcane free from yellow leaf virus developed from Meristem sugarcane aseptically. Ghag *et al*., (2015) isolated and developed from embryogenic cultures derived from banana shoot tip which showed good resistance against Fusarium oxysporium var.cubense race. Mutandwa (2008) reported that the use of tissue cultured sweet potatoes in Zimbabwe have been shown to improve household food security. Farmers who used tissue cultured sweet potato obtained yields of up to 25 tons per hectare against a national average of 6 tons per hectare. Fuglie *et al*, (1998) reported that the rapid diffusion of virus free sweet potato planting material in Shandong province of china, reaching 80% of the province`s small growers within the only four years. Njuguna *et al*. (2003) Reported that Banana Tissue in

Kenyan free of diseases and pests for a longer time than the traditional suckers. other many plants produced by meristem tissue culture such as Apple free from Black spot virus disease (Raman and Goodwin, 2000), Strawberry free from Crown rots disease (Chandra *et al.*, 2010),

2.6.5. CRISPR/Cas9 and CRISPR/Cpf1 technology used in modern plant breeding

Clustered regularly interspaced short palindromic repeats (CRISPR) are DNA sequences, approximately 25-50 nucleotides long, separated by short sequences called spacers (Marraffini, 2010). This is an exciting technology for the plant breeding field, and has the potential to bring new products to the market in a much quicker. CRISPR is a relatively new gene-editing technology that has the possibility of making a large impact to plant breeding. CRISPR-Cas technology may greatly benefit plant breeding and completely change the way plant breeding is currently being conducted. With CRISPR-Cas technology, changes to the plant genome can be done in a nucleotide-specific manner; modifying, inserting or knocking out a gene (Borte, 2015). CRISPR/cas9 tools have mainly been explored against virus infection, followed by efforts to improve fungal and bacterial diseases and nematode resistance (Peng *et al*, 2017). Virus resistance can be accomplished by targeting host factors that are involved in the replications of the virus or targeting and destroying the viral genome itself and thus preventing the replication. Many studies have been carried out to directly target geminiviral genomic DNA with CRISPR/SpCas9 (Gomez *et al,* 2019). Bastes *et al* (2019) reported that design and Constructs SpCas9 and sgRNAs which targeted the replication-associated protein gene beet severe curly top virus and bean yellow dwarf virus were transformed into different plant. The resulting plants exhibited high levels of resistance to the targeted virus. During the infection process of bacteria, type III effectors are secreted into the plant cell (Büttner and He, 2009). These effectors primarily interrupt the host's defense pathways and/or activate the S genes for disease development (Zaidi *et al.* 2018). Therefore, both S genes and negative regulators of plant innate immune response are good target sites for CRISPR/Cas9-mediated gene editing to improve plant disease resistance. Zhou *et al.,* (2015) reported that rice resistance to bacterial blight disease CRISPR /cas9 genome editing technology by target editing SWEET13 susceptibility gene, the gene encoding a sucrose transporter involved host pathogen infraction. Chandrasekaran *et al*., (2016) developed cucumber plants resistance to potyviruses by target editing elF4E susceptibility gene by CRISPR/Cas 9 technology.

3. CONCLUSION

With the advancement of new technologies and the discovery of molecular tools it paved a new pathway for discovering the disease resistant traits. Modern plant breeding techniques helps to produce different crop varieties resistant to different diseases. Currently, modern plant breeding techniques for wheat resistance to leaf rust disease and stem rust disease; maize resistance to rough dwarf disease and leaf blight; rice resistance to blast disease and bacterial blight disease; tomato resistance to mosaic virus are being implemented. Modern plant breeding offers large potentials to contribute to sustainable agricultural development and food security.

4. RECOMMENDATION

Applications of molecular marker assisted selection to plant breeding will be a valuable supplement to conventional breeding. Integration of marker assisted into conventional breeding programs represents an optimistic strategy for future crop improvement. Though, plant breeding has numerous contributions for crop improvement, the development of prefect varieties in crop plants is still under study. The recent developments in genetic engineering techniques further equipped the plant breeders to reshuffle genes even across the organisms. Genetically engineered varieties can be produced by this method for commercial purpose.

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