Biotechnological Approaches for Management of Diseases in Plants

Abstract

Plant diseases cause substantial yield losses in cultivated crops and continue to be a serious threat to agriculture and food security at the global level. As a means of improving production and productivity, the development of appropriate methodologies for the management of disease-causing plant pathogens is indispensable. Generally, plant disease management practices involve cultural, mechanical, biological and chemical approaches, of which the development of varieties and hybrids resistant to diseases is viewed as the most cost-effective way to manage plant diseases without incurring additional expenditure. Over the years, conventional plant breeding has contributed significantly towards the development of disease resistant cultivars through appropriate screening and selection on the basis of phenotypic traits. However, the limited availability of resistant germplasm along with the requirement for extensive resources and a longer duration, have left little significance for continued improvement through this means. Emerging diseases along with erratic climate conditions affecting crops around the globe force scientists to find alternative solutions to swiftly overcome such threats. The subsequent evolution of innovative biotechnological tools, techniques and methods has brought revolutionary

Authors

Manoj M S
Ph.D. Scholar,
Department of Genetics and Plant Breeding, University of Agricultural Sciences, Dharwad, Karnataka, India.
E-mail: manojms005@gmail.com

Revanasiddayya
Ph.D. Scholar,
Department of Genetics and Plant Breeding, University of Agricultural Sciences,Raichur, Karnataka, India.

Abhishek K Gowda
Ph.D. Scholar,
Department of Genetics and Plant Breeding, University of Agricultural Sciences, Dharwad, Karnataka, India.

Mallikarjun P K
Ph.D. Scholar,
Department of Genetics and Plant Breeding, University of Agricultural Sciences, Dharwad, Karnataka, India.

changes in the way of managing plant diseases in a much more accelerated and rapid manner. Various biotechnological and genetic engineering approaches such as tissue culture, transgenic technology, marker assisted breeding, RNA interference and genome editing have successfully addressed numerous challenges that arose from diseases causing plant pathogens. They are equally complemented with new-age technologies like sequencing of genome, transcriptome and proteome along with metabolomics profiling and target site modification. Judicious management of plant diseases demands the blending of conventional plant breeding strategies with modern technologies of molecular genetics to enhance crop production and productivity.

Keywords: *Biotechnology, CRISPR, Disease, Pathogen, RNAi, Tissue culture*

I. INTRODUCTION

Plant disease is an abnormal pathogenic condition that causes plant suffering and leads to the expression of symptoms. Plant pathogens like bacteria, fungi, nematodes, phytoplasma, viruses and viroids cause harmful and economically significant diseases in a wide variety of plant species around the world (Kumar *et al.*, 2018). Pathogens discharge organic acids, enzymes, growth regulators, toxins and other harmful substances into the plant system, which exhibit various symptoms in the affected plants. Destruction of vascular bundle tissues, stunting, chlorosis, necrosis, drying of the leaves, destruction of floral parts and witling are a few kinds of symptoms. Subsequently, the ability of the plants to absorb the water and minerals from the soil and their conductance to plant tissues is inhibited, which results in reduced photosynthesis and the destruction of plants (Prince *et al.*, 2015). Plant diseases have been a threat to agriculture and food security at the global level as phyto-pathogens cause substantial yield losses in most of the food crops, which estimates the average global yield loss to be around 16%, which can be as high as 40% to 50% in some crops. (Savary *et al.*, 2019).

Anticipating the onset of infection and addressing weak areas in the disease cycle are the cornerstones of management strategies for plant diseases. Therefore, accurate diagnosis of a disease is essential to recognise the invading pathogen, which is the actual target of any disease management practice. A complete knowledge of the cultural requirements of the host plant, disease cycle, including climatic, edaphic and other environmental factors that influence the cycle, is essential for the management of any disease in an effective manner. Generally, disease management practices in plants can be distinguished into cultural, mechanical, biological and chemical approaches. Several of these methods are used in integrated plant disease management to control specific plant diseases or pathogen vectors. (Fry, 1982).

