Journal of Natural Sciences Research www.iiste.org
ISSN 2224-3186 (Paper) ISSN 2225-0921 (Online)
Vol.13, No.17, 2022

Advances on the Application of Non-Codding RNA in Crop Improvements

Diriba Guta Dekeba
Ethiopian Institute of Agriculture Research, National Agricultural Biotechnology Research center,
Holeta, Oromia, Ethiopia

Abstract

The worldwide population is expanding exceptionally quickly coming about in a challenge of food security. Food security is an exceptionally serious worldwide issue, particularly within a developing country. RNAi applications are the modern innovation that can contribute to the solution for these issues. Non-coding RNAs are molecules that can't be transcribed to protein or inhibiting protein transcribed. There are numerous types of non-coding RNAs include transfer RNAs (tRNAs) and ribosomal RNAs (rRNAs), as well as small RNAs and the long ncRNAs. Crop yield improvement is the ultimate objective of molecular crop breeding. There are multiple physiological traits influencing crop yield, and several studies have found ncRNAs and their target genes are involved in those traits. The non-coding RNA interferes with the physiological traits which influence the crop yield are; plant architecture, abiotic stress, biotic stress, and fruit improvement.

Keywords: Crop improvement, long RNA, ncRNA, RNA interference, small RNA
DOI: 10.7176/JNSR/13-17-01
Publication date: October 31st 2022

1. INTRODUCTION

The worldwide population number has been rapidly expanding. In any case, worldwide food security is still challenged by around the world climate changes, human population expansion, and utilization of crop plants (FAO, 2020). But as the area of arable land and cultivated land continues to decrease, the future capacity to meet the world's food security needs has come under a cloud of instability. These issues and threats have caused scientists to explore for solutions to move forward the productivity of the crops. Advocates of new technologies including DNA recombination have promised a modern green transformation, with genetically modified crops including transgenes accomplishing targeted traits (Wheeler and von Braun, 2013, Sang and Chanseok, 2016, Bader *et al*., 2020, Mezzetti *et al*., 2020).

However, presently, utilizing non-coding RNA (ncRNA) to improve crops gives a modern alternative. Non-coding RNAs refer to transcripts that don't code for proteins but play important regulatory parts within the cell, which excludes the possibility of producing exogenous protein products. Non-coding RNA may be a gene silencing phenomenon that involves sequence-specific gene regulation include by double-stranded RNA resulting in inhibition of protein generation (Zheng and Qu, 2015; Brant and Budak, 2018; Yan *et al*., 2020; Yan *et al*., 2021).

Some classes of regulatory ncRNAs regulate single target gene specifically, while the others modulate multiple genes at a genome-wide level, via various molecular mechanisms. These accumulative findings suggest that regulatory ncRNAs could be potential targets for molecular crop breeding (Zhou and Luo, 2013).

A division of ncRNAs is processed in to functionally important RNAs such as transfer RNA (tRNA), ribosomal RNA (rRNA), small non-coding RNA including; microRNAs(miRNAs), small interfering RNAs (siRNAs), and PIWI-interacting RNAs (piRNAs), and the long non-coding RNAs (lncRNAs). Two widely studied lncRNAs are X inactivation specific transcript (XIST) and HOX antisense intergenic RNA (HOTAIR) (Renyi and Jiankang, 2014; Santosh *et al*., 2014; Brant and Budak, 2018).

Post-transcriptional gene silencing (PTGS) is reported in many organisms; fungi, animals, and plants. These products are the results of non-coding dsRNA, the DICER or Dicer-like enzyme that carries out this cleavage, small non-coding RNA involves an RNA-induced silencing complex (RISC), and Argonaute proteins (AGOs). Drosha and Pasha are part of the “Microprocessor” protein complex. Drosha and Dicer are RNase III enzymes, Pasha is a dsRNA binding protein when Argonautes are RNase H enzymes (Wilson and Doundna, 2013, Kamthan *et al.,* 2015, Brant and Budak, 2018).

