BHARATHIDASAN UNIVERSITY
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Programme: M.Sc., Biotechnology (Environment)

Course Title : Plant and Animal Biotechnology
Course Code: CO 03

Unit-III
Stress tolerance and resistance

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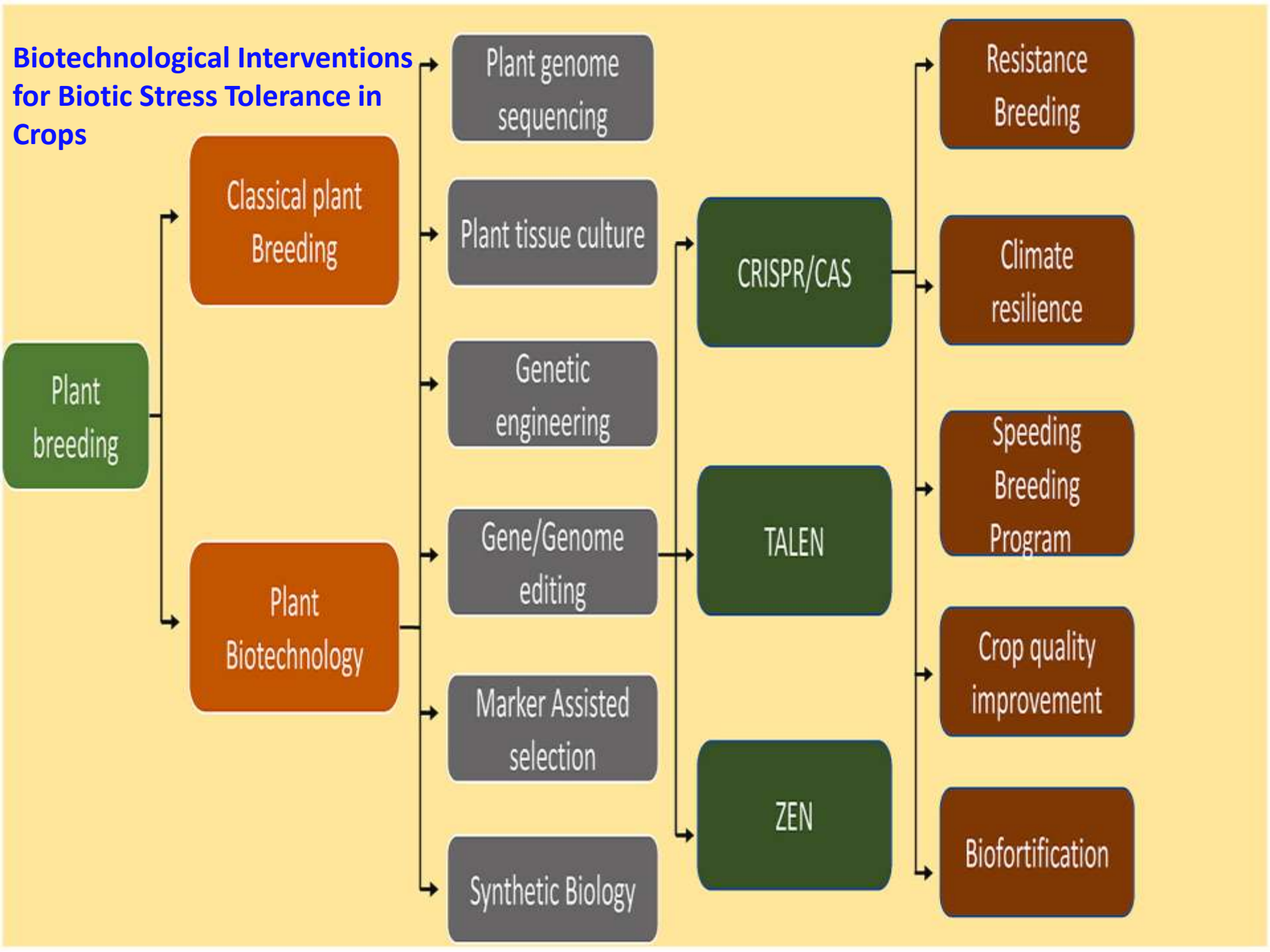
Biotechnological Interventions for Biotic Stress Tolerance in Crops



- Plants are constantly exposed to biotic stress factors that affect their growth, development, and productivity.
- This interaction involves complex molecular mechanisms of resistance, tolerance, susceptibility, and sensibility
- plants are limited by biotic factors that are constantly challenging their integrity, such as viroids, viruses, bacteria,fungi, oomycetes, protists, mycoplasmas, nemato des, invertebrates and other plants.
- The interaction of plants with these biotic factors has been a focus of attention because of their phytosanitary impact for crop production.
- How plants defend themselves against diseases and pests is the basis to develop sustainable control methods