Cultural and mechanical methods are able to manage a limited number of diseases in comparison to others. A variety of registered chemicals are available against diseases caused by fungi, but the chemical products currently available for the control of bacteria are less efficient and limited in number, whereas no case has been reported for the control of viruses through chemicals (Kumar *et al.*, 2018). The development of varieties and hybrids exhibiting disease resistance has proven to be the most economical approach to manage pathogen attack via biological means. Conventional plant breeding methods have made significant contributions to the development of cultivars resistant to various diseases. However, the limited availability of resistant germplasm, along with

the requirement for extensive resources and a longer duration, have left little significance for continued improvement through this means. Under such circumstances, various evolving approaches of biotechnology have paved the way for accelerated management of plant diseases through the development of crop varieties that can resist numerous diseases.

II. MANAGEMENT OF PLANT DISEASES THROUGH BIOTECHNOLOGICAL APPROACHES

Biotechnology uses cutting-edge technologies to enhance or create new species and products that may be utilized in various ways by genetically manipulating, altering and multiplying biological organisms. Plant biotechnology assists plant pathology in the development of parental lines devoid of pathogens through rapid clonal propagation, employing genetic engineering techniques to insert resistance genes into plants, editing the DNA sequence and in many other ways. In this chapter, we will discuss various biotechnological methodologies, tools and techniques that are being used for the management of diseases in plants.

Tissue Culture

A plant cell can be cultured in an artificial nutrient medium and a whole plant can be regenerated from cultured cells. This technique of raising plants under controlled conditions is known as tissue culture. Various methods viz., callus culture, invitro shoot grafting, meristem culture, somatic embryogenesis, protoplast culture and somaclonal variation, can be utilised for the development of disease resistant plants. Since rapidly developing plant meristems are typically virus- free or at least have considerably lower viral concentrations than non-meristem cells, meristem or shoot tip culture techniques can be employed to remove viruses from affected germplasm and are frequently used in vegetatively propagated plants.

Vegetative buds that are developing quickly are excised, cleaned with sterile distilled water, and then disinfected by submerging them in a mercuric chloride solution (0.1%) for a couple of minutes. After that, sterile distilled water is used to rinse the buds numerous times. A sterile scalpel is used to cut 3–4 leaf primordia (0.3–0.6 mm in size) off the bud under the microscope. The buds are then aseptically placed in test tubes filled with Murashige and Skoog medium and incubated at 25 ± 2 ºC in light for 40 to 45 days. After being taken out of the test tubes, the plantlets are rinsed with tap water and placed in the Hoagland solution for 72 to 96 hours to harden. The plantlets are moved to pots with a 3:1 ratio of peat soil to vermiculite and placed in a mist chamber for a week.

Thereafter, the plants are grown under glasshouse conditions for further investigation.

Ramgareeb *et al.,* (2010) used apical meristem culture to develop virus free sugarcane plants from a South African cultivar, NCo376, infected with two viral diseases viz., mosaic virus and yellow leaf virus. The development of virus-free plants was largely attributed to the treatment of nodes with hot water, followed by the germination of vegetative buds at 40°C. However, difficulty in isolation, a low survival rate, the requirement for aseptic conditions and long duration for regeneration are some of the limitations associated with this technique.

A desired gene obtained from an organism that is incorporated into the genome of a related or unrelated species of another organism is called a transgene, and the process involved is called transgenecis. Genetic engineering techniques have allowed the transfer of genes across different genera and species, offering rapid insertion of disease resistant genes from a source organism into the genome of a crop plant, followed by precise manipulation of the temporal or tissue specific expression of a desired trait. Sometimes a disease-causing pathogen itself acts as a source of resistance, as observed in the case of resistance against plant viruses mediated by coat protein, which proved successful in controlling the papaya ring spot virus on the Hawaiian Islands. The transfer of genes across species has become routine in disease resistant breeding and subsequent biotechnological advances have enabled the incorporation of novel genes imparting disease resistance into crop plants. Such gene transfer could be accomplished either through a direct method using biolistics or by a vector method via *Agrobacterium tumefaciens.*