The objectives of this review are to discuss non-coding RNA and its use in crop improvements. The RNA interferences in the environmental adaptation of crops are for tolerance to abiotic stress and plant protection against biotic stresses. The RNA interferences are increasing yield potential in plants including manipulation of crop developments and nutritional improvements.

1.1. NATURES OF NON-CODING RNAs (ncRNAs)

1.1.1. RIBOSOMAL RNA (rRNA)

Ribosomal RNA is the most abundant small non-coding RNA molecule, consist of 4-10% of cellular RNA. They are translation machinery in that they translate the genetic information in an mRNA template-directed manner into a corresponding polypeptide chain (Rodnina and Winter Meyer, 2011). The mRNA sequence starting from the start codon of AUG and is followed by the codon which produces proteins which are followed by termination codon signaling that the protein is ready for dissociation from the ribosome for subsequent folding into its functional state (Crick, 1970). The ribosomes in the eukaryotes are more complex than prokaryotic, in general, ribosomes have three different binding sites; A (aminoacyl) site, the P (peptidyl) site, and the E (exit) site, formed in the intersubunit interface. The mRNA binding to the 30S subunit can move stepwise manner, one codon at a time during the peptide elongation (Crick, 1970).

The ribosomal RNA constitutes approximately 60% rRNA and 40% protein by weight and also it contains two major rRNAs and 50 or more proteins. The subunit 60S holds (three rRNAs 5S, 5.8S, 28S, and about 40 proteins) and the 40S subunit contains (an18S rRNA and about 30 proteins). The LSU rRNA acts as a ribozyme, catalyzing peptide bond formation (Anita *et al*., 2013).

1.2. TRANSFER RNA (tRNA)

Transfer RNA is the link between mRNA and the peptide sequence in transfer RNA (tRNA), there is the enzyme that can recognize both tRNA and an anticodon complementary to the mRNA codon cognate to this amino acid and couples them together at the expense of ATP hydrolysis to a high standard free energy complex called aminoacyl-tRNA (Sharp *at el,* 1985).

The mRNA binds to the 40s ribosomal subunit, then it is followed by binding of the initiator tRNA, charged with formylated methionine, to the P site in a reaction step greatly accelerated by the three initiation factors. Once the mRNA and initiator tRNA is correctly bound, the 60S large subunit binds to form an 80s initiation complex with a release of the eIF factors. With the mRNA in the correct reading frame, initiator tRNA in the P site, and the empty A site programmed with the first internal codon of the protein to be synthesized. The ribosome has now left the initiation phase and entered the peptide elongation phase (Valle *et al*., 2003).

1.3. SHORT NON-CODING RNAs

1.3.1. Micro RNA (miRNA)

Micro RNA is the party of small non-coding RNA (20-22nt) having partially double-stranded stem–loop structures, regulating negative gene expression in both animals and plants. It's transcribed by RNA polymerase II enzyme and after transcription cleaved by Dicer-like 1(DCL1) enzyme and transported to the cytoplasm and incorporated into Argonauts (AGO) protein. Mature miRNA is produced in different methods and incorporates into the protein RISC (RNA-induced silence complex). Matured single miRNA incorporated with the protein RISC are binding with other complementary mRNA sequences to produce the protein (Khraiwesh *et al*., 2012).

1.3.2. Small interfering RNA (siRNA)

Small interfering RNA are the parts of small non-coding RNA that have the function to facilitate the post-transcriptional gene silencing (PTGS) by degradation of mRNA. Small interfering RNA is generated from double strands of RNA which exhibit near-perfect complementary and cleaved by DCL2, DCL3, and DCL4 to produce siRNA duplex 22nt, 24nt, and 21nt respectively (Sang and Chanseok, 2016). The other cofactors of siRNA are RNA-dependent RNA polymerases 2 and 6 (RDR2, RDR6), SUPPRESSOR OF GENE SILENCING 3 (SGS3), and plant-specific DNA-dependent RNA polymerases IV and V (Bologna and Voinnet, 2014).