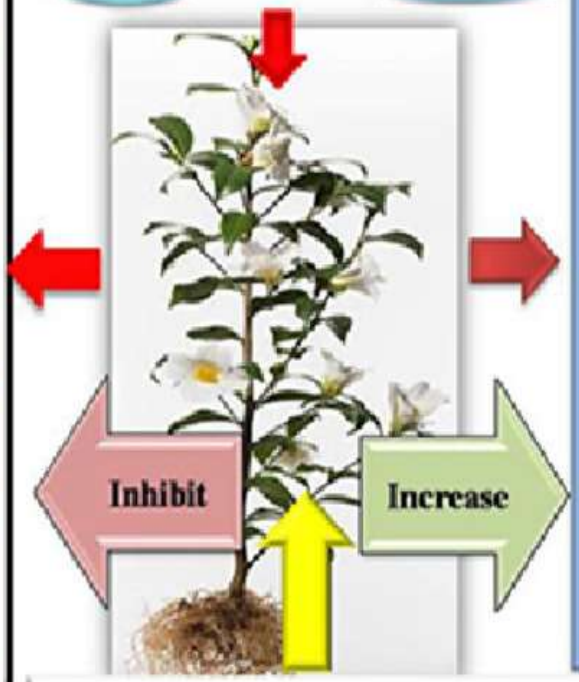
- Biotic stress triggers loss of essential nutrients that finally lead to cell death.
- Plants have a series of defense mechanisms to combat ill effects of biotic stress.
- **Phytohormones** strengthen plant immunity by regulating stress responsive compounds.
- **Induced mutation** enhances gene expression patterns thereby tolerance to stressors.
- **Gene editing by CRISPR-Cas9** is an efficient approach for site-specific mutation.
- **TILLING** is an effective process to characterize single base pair change in genome.

Physiological and Biochemical Effects

- Clorosis
- Disease susceptibility
- Impaired cell division
- Necrotic lesion
- Oxidative stress
- Nutrient deprivation
- Hormonal imbalance

BIOTIC STRESSORS

Phytoplasma | Bacteria | Virus | Nematode

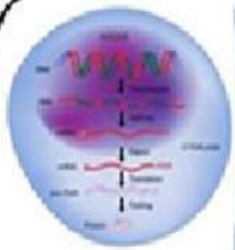


Natural Defense Mechanism

Change in Genetic Constituents

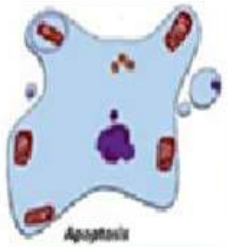
Mutagenesis

Physical mutagen	Chemical mutagen	Gene editing
Gamma rays, ultraviolet rays, Fast neutron, *etc.*	Alkylating agent, Sodium azide, *etc.*	ZFN, TALEN, CRISPR



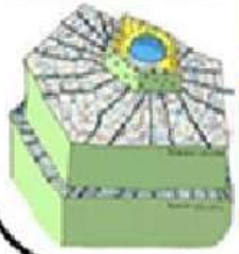
Expression of defence and pathogenesis related genes

