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Gene Silencing in Horticultural Transgenic Plants: Mechanisms, Technologies and Applications in Horticultural Crops

S. Choudhary¹, S. K. Barolia^{1*}, R. Sharma², R. S. R. Krishnan³,
M. R. Hindala⁴ and M. Sewda⁴

¹ICAR-National Research Centre on Seed Spices Tabiji Ajmer, Rajasthan, India

²Biotechnology Centre, Jawaharlal Nehru Krishi Vishva Vidhalya,
Jabalpur (Madhya Pradesh) India

³Jawaharlal Nehru Krishi Vishva Vidhalya, Jabalpur (MP) India

⁴Department of Botany, University of Rajasthan, Jaipur, India

*Corresponding author

ABSTRACT

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The gene silencing in horticulture transgenic crops is too crucial to know about the internal mechanism of plant at genomic level. Horticulture involves a variety of plants and crops including fruit, vegetables, spices, and ornamental plants. Gene silencing refers to the ability of a cell to prevent the expression of a certain gene. With the cleavage or translational repression of the messenger RNA (mRNA) molecules, the genes that form them are essentially inactive. Horticultural plants have evolved a variety of gene silencing pathways in which small RNAs (20–30 nucleotides) repress the expression of genes at the transcriptional or post-transcriptional level. Gene silencing using RNA interference technology, transcriptional gene silencing, and virus-induced gene silencing has been used in horticultural crops. The presence of double-strand RNAs inside the cell lead to the production of small interfering RNAs, short hairpin RNAs, and micro-RNAs. The production of their RNAs leads to the silencing of mRNA (actual protein coating RNA). This technique is a very effective experimental tool to silence specific genes for better stress tolerance, enhance insect/pest/pathogen resistance, and improve nutritional status. This technique and mechanism can be controlled by artificial means and applied for the improvement of cultivars of a variety of horticultural crops.

Introduction

Using RNAi technique, two ripening-specific ethylene-induced N-glycoprotein modifying enzymes, α -mannosidase (α -Man) and β -D-N-

acetyl hexosaminidase (β -Hex) were targeted and the shelf life of tomato enhanced by 2–2.5 fold with firmer fruits (Meli *et al.*, 2010). The recent study shows, the role of micro-RNAs in tomato fruit development and

ripening (Molesini *et al.*, 2012; Karlova *et al.*, 2013). The recent study shows that CNR is also negatively regulated by APETALA2a (a target of miR172). One way, it positively regulates fruit ripening. In another way, it negatively regulates ethylene synthesis (Karlova *et al.*, 2013). The silencing of ripening-related chalcone synthase (CHS) gene in strawberry fruits (*Fragaria x ananassa* cv. Elsanta) by a ihp-RNA construct containing the partial sense and corresponding antisense sequences of CHS separated by an introns obtained from a *F. ananassa* quinone oxidoreductase gene. The reduced levels of CHS mRNA and enzymatic CHS activity, the levels of anthocyanins were down-regulated and precursors of the flavonoids pathway were shunted to the phenylpropanoid pathway leading to large increases in levels of (hydroxy) cinnamoyl glucose esters. This technique in combination with metabolite profiling analysis will be useful for the development and ripening of strawberry fruit (Hoffman *et al.*, 2006).

RNAi for an enhancement of shelf life

Horticultural crops are more prone to post-harvest losses and deterioration in the quality and economic viability of many plant species. India is the largest producer of horticultural crops and ~30% post harvest losses in fruits and vegetables is due to spoilage (Agricultural Research Data Book, 2004). Therefore, there is an urgent need to increase shelf life of horticultural crops to minimize the deterioration and post harvest losses. The traditionally farmers used to harvest green fruits and middle man/ sale personal spray ethylene to fetch higher prices of ripen fruits but there is substantially decrease in flavor content. The shelf life in tomato has been increased by silencing of ethylene producing genes through RNAi technology. As tomato being a climacteric fruit, has autocatalytic activities of ethylene during its ripening

period. Therefore, an increase in self life or delaying in ripening process can be achieved by introduction of ACC oxidase ds RNA in tomato which suppress the expression of ethylene genes. In transgenic tomato plants targeting more homolog than a single unit of ACC oxidase (1aminocyclopropane -1-carboxylate) by using RNAi technology during ripening stage would be more effective rather than single homolog (Gupta *et al.*, 2013). The chimeric RNAi-ACS construct designed to target ACS homologs, effectively repressed the ethylene production in tomato fruits enhanced the tomato self-life by 45 days in tomato.