Agrobacterium mediated transformation is an extensively used method for plant genetic engineering that employs the soil bacteria *Agrobacterium tumefaciens*, which is known to cause crown gall disease. A. *tumefaciens* has an exceptional ability to transfer a particular DNA segment of the tumour inducing plasmid into the nuclear genome of infected plant cells, where the segment is stable integrated and transcribed subsequently (Kumar *et al.,* 2018). The hevein-like gene that provides broad-spectrum resistance to stem wilt was inserted into the asparagus genome using Agrobacterium mediated transformation. Successful transformation in the transgenic plants was identified by polymerase chain reaction and confirmed by Southern blot hybridization. Significant enhancement of resistance to stem wilt was observed in the transgenic asparagus plants compared to non-transgenic plants (Chen *et al.*, 2019).

Biolistic transformation, also known as particle bombardment, is a potent tool that allows the direct transfer of a gene to a wide range of cells and tissues that have proven difficult to transform by the Agrobacterium technique. It is more appreciated for the improvement of species with high levels of heterozygosity (Gardner, 1993). The particle bombardment device primarily contains a mechanism to accelerate the particles to the proper speeds and adjust their penetration into the receiver cells. A biolistic transformation method was used to transfer the stilbene synthase gene (Vst1) into the genome of spring wheat Jinghong 5. A phytoalexin stilbene produced by this gene inhibits the germination of fungal spores and arrests the growth of powdery mildew causing pathogens (Liang *et al.,* 2000).

Agrobacterium mediated transformation offers stable transformation with high efficiency but suffers from the host specific nature and requirements of the protoplast. These limitations can be overcome by adopting the biolistic transformation method, which is associated with lower transformation efficiency, random integration of transgenes and additional expenditure (Kikkert *et al.,* 2005).

Marker Assisted Selection

The advent of molecular markers has revolutionised plant breeding programmes over the past 15 to 20 years, followed by the evolution of advanced molecular techniques that have made it possible to use markers and probes to track numerous resistance genes introgressed into a single cultivar from different sources through a crossing programme. The ability of DNA based molecular markers to identify allelic variation in the genes governing economic traits has the potential to increase the effectiveness of conventional plant breeding through marker assisted selection (MAS) by reducing the reliance on laborious and time-consuming phenotype-based screening procedures (Kumar *et al.,* 2023).

Marker assisted selection (MAS) involves the selection of plants from a large population at an early generation with a desired genetic background at specific loci while maintaining adequate allelic variants in the population. Soon after selection through DNA markers, the genetic diversity at unselected loci may enable breeders to develop novel varieties and hybrids through conventional breeding with respect to the traits under consideration (Kumar *et al.,* 2015).

MAS employs suitable DNA markers that help in identifying resistance genes by screening a large germplasm, selecting parents for hybridization,

introgression and pyramiding of genes for resistance to diseases. Though DNA markers are free of environmental effects, traits may interact with the environment. Therefore, while developing markers, phenotyping should be carried out in multiple environments and validated across breeding populations.

Marker Assisted Backcross

The most effective way to introgress a single gene or a quantitative trait locus affecting the trait of interest while maintaining the good agronomic traits of the recurrent parent in a backcross programme is to use marker assisted backcrossing (MABC). The selection of a target locus with a reduced length of donor DNA segment in order to accelerate the recovery of the recurrent parent genome during backcrossing is called MABC. The use of molecular markers accelerates the process of backcrossing by decreasing the number of backcrosses required to recover the recurrent parent phenotype. MABC operates through foreground, recombinant and background selections.

The purpose of foreground selection is to maintain the target locus in a heterozygous condition until the completion of the final backcross. Here, plants carrying the marker allele of the donor parent at the target locus are selected. Recombinant selection aims to reduce the size of the donor chromosome segment containing the target locus. It involves the selection of backcrossing progeny with the target gene and recombination events between the target locus and linked flanking markers. The objective of background selection is to reduce linkage drag created by the donor parent and it aims at selecting all genomic regions using recurrent parent marker alleles, excluding the target locus, which is selected on the basis of phenotype (Singh and Singh, 2015).