Increase in level of cellular hormones

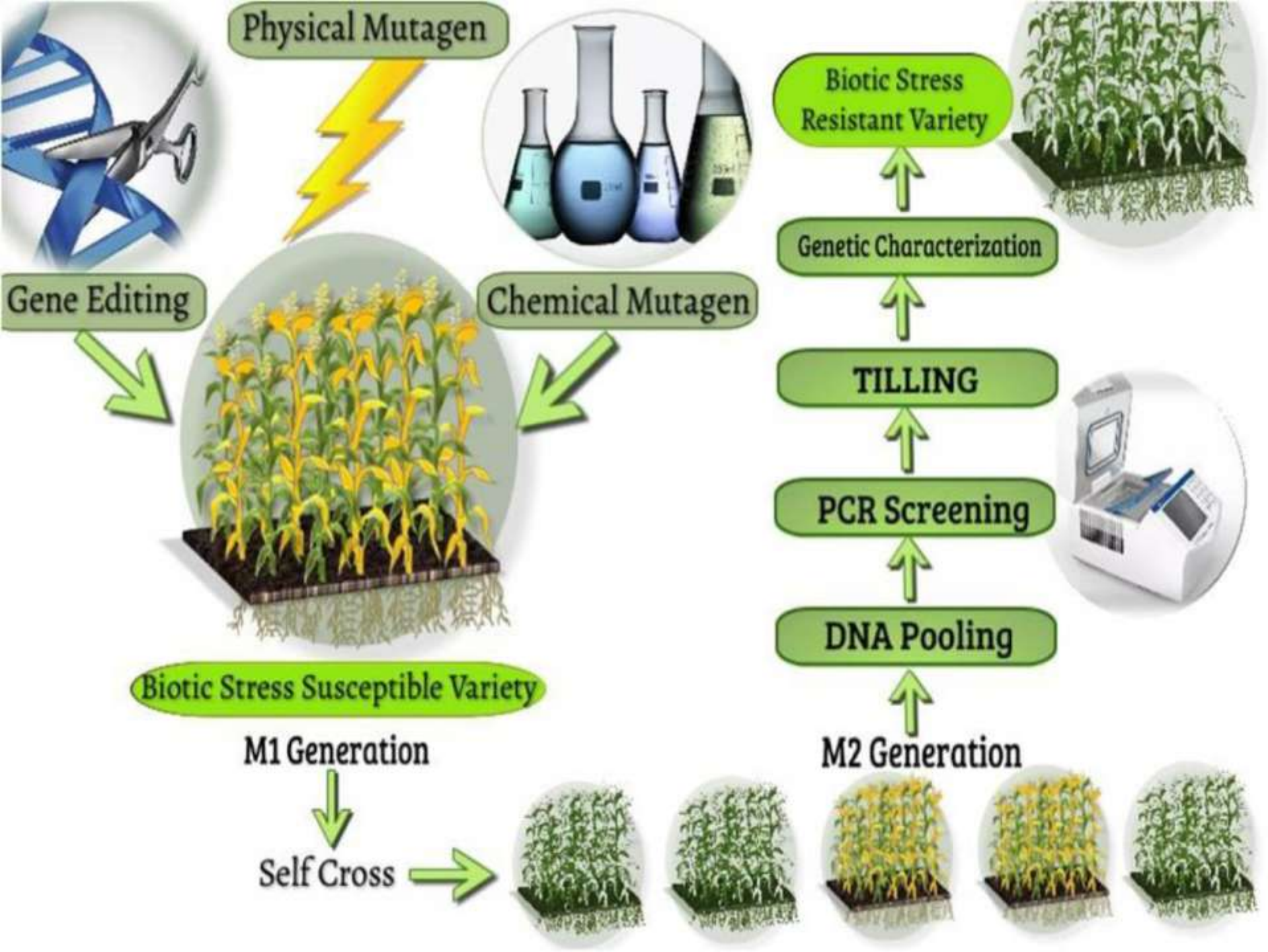


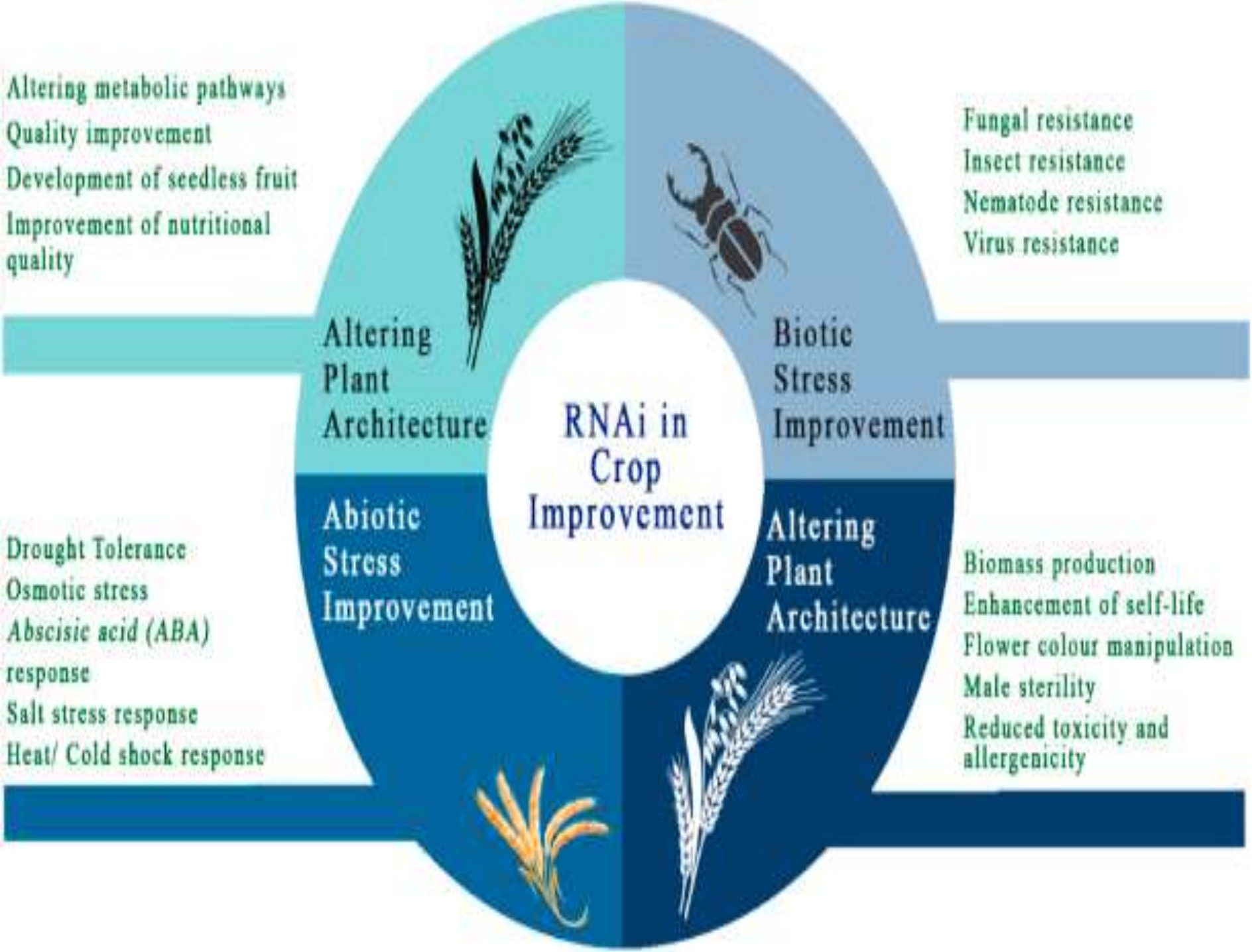