The ethylene suppression brings compositional changes in the fruits by enhancing polyamine (PA) levels. At same time, decreased levels of ethylene in RNAi-ACS fruits lead to the altered levels of various ripening-specific transcripts (i.e. up-regulation of PA biosynthesis, ascorbic acid (As-A) metabolism genes & down-regulation of cell wall hydrolysing enzyme genes). The down-regulation of ACS homologs using RNAi can be an effective approach for obtaining delayed ripening with longer shelf life and an enhanced processing quality of tomato fruits, owing to the reduced rate of softening.

The silencing of a ripening-related chalcone synthase (CHS) gene in strawberry fruits (*Fragaria X ananassa* CV. Elsanta) by a ihp RNA construct containing the partial sense and corresponding antisense sequences of CHS separated by an intron obtained from a *F. ananassa* quinone oxidoreductase gene. The reduced levels of CHS mRNA and enzymatic CHS activity, the levels of anthocyanins were down regulated and precursors of the flavonoids pathway were shunted to the phenylpropanoid pathway leading to large increases in levels of (hydroxy) cinnamoyl glucose esters. This technique in combination

with metabolite profiling analysis will be useful for the development and ripening of strawberry fruit (Hoffman *et al.*, 2006).

RNAi for nutritional quality improvement-Bio-fortification

Most of the world's population lacks one or more essential mineral elements in his food supplements (White and Broadley, 2009). RNAi being a promising technology in recent year has great potential to improve better quality and nutritional quality traits by modifying the expression of genes. Bio-fortification of plant using RNAi provides essential elements like Cu, Se, Ca, Fe, I, S, P, Zn, Mg, etc., (Table 1) and dietary antioxidants such as in tomatoes (Niggeweg *et al.*, 2004). RNAi play key role in development of fruit & vegetables with seedless-ness with desired agronomic traits, with enhanced nutritional quality such as carotenoid and flavonoids contents, both are highly beneficial for human health. RNAi mediated bio-farming or molecular farming is another approach for production of plant derived proteins, essential oils, starch, vitamins, antioxidants etc. for pharmaceutical industry. RNAi in combination with fruit specific promoter used to suppress an endogenous photo morphogenesis gene DET1 in tomato, a regulatory gene involved in repression of several light signalling pathways. DET1 was effectively degraded in transgenic tomato with suppressed DET1, with the increase in the level of flavonoids and carotenoid content (Davuluri *et al.*, 2005). Abscisic acid (ABA) plays very significant roles at the time of fruit ripening in tomato. The SINCED1 gene in tomato encodes 9-cis-epoxycarotenoid dioxygenase, which is an important enzyme in the ABA biosynthesis, was suppressed by RNAi. The fruits showed more accumulation of upstream compounds, chiefly lycopene and β carotene from these RNAi lines. Similarly, in apple the fruit

quality has been improved by enhanced self-life using RNAi approach (Dandekar *et al.*, 2004), reducing accumulating amount of a major apple allergen (Gilisen *et al.*, 2005) and silencing the leaf sorbitol synthesis process, which affects starch accumulation and sugar-acid balance there by the fruit quality. RNAi based production potential of for these products have been published by several authors, some of which related to horticultural crops are discussed in this article.

CaMV 35S promoter-driven hairpin RNAi construct is part of the coding region of the tobacco NtSPP2 gene to reduce SPP expression in transgenic potato tubers (Chen *et al.*, 2008). They reported that Suc6P accumulates in RNAi-silenced sucrose phosphatase (SPP) potato tubers upon cold storage at 4 °C. They have revealed from northern analysis that cold-induced expression of vacuolar invertase (VI) was blocked in SPP-silenced tubers explaining a reduced sucrose-to-hexose conversion. Suc6P levels were found to be negatively correlated with VI expression.

