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*Review Article*

# Current Status of RNAi Based Crop Breeding

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## Abstract

RNA interference studies have provided new opportunities for high-efficiency and high-throughput technology for gene suppression in plants with an aim to achieve desirable traits. RNAi which is a conserved mechanism of post transcriptional gene silencing have advantage for functional genomics. RNA construct can easily target to specific gene from any background in a dominant manner by initiating sequence specific RNA degradation pathways. Gene silencing in either way such as tissue specific silencing, inducible silencing or host delivered RNAi demonstrated to serve as a defense mechanism for an improvement of crops against nematodes, bacteria, fungi, insect, pest, parasitic weeds, and viruses. Many breeding goals have been achieved in different crops which were nightmares in the past for the researchers. But the advent of new technology has paved the way to develop resistant crops to combat biotic and abiotic stresses. It also gives a hope to attain world food security, enhances nutritional quality (in terms of bio-fortification and bio-elimination) for mankind and proving itself as an ecofriendly tool for plant protection. Especially, host gene silencing hairpin RNAi is reported as more stable gene silencing strategy in plants against different pathogens and also provide protection from invasion by foreign nucleic acid.

**Key Words:** RNA Interference, Crop Breeding, RNAi Mechanism, Diseases, Crop Improvement.
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**Abbreviations:** RNAi: RNA interference, PTGS: Post Transcriptional gene silencing, HGS: Host gene silencing, dsRNA: Double stranded RNA, siRNA: Small interfering RNA, TIGS: Transient induced gene silencing, GST: Glutathione S-transferase, VSR: Viral suppressor of RNA, TYMV: Turnip yellow mosaic virus, TuMV: Turnip mosaic virus, BGMV: Bean golden mosaic virus, CMV: Cucumber mosaic virus, BYDV: Barley yellow dwarf virus, SLCMV: Sri-Lankan cassava mosaic virus, SQS: Squalene Synthase, ACC: 1-Aminocyclopropane-1-carboxylate

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## Introduction

Conventional breeding strategies have assisted the researcher to generate high yielding and better quality varieties but many unavoidable factors have led to slow down the variety development process including infertility constraints in crops (Hussain, 2002). Crop productivity has been severely affected by a variety of diseases since the dawn of agriculture. Approximately 67,000 pest species, 9000 insect species, >4100 identified parasitic species of nematode, 26 families of viruses have been able to damage crop improvement from conventional agriculture to modern agri-sytem (Nicol, *et al.* 2011; Ross and Lembi, 1985) After all, many common strategies have been developed to achieve resistance in plants as toxic proteins, lutein and inhibitors, however, RNAi mediation technology can be done by several methods such as RNAi vectors, in vitro dicing and synthetic molecules. The term RNAi was devised by Craig Mello and his co-workers (Rocheleau *et al.*, 1997) and commonly used as a key strategy in functional genomics.

In most of cases, reduce or enhance activity of a genes are required for crop improvement. RNA interference phenomena is being used in plants for the disruption of gene activity in which a construct is introduced in to plants that generate double stranded RNAs. dsRNAs degrades mRNA of target gene by sequence specific degradation mechanism that may produce a phenotype (Dennis *et al.,* 2008). RNAi based gene silencing have been used widely in an attempt to suppress essential endogenous genes involved in vacuole formation, amino acid, fatty acid and nucleotide biosynthesis (Framond *et al.,* 2007). It is a conserved mechanism of post transcriptional gene silencing (PTGS) which controls different processes including development, maintenance of genome stability and defense against molecular parasites (Joseph *et al.,* 2012). RNAi technology leads to reduce the allergic proteins and toxic compounds from food and feed crops including tomato, wheat, apple, rice and peanuts by altering their nutritional value. It has been successfully exploited in plants against bacteria, viruses, nematodes, fungus, insects, pests, parasitic weeds and abiotic stresses.

RNAi mediated silencing have been carried out widely against fungal pathogens (*Magnaporthe grisea, M. oryzae, Phytophthora infestans, P. parasitica, Blumeria graminis, Fusarium graminearum*) in wheat (Riechen, 2007; Yin *et al.*, 2011), tobacco (Hernandez *et al.*, 2009), barley (Weis *et al.,* 2013; Koch *et al.,* 2013), potato (Eschen-Lippold *et al.,* 2012) and rice (Jiang *et al.,* 2009; Yara *et al.,* 2007; Campo *et al.,* 2013). Similarly, RNAi mechanism have been exploited against various viruses to silence gene of interest by introducing gene sequence into cells or organisms in *Arabidopsis thaliana* (Niu *et al*., 2006), *Phaseolis vulgaris* (Bonfim *et al*., 2007), *Manihot esculenta* (Ntui *et al*., 2015), cantaloupes (Krubphachaya *et al*., 2007), rice (Tyagi *et al*., 2008; Liang *et al*., 2004;), Pepper (Lee *et al*. 2009), Sugarcane (Guo *et al.* 2014), tobacco (Ntui *et al*., 2013), tomato (Ntui *et al.,* 2014), barley (Wang *et al.,* 2000), potato (Missiou *et al*., 2004; Ntui *et al.,* 2013;). RNA interference pathway also helped as a natural antibacterial defense mechanism by generating dsRNA to induce suppression of target gene in *Arabidopsis thaliana* (iaaM and ipt, PPRL, T1R1), *C. limon* (PDS & CalS1), tomato (Iaam and ipt) and rice (OsSSI2). Host derived RNAi appears to be most promising strategy for the control of nematode by broadly categorizing dsRNA in tobacco (Yadav *et al.,* 2006; Fairbairn *et al.,* 2007), soybean (Steeves *et al.,* 2006) and *Arabidopsis thaliana* (Huang *et al.,* 2006; Sindhu *et al.,* 2009; Patel *et al.,* 2010; Dinh *et al.,* 2014).

RNAi mediated silencing in insects by ingestion in gut system give a long lasting and cost effective method. Therefor researcher generated many plants, which were resistant to insect and pests attack such as tobacco (Thakur *et al*., 2014; Mao *et al.,* 2007), *Arabidopsis thaliana* (Coleman *et al.,* 2014; Mao *et al.,* 2007) and maize (Baum *et al.,* 2007). Tomato (Aly, 2010), tobacco (Aly, 2010), lettuce (Tomilov *et al.,* 2008) and maize (Framond *et al.,* 2007) plants were generated against parasitic weeds that's why tomato, lettuce and tobacco showed effective resistance but maize transgenic plants did not show effective resistance against parasitic weeds. Like all others, canola (Wang *et al.,* 2009), rice (Manavalan *et al.,* 2012; Li *et al.,* 2009; Park *et al.,* 2010; Ning *et al.* 2011) and barley (Martin, 2015) plants were exploited against drought stress, wheat (Gil-Humanes *et al.,* 2008), cotton (Sunikumar *et al.,* 2006), *Arabidopsis thaliana* (Zhu and Galili, 2004), maize (Segal *et al.,* 2003), tomato (Jong *et al.,* 2011; Meli *et al.,* 2010; Xiong *et al.,* 2005) and potato (Eck *et al.,* 2007) plants were generated to improve nutritional quality, alfalfa (Reddy *et al.,* 2005) plants were mediated to improve digestibility meanwhile wheat (Feldmann, 2006) and rice (Qiao *et al.,* 2007) plants were also transformed to improve grain yield with this novel strategy of RNAi.

Therefore, RNAi is an important area of molecular research all over the world but it needs more intentions and clear understanding. The application of RNAi mechanism range from simple molecular biology to gene therapy. It is now in advance state but still did not come out from its infancy (Kumar *et al.,* 2012). After all, there is a need to establish specific role of RNAi pathways against non-viral pathogens. But it can be anticipated that gene silencing based technologies can be developed for important crop

species to enhance resistance against pathogenic infections.