Activates programmed cell death

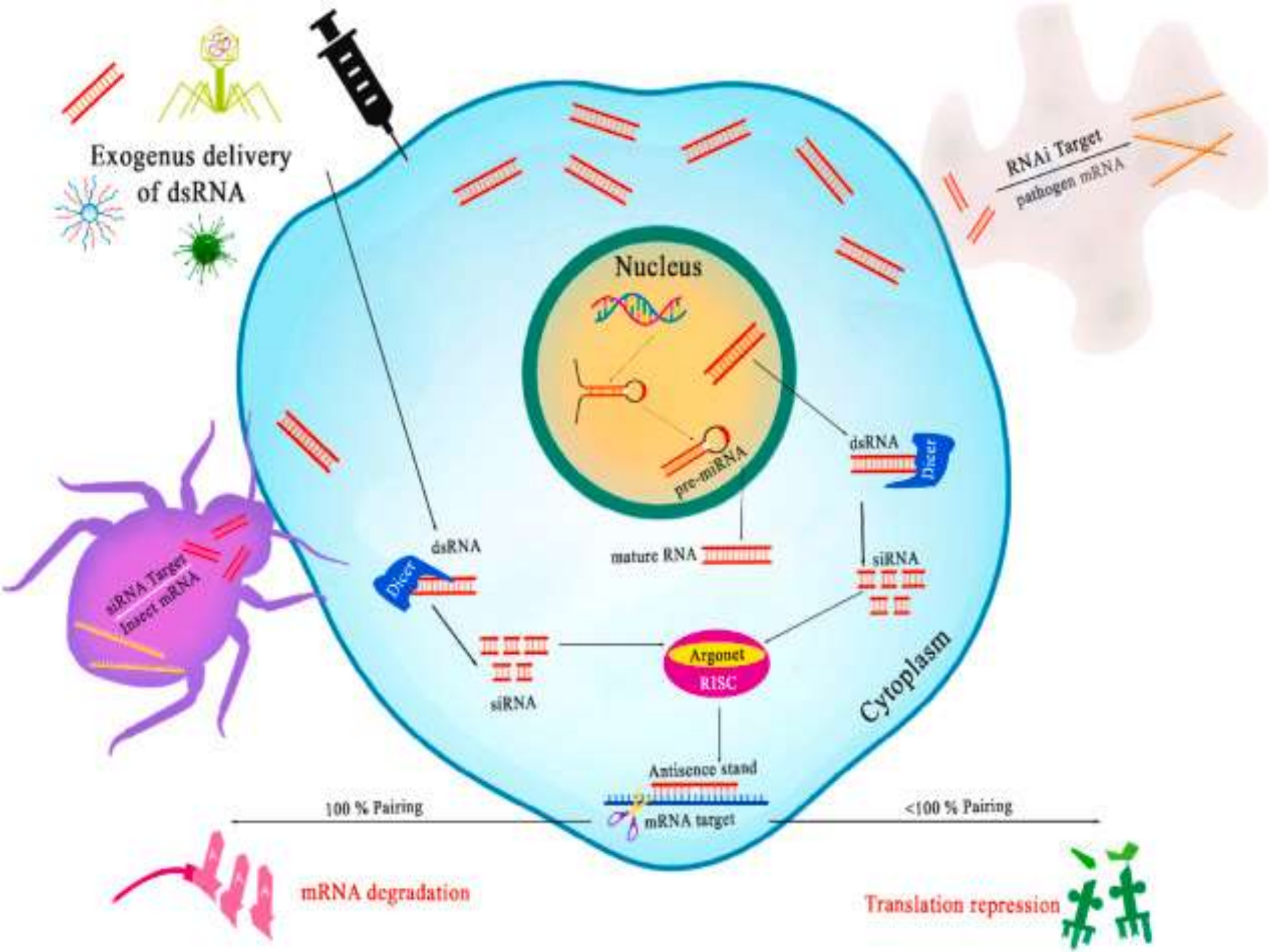
Accumulate Some Potent Low Molecular Weight Osmolites