In opium poppy (*Papaver somniferum*), through the use of this technology the morphine line has been replaced with non-narcotic alkaloids. RNAi used to interfere with multiple steps of a complex biological pathway. An hnRNA construct was designed to silence of the multigene codeine reductase (COR) gene family. The transgenic plant accumulated (S) reticuline, a precursor non-narcotic alkaloid which occur seven enzymatic steps up stream of codeinone in the pathway. It occurs at the expense of morphine, codeine, opium and baine (Allen *et al.*, 2004).

In sweet potato (*Ipomoea batatas*), the range in amylose contents is narrow (10 - 25%) compared with other crops. The amylose content has been markedly increased utilizing

this approach by suppressing the expression of endogenous IbSBEII gene. The introduction of construct encoding dsRNA of SBEII into sweet potato genome to inactivate IbSBEII gen, which resulting in increase in apparent amylose content in the sweet potato starch. The ratio of amylose to amylopectin has a great influence on the physicochemical properties of starch. It the improved sweet potato has new dietary and industrial applications. Similarly, in potato through the use of RNAi approach the carotenoids such as β -carotene and total carotenoid production has been increased through silencing of β -carotene hydroxylase gene. As potato tuber contain low level of carotenoids which mainly composed of xanthophylls lutein and viloxanthin. But none of these compounds has provitamins A activity. Only β -carotene is main precursor of vitamin A.

Utilization of RNAi for seedless organic product (Parthenocarpy)

Phytohormones assume an urgent job in guideline of change between blossoming, preparation and fruiting. Parthenocarpy is fundamental for creating plant crops when fertilization and preparation is severally influenced by abiotic stress and yield is diminished. Accordingly to guarantee yield dependability in troublesome climatic condition parthenocarpic organic products required. An examination demonstrates that seedlessness in watermelon and brinjal increment the surface and self existence of natural products (Pandolfini. 2009).

Two individual from ARF family ARF8 and ARF7 show significant level of articulation in non-pollinated blossoms and down controlled after fertilization. In tomato plants, RNAi empowers constraint of gibberellic corrosive and auxin signal pathways after a decrease in the degree of SlARF7 transcript, which is answerable for fertilization and preparation

Table-1 (Jong *et al.*, 2009). It by-pass the auxin flagging preparation pathway in tomato that prompts the advancement of parthenocarpic organic products, which has incredible business esteem in the current rural market and better return can be accomplished even natural conditions bothersome for fertilization and treatment. By down-managing a chalcone synthase, a quality associated with flavonoid biosynthesis, seedless organic products have been accomplished in tomato. Phytohormones, for example, auxin and gibberellins are firmly connected with parthenocarpy in tomato which thusly is controlled by numerous miRNAs (Molesini *et al.*, 2012). Accordingly, controlling the degree of phytohormones by controlling exercises of miRNAs or their objectives could end up being a viable way to deal with to get parthenocarpy natural products. Parthenocarpic natural products were likewise seen in tomato in which qualities of the AUCSIA family coding for 53-amino-corrosive long (protein or peptide) were practically smothered by RNAi (Molesini *et al.*, 2009).

RNAi for changed bloom shading

By and by, request of blossoms has expanded for improvement and fragrance reason. Assortment of blossoms and fragrance has accessible in showcase as per purchaser need and inclination. A ton of research has been brought out to improve the blossoms through quality hushing (Table 1).

In chrysanthemum work has been done to expand the splendor in petals through RNAi. Two chrysanthemum cultivars, C \times morifolium 'LPi' and C \times morifolium 'LPu', that just gather flavonoids in their ligulae blossoms used to disengaged seven anthocyanin biosynthesis qualities for example CmCHS, CmF3H, CmF3'H, CmDFR, CmANS, CmCHI and Cm3GT in

these cultivars. RT-PCR and qRT-PCR examinations demonstrated that CmF3'H was the most significant compound required for cyanidin biosynthesis. To remake the delphinidin pathway, the down guideline of CmF3'H utilizing RNAi and over communicated the *Senecio cruentus* F3'5'H (PCFH) quality in chrysanthemum exhibited an altogether expanded substance of cyanidin and more splendid red blossom petals however didn't aggregate delphinidin. These outcomes showed that CmF3'H in chrysanthemum is significant for anthocyanin gathering, and *Senecio cruentus* F3'5'H just displayed F3'H action in chrysanthemum however didn't modify the delphinidin pathway to shape blue bloom chrysanthemum (Huang *et al.*, 2013).