## RNA Interference brief history

RNAi has been elucidated in many organisms such as Protozoa, Nematodes, Mouse, Human and Insects cells (Hammond *et al*., 2001; Agrawal *et al*., 2003; Baulcombe, 2004; Tang and Galili, 2004). Before the accidental discovery of RNAi technology, other techniques such as transposons elements, physical and chemical mutagens, antisense strand of RNA and T-DNA insertion were used overwhelmingly for suppression to generate gene loss of function. In 1990, scientists discovered RNAi mechanism accidently in petunia plant in an attempt to up-regulate a gene for the production of an enzyme (chalcone synthase, chsA) to get a more purple plant. Then it became an important method for analyzing the effect of gene silencing (Hannon and Rossi, 2004; Meister and Tuschl, 2004; Pradhan *et al.,* 2015). In 1998, Fire *et al.* induced RNA both strand (sense and antisense) in *C. elegans* and found gene silencing phenomena ten time greater than injecting sense and antisense strand separately. Because injecting both stand at the same time triggered dsRNA processes required for RNAi. Once this novel strategy helped to produce dsRNAs in the cell then DICER (enzyme) recognized automatically for further cleaving them into siRNAs (Carneiro and Carneiro, 2011). Meanwhile, RISC complex (discovered by Hammond *et al*., 2001) uses siRNAs to degrade complementary mRNAs for knock down of gene expression.

## Components of RNAi

There are many components of RNAi which serve as an initiator, effectors, amplifiers and transmitters. Such as dsRNA, Dicer-a protein (discovered by Bernstein *et al.,* 2001) consist of multiple RNA-interacting four functional domains (N-terminal helicase, dual RNase III motifs, C-terminal dsRNA binding domain and piwi/argonaute/zwille domain). Dicer that degrades long dsRNAs into small effector molecules called siRNAs. RISC is an abbreviation of RNA Induced Silencing Complex (discovered in *Drosophila* by Hammond *et al.,* 2001) which form from TRBP and Argonaute-2 protein. Passenger strand is a sense strand that will be degraded; Guide strand is an antisense strand that will be incorporated into RISC. (Mukherjee *et al*., 2012; Chendrimada and Gregory, 2005; Whitehead *et al*., 2009; William *et al.,* 2004; Agrawal *et al*., 2003). These are the main components of RNAi mechanism. But four classes of RNAs plays important role in RNAi such as dsRNA, siRNA, miRNA and hpRNA.

## RNAi working mechanism

RNAi is a conserved and integral aspect for the silencing of gene expression at the transcriptional and translational level; protection against pathogens, regulation of epigenetic modifications, control of genome stability, curbing of transposon movement and regulation of heterochromatin formation. RNAi can drift from one cell to another cell with siRNAs and high molecular weight RNA being responsible for systematic post transcriptional gene silencing. RNA dependent RNA polymerase plays an important role to trigger silencing effect because it recognizes RNAs as a template and synthesize antisense RNAs to form dsRNAs (Depicker and Van-Montagu, 1997) as shown in figure 1. In an initial step, long dsRNA is cleaved into 21-23bp small RNA fragments by a ribonuclease (RNase) III enzyme called as Dicer. (Kumar *et al.,* 2012). Dicer enzyme further divided the dsRNA into dsSiRNA with an ATP depended reaction. In a 2$^{nd}$ effector, siRNA generated by dicer is incorporated into multinucleate effector complex i;e RNA induced silencing complex (RISC) which is inactive to carry out RNAi. At this stage, 2$^{nd}$ ATP dependent reaction starts with the help of helicase that mediates unwinding of siRNA duplex to create an active form of RISC. In a last step, single stranded siRNA (guide/antisense strand) and Argonaute protein plays a vital role to perform silencer activity to RISC. The target mRNA is degraded by activated RISC at a single site where 5` end of guide strand is bound to complementary mRNA targeted sequence. After degradation of mRNA, RISC set off and siRNA can be recycled for mRNA recognition and cleavage again (Kumar *et al.,* 2012; Nykanen *et al.,* 2001).

## Application of RNAi for crop breeding

Now a day, small interfering RNA based gene silencing is very common in plants to control diseases (Rahman *et al*., 2008). This technology holds the key to future technological applications in crop plants. Gene knockdown studies are being carried out efficiently, when transgenes are present in the form of hairpin constructs (Agrawal *et al.,* 2003). Gene silencing has been successfully carried out in many important crops including wheat, barley, banana and *medicago* against many fungal and oomycete pathogens (Koch and Kogel, 2014; Vega-Arreguin *et al.,* 2014) as shown in figure 2. RNAi based breeding against viral diseases seems to be most promising for the development of transgenic plants in different crops such as soybean, rice, maize, tobacco, potato, tomato and barley (Kim *et al*., 2013; Zhang *et al*., 2011; Niu *et al.,* 2011; Ntui *et al.,* 2013; Lin *et al.,* 2011; Wang *et al.,* 2000; Bucher *et al*., 2006). Many breeding goals have been achieved with the application of RNAi such

as increasing the concentration of unique secondary metabolites, enhancing shelf life of fruit, improving the yield of crops, extending diseases, insect and pest resistance in crops (Xiong *et al.,* 2015). Plants being sessile in nature, it is a surprising phenomenon that they are in constant communication with other interacting organism and environments through mobile small RNAs (sRNAs). sRNAs and their forerunner RNAs possibly plays a role as a mobile signal that spread gene silencing information to influence the interacting organisms (Weiberg *et al.,* 2015). However, many crop species have been improed with the help of this technology as shown in figure 2. Interestingly, many genes have been silenced in one specie in separate experiments to get resistance against desired pathogenic species.

## Improvement of disease resistance

RNAi has been used extensively by breeders and geneticists to induce gene silencing for the development of resistant plants against biotic and abiotic stresses. It is mostly used against RNA viruses but rarely used against DNA viruses (Hussain, 2015). Inter-organism RNAi is aimed at a gene in attacking pest or pathogen, if it is successful then off target gene silencing can be easily addressed. This cross species or host induces gene silencing could replace chemical fungicide and insecticide for the control of major pests in crops (Jones, 2015). Most of eukaryotic microbial organisms that come in a contact with plants possess functional RNAi pathways also generate regulatory siRNAs. Interestingly, scientist have successfully reported a disease control strategy called as host induced gene silencing by generating transgenic plants that can express exogenous RNAi; by silencing targeted genes in pathogens and pests (Nunes and Deans, 2012). In plants, viral and bacterial contagious diseases suppress miR482 leads to down regulation of R-genes. When these miRNAs overexpress as negative regulators then transgenic plants becomes more prone to bacterial diseases. Conversely, these miRNAs or a miRNA over express and act as positive regulators then transgenic becomes more resistant to bacterial diseases. In these cases, over-expression of target gene might be an effective strategy for improving plant resistance (Kamthan *et al*., 2015).

## Bacterial diseases

Bacterial diseases spread rapidly that's why it is very difficult to control all of sudden. RNAi pathways helps as a natural antibacterial defense mechanism to generate resistance against bacterial pathogens as an alternative strategy to control plant diseases. Crown gall disease developed in *Arabidopsis thaliana* and *Lycopersicon esculentum* which can reduce production of crops significantly. RNAi mediated silencing of iaaM and ipt oncogenes of *Agrobacterium tumefaciens* leads to develop highly resistant lines against crown gall disease. One tomato line was completely resistant to *A. tumefaciens* strains but 01/29 and 01/33 line of *A. thaliana* showed 0.0% and 1.5% tumor-genesis; that was a landmark achievement against bacterial disease (Escobar *et al.,* 2001). A new class of 30 to 40-nt of small RNAs are identified in *A. thaliana* called as long-siRNAs (lsiRNAs) which were induced in response to pathogen attack of *Pseudomonas syringae.* induction of AtlsiRNA-1 silences the AtRAP possibly by promoting mRNA decapping and 5′ to 3′ degradation. The knockout mutant of AtRAP (a negative regulator of plant defense) showed high resistance to virulent and avirulent infection of bacterial pathogens. As a result, the induction of nat-siRNAATGB2 enhances the RPS2-mediated race-specific resistance in *A. thaliana* (Katiyar-Agarwal *et al.,* 2007). *A. thaliana* activate its defense system after perceiving pathogen associated molecular patterns (PAMPs) as bacterium (*Pseudomonas syringae)* flagellin.