Strengthens Cell Walls


Physical Mutagen
Gene Editing
Chemical Mutagen
Biotic Stress Susceptible Variety
M1 Generation
Self Cross
M2 Generation
DNA Pooling
PCR Screening
TILLING
Genetic Characterization
Biotic Stress Resistant Variety

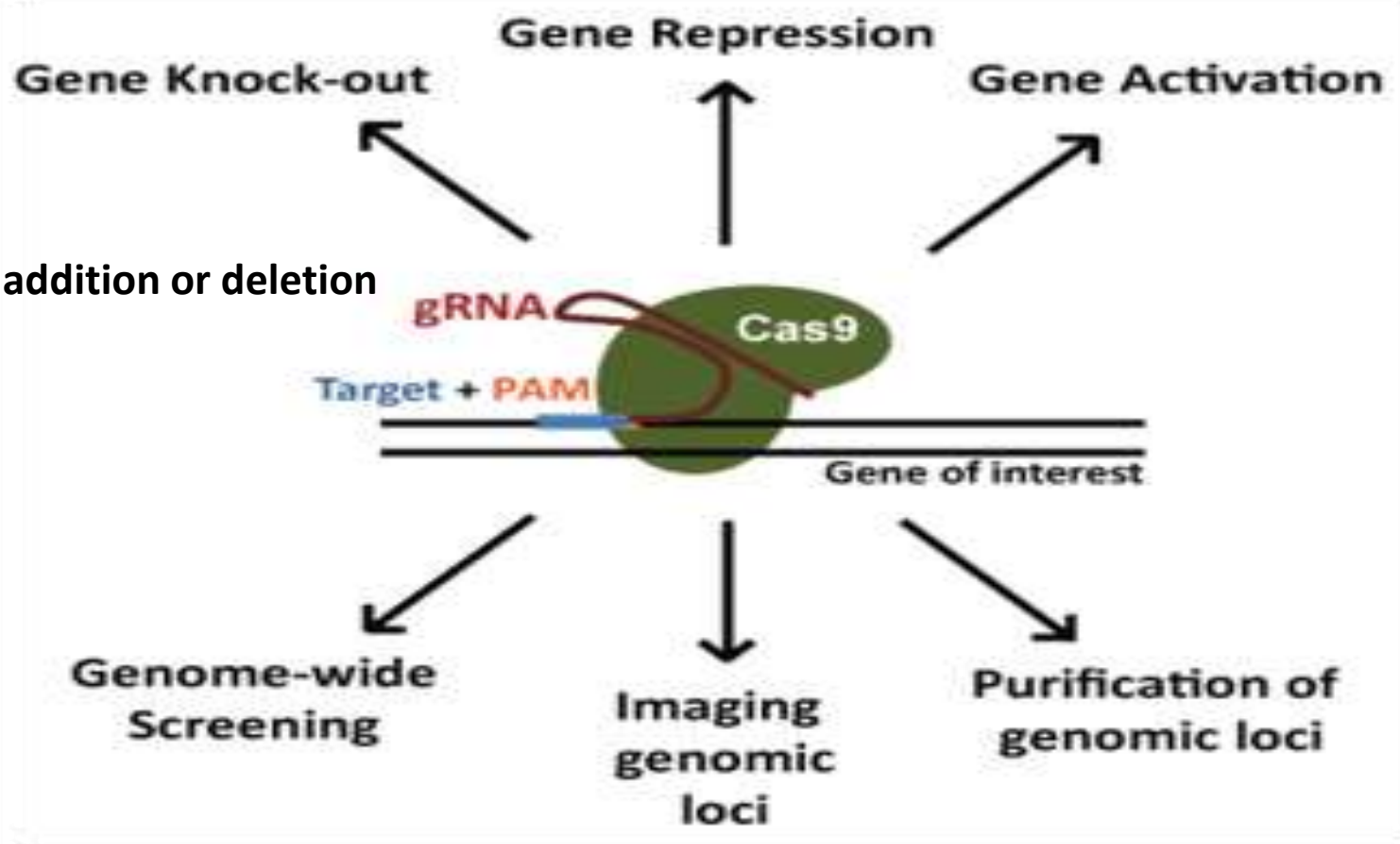

Altering metabolic pathways
Quality improvement
Development of seedless fruit
Improvement of nutritional quality
Altering Plant Architecture
Fungal resistance
Insect resistance
Nematode resistance
Virus resistance
Biotic Stress Improvement
RNAi in Crop Improvement
Abiotic Stress Improvement
Drought Tolerance
Osmotic stress
Abscisic acid (ABA) response
Salt stress response
Heat/ Cold shock response
Altering Plant Architecture
Biomass production
Enhancement of self-life
Flower colour manipulation
Male sterility
Reduced toxicity and allergenicity

