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Role of RNAi in Crop Improvement

Amaresh*[1] and Aswini Nunavath[2]
[1]Ph.D Scholar, Division of Genetics,Indian Agricultural Research Institute, New Delhi
[2]Ph.D Scholar, Department of Genetics and Plant Breeding, Professor Jayashankar Telangana State Agricultural University, Hyderabad

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Introduction

The RNA interference (RNAi) is involved in target-specific gene regulation driven by the introduction of dsRNA resulting in transcriptional repression or inhibition of translation. The discovery of RNAi and its regulatory potentials, it has become obvious that RNAi has huge potential in opening a new outlook for crop improvement. RNAi technology is accurate, stable, efficient and better than antisense technology. It has been employed successfully to alter the gene expression in plants for better biotic and abiotic stresses and the nutritional improvement in terms of bio-fortification and bio-elimination. Thus, there is huge potential of RNAi to improve the agriculture crop productivity.

Andrew Fire and Craig Mellow (1998) proved that dsRNA is responsible for endogenous silencing of mRNA. Play important role in eukaryotic gene regulation at both the transcriptional(by triggering epigenetic modifications, including DNA methylation and histone modifications) and post-transcriptional (controlling mRNA stability or preventing translation) levels. Different types of small RNA involved in plant response with typical biogenesis mechanism play significant role in gene regulation of crop plants (Table 1).

miRNA

Produced from long primary transcripts of miRNAs (pri-miRNAs) containing at least one hairpin-like miRNA precursor processed by RNase III (in nucleus) & generate precursor miRNA (pre-miRNA.This pre-miRNA transferred to cytoplasm, forms a stem & loop structures and cleaved by RNase III (i.e.Dicer) to generate mature miRNAs (~22 nt). The miRNAs with Argonaute protein & RNA-induce silencing.

siRNA

The dsRNA (~21 nt), either artificially synthesized or transferred from nucleus to cytoplasm, processed by DCR-1 to form ds-siRNA. The ds-siRNAs are connected to siRISC by RISC. Unfolded by a helicase to activate the siRISC. Activated siRISC is associated with the target mRNA.Degradation of complementary target mRNA (translation inhibition) will occur.

Table 1. Types of sRNAs involved in plant response

S. no	Class	Full form	Length (nt)	Originating loci	Biogenesis	Function
1	miRNA	microRNA	20–22	MIR genes	The hairpin structures of ssRNA transcripts are cleaved by DCL1 and HYL1	Silences expression of target genes by mRNA degradation and translational repression
2	siRNA	Short interfering RNA	24–30	Repeats, transposons, and retroelements (endogenous). Transgenes and viral RNAs (exogenous)	RDR-generated dsRNAs are cleaved by DCL-4 and HYL1	Silences gene expression by RNA-dependent DNA methylation and chromatin modification
3	ta-siRNA	Trans-acting-Short interfering RNA	21	TAS loci	miRNAs mediated cleavage of TAS genes, transcribed by RDR into dsRNA, and then processed by Dicers	Silences target gene expression through mRNA cleavage
4	nat-siRNA	Natural antisense transcript derived siRNA	20–22; 23–26	Loci generating pairs of sense-antisense transcripts	dsRNA derived from overlapping transcripts are cleaved by DCL1 and binds to AGO1	nat-siRNA silences target gene expression through mRNA cleavage

Difference between siRNA and miRNA

Properties	siRNA	miRNA
Origin	Encoded by transposons, viruses, heterochromatin	Encoded by their own genes (MIR genes). Transcribed by RNA Pol. II
Biogenesis	Long bimolecular RNA duplexes or extended hairpins	Single RNA molecules- include an imperfect stem–loop secondary structure
Evolutionary conservation	Rarely conserved in related organisms	Nearly always conserved in related organisms
Natureof Regulatory targets	Mediate the silencing of the same (or similar) genes from which they originate	Regulate different genes
Role	Plant immune mechanism	Regulation of a wide range

	as they help to prevent the spreading of foreign nucleic acid	of physiological responses and developmental processes

Components of gene silencing

DCL: It is RNAase III enzyme, It cleaves long dsRNA into 21-25 nt fragments. Eg. DCL1, DCL2, DCL 3, DCL 4 etc.

AGO: Agronaute protein binds small RNA and their targets. Eg. AGO1, AGO2, AGO3 AGO4, AGO5, AGO7

HEN 1 PROTEIN: It protects miRNA and siRNA from exonuclease activities by methylation of these RNA.

RDRP: RNA dependent RNA Polymerase is involved in amplification of siRNA amount. RDR2 and RDR6 are required for the production of 24nt siRNA.