A bacterial PAMP down-regulates auxin signaling in *A. thaliana* by targeting auxin transcripts. Overexpression of *T1R1* (Auxin signaling pathway) increases the susceptibility to virulent Pto DC3000. Because auxin promotes susceptibility to bacterial diseases. But down regulation of auxin signaling through miR393 resulting ARF (Auxin response factors) inactivation and consequently increases resistance against bacterium (*Pseudomonas. syringae)* (Navarro *et al.,* 2006). In rice (*Oryza sativa* L.), *OsSSI2-kd*, ortholog of *SSI2* (Os01g0919900) plays its role in the defense response of rice bacterial leaf blight disease; the most devastating disease worldwide. RNAi mediated knockdown of *OsSSI2* decreases the oleic acid level and increased stearic acid which indicate that *OsSSI2* is involved in fatty acid desaturation activity. Moreover, this suppression of fatty acid by desaturase gene enhances resistance against leaf blight of bacterial pathogen *Xanthomonas oryzae* (Jiang *et al*., 2009).

## Fungal diseases

Plant fungal diseases caused by fungal pathogen is often distinguishable, particularly from infected plant organs (Brown and Ogle, 1997). Against fungal diseases, targeting of fatty acid genes through RNA interference proved as an important strategy to generate disease resistant or at least disease tolerant crops. In rice, *OsSSI2-kd* plants showed high resistance against blast fungus *Magnapothe grisea.* Down regulation of *OsSSI2* indirectly increases the resistance of rice to fungal pathogens (Jiang *et al.,*

2009). Moreover, resistance was also enhanced against causal agent *Magnapothe grisea* by RNAi mediated co-suppression of two omega-3 fatty acid (*OsFAD7 & OsFAD8*). Transgenic rice plants (*F78Ri*) showed high resistance against *Magnapothe grisea (*Yara *et al.,* 2007). In potato, the late blight is the most devastating disease caused by Phyto-pathogen i.e *Phytophthora infestans.* RNAi mediated targeting of genes (*SYR1)* involved in late blight disease led to attain enhanced resistance against this infectious disease. Transgenic plants (*StSYR1-RNAi)* with down regulation of syntaxin gene showed improvement in defense status of potato against late blight disease (Eschen-Lippold *et al.,* 2012*).* In rice (*Oryza sativa),* transgenic lines were generated for overexpression of *osa-MIR7695* precursor. It was reported that overexpression of a novel *osa*-miR7695 (negatively regulates natural resistance-associated macrophage protein 6, *Nramp6*) precursor which confers resistance against blast fungus *Magnaporthe oryzae* (Campo *et al.,* 2013). In wheat (*Triticum aestivum),* RNAi mediated down regulation of Mildew locus O (MLO) led to generation of *T. aestivum* line attaining stable broad-band resistance against *Blumeria graminis* (powdery mildew) (Riechen, 2007). In barley, RNA interference-mediated transient induced gene silencing of HvLFGa (*Hordeum vulgare LIFEGUARG*) construct reduces fungal growth of *Blumeria graminis* (powdery mildew). But the overexpression of HvLFGa TIGS constructs enhances the susceptibility to fungus of powdery mildew (Weis *et al.,* 2013). RNAi mediated technology has proven by inducing resistance to some bio-trophic pathogens genes. By host induced RNA interfering system, genes were silenced in wheat against stripe rust fungus (*Puccinia striiformis* F. *sp. tritici*). Therefore, Zak (local variety of wheat) plants showed reduced expression level of *PSTha12J12 transcript* and hence proved to enhance resistance against rust fungus (Yin *et al.,* 2011). The disease of *Fusarium* head blight (FHB) caused by casual pathogen *Fusarium graminearum (Fg);* is one of the most devastating disease of barley crop. To tackle this disease, HIGS was exploited by targeting fungal cytochrome P450 lanosterol C-14A-demethylase (CYP51) genes to reduce the growth of infection. Transgenic plants were generated by transforming Golden Promise cultivar with p6i-*CYP3RNA* that produces sense and antisense copies of *CYP3RNA* in plants. Transformed plants expressing *CYP3RNA* showed resistance against Head Blight and growth of fungal colonization (Koch *et al.,* 2013). In tobacco plants, black shank (caused by *Phytophthora parasitica var. nicotianae*) disease affect tobacco plants on all growth stages and can be devastating with 100 % losses in the fields. During infection of pathogens, plant cells responds by increasing their expression of GST genes followed by increased infection of pathogen. Transgenic tobacco plants were generated by down regulation of GST genes. RNAi vector induced gene silencing reduced the expression of GST in transgenic plants. Silencing of GST enhances the resistance of *Nicotiana tobacum* against by *Phytophthora parasitica var. nicotianae* (Hernandez *et al.,* 2009).

## Viral diseases

RNA interference employs robust and selective pathway for battling with various viral diseases that cause significant economic losses. Viral suppression of the immune system is a widespread phenomenon and many viruses inhibit the viRNA pathway by expressing viral suppressors of RNA interference (Marques and Carthew, 2007; Ding & Voinnet, 2007). Transgenes encoding hpRNA are greatly effective for the silencing of both endogenous genes and transgenes which are employing virus resistance in plants. Transgene encoding hpRNAs showed more efficient than sense and antisense viral transgene against viral diseases in plants (Wang and Waterhouse, 2001). Keep in mind that many viruses have VSR silencing protein to encounter plant defense mechanism. Viral suppressor of RNA (VSR) suppresses gene silencing mechanism by improving the component of RNAi or by binding siRNAs (Duan *et al.,* 2012). Transgenic *A. thaliana* plants expresses artificial miR-P69 that exactly target *P69* gene of *TYMV*. Transgenic *A. thaliana* plants expressing amiR-P69$^{159}$ were resistant to *TYMV*. But transgenic plants of *A. thaliana* expressing amiR-HC-Pro$^{159}$ were resistant to *TuMV*. However, *A. thaliana* transgenic plants that carried amiR-P69$^{159}$ and amiR-HC-Pro$^{159}$ transgene were resistant to both viruses of *TYMV* and *TuMV* (Niu *et al*., 2006). In a same way, by using RNAi, a transgenic common bean lines were developed with hairpin construction to induce PTGS against the *AC1* viral gene. By inducing gene silencing, highly resistant common bean plants were generated against BGMV (Bonfim *et al*., 2007). Transgenic tobacco (*Nicotiana benthamiana*) displayed resistance against *TuMV* while expressing artificial miRNA (amiR$^{159}$-P69) directed against the regions in virus genome. Induction of amiR$^{159}$-P69 employed resistance against *TuMV-GP69* (Lin *et al.,* 2009). In another experiment, RNAi mediated construct keeping inverted repeat of defective gene was used to generate transgenic tobacco carrying CMV dsRNA. Transgenic tobacco lines expressing defective CMV replicase-derived dsRNA were highly resistant to CMV of strain O and Y (Ntui *et al.,* 2013). Tomato (*Lycopersicon esculentum* Mill.) widely grown as a vegetable or as a fruit in some parts of the