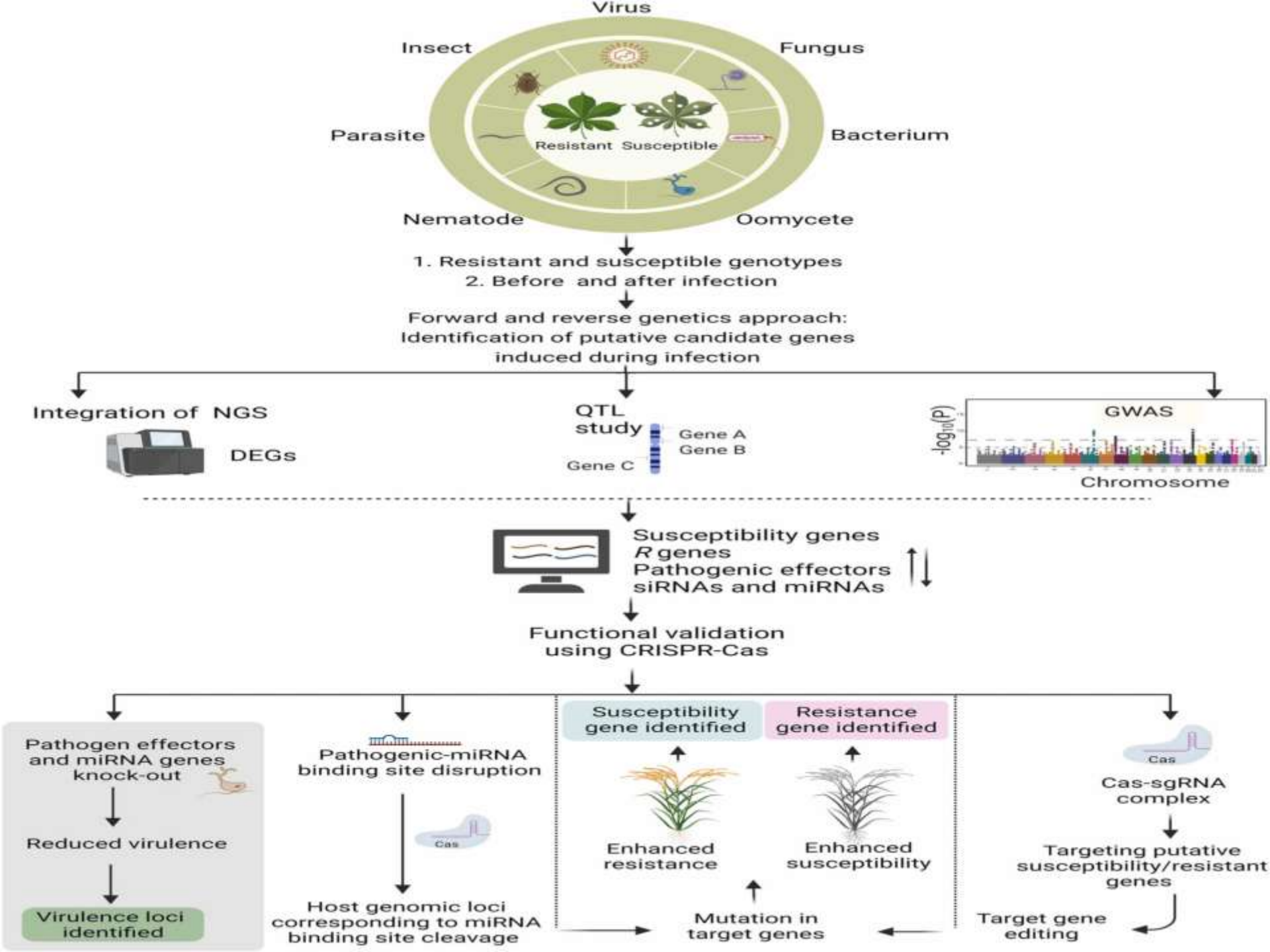

Exogenus delivery
of dsRNA
RNAi Target
pathogen mRNA
Nucleus
pre-mRNA
mature RNA
dsRNA
Dicer
siRNA
Argonet
RISC
Cytoplasm
siRNA Target
Insect mRNA
Antisence stand
mRNA target
100 % Pairing
<100 % Pairing
mRNA degradation
Translation repression

Genome engineering is a term used to describe the process of making specific, targeted alterations in the genome of a living organism.

- **Genetic engineering** is the direct manipulation of an organism's DNA using any number of methods.
- **GMO** is the genetic modification of organisms. It's been around for a while and uses imprecise methods of genetic engineering.
- **Genome editing/engineering** is now a more precise method of genetic engineering which hopes to avoid any bad associations with GMO.

CRISPR-Cas9 Application




Virus
Insect
Fungus
Parasite
Bacterium
Resistant
Susceptible
Nematode
Oomycete
1. Resistant and susceptible genotypes
2. Before and after infection
Forward and reverse genetics approach:
Identification of putative candidate genes
induced during infection
Integration of NGS
DEGs
QTL
study
Gene A
Gene B
Gene C
GWAS
-log10(p)
Chromosome
Susceptibility genes
R genes
Pathogenic effectors
siRNAs and miRNAs
Functional validation
using CRISPR-Cas
Pathogen effectors
and miRNA genes
knock-out
Reduced virulence
Virulence loci
identified
Pathogenic-miRNA
binding site disruption
Cas
Host genomic loci
corresponding to miRNA
binding site cleavage
Susceptibility
gene identified
Resistance
gene identified