Infection resistance through RNAi

Plant pathogen inferred (PDR) is the most remarkable way to deal with battle infection disease in plants. The utilization of PDR idea has assisted with designing infection safe plants (Simon-Mateo and Garcia 2011). Another system dependent on the utilization of miRNA develop that express various fake miRNA (amiRNA) focusing on different district of a viral quality. RNAi interceded coat protein (CP) quality of infections is likewise seen as very compelling in prompting protection from the plant against infections. Infection obstruction has been built effectively by focusing on the coat protein (CP) quality through RNAi in numerous green plants. Transgenic tobacco communicating the CP quality of Tobacco Mosaic Virus (TMV) was impervious to TMV and that the obstruction was because of the communicated Coat Protein as appeared by Powell-Abel *et al.*, (1986). After, this technique was applied to produce obstruction against a few distinctive infections, for example, potato impervious to Potato Virus Y (PVY) by Missiou *et al.*, 2004. Cucumis cv.

melo impervious to Papaya Ring Spot Virus type W (PRSV-W) by Krubphachaya *et al.*, 2007 and *Prunus domestica* impervious to Plum Pox infection (PPV) (Hily *et al.*, 2007).

Comparative technique was utilized to effectively design obstruction in cassava plants against African Cassava Mosaic Virus (ACMV); Vanderschuren *et al.*, 2009). RNA quieting procedure isn't restricted to RNA infections yet can effectively be applied to DNA infections to design obstruction. In blackgram plants recouped effectively from geminivirus *Vigna mungo* yellow mosaic infection (VMYMV) contamination when immunized with hpRNA develop containing the advertiser succession of VMYMV heavily influenced by the 35S advertiser (Pooggin *et al.*, 2003). RNAi strategy has been utilized to produce basic bean impervious to geminivirus Beans Golden Mosaic Virus (BGMV) (Bonfim *et al.*, 2007). A wide-range of opposition has been created against tospoviruses by focusing on different districts of a viral quality in tomato plants (Bucher *et al.*, 2006).

RNAi for Insect and nematode opposition

Creepy crawly bugs cost billions of dollars in type of yield misfortunes and pesticide. Still people groups face an ever present danger of bug spray and host opposition. Every year crop loss of worth US\$125 billion was accounted for by unmanageable phytoparasitic nematodes. Gheysen and Vanholme (2007) showed that ds-RNA articulation in a host plant against housekeeping or parasitism qualities in the root-tie nematode brings about nematode safe plants.

Huang *et al.*, (2006) were first to show protection from more than one nematode animal types, focusing on a quality engaged with parasitism instead of a nematode

housekeeping quality. All things considered, late sequencing of the *Meloidogyne* hapla genome will uncover new focuses for HD-RNAi (Oppermana *et al.*, 2008).

Through host-instigated RNAi (Sindhu *et al.*, 2009) directed every one of the four nematode parasitism qualities of sugar beet sore nematode (*Heterodera schachtii*), 3B05, 4G06, 8H07 and 10A06, having host *A. thaliana*. They revealed that no total obstruction was watched, however it prompted 23–64 % decrease in the quantity of develop nematode females in various RNAi lines.

Ibrahim *et al.*, (2011) they directed four distinct qualities for RNAi develops (Ibrahim *et al.*, 2011). The qualities have high comparability with *Heterodera glycines* (basic soybean blister nematode) and *C. elegans* to decide their viability to diminish nerves shaped by *Meloidogyne incognita* in soybean roots. Of the four, two develops focusing on the qualities encoding tyrosine phosphatase (TP) and mitochondrial stress-70 protein antecedent (MSP) had the option to decrease nerve arrangement by 92 and 94.7 %, separately.

Colorado potato bugs are a feared bug of potatoes shielded from herbivory utilizing RNA obstruction (RNAi). They hereditarily changed plants to empower their chloroplasts to aggregate twofold stranded RNAs (dsRNAs) directed against fundamental bug qualities.