world. But its quality and production is greatly affected under the attack of CMV. To generate transgenic tomato resistant lines against CMV, Plants were engineered with RNAi mediated inverted repeat of 1138 bp fragment with CMV-Rep gene of CMV with strain O. Transgenic plants expressing CMV specific dsRNA of the replicase gene were subjected to CMV-O inoculant. Some plants showed high resistance to viral infection while some transgenic plants showed mild symptoms but later recovered themselves. However, some plants showed susceptibility to CMV of strain O (Ntui *et al.,* 2014). In a same way, a transformation system was used to develop CMV resistant line (CMVP1) of pepper by inducing coat protein gene (CMVP0-CP) (Ntui *et al.,* 2014; Lee *et al.,* 2009). Transgenic lines have been generated in barley against barley yellow dwarf virus (BYDV-PAV). Plants were transformed with a transgene to produce hairpin RNA carrying BYDV-PAV sequences. Among 25 transgenic barley lines, 9 transgenic lines showed extreme resistance to BYDV-PAV (Wang *et al.,* 2000). With the help of RNAi, Cassava (*Manihot esculanta*) plants were engineered with a construct carrying inverted repeats of 527 bp DNA fragment of Sri-Lankan cassava mosaic virus (SLCMV). Transgenic cassava (KU50) lines expressed dsRNA homologous to AV2 and AV1 of DNA of SLCMV. These KU50 transgenic lines showed high resistant to SLCMV as compared to wild type plants (Ntui *et al*., 2015). To develop RNAi mediated potato line against CMV, most susceptible local cultivar called as Danshaku was transformed by using two constructs carrying inverted repeats of 1138bp of defective replicase gene derived from RNA of CMV-O. Danshaku transgenic lines showed complete resistant to both strains (O & Y) of CMV (Ntui *et al*., 2013). RNA interference phenomena was used to generate transgenic line of potato against the potato virus Y (PVY). Potato lines showed dsRNA derived from 3´ terminal of CP gene of PVY and lines were also examined for generation of transgene derived siRNAs prior to viral infection. Among fifteen transgenic lines, twelve lines produced siRNAs and, that twelve transgenic lines were highly resistant to three strains of potato Virus Y ($PVY^{N}$, $PVY^{O}$ and $PVY^{NTN}$) (Missiou *et al*., 2004). Post transcriptional gene silencing was carried out in Cantaloupes (cultivar-sun lady) against the papaya ring-spot virus type W (PRSV-W). Binary vectors were constructed containing inverted repeats of CP gene coding region (pSA1304) or CaMV promoter (pSA1175). Transgenic lines carrying pSA1175 were susceptible to PRSV-W and the lines containing pSA1304 were highly resistant to PRSV-W (Krubphachaya *et al*., 2007). Post transcriptional gene silencing was carried out with hpRNA in rice (*Oryza sativa ssp. Japonica*) cultivar locally called as *Zhonghua* against the rice dwarf virus (RDV). A construct was generated with 128-754 bp against rice dwarf virus under the control of promoter CaMV35S. Consequently, hpRNA confers high resistance to RDV in transformed lines of rice cultivar (Zhonghua variety) (Liang *et al*., 2004). Rice tungro disease is caused by *Rice tungro bacillform virus* (RTBV) and seriously damages rice production in Southeast Asia. To develop transgenic lines against RTBV, transgenic rice plants expressing DNA encoding of RTBV in sense and antisense orientation were generated. Resultantly, transgenic plants were developed carrying dsRNAs. Among all lines, only one transgenic line (RTBV-O-Ds1) showed extreme resistance against rice tungro bacilliform virus (Tyagi *et al.,* 2008). Sugarcane mosaic disease is also a devastating disease of sugarcane which mostly damages photosynthesis and thus resulting of decline in sugar content and yield. Sugarcane mosaic virus is mainly caused by potyvirus sugarcane mosaic virus or sorghum mosaic virus. RNA interference vector pGII00-HACP carrying an expression cassette of hairpin interference and *cp4-epsps* herbicide tolerant gene was transferred to sugarcane cultivar ROC22. Transgenic line *ROC22* showed high resistance against sorghum mosaic virus (SrMV) and serve as a resistant germplasm for breeding lines (Guo *et al.* 2014).

## Insect and Pest resistance

Plant and insects are living together for more than 400 million years ago till now with diverse and complex relationship with microbial associates (Sugio *et al*., 2014). Plants recognize insects and start their downstream signaling with strong defense mechanism. Meanwhile, Insects also recognize their host plants and try to overcome plant defense mechanism (Bruce, 2015) leading to heavy losses (10-20%) of crop production (Ferry *et al.,* 2006). RNA interference technology has emerged a new horizon to identify insect effectors. These plants also possess many characters over transgenic Bt. (*Bacillus thuringiensis*) crops because it is dominant, specific, sequence based, ecofriendly and minimum off target effects (Jagtap *et al.,* 2011). Meanwhile, these concepts can be applied for tissue specific silencing, inducible silencing and host delivered RNAi (hdRNAi) in which silencing is induced in plant feeding insects. That's why hdRNAi technology is also called as species-specific insecticide (Whyard *et al.,* 2009; Senthil-Kumar and Mysore, 2010).

Cotton bollworm (*Helicoverpa armigera*) required elevated expression of gossypol-inducible P450 gene (*CYP6AE14*). After RNAi-mediation, larvae were fed on double stranded RNA (dsRNA)

expressing plants (*A. thaliana, N. tobacum*) specific to *CYP6AE14*; larval growth was retarded because the larval tolerance was greatly reduced to gossypol (Mao *et al.,* 2007). In aphid (*Myzus persicae)*, gene was silenced by plant mediated RNAi in which dsRNAs were induced into the aphids by feeding the insects on transgenic plants of *A. thaliana*, generated by RNAi that stably produced dsRNAs. RNAi down regulation of GPA genes decreases GPA fertility for many generations and further played a key role to reduce the aphid population. The aphids, which were reared on dsMpC002 transgenic plant experienced, 60% reduction in aphid reproduction (Coleman *et al.,* 2014). Sequence specific gene silencing was triggered in western corn rootworm (WCR) *Diabrotica virgifera virgifera* L. by feeding transformed maize plants (pMON94805) that was expressing dsRNA. Double stranded RNA exhibited significant activity resulting in mortality and stunting of larvae. RNAi in coleopterans (WCR) by oral delivery of dsRNAs increases the efficacy and durability of insect protected crops designed to control this important pest (Baum *et al.,* 2007). Transgenic tobacco lines were generated to produce dsRNAs to knock down the v-ATPase mRNA of feeding whiteflies. Host plant mediated pest resistance was attained against whiteflies by developing transgenic plants. Sufficient siRNAs were delivered to whiteflies to knockdown expression of v-ATPase, resultantly target genes was successfully silenced for effective mortality (Thakur *et al.,* 2014). Injection and feeding of double stranded RNA have proven to be effective strategy to control many species of insects. But oral delivery of in-vitro synthesized dsRNA is very easy to use.

## Nematode resistance

Plant parasitic nematodes always shows different feeding habits that's why each species effect on plant roots differently. Almost all plant species can tolerate minor nematode attack but it becomes a problem when its population increases from threshold level. Guo and Kemphues investigated the function of par-1 gene in nematode (*Caenorhabdites elegans*). They revealed that injecting either sense or antisense RNA for the par-1 gene would result in down-regulation of gene. Further, Andrew Fire and Craig Mello revealed that by injecting both strand (sense and antisense) together resulted more efficient silencing of target gene. This investigative research was a defining moment for silencing of targeted gene then called as RNA interference (Fire *et al.,* 1998; Ali *et al.,* 2010). Host induced RNAi (hdRNAi) is a leading method to engineered crops with hpRNA vector to produce dsRNA against the target organism so that dsRNA is being ingested into the organism while feeding. Feeding helps to starts RNAi mechanism and silencing of target genes in the organism. Root knot nematodes (*Meloidogyne* spp.) and cyst nematodes (*Heterodera* ssp. and *Globodera* ssp.) occupy host roots and introduce secretions into plant cells to carry out morphological and physiological changes. Finally, nematode modifies them to metabolically active site for feeding. Nematode obtain nutrients from active site of plant for their development and reproduction (Vanholme *et al.,* 2004). To suppress the plant parasitic nematodes, RNAi is powerful technique for exploration of function of nematode gene and identification of target genes (Niu *et al.,* 2010). In tobacco, hair-pin shaped double stranded RNA constructs were introduced into plants. Two genes were selected as a target, one gene coding for splicing factor and other coding for integrase of root knot nematode. Transgenic plants were highly resistant against nematode *Meloidogyne incognita* and dsRNA delivered through tobacco plant completely degraded target mRNA and provides host effective resistance against the parasite (Yadav *et al.,* 2006). Soybean plants were transformed containing sense and antisense strand of major sperm gene protein (MSP) from *Heterodera glycines.* Transgenic plants produced MSP siRNA significantly, and suppresses the reproductive potential of nematodes up to 68% reduction in egg $g^{-1}$ root tissue (Steeves *et al.,* 2006). Arabidopsis thaliana plants were also transformed against the root knot nematode genes (*16D10*) because plant parasitic nematodes infect nearly >1700 plant species worldwide. Transgenic plants were generated to silence root knot nematode (RKN) parasitism gene *16D10* by expressing dsRNAs. Secreted *16D10* peptide is an important molecule to regulate the interaction of root knot nematode and host plant. In vitro ingestion of dsRNAs, silence the target parasitism gene of root knot nematode. Resultantly, transgenic plants were effectively resistant to four species (*M. incognita, M. Javanica, M. arenaria and M. hapla*) of RKN (Huang *et al.,* 2006). In Arabidopsis thaliana, host induced gene silencing was also carried out against cyst nematodes (*Heterodera schachtii*) that is a devastating pathogen which infect almost every food and fiber crop in the world. Cyst nematode used parasitism protein to successfully infect the plants to establish active feeding site. Transgenic plants expressed dsRNA that targeted mRNA of parasitism gene to disrupt parasitic cycle. Host induced RNAi for all four (3B05, 4G06, 8H07, 10A06) nematode parasitism gene led to reduction in the number of mature cyst nematode. However, complete resistant to cyst nematode was not observed (Sindhu *et al.,* 2009). Transgenic tobacco (*Nicotiana tabacum*) plants were generated, expressing dsRNAs construct against the root knot nematode putative gene (MjTis11).