Enhanced
resistance
Enhanced
susceptibility
Mutation in
target genes
Cas
Cas-sgRNA
complex
Targeting putative
susceptibility/resistant
genes
Target gene
editing

Plant	Gene	Pathogen	Plant	Gene	Pathogen	Plant	Gene	Pathogen
Rice	*OsSWEET 11/13/14* *OsCUL3a*	Bacterium	Tomato	*SlJaz2* *SlDmr6*		Arabidopsis	*AtIAN9*	
	OsERF922 *OsBSR-K* *OsSec3A* *OsPid2* *Osmes1* *OsPP2A-1*	Fungus		*SlPMR4* *Slmlo1* *SlyFRG4* *miR482b* *miR482c* *SlWAT1*			*AtErf019* *AtRaf36*	
	Viral RNA *OseIF4G*	Virus		Virus DNA, IR,Rep,CP *SleIF4E1* *SlPelo* *SleIF4E2*			Viral DNA, RNA Rep,IR,CP *AtEIF4E* *AtEIF(iso)4E* *AteIF4E1*	
	OsCYP7A1	Insect		*SlMAX1*				
Wheat	*TaMLO-B1* *TaMLO-A1* *TaEdr1* *TaNFXL1*		Grape	*VvMlo6* *DMR* *VvWRKY52* *VvMlo-7* *VvMlo3* *VvPR4b*		Potato	*StDND1* *StCHL1* *StDMR6-1* *StDMR6-2*	
							Viral P3,CI Nib,CP *StCoilin*	
Soybean	*GmF3H1/2,* *GmFNSII-1*		Maize	*LOX3*		Oilseed rape	*BnWRKY11* *BnWRKY70* *BnCRT1a* *BnHK* *BnF5H* *BnQCR8*	
				ZnGD1a				
Cotton	*Gh14-3-3d*		Cocoa	*TcNPR3*	Oomycete	Apple	*MdDIPM 1/2/4*	
Tobacco	Viral RBS,IR hairpin,RCRII Rep,IR,CP,Hc-Pro		Barley	*HvMorc1* *HvMorc 6a*		Banana	*MusaDMR6*	
	P3,CI, NIB,CP,1A, 3'UTR-A *CLC-Nb1a/b* *CLCuMuV C1*			*HvEIF4E* MP/CP, Rep/RepA, LIR			Viral DNA	
Cucumber	*CseIF4E*		Citrus	*CsLOB1* *CsWRKY22*		Watermelon	*Clpsk1*	
Sorghum	*SbLGS1*	Parasitic plant	Cassava	*MeSWEET10a*		Sweet Basil	*ObHSK*	
				MenCBP-1,2 *Men-eIF4E*			*ObDMR1* *ObDMR6*	

Table 2 Technologies in use of improving biotic stress-tolerant agricultural plants.