RNAi for parasitic and bacterial opposition

Organism and microorganisms are most dangerous plant pathogen and perhaps the greatest test to specialists. They spread exceptionally quick and become hard to control. RNAi is one way to deal with upgrading parasitic and bacterial obstruction in plants. Escobar *et al.*, (2001) demonstrated

that hushing of two bacterial qualities (*iaaM* and *ipt*) could diminish the creation of crown nerve tumors (*AGROBACTERIUM TUMEFACIENS*) to about zero in *Arabidopsis* proposing that protection from crown nerve sickness could be designed in trees and decorative plants. In 2010, Xin *et al.*, announced that 24 miRNAs were receptive to fine buildup disease brought about by the commit biotrophic parasite *Blumeria graminis f. sp. tritici*. What's more, further anti-cipated 149 objective qualities which were conceivably managed by the novel wheat mi-RNA.

Decreased allergenicity and lethality through RNAi

Nourishment hypersensitivity is a misrepresented insusceptible reaction of our body activated by allergens present in nourishment, for example, peanuts, apple, mango or some other explicit nourishment. Along these lines, there is a need to diminish or take out the substance of allergens from our nourishment. Not just this, there is likewise a need to create plants liberated from dangerous substances as the regular poisons are available in a wide assortment of plants usually devoured as nourishment. These lethal substances when ingested in noteworthy sum or when they are not handled suitably can be possibly hurtful to human wellbeing causing food contamination. The end of allergens and harmful substances can be accomplished by utilizing RNA obstruction that may adjust the biosynthesis of allergens by changing its biochemical pathway to improve the nourishment quality by limiting the danger of nourishment hypersensitivity and harmfulness.

Nourishment hyper-sensitivity is an exaggerated invulnerable reaction of our body activated by allergens present in nourishment and green plants. So there is expected to wipe out of allergens and harmful substances by

utilizing RNAi that modify the biosynthesis of allergens by adjusting its biochemical pathway to improve the nourishment quality. RNAi has fundamental and intense innovation to build's plant with diminished allergenicity and harmfulness. To quiet the particular allergens and poisonous metabolites, RNAi is profoundly productive due its succession explicitness to specific allergens without hampering the fundamental cell compound. In apple (*Malus domestica*), an allergen known as mal d1 articulation has been diminished through RNAi approach. Mal d1 cross response immunizer is IgE, cause unfriendly response in patients. To manufacture the RNAi build for powerful hushing mal d1 quality, an intron containing mal d1 quality was confined from cultivars known as Gala. The outcomes show that a wild-type plantlet had altogether (P < .05) higher allergenicity than 5 of the change plants. The decrease of articulation of Mal d 1 was affirmed by

immunoblotting and skin prick trial of apple pamphlet (Gilissen *et al.*, 2005). In another examination, the outflow of allergen Lyc e 3, which encodes a vague lipid move protein (ns-LTP) in tomato plants, was decreased through explicit dsRNAi build of LTPG1 and LTPG2 (Le LQ *et al.*, 2006).

Smothered lfs quality by hp-RNAi utilizing 35S CaMV advertiser decreasing LFS movement in injured onion bringing about the creation of tearless onion (Eady *et al.*, 2008).

An utilized RNAi to stifle the cytochrome P450 protein creation hindering the biosynthesis of linamarin and lotaustralin, and produced transgenic cassava (*Manihot esculenta*) plants with disposal of under 1% of cyanogenic glucosides from leaves and 92 % decrease of cyanogenic glucosides from tubers (Jorgensen *et al.*, 2005) (Fig. 1).