Concerned gene (MjTis11) was successfully silenced in nematode, when nematode fed on the roots of transgenic plants. But the silencing of novel gene did not render disease resistance but it gives a strategy to control other feeding pests by host delivered RNAi (Fairbairn *et al.,* 2007). Plant host triggered RNAi was used to silence parasitism gene by generating transgenic plants of *Arabidopsis thaliana* that were expressing hairpin dsRNA structure. When nematode fed on roots of plant, complementary Hs4F01 construct induce silencing and significantly reduced the pathogen infection level (Patel *et al.,* 2010). A considerable tolerance was found in transgenic plants of Brassica rapa (ssp. oleifera) against the root parasitic pathogen (*Heterodera schachtii)* gene (8H07). To enhance the plant resistance, dsRNAs were expressed in both plants and pathogen for the silencing of gene (8H07) as an effective strategy against the infection. Silencing of gene activity increases the tolerance in transgenic rape plants against *Heterodera schachtii* parasitizing on roots (Tsygankova *et al.,* 2013).

*Meloidogyne chitwoodi* is a devastating pathogen worldwide and continuously posing threat to potato (*Solanum tuberosum*) and *Arabidopsis thaliana*. With the help of RNAi, transgenic lines of potato and Arabidopsis were developed by introducing a construct pART27 (16D10i-2) to produce dsRNAs, complementary to *Meloidogyne chitwoodi* effector gene *Mc16D10L*. In this way, plant mediated RNAi led to produce significant resistance in *Arabidopsis thaliana* and potato against the pathogen (*Meloidogyne chitwoodi*) infection (Dinh *et al.,* 2014).

## Resistance against parasitic weeds

Naturally, host produced signals play important role for weed seed germination and development. Parasitic weed families (Orobanchaceae and Scrophulariaceae) consists of >4000 species which cause most devastating irreversible damage to agricultural production including yield and quality. Most of the weeds have evolved themselves according to the specificity of plants in natural ecosystem. *Striga hrmonthica* and *Striga asiatica* showed them almost specific to grasses such as sorghum, maize, pearl millet, rice and sugarcane. But *Kuntze* parasitic have evolved them to parasitize cowpea, tobacco and sweet potato. *Orobanche* is a parasitic weed which regulate the mannitol by Mannose 6-Phosphate reductase (M6PR) to uptake of water and nutrients from the host plant. Transgenic plant of tomato was generated to silence the mannitol activity in parasite. To provide the resistance to host plant against parasite, an inverted repeats technique was used to silence the M6PR mRNA. Tubercles or shoots of parasitic weed (*O. aegyptiaca*) showed reduced growth (almost dead) on transgenic plants of tomato by 60-80% due to enhanced resistance of transgenic lines. In a same way, Tobacco transgenic plants were produced to express cecropin peptide (*Sarcotoxin IA*) under HMG2 promoter. It was observed that *Orobanche* biomass decreased and host transgenic plants biomass increased which was a clear indication of plants resistance against parasitic weed (Aly, 2010). When a parasitic plant (*Triphysaria versicolor*) displaying reporter gene (GUS) was allowed to parasitize lettuce plants (control). GUS showed full activity but same parasitic weed did not show GUS activity with RNAi mediated transgenic lettuce. These results showed that silencing signal from host moved to down-regulate the target gene in parasite (Tomilov *et al.,* 2008). RNAi mediated gene silencing was also carried out in maize (*Zea mays* L.) against the parasitic weed *Striga asiatica* infection. Maize plants were generated with RNAi constructs targeting 5 different *Striga* genes but no one showed effective resistance against parasitic weed *Striga asiatica* (Framond *et al.,* 2007). However, further research is needed to draw a final conclusion about RNAi control of maize parasitic weed.

## Drought tolerance

Drought stress is a major devastating constraint for production and stability of every crop. That's why considerable efforts have been exploited to identify the traits associated with drought resistance in plants. It is believed that drought stress caused by climate change will affect the crop yield in many parts of world. Drought tolerant maize based on *Csp* gene, is the most advanced drought tolerant crop which is expected to be launched by 2017 in Africa. To overcome all drought devastation, drought silencing has been carried out to generate drought resistant crops. RNAi mediated knockdown of canola farnesyl-transferase (FTA) for drought tolerance was totally dependent on the amount of available water. Transformed plants were resistant to seed abortion and lose less water through transpiration. Conditionally, knockdown of FTA under AtHPR1 resulted in drought resistance without affecting yield (Wang *et al.,* 2009). RNAi mediated down-regulation of farnesyltransferase/SQS by maize squalene synthase in rice plants enhances the drought tolerance. Transgenic RNAi lines loss water more slowly by reducing stomatal conductance and delayed wilting by retaining leaf water. That's why transgenic RNAi lines showed drought tolerance at both stages either vegetative or reproductive (Manavalan *et al.,* 2012). RNAi construct was developed by cloning sense and antisense fragments of *OsDIL* (*Oryza sativa* drought-induced *LTP*) into the pGEM-RNAi vector.

Overexpression on *OsDIL* decreases the down-regulation of anther development genes by drought. This mechanism significantly supported anther development genes (*OsC4*, *CYP704B2* and *OsCP1*) and pollen fertility in transgenic rice plants under drought conditions (Guo *et al.* 2013). RNAi-mediated rice plants were generated to down-regulate the expression of *RACK1* gene. Transgenic plants showed 50% less expression as compared to the normal rice plants. That's why transgenic plants showed higher tolerance to drought stress (Li *et al.,* 2009). Similarly, in rice (*Oryza* sativa), silencing of RING finger E3 ligase gene-*OsDSG1* and RING finger E3 ligase C3HC4-*OsDIS1* (drought induced SINA protein) potentially showed tolerance to drought stress (Park *et al.,* 2010; Ning *et al.,* 2011). In barley, virus induced gene silencing was carried out to examine down-regulation of gamma-aminobutyric acid transaminase (GABAT) gene under drought stress condition. RNAi-mediated plants produced dsRNAs which silenced the GABAT gene. GABAT gene was down-regulated by 65-77% in silenced plants and remained more susceptible to drought stress. Silenced barley plants showed severe symptoms of drought due to very low amount of GABAT enzyme (Martin, 2015).