Two genes, Xa13 and Xa21 obtained from the IRBB 55 rice variety, which confer resistance to bacterial leaf blight on rice, were incorporated into the genetic background of Pusa Basmati 1 through marker assisted backcross breeding, which led to the development of Improved Pusa Basmati 1 (Singh *et al.*, 2011). Similarly, Liu *et al.* (2021) transferred an executor gene Xa7 into the background of the agronomically superior but bacterial leaf blight susceptible rice cultivar CH448 through marker assisted breeding.

RNA Interference

Diverse RNA-based processes that result in sequence specific inhibition of gene expression that occur at the level of transcription or translation are called RNA interference (Dayou, 2018). Or gene silencing is renowned as post-

transcriptional gene silencing in plants, quelling in fungi and RNA interference (RNAi) in animals (Vaucheret and Fagard, 2001). Specific RNA silencing pathways present in plants are involved in controlling the expression of developmentally regulated genes, suppressing the mobility of transposable elements within the genes and defending against viral infection.

The presence of hairpin RNA or double stranded RNA (dsRNA) in cells induces RNA silencing, which is then cleaved into small (21 to 25 nt) products known as micro/small interfering RNA (mi/siRNA) by the dsRNA specific RNAseIII enzyme Dicer (Bernstein *et al.,* 2001). Subsequently, the mi/siRNAs associate with an RNA induced silencing complex that uses one of the mi/siRNA strands to scan endogenous RNA molecules and cleaves those that have homology with the mi/siRNA (Filipowicz, 2005). Five kinds of naturally occurring small RNAs, including microRNA, small interfering RNA, natural antisense transcript siRNA, repeat associated siRNA, and trans acting siRNA, have been described in *Arabidopsis thaliana* (Matsui *et al.,* 2013; Sharma *et al.,* 2023).

RNAi technology has emerged as one of the most promising strategies to enhance the resistance in plants to fight against several diseases caused by plant pathogens. The nature of this phenomenon has been evaluated in various host-pathogen systems and efficiently used to silence the action of pathogens. Host induced RNA interference technology was used to silence three pathogenicity genes viz., *FOW2, FRP1* and *OPR* in *Fusarium oxysporum* f. sp. *conglutinans*. Transgenic Arabidopsis lines with F. oxysporum infection showed significantly lower mRNA levels in all three of the targeted genes, which also improved resistance and delayed the onset of disease symptoms (Hu *et al.,* 2015).

Omics Technologies

Crop improvement is facilitated with new age technologies like genome sequencing, transcriptome sequencing, proteome sequencing, metabolomics profiling, target site modification, the development of candidate gene and genome wide markers to address various biotic and abiotic stresses in a better manner. Analysis of sequencing data from genome wide association studies and transcriptomics in combination with data from proteomics and metabolomics can provide an integrated approach to understanding plant-pathogen interaction at various levels. A combined approach of accelerated gene discovery with the assistance of genomics, transcriptomics, proteomics and metabolomics is proving to be an effective way to speed up disease resistance breeding programmes across the globe (Eldakak *et al.,* 2013; Kumar *et al.,* 2023).

Genomics

Genomics is the study of the organisational structure and functional role of an entire genome, including all the genes present in an organism. Genome editing is a kind of genetic engineering in which a DNA segment is either inserted or replaced or removed from the desired genomic region using artificially engineered nucleases. The success of genome editing depends on two natural DNA repair mechanisms viz., Non homologous end joining and Homology directed repair. Engineered nucleases like CRISPR/Cas (Clustered Regularly Interspaced Short Palindromic Repeats), Mega- nucleases, TALENs (Transcription Activator like Effector Nucleases) and ZFNs (Zinc Finger Nucleases) can cleave any targeted region in the genome and induce modifications that are impossible using conventional RNAi.

CRISPR

Clustered regularly interspaced short palindromic repeats are the segments of prokaryotic DNA containing repetitive base sequences. Basically, CRISPR plays a crucial role in a bacterial defence system, which forms the cornerstone of a genome editing technology known as CRISPR/Cas9 that allows permanent modification of desired genomic regions within organisms (Gowda *et al.*, 2020).