Plant species	Technologies	Targets	Resistance against
Arabidopsis thaliana	Artifical miRNA	TuMV coat protein sequences	Turnip yellow mosaic virus (TYMV) and turnip mosaic virus (TuMV) [114]
	CRISPR/Cas9	eIF(iso)4E	Turnip mosaic virus (TuMV) [115]
Solanum lycopersicum	Artificial miRNA	CMV 2a/2b genes and the 3′ UTR [44]; coat protein (AV1) and the pre-coat protein (AV2) gene sequences	Cucumber mosaic virus (CMV) [44]; Tomato leaf curl New Delhi virus (ToLCNDV) [116]
	CRISPR/Cas9	Mlo genes	Powdery mildew fungus (*Oidium neolycopersici*) [117]
	Hair-pin RNA	AC1 and AC4 genes [118]; mature viroid RNA	Tomato Leaf Curl Virus [118]; Potato spindle tuber viroid [119]
Triticum aestivum	Artificial miRNA	5′ UTR region, ORF pipo region of P3 cistron, P1 gene, P3 cistron and HCpro gene	Wheat streak mosaic virus (WSMV) [30]
	CRISPR/Cas9	Mlo genes	Powdery mildew fungus (Blumeria graminis) [120]
Nicotiana tabacum	Artificial miRNA	HC-Pro and p25 gene	Potato Virus X (PVX) and Potato Virus Y (PVY) [29]
	Hair-pin RNA	acetylcholinesterase 2 coding gene (MpAChE2)	*Myzus persicae* [111]
Nicotiana benthamiana	Artificial miRNA	V2 gene sequence; P1 and Nlb genes; nucleoprotein (N) and silencing suppressor (NSs) genes; L (replicase) gene	Cotton leaf curl Burewala virus (CLCuBuV) [121]; Cassava brown streak virus (CBSV) [122]; Tomato spotted wilt virus (TSWV) [123]; Watermelon silver mottle virus (WSMoV) [124]
	CRISPR/Cas9	tomato yellow leaf curl virus (TYLCV); AGO genes	Tomato yellow leaf curl virus (TYLCV) [125]; Cymbidium ringspot virus (CymRSV), Carnation Italian ringspot virus (CIRV) and turnip crinkle virus (TCV) [126]
	Hair-pin RNA	MP gene and Rep gene	Tobacco mosaic virus and Cucumber mosaic virus [127]

Table 2 Technologies in use of improving biotic stress-tolerant agricultural plants—cont'd

Plant species	Technologies	Targets	Resistance against
Hordeum vulgare	Artificial miRNA	Rep and/or RepA proteins (C1 and/or C2 genes) and movement protein (MP) (V1 gene)	Wheat dwarf virus (WDV) [128]
	Hair-pin RNA	hpBYDVpol gene	Barley yellow dwarf virus-PAV (BYDV-PAV) [129]
Vitis vinifera	Artificial miRNA	CP gene [130]; GVA ORF1 and ORF5	Grapevine fanleaf virus (GFLV) [130]; Grapevine virus A (GVA) [131]
Glycine max	Artificial miRNA	Rhg1 genes; Glyma18g02680.1 gene; J15, J20, and J23 genes	Soybean cyst nematode (SCN) [132–134]
	Hair-pin RNA	Rhg1 genes	Soybean cyst nematode (SCN) [132]
Oryza sativa	Artificial miRNA	xa13 gene	Rice bacterial blight (*Xanthomonas oryzae*) [135]
	CRISPR/Cas9	OsERF922 gene	Rice blast (*Magnaporthe oryzae*) [136]
Solanum tuberosum	Artificial miRNA	Avr3a avirulence effector	*Phytophthora infestans* [137]
	Hair-pin RNA	coat protein gene	potato virus Y(PVY) [138]
Citrus sinensis	CRISPR/Cas9	CsLOB1 promoter	Citrus canker (*Xanthomonas citri*) [139]

Thank You