## Improvement of nutritional quality

RNA interference technique has been used in many plants to improve their nutritional quality, which is consumed as food. Gluten is a major protein in wheat grain, which mainly imparts its role in dough making process. Mainly gliadins (polymeric glutenins) give extensibility and elasticity to gluten and dough. Expression of gamma-gliadins was down regulated by RNA interference to examine the silencing of specific groups of gluten protein. Gliadins was successfully silenced in transgenic plants of bread wheat (Gil-Humanes *et al.,* 2008) to improve the nutritional quality because Gliadin of 2012 is far different from gliadin of 1960 due to genetic disorders because researcher transformed plants just to get high yield per acre without considering off target effects in crops. But, by the way, gliadins can bind to antibodies, which may contribute to immune mediate neurological impairment (Davis, 2012). Globally cotton seed can provide protein to half of a billion people per year but its nutritional components (gossypol content) are very toxic. RNAi was successfully induced to silence the gossypol biosynthesis in cotton seed by disrupting ō-cadinene synthase gene. As a result, gossypol terpenoid contents were greatly reduced in cotton seed in a stable and heritable manner (Sunikumar *et al.,* 2006). In wheat, RNAi-mediated plants were generated by down-regulating the two-isoforms of starch branching enzyme (SBEIIa and SBEIIb). It was just to undermine the prevalent diseases including obesity. Silencing of both enzymes in wheat resulted in high-amylose (>70%) phenotype (Regina *et al.,* 2005). Transgenic *Arabidopsis thaliana* plants were generated by introducing a bacterial feedback insensitive dihydrodipicolinate synthase (DHPS) and RNAi construct *AtLKR/SDH* with seed specific expression. Down-regulation of *AtLKR/SDH* gene (regulate lysine catabolism) boosted lysine level in mature seeds. But those seeds with elevated level of lysine germinate very poorly because excessive lysine was not degraded efficiently during seed germination. However, reduction of lysine degradation during seed development enhances the seed germination (Zhu and Galili, 2004). With the help of seed specific RNAi approach, high lysine corn was generated by silencing of 22-kD maize zein storage protein. RNAi triggered silencing of 22-kD zein gene did not alter function of Opaque-2 transcription factor, however it produced high quality corn (lysine rich protein) and a free lysine corn (Segal *et al.,* 2003). Seedless-ness is also a desired quality trait for some fruits and vegetables that's why RNAi mediated suppression of *SIARF7* transcript leads to reduction of pollination and fertilization in tomato plants. As a result, silencing of auxin signaling pathway, parthenocarpic fruit was generated with great commercial value (Jong *et al.,* 2011). Softening of fruit and vegetables is also a check point between the seller and consumer. Softening can be the result of excessive ripening of fruit and vegetables because it directly affects the palatability, consumer like/dislike, shelf life and effectively it plays a major role in cost determining factors. Transgenic tomato lines generated by RNAi by suppressing two ripening gene especially *N-glycoprotein* modifying enzyme (α-man) and β-D-N-acetyl-hexo-saminidase (β-Hex). Results showed that 30 days shelf life was increased after the silencing of both ripening enzymes (Meli *et al.,* 2010). But shelf life of tomato was prolonged up to 120 days by down-regulating the *ACC* oxidase gene. The dsRNA was successfully expressed in transgenic tomato plants derived from a known cultivar (Hezuo 906). Transgenic tomato plants were generated by down-regulating of *ACC* oxidase gene (Xiong *et al.,* 2005). Plant carotenoids plays an important role in human diet and helpful in reducing certain diseases. Potato is a main source of beta-carotene and also used as a staple food especially in Ireland. RNA interference mediated silencing was carried out in different potato lines (Yema de Huevo, 91E22, Desiree) for beta-carotene hydroxylase gene (bch) which alters the beta-carotene in to zeaxanthin. After successfully silencing of beta-carotene hydroxylase gene (which converts beta-carotene to zeaxanthin), B-carotene content was increased from trace amount to 331 μg $100g^{-1}$ fresh

weight by establishing highly nutritious crop (Eck *et al.,* 2007).

## Improvement of Forage Quality and Digestibility

Improving forage quality and digestibility are an important objective of forage breeding program. Lignin is a polymer and found in thickened cell wall triggered a negative influence on digestibility. Down-regulation of lignin enhances the digestibility and make forages easy to use, digest, enhanced animal performance and protect the environment from excessive animal dung. Transgenic Alfalfa (*Medicago sativa* L.) lines were generated by down-regulating the cytochrome P450 enzymes of the lignin. Down-regulation of P450 enzymes of lignin improves the digestibility in animals yet seen in forage crop (Reddy *et al.,* 2005).

## Improvement of grain yield

RNAi has been triggered in crop plants directly for silencing of genes, which is involved in yield and yield related components. No doubt, above all facts, all experiments are going to be practiced directly or indirectly in crop plants just to feed the masses or just to get high yield. But here, RNAi is especially exploited just to silence that gene which is directly involved in agronomic components. Silencing of gene (*OsDWARF4)* in rice resulted in dwarf plants with erect plant leaves. Erect leaf helps to increase photosynthesis in plants that's why such plants have a potential to improve grain yield (Feldmann, 2006). In rice, semi-dwarf line was generated by silencing of *OsGA20ox2* expression in taller rice variety (QX1). RNAi semi dwarf line showed increase in panicle length, seeds per panicle and 1000 grain weight (Qiao *et al.,* 2007). RNAi approach has enormous potential to integrate with crop breeding strategies and silencing can be induced "at will" by protecting possible adverse effects (Senthil-Kumar and Mysore, 2010).

## Conclusion and way forward

The field of RNAi is passing through an impressive phase. It emerged as a potent technology for crop protection and modifying existing crops for the benefit of mankind. It has established far reaching consequences by silencing gene expression and finding the potential of novel genes in plants to combat biotic/abiotic stresses, and also improve nutritional quality. It kindled a hope for the treatment of several diseases and give confidence to deliver stable and quality food for million masses of the world. RNAi is a very cost effective, high throughput and a reliable technique to down-regulate gene expression, which can be used to know the function of gene in all organisms (Kumar *et al.,* 2012). This technique can be exploited to produce siRNAs based drugs and to silence viral genes (Rahman *et al.,* 2008). The regulatory role and signaling pathway of miRNAs are definitely requiring much more attention of researchers to investigate in plant biology. Likewise, silencing of expression of target gene or miRNA might lead to pleiotropic changes in plant development. That's why transforming mechanism or strategies should be designed after clear knowledge and understanding of miRNA regulation. Instead of few limitations, crop improvement strategies based on small non-coding RNAs have enormous potential to increase productivity as well as nutritional value. Once this technology is finalized, it will create a new era in commercial crops.

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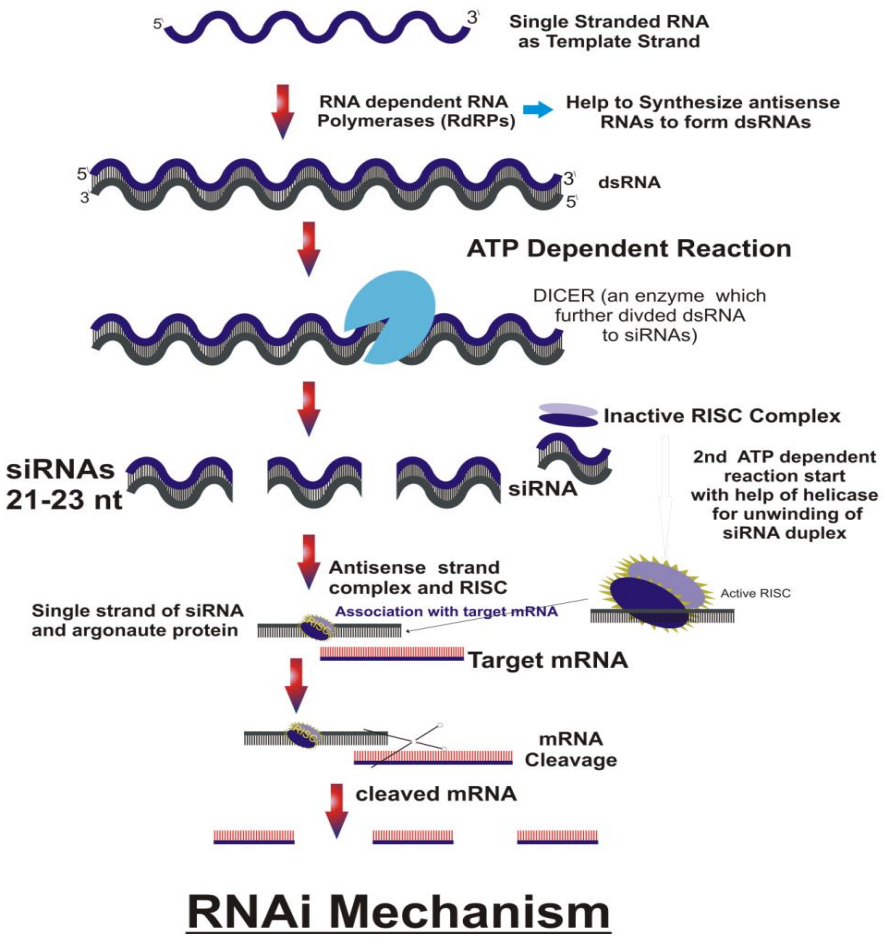


**Figure 1:** RNAi pathway and formation of siRNAs by DICER complex. Following cleavage, RISC loads, unwinding of siRNAs and binding to complementary mRNA, resultantly mRNA degraded.