The general protocol for employing CRISPR/cas9 as a genome editing tool involves the selection of the target genomic region that has to be edited, followed by the design of guide RNA. Then both the Cas protein complex and guide RNA construct are assembled into a suitable transformation vector and subsequently delivered to plants. Later, the transformed plants are regenerated and screened for the edited region of the genome.

The complementary sequence based interaction between the DNA of the target site and the guide (noncoding) RNA allows the CRISPR/Cas9 system to recognise a target site. The guide RNA and Cas protein complexes possess the necessary nuclease activity for exact cleavage of double stranded DNA using Cas9 endonuclease. CRISPR/Cas9 is highly efficient in target genome editing and much cheaper than other genome editing tools like ZFNs and TALENs, as they are costlier and require protein engineering.

Li *et al.*, (2017) demonstrated the generation of DNA level mutations on allotetraploid cotton using CRISPR/Cas9 with high efficiency and specificity, resulting in the development of transgenic plants with resistance against verticillium wilt. Tashkandi *et al.*, (2018) imparted resistance in Nicotianabenthamiana and Solanumlycopersicum against the tomato yellow leaf

curl virus through the CRISPR/Cas9 system. Wei *et al.* (2021) employed a CRISPR/Cas9 mediated precise homology directed repair system to insert an effector binding element, AvrXa23, in the promoter region of the susceptible xa23 allele in order to develop resistance to bacterial leaf blight in the rice cultivar, Nipponbare and established a new strategy for engineering broad spectrum resistance for Xanthomonas species in rice.

Transcriptomics

The study of RNA transcripts produced by the genome within a cell at a specific developmental stage using high throughput sequencing approaches is called transcriptomics. Techniques like microarrays and RNA sequencing are used to obtain transcriptomic data. Transcriptome profiling helps to find out the biochemical and signalling pathways, manipulated during plant-pathogen interactions, signifying the difference between compatibility and incompatibility phenomena in such interactions (Nibedita and Jolly, 2017). Transcriptomics enabled the discovery of several disease resistance genes, which resulted in substantial advances in the management of diseases in plants (Horgan and Kenny, 2011; Lowe *et al.*, 2017).

A transcriptomic study was carried out on Oryza meyeriana leaves infected with Xanthomonas oryzae by employing RNA sequencing to investigate the transcriptional responses and interactions between the host and the pathogen, (Cheng *et al.,* 2016). The development of transcriptomics technology has envisioned the usage of RNA sequencing for transcripts or gene expression profiles in the management of numerous diseases in plants (Prabha *et al.,* 2013). The understanding of the interactions between various diseases and host plants has improved as a result of RNA sequencing. One such study was conducted by Yang *et al.,* (2017) to compare transcriptional changes in the stems of Nicotianatabacum infected with Phytophthoranicotianae.

Proteomics

Genome sequence gives an idea about the sequence of proteins, which thereafter undergo some post-translational modifications in eukaryotic cells. Genomics fails to explain such modifications, whereas proteomics can interpret the biological relevance of post-translational modifications and transcript advances (Park, 2004 and Thurston *et al.,* 2005). Through proteomics, an entire set of proteins produced in a cell during a particular developmental stage in a given environment, as well as their functional roles and interactions, can be investigated (Zulkarnain *et al.*, 2015, Singh and Singh, 2015). This approach is

useful to identify the pattern and specificity of a particular protein released in a plant cell when there is a pathogen attack. Typical methods for proteomics analysis include Two dimensional electrophoresis, Fluorescence two dimensional difference gel electrophoresis, Mass spectrometry and Multidimensional protein identification technology (shotgun approach) (Chandramouli and Qian, 2009).

The Proteomics approach was used by Li *et al.,* (2012) to identify nearly 1500 proteins that are upregulated after bacterial leaf streak infection in leaves of rice crop of which 23 were significantly associated with disease resistance. Proteomic studies help to elucidate the molecular mechanisms underlying plant disease and the functions of relevant genes.