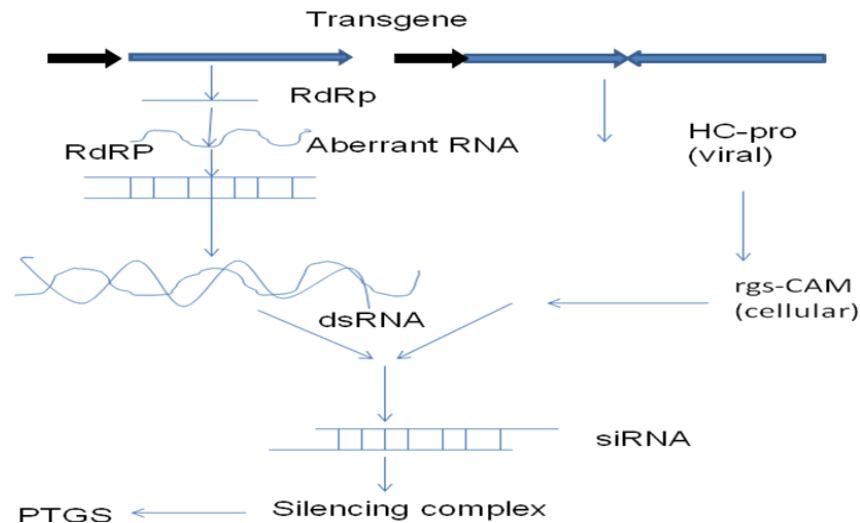
Table.1 Gene targeted for RNAi-mediated crop improvement in horticultural plants

S. No.	Crop improvement	Plant used	Traits improved	Gene targeted	References
1	Bio-fortification	Tomato	β-Carotene & lycopene	NCED1	(Sun <i>et al.</i> , 2012)
		Tomato	Carotenoid & flavonoid	DET 1	(Davuluri <i>et al.</i> , 2005)
		Potato	β-Carotene & lutein	BCH	(Eck <i>et al.</i> , 2007)
		Tomato	Vitamin C	APX	(Zhang <i>et al.</i> , 2011)
2	Bio-elimination	<i>Coffea canephora</i>	Caffeine	CaMXMT 1	(Ogita <i>et al.</i> , 2003)
		<i>Papaver somniferum</i>	Morphine	Codeine Reductase (COR)	(Allen <i>et al.</i> , 2004)
3	Altered phenotype	Torenia hybrida cv. Summerwave Blue	Flower colour: blue to white	CHS	(Fukusaki <i>et al.</i> , 2004)
		Petunia	Scent profile modification	PhBSMT	(Underwood <i>et al.</i> , 2005)
		Tomato	Parthenocarpy	AUCSIA	(Molesini <i>et al.</i> , 2009)
		Tomato		CHS	(Schijlen <i>et al.</i> , 2007)

Table.2 Name of horticultural crops and varieties in which transgenic developed (Xiong *et al.*, 2015)

S.No	Crops	Variety	Characters
1	Papaya	Rainbow, SunUp	Disease resistance
		Huanong No. 1	
2.	Apple	Golden Delicious	Modified product quality
		Granny Smith	Non-browning phenotype
3.	Plum	C-5 (NA)	Disease resistance
	Grape		
4.	Tomato	Da Dong No 9 (NA), Huafan No 1 (NA), FLAVR SAVR	Modified product quality
5.	Brinjal	Bt Brinjal Event EE1	Insect resistance
6.	Potato	Lugovskoi plus, Atlantic NewLeaf2 potato)	Insect resistance
		Starch Potato, Innate2 Russet Burbank Potato	Modified product quality
		New Leaf2 Y Russet Burbank potato, New Leaf2 Plus Russet Burbank potato)	Insect + disease resistance
7.	Sweet pepper	PK-SP01 (NA)	Disease resistance
		New Leaf2 Plus Russet Burbank potato	
8.	Rose	WKS82/130-4-1 (NA)	Modified product quality
9.	Carnation	Moondust	Modified product quality
		Moonshadow	Herbicide tolerance 1 modified product quality
		Moonshade	
		Moonlite	
		Moonaqua	
		Moonvista	
		Moonique	
		Moonpearl	
		Moonberry	
		Moonvelvet	
10.	Petunia	Petunia-CHS	Modified product quality
11.	Creeping Bentgrass	Roundup Ready Creeping Bentgrass	Herbicide tolerance

Fig.1 Post-transcriptional gene silencing (PTGS) in plants triggered by single transgene expression above a threshold level or by inverted repeat transcription. The single transgene transcript is converted into aberrant RNA and subsequently transformed into double-stranded RNA (dsRNA). The viral PTGS inhibitor acts by activation of regulator of gene silencing- Calmodulin-related protein (rgs-CAM), a cellular PTGS inhibitor