Role RNAi in crop improvement

RNAi has several applications in crop improvement such as, Defence improvement against insect, virus, nematode, bacteria and fungi, Biofortification for β-Carotene and lycopene, carotenoid and flavonoid, starch, lysine, amylose and vitamin C, Bio-elimination for caffeine, cadmium, and morphine. Altered phenotype for flower colour, scent profile modification, parthenocarpy, enhanced shelf life, plant architecture, and Male sterility and fertility (Table 2 & 3).

Table2.RNAi-mediated improvised defence

S. no.	Defence improvement	Resistance against	Targeted gene	Plant used	References
1	Insect resistance	*Helicoverpa armigera*	*CYPAE14*	Cotton	Mao et al. (2007)
		Corn rootworm	V-ATPase A	Maize	Baum et al. (2007)
2	Virus resistance	Rice Dwarf Virus (RDV)	PNS12	Rice	Shimizu et al. (2009)
		Bean golden mosaic virus (BGMV)	AC1 gene	Bean	Bonfim et al. (2007)
		BYDV (Barley Yellow Dwarf Virus)	BYDV-PAV	Barley	Wang et al. (2000)
3	Nematode resistance	*Meloidogyne incognita*	splicing factor and integrase	Tobacco	Yadav et al. (2006)
		Meloidogyne	16D10	Arabidopsis	Huang et al. (2006)
		Meloidogyne javanica	Tis11	Tobacco	Fairbairn et al. (2007)
4	Bacteria resistance	*Xanthomonas citri* subsp. *citri* (Xcc)	PDS and CalS1	Lemon	Enrique et al. (2011)
5	Fungus resistance	*Phytophthora infestans*	SYR1	Potato	Eschen-Lippold et al. (2012)
		Phytophthora parasitica var. *nicotianae*	GST	Tobacco	Hernández et al. (2009)
		Blumeria graminis f. sp. *tritici*	MLO	Wheat	Riechen (2007)

Table 3. The gene targeted for RNAi-mediated crop improvement

S. no.	Crop improvement	Traits improved	Gene targeted	Plant used	References
1	Biofortification	β-Carotene and lycopene	NCED1	Tomato	Sun et al. (2012)
		Carotenoid	ε-CYC	*Brassica napus*	Yu et al. (2007)
		Carotenoid and flavonoid	DET 1	Tomato	Davuluri et al. (2005)
		Carotenoid with decreased sinapate esters	DET1	*Brassica napus*	Wei et al. (2009)
		β-Carotene and lutein	BCH	Potato	Eck et al. (2007)
		Starch	AtGWD	Maize	Weise et al. (2012)
			AtGWD and AtSEX4	Arabidopsis	Weise et al. (2012)
		Lysine	ZLKR and SDH	Maize	Houmard et al. (2007)
			Maize zein storage protein	Maize	Segal et al. (2003)
		Amylose	SBE IIa and SBE IIb	Wheat	Regina et al. (2006)
			SBE IIa and SBE IIb	Barley	Regina et al. (2010)
		Stearic- and oleic- fatty acids	Stearoyl-acyl-carrier protein Δ9-desaturase and oleoyl-phosphatidylcholine ω6-desaturase	Cotton	Liu et al. (2002)
		Vitamin C	APX	Tomato	Zhang et al. (2011)
2	Bio-elimination	Caffeine	CaMXMT 1	*Coffea canephora*	Ogita et al. (2003)
		Cadmium	PCS	Rice	Li et al. (2007)
		Morphine	Codeine Reductase (COR)	*Papaver somniferum (Opium poppy)*	Allen et al. (2004)
3	Altered phenotype	Flower colour: blue to white	CHS	Torenia hybrida cv. Summerwave Blue	Fukusaki et al. (2004)
		Scent profile modification	PhBSMT	Petunia	Underwood et al. (2005)
		Parthenocarpy	AUCSIA	Tomato	Molesini et al. (2009)
			CHS	Tomato	Schijlen et al. (2007)
		Male sterility	TA29	Tobacco	Nawaz-ul-Rehman et al. (2007)
			GEN-L	Rice	Moritoh et al. (2005)
			BCP1	*Arabidopsis thaliana*	Tehseen et al. (2010)
		Fertility restored	orfH522	Tobacco (male sterile)	Nizampatnam and Kumar (2011)

Conclusion

RNAi can be largely effective for functional genomics and biotechnology of annual as well as perennial plants. To meet the worldwide demand for food, fibre and fuel the production of improved crops are looked-for, which will be provided with the advances in RNAi technology. There are numerous opportunities for the applications of RNAi for its improvement such as stress-tolerance, enhanced nutritional level and higher yield. Thus, there is vast potential of RNAi to improve the agricultural crop yield significantly.

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