Metabolomics

Metabolomics is the large scale study of small molecules (metabolites) present in the cells or tissues of an organism that represent the end products of cellular processes. These metabolites are not solely the end product of gene expression patterns but can also serve as regulatory molecules (Rochfort, 2005). Interactions between plants and pathogens could be precisely studied through the identification and quantification of various metabolites (Rojas *et al.,* 2014; Jayhoon *et al.,* 2023). Identification and quantification of various metabolites produced during the infection process could help to understand the interactions between plant and pathogen in a much better manner. The most commonly used techniques in metabolomics analysis are gas chromatography, high performance liquid chromatography, mass spectrometry and nuclear magnetic resonance spectroscopy (Kasture *et al.,* 2012).

Investigation of altered metabolites isolated from tomatoes infected with Botrytis cinerea and Pseudomonas syringae revealed that resistance in the host plant is linked with metabolomics reprograming in the host and leads to biochemical changes in tomatoes (Gemma *et al.,* 2015). Omics technologies offer several advantages over traditional methods but are associated with certain limitations like biassed results, complex methodologies, the requirement of huge initial investments and high end laboratories, difficulty in operation, data analysis and management (Lay *et al.,* 2006).

III. CONCLUSION

The scientific community is witnessing how plant diseases are threatening crop yields worldwide through their continuous evolution and hence there is a need

to implement all possible disease management strategies to tackle such challenges. The advancements in the area of plant pathology, in conjunction with biotechnology and molecular biology, have opened new avenues for formulating effective strategies for the management of plant diseases. Genetic engineering, transgenics and tissue culture have greatly enhanced the ability of crop plants to withstand or resist pathogen attack. Subsequent progress in DNA marker technology and marker assisted selection provides new solutions for the selection of disease resistant genotypes in early segregated populations, to produce varieties with multiple disease resistance and to incorporate resistance genes into agronomically superior but disease susceptible cultivars. Various omics technologies are strengthening plant disease management by unleashing the molecular basis of host-pathogen interaction and disease resistance mechanisms. They assist in engineering plants to express resistance that can either recognise pathogen molecules or finely tune hormone signalling for the benefit of crop yield. Although there are several tools, techniques and methods available, each has its own advantages and limitations. Upcoming research and technological advancement need to concentrate on the practical application of new tools and techniques in addition to addressing shortcomings associated with them. Reliance on biotechnology alone may not yield desirable results. Therefore, both conventional breeding and biotechnology assisted molecular breeding need to go hand in hand to develop crop varieties and hybrids that can resist multiple diseases without losing economic yield, so as to enable the farmers to minimise the expenditure on management aspects of plant diseases.

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Questions

1. Elaborate on the use of RNA interference (RNAi) as a biotechnological tool for controlling plant diseases.
2. Explain the concept of marker assisted backcrossing with examples.
3. Discuss the key steps involved in using CRISPR/Cas9 for targeted genome editing in plants.
4. Explain the concept of genetic engineering in the context of plant disease management. How can the manipulation of plant genes contribute to enhanced disease resistance?
5. Discuss the significance of omics technologies in understanding the molecular interactions between plants and pathogens.

Self Assesment

1. The technique of raising plants under controlled conditions known as?
   a. Hydroponics
   b. Tissue culture
   c. Greenhouse cultivation
   d. Organic farming

2. Tissue culture method commonly used to obtain virus free plants in vegetatively propagated plants is
   a. In vitro shoot grafting
   b. Somaclonal variation
   c. Meristem culture
   d. Protoplast culture

3. Which technique allows the direct transfer of genes to a wide range of cells and tissues and is appreciated for species with high levels of heterozygosity?
   a. Agrobacterium-mediated transformation
   b. Somaclonal variation
   c. Particle bombardment
   d. In vitro shoot grafting

4. What soil bacteria are extensively used in Agrobacterium-mediated transformation?
   a. Escherichia coli
   b. Pseudomonas aeruginosa
   c. Bacillus subtilis
   d. Agrobacterium tumefaciens

5. Transformation method that offers stable transformation with high efficiency but suffers from host specificity and protoplast requirements?
   a. Agrobacterium-mediated transformation
   b. Particle bombardment
   c. Biolistic transformation
   d. Somaclonal variation