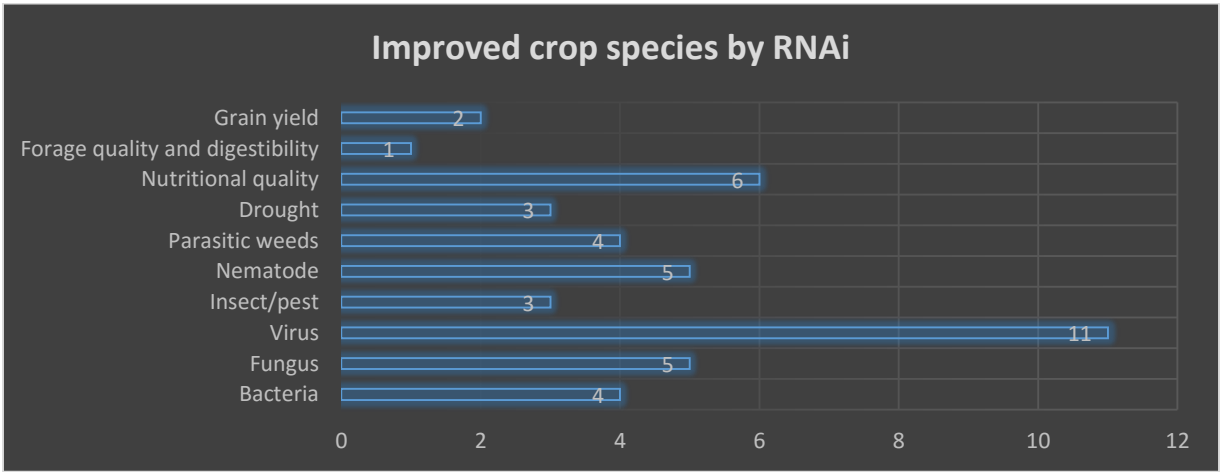


**Figure 2:** Through RNAi; resistance was achieved against bacteria, fungus, viruses, insect/pest, nematode, drought and parasitic weeds. Grain yield, nutritional quality and forage digestibility were also improved in above mentioned crop species, such as maximum 11 different crops gained resistance against viruses and minimum 1 specie was improved for forage digestibility.

**Table 1: Use of RNAi in plant species against bacterial attack**

| Crop | Targeted Gene | Bacterial Resistance | Transformation success | Plant Line | Reference |
|---|---|---|---|---|---|
| *A. thaliana* | iaaM and ipt | *A. tumefaciens* | 87.5% | Arabidopsis-01/33 | Escobar *et al.* 2001 |
| *L. esculentum* | Iaam and ipt | *A. tumefaciens* | 76.9% | Tomato-01/6 | Escobar *et al.* 2001 |
| *A.s thaliana* | PPRL | *Pseudomonas syrinage* | Enhanced resistance | *Arabidopsis thaliana* | Katiyar-Agarwal *et al.* 2007 |
| *A. thaliana* | T1R1 | *Pseudomonas syrinage* | Enhanced resistance | *Arabidopsis thaliana* | Navarro *et al.* 2006 |
| *C. limon* | PDS & CalS1 | *Xanthomonas citri* | Enhanced resistance | *CalS1 C. limon* | Enrique *et al.* 2011 |
| *O. sativa* L. | OsSSI2 | *Xanthomonas oryzae pv. oryzae* | Enhanced resistance | *Oryza sativa* L. | Jiang *et al.* 2009 |

**Table 2: Use of RNAi in various plant species against fungal attack**

| Crop | Targeted Gene | Fungal Resistance | Transformation success | Plant Line | Reference |
|---|---|---|---|---|---|
| *O. sativa* L. | OsSSI2 | *Magnaporthe grisea* | Enhance Resistance | OsSSI2-kd | Jiang *et al.* 2009 |
| *O. sativa* L. | OsFAD7 & OsFAD8 | *Magnaporthe grisea* | High resistant | F78Ri | Yara *et al.* 2007 |
| *O. sativa* L. | Nramp6 | Blast fungus *Magnaporthe oryzae* | Enhanced resistance | Rice lines | Campo *et al.* 2013 |
| *S. tuberosum* | SYR1 | *Phytophthora infestans* | Enhanced resistance | StSYR1-RNAi | Eschen-Lippold *et al.* 2012 |
| *T. aestivum* | MLO | *Blumeria graminis f. sp. tritici* | Resistant | *Triticum aestium* | Riechen, 2007 |
| *H. vulgare* | HvLFGa | *Blumeria graminis f. sp. hordei* | Enhances resistance | Barley transformed plants | Weis *et al. 2013* |
| *T. aestivum* | PSThaJ12 | *Puccinia striiformis f. sp. tritici* | Enhance resistance | Zak wheat | Yin *et al.* 2011 |
| *H. vulgare* | CYP51 | *Fusarium graminearum* | Resistant | Golden Promise Cultivar | Koch *et al.* 2013 |
| *N. tabacum* | Glutathione S-transferase (GST) | *Phytophthora parasitica var. nicotianae* | Resistant | Transgenic lines of tobacco | Hernandez *et al.* 2009 |

**Table 3: Use of RNAi in various plant species for virus resistance**

| Crop | Target region | Viral Resistance | Transformation success | Plant Line | Reference |
|---|---|---|---|---|---|
| *A. thaliana* | Viral mRNA | *Turnip yellow mosaic virus* | Highly resistant | Transgenic *A. thaliana* | Niu *et al.* 2006 |
| *A. thaliana* | Viral mRNA | *Turnip Mosaic Virus* | Highly resistant | Transgenic *A. thaliana* | Niu *et al.* 2006 |
| *P. vulgaris* | *AC1* | *Bean golden mosaic virus (BGMV)* | 93% | Bean lines | Bonfim *et al.* 2007 |
| *N. tabacum* | Viral mRNA | *Cucumber mosaic virus with strain O and Y* | >95% | Transgenic tobacco lines | Ntui *et al.* 2013 |
| *L. esculentum* | Viral mRNA | *Cucumber mosaic virus with strain O and Y* | Some transgenic plants were highly resistant | Tomato transgenic lines | Ntui *et al.* 2014 |

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|---|---|---|---|---|---|
| *H. vulgare* | Viral mRNA | *Barley yellow dwarf virus-PAV (BYDV-PAV)* | Highly resistant | Barley transgenic lines | Wang *et al.* 2000 |
| *M. esculenta* | Viral mRNA | *Sri-Lankan cassava mosaic virus (SLCMV)* | Highly resistant | KU50 | Ntui *et al*. 2015 |
| *S. tuberosum* | Viral mRNA | *Cucumber mosaic virus with strain O and Y* | 100% resistant to both strains | Danshaku transgenic line | Ntui *et al.* 2013 |
| *S. tuberosum* | Viral mRNA | *Potato virus Y* | Highly resistant | Potato transgenic lines | Missiou *et al*. 2004 |
| *C. melo* | Viral mRNA | *Papaya ringspot virus type W* | Resistant | Transgenic line of Cultivar Sun lady | Krubphachaya *et al*. 2007 |
| *O. sativa* L. ssp. *Japonica* | Viral mRNA | *Rice dwarf virus* | Resistant | Cultivar Zhonghua 11 | Liang *et al*. 2004 |
| *O. Sativa* L. | RTBV-Os/O-Ds | *Rice tungro bacilliform virus (RTBV)* | Disease resistant | Line RTBV-O-Ds1 | Tyagi *et al*. 2008 |
| *Capsicum* | CMVP0-CP | *Cucumber Mosaic virus* | Highly resistant | CMVP1 line | Lee *et al*. 2009 |
| *S. officinarum* | CP | *Sorghum mosaic virus (SrMV)* | Enhance resistance | ROC22 cultivar | Guo *et al.* 2014 |