RNAi for abiotic stress resistance

Abiotic stress is a genuine danger for the life on earth, especially plants whose development and yield influenced contrarily. Plants have adjusted various physiological, bio-compound and metabolic methodologies to experience the abiotic stresses. Typically, it is dubious to conceive the convoluted pathway of flagging that are animated and killed in light of various abiotic stresses (Chawla *et al.*, 2011). Current discoveries showed that RNAi is assuming a basic job in abiotic stresses incitement in various harvests. The capacity of miRNAs (microRNA) corresponding to abiotic stress like oxidative pressure, cold, dry spell, and saltiness were accounted for by Sunker and Zhu (2004). Moreover, miR402, miR319c, miR397b, and miR389a were constrained by abiotic worry under differing levels in *Arabidopsis* (Jagtap *et al.*, 2011).

Quality hushing forms have been generally misused as adaptable exploratory and

biotechnological instruments for utilitarian quality examinations and transgenic ways to deal with crop improvement, infection obstruction, and metabolic building. In number of cultivation transgenic crops has created against a few biotic and abiotic stress resistances (Table 2).

Application of RNAi in transgenic plants

RNA interference (RNAi) is an evolutionary conserved gene regulatory approach that has significant impact on crop improvement; it permits down-regulation in gene expression with greater precise manner without affecting the expression of other genes. In the recent year the RNAi mediated technology has been used in metabolic engineering of plants with respects to improvement of various traits and to targets the genes linked to different undesired characters. In several plants RNAi has been used to improve their nutritional value, flavour, genetically modification of fatty acid composition and reduction toxicity/allergency in plants. The several application

of RNAi approach has been an elaborated here in major consumed crops.

In conclusion all technology has its limits. Post-transcriptional RNA silencing generally does not lead to complete gene silencing. Furthermore, it is unclear whether RNA silencing technologies can be used to consistently inactivate endogenous gene promoters in plants, which, unlike transgene promoters, appear to be resistant to siRNA-directed transcriptional silencing. This would limit the applications of RNA silencing technologies, particularly in cases where complete and stable genetic deletion is required. Combinations of RNA silencing technologies based on different RNA silencing pathways could improve silencing efficiency. For example, combining PTGS with TGS-based technologies, siRNA with miRNA-based technologies, or technologies based on all the different RNA silencing pathways, could potentially lead to more potent gene silencing than using technologies based on a single track. Recent years have seen great advances in the development of technologies for site-directed mutagenesis in plants. Technology can now be used to mutagenesis or modify nucleotide sequences of selected genes or genomic loci in plants (CRISPR / Cas9). The complete deletion of essential genes is lethal to plants and therefore such mutants cannot be recovered by this technology like mutagenesis technologies.

However, mutants of such genes could be recovered for genetic function analysis by incomplete deletion of genes with RNA silencing technologies. This is demonstrated in rice, where transformation with shRNA libraries results in the recovery of essential genetic mutants. RNA silencing technologies allow for tissue-specific silencing of a gene using a transgene expressly expressed in the tissue, while the genetic mutation causes inactivation of the gene in all tissues. iv) RNA

silencing technologies can be used to simultaneously silence multiple genes using transgenes that contain a conserved sequence or a composite sequence of multiple genes, while this would be difficult to achieve using CRISPR / Cas9-like mutagenesis methods. With continued efforts to better understand RNA silencing mechanisms in plants, it can be expected that RNA silencing technologies will be further improved to overcome the potential limitations that allow for wider applications in agriculture. RNAi has become a highly effective experimental tool in functional genomics for silencing genes for both basic and applied biological studies in various organisms including plants. RNAi deploys small RNAs, mainly siRNAs, to mediate the degradation of mRNA for regulating gene expression in plants. However, RNAi stability in plants is critical, but the RNAi-mediated gene suppression approach opens new avenues for the development of eco-friendly biotech approaches for crop improvement. By way of knocking out of the specific genes for better stress tolerance and integrating novel traits in different plant species for insect/pest/pathogen resistance and enhanced nutritional status become more convenient rather than conventional practices. This technology having revolutionary capabilities could be further exploited for functional analysis of target genes and regulation of gene expression for crop improvement.

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