6. Solution that is used to disinfect vegetative buds in the tissue culture process is
   a. Mercuric chloride
   b. Aluminium chloride
   c. Magnesium chloride
   d. Zinc chloride

7. A desired gene obtained from an organism that is incorporated into the genome of a related or unrelated species of another organism is called
   a. Transgene
   b. Jumping gene
   c. Housekeeping gene
   d. Basic gene

8. What is a potential drawback of Agrobacterium-mediated transformation?
   a. Low transformation efficiency
   b. Random integration of transgenes
   c. High specificity to host plants
   d. Additional expenditure

9. Coat protein mediated resistance was developed in which of the following disease
   a. Papaya leaf curl
   b. Tomato leaf curl
   c. Pigeon pea mosaic
   d. Papaya ringspot

10. Agrobacterium tumefaciens, which is known to cause which of the following disease
    a. Root rot
    b. Root knot
    c. Crown gall disease
    d. Leaf blight

11. Method that involves the selection of plants from a large population at an early generation with a desired genetic background at specific loci while maintaining adequate allelic variants
    a. Tissue culture
    b. Marker assisted selection
    c. RNA interference
    d. Anther culture

12. The most effective way to introgress a single gene or a quantitative trait locus affecting the trait of interest while maintaining the good agronomic traits of the recurrent parent in a backcross programme is
    a. Test cross
    b. Marker assisted backcrossing
    c. Single cross
    d. None of these

13. What is Marker Assisted Backcrossing (MABC) used for in plant breeding programs?
    a. Accelerate recovery of the donor parent genome
    b. Increase the number of backcrosses
    c. Enhance the length of the donor DNA segment
    d. Slow down the backcrossing process

14. Marker Assisted Backcrossing involves which of the following
    a. Foreground selection
    b. Recombinant selection
    c. Background selection
    d. All of these

15. Foreground selection in MABC aims to maintain target locus in a condition
    a. Homozygous
    b. Heterozygous
    c. Hemizygous
    d. None of these

16. What is the purpose of background selection in MABC?
    a. Maintain the target locus
    b. Reduce linkage drag
    c. Select plants with the marker allele
    d. Induce recombination events

17. Improved Pusa Basmati 1 wasincorported with the following genes that confer resistance to bacterial leaf blight
    a. xa12 and Xa21
    b. xa13 and Xa21
    c. xa13 and Xa22
    d. xa21 and Xa31

18. Diverse RNA-based processes that result in sequence specific inhibition of gene expression that occur at the level of transcription or translation are called
    a. RNA interference
    b. gene silencing
    c. post-transcriptional gene silencing
    d. All of these

19. What enzyme cleaves double-stranded RNA into small products in RNA interference?
    a. DNA polymerase
    b. Helicase
    c. Dicer
    d. Ligase

20. Which type of RNA is associated with an RNA-induced silencing complex in RNA interference?
    a. Messenger RNA (mRNA)
    b. Small Interfering RNA (siRNA)
    c. Ribosomal RNA (rRNA)
    d. Transfer RNA (tRNA)

21. What is the significance of Host-induced RNA interference technology in plant disease resistance?
    a. Enhances pathogen action
    b. Suppresses host genes
    c. Silences the action of pathogens
    d. Promotes disease symptoms

22. Genetic engineering technique in which a DNA segment is either inserted or replaced or removed from the desired genomic region using artificially engineered nucleases is
    a. Genome sequencing
    b. Genome editing
    c. Gene silencing
    d. Gene expression

23. Which DNA repair mechanisms does the success of genome editing depend on?
    a. Non-homologous end joining
    b. Homology directed repair
    c. Both a and b
    d. None of these

24. What is metabolomics?
    b. Study of proteins in a cell
    c. Investigation of small molecules in cells or tissues
    d. Analysis of RNA transcripts
    e. Examination of post-translational modifications

25. The study of RNA transcripts produced by the genome within a cell at a specific developmental stage using high throughput sequencing approaches is called
    a. Metabolomics
    b. Proteomics
    c. Transcriptomics
    d. None of these