**Table 4: Use of RNAi in various plant species against insect & pest attack**

| Crop | Targeted Gene | Insect/Pest Resistance | Transformation Success | Plant Line | Reference |
|---|---|---|---|---|---|
| *N. tabacum* | V-ATPaseA | Whitefly | Resistance enhanced | Transgenic Tobacco lines | Thakur *et al*. 2014 |
| *A. thaliana* | GPA | *Myzus persicae* | 60% decline of insect population | dsMpC002 | Coleman *et al.* 2014 |
| *A. thaliana, N. tobacum* | P450 (CYP6AE14) | *Helicoverpa armigera* | Growth retarded | *AtdsCYP6AE14-2* *NtdsCYP6AE14-2* | Mao *et al.* 2007 |
| *Z. mays* L. | WCR | *Western Corn Rootworm* | Mortality and Stunting larvae | pMON94805 | Baum *et al.* 2007 |

**Table 5: Use of RNAi in various plant species against nematode attack**

| Crop | Targeted Gene | Nematode Resistance | Transformation Success | Plant Line | Reference |
|---|---|---|---|---|---|
| *N. tabacum* | Integrase gene/Splicing factor | *Meloidogyne incognita* | Complete resistance | Tobacco | Yadav *et al.* 2006 |
| *G. max* | Major Sperm Protein (MSP) | *Heterodera glycines* | 68% reduction in egg production | Soybean | Steeves *et al.* 2006 |
| *A. thaliana* | Parasitism gene 16D10 | Meloidogyne species | Highly resistant | *Arabidopsis thaliana* | Huang *et al.* 2006 |
| *A. thaliana* | 3B05, 4G06, 8H07, 10A06 | *Heterodera schachtii* | Not completely resistant | Transgenic *A. thaliana* plants | Sindhu *et al.* 2009 |
| *A. thaliana* | Hs4F01 | *Heterodera schachtii* | Reduce the infection level and no. of females | Transgenic *A. thaliana* plants | Patel *et al.* 2010 |

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|---|---|---|---|---|---|
| *A. thaliana* | Mc16D10L | *Meloidogyne chitwoodi* | Significantly resistant | Transgenic Arabidopsis line | Dinh *et al.* 2014 |
| *N. tabacum* | MjTis11 | *Meloidogyne javanica* | Gene was silenced but silencing did not render resistance | Transgenic Tobacco plants | Fairbairn *et al.* 2007 |
| *B. rapa* ssp. Oleifera | 8H07 | *Heterodera schachtii* | Sufficient tolerant | Transgenic rape plants | Tsygankova *et al.* 2012 |
| *S. tuberosum* | Mc16D10L | *Meloidogyne chitwoodi* | Significantly resistant | Transgenic Potato line | Dinh *et al.* 2014 |

**Table 6: Use of RNAi in various plant species against parasitic weeds attack**

| Crop | Targeted Gene | Parasitic weed Resistance | Transformation Success | Plant Line | Reference |
|---|---|---|---|---|---|
| *L. esculentum* | Mannose 6-phosphate reductase (M6PR) | *Orobanhe aegyptiaca* | Enhanced resistance | Tomato transformed line | Aly, 2010 |
| *N. tabacum* | Sarcotoxin *IA* | *Orobanche* | Enhanced Resistance | Engineered tobacco line | Aly, 2010 |
| *L. sativa* | GUS | *Orobanchaceae* | Enhanced resistnce | Transgenic Lettuce | Tomilov *et al.* 2008 |
| *Z. mays* | 5 Striga | *Striga asiatica* | No effect | Transgenic Maize | Framond *et al.* 2007 |

**Table 7: Use of RNAi in various plant species against drought stress**

| Crop | Targeted Gene | Drought Tolerance | Transformation Success | Plant Line | Reference |
|---|---|---|---|---|---|
| *Brassica* | Farnesyl-transferase (FTA) | Drought Resistance | Enhanced Resistance | Transgenic canola plants | Wang *et al.* 2009 |
| *O. sativa* L. | Squalene synthase (SQS) | Drought tolerance | Enhanced Tolerance | Transgenic RNAi plants | Manavalan *et al.* 2012 |
| *O. sativa* L. | Receptor for actiated C-Kinase 1 (RACK1) | Drought tolerance | Enhanced tolerance | Transgenic rice plants | Li *et al.* 2009 |
| *O. sativa* L. | E3 ligase gene-OsDSG1 | Drought tolerance | Enhanced tolerance | *Oryza sativa* L. | Park *et al.* 2010 |
| *O. sativa* L. | C3HC4 RING finger E3 ligase OsDIS1 | Drought Tolerance | Enhanced Tolerant | *Oryza sativa* L. | Ning *et al.* 2011 |
| *H. vulgare* | Gamma-aminobutyric acid transaminase (GABAT) | Drought tolerance | Susceptible to Drought | Transgenic barley plants | Martin, 2015 |

**Table 8: Use of RNAi in various plant species to improve nutritional quality**

| Crop | Targeted Gene | Nutritional quality | Transformation Success | Plant Line | Reference |
|---|---|---|---|---|---|
| *T. aestivum* | Gamma Gliadin | Gliadins | 55-80% and 33-43% gliadin contents reduced | Transgenic lines (BW2003, BW208) | Gil-Humanes *et al.* 2008 |
| *G. hirsutum* | Ō-cadinene synthase | Gossypol | Gossypol content significantly reduced | Transgenic cotton plant | Sunikumar *et al.* 2006 |
| *A. thaliana* | *AtLKR/SDH* and DHPS | Lysine | Reduction of Lysine degradation | Transgenic Arabidopsis plants | Zhu and Galili, 2004 |

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|---|---|---|---|---|---|
| *Z. mays* L. | 22-kD zein gene | Lysine | High lysine content | Transformed corn plants | Segal *et al.* 2003 |
| *S. lycopersicum* L. | SIARF7 | Auxin and GA | Trigger the Auxin and GA pathway | Transgenic tomato plants | Jong *et al.* 2011 |
| *S. lycopersicum* L. | A-Man and β-Hex | Delayed ripening | Enhanced shelf life up to 30 days | Transgenic Tomato lines | Meli *et al.* 2010 |
| *S. lycopersicum* L. | ACC oxidase | Delayed ripening | Enhanced shelf life up to 120 days | Transgenic tomato plants (Hezuo 906) | Xiong *et al.* 2005 |
| *S. tuberosum* | Bch gene | β-carotene content | Enhanced β-carotene content | Yema de Huevo, 91E22, Desiree | Eck, *et al.* 2007 |

**Table 9: Use of RNAi in various plant species to improve forage and its digestibility**

| Crop | Targeted Gene | Forage and its digestibility | Transformation Success | Plant Line | Reference |
|---|---|---|---|---|---|
| *M. sativa* L. | P450 | Digestibility increased | Improves digestibility | Alfalfa | Reddy *et al.* 2005 |

**Table 10: Use of RNAi in various plant species to enhance grain yield**

| Crop | Targeted Gene | Grain Yield | Transformation Success | Plant Line | Reference |
|---|---|---|---|---|---|
| *T. aestivum* | OsDWARF4 | Yield | Yield enhanced | Wheat | Feldmann, 2006 |
| *O. sativa* L. | OsGA20ox2 | Enhanced panicle length, seeds/panicle, 1000 grain weight | Enhanced Yield | RNAi semi dwarf lines | Qiao *et al.* 2007 |

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