After the siRNAs are cleaved by DCL, it is loaded to AGRONAUTEs (AGOs) and corporates with complex forming gene silence machinery which is called RISC (RNA induced silence complex). The RISC recognizes the complementary sequence of mRNA and AGO-loaded small RNA (also known as 'guide RNA'), then RISC suppresses the target mRNA via cleavage or transcriptional gene silencing (TGS) mechanism. The molecular mechanisms of RISC-mediated gene suppression depend on which small RNA species and particular AGO protein that forms RISC; miRNA- or 21-nt siRNA-loading AGO1 participates in slicing and degradation of target endogenous mRNA, whereas 24-nt siRNA-loading AGO4 and AGO6 mediate transcriptional gene silencing through heterochromatin formation (Sang and Chanseok, 2016).

Small interfering RNA is naturally very important in the organism to protect it from different natural enemies. So, RNA interference (RNAi) was considered to be a natural defense mechanism that used exogenous siRNAs to protect organisms from viruses, but it soon became evident that endogenous siRNAs (endo-siRNAs) also play a role in regulating genome functions (Kamthan *et al*., 2015).

1.3.3. Piwi-interacting RNAs (piRNA)

Piwi-interacting RNAs are small non-coding RNAs that have 20-31nt; it is named after forming a complex with Piwi proteins of the Argonaut family. Piwi-interacting RNAs were first discovered in *Drosophila* as repeat-associated siRNAs (rasiRNA), which show complementarities to a variety of transposable and repetitive elements (Minna *et al.* 2011).

The main function of these RNA molecules involves chromatin regulation and suppression of transposon activity in germ-line and somatic cells. piRNAs are antisense to expressed transposons target and cleave the transposon in complexes with PIWI proteins. This cleavage generates additional piRNAs which target and cleave additional transposons. This cycle continues to produce an abundance of piRNAs and augment transposon silencing (Sato and Siomi, 2013).

1.4. LONG NON-CODING RNA (lncRNA)

LncRNAs are transcripts generally longer than 200nt having little or no potential of encoding proteins. Most of the lncRNAs have similar characteristics within the mRNA, transcribed by RNA polymerase II. In addition to RNA Pol II-derived lncRNAs, there are other classes of lncRNAs transcribed by two plant-specific DNA-dependent RNA polymerases, RNA Pol IV and RNA Pol V, which play critical roles in transcriptional gene silencing mediated by RNA-dependent DNA methylation (RdDM) (Sang and Chanseok, 2016).

LncRNA can be divided based on genomic location, sense lncRNAs overlap with one or more exons of a transcript on the same strand, antisense lncRNAs overlap with one or more exons of a transcript on the opposite strand, intronic lncRNAs derive from an intron within another transcript, and intergenic lncRNAs occur in the interval between two genes on the same strand (Ma *et al*., 2013).

Although only a small number of lncRNAs are identified and functionally investigated until now, plant lncRNAs play important roles as regulators in complex gene regulatory networks involved in plant development and stress management (Zhang *et al*., 2014). The human genome has only 3% of the coding region but more than 85% of the genome is actively transcribed and the biggest challenge understand the functional role for these transcripts (Hangauer *et al*., 2013).

The role of lncRNA through regulating the expression levels of target genes ranging from transcription to translation. Some examples of their functions are; lncRNA, called LDMAR (for long-day specific male-fertility-associated RNA), which regulates the photoperiod-sensitive male sterility of the rice variety whose pollen is completely sterile in a long-day condition. Genome-wide investigation on rice also identified a set of lncRNAs that are specifically expressed during the reproduction stage (Zhang *et al.* 2014). On the Flowering Locus C (FLC) in *Arabidopsis*, an antisense lncRNA, COOLAIR, and a sense lncRNA, COLD AIR, are produced during vernalization to form an epigenetic switch for silencing the expression of the FLC gene to promote flowering (Fatica and Bozzoni, 2014, Renyi and Jiankang, 2014).

2. APPLICATION OF RNAi IN CROP IMPROVEMENT

Crop yield improvement is the main ultimate goal of molecular crop breeding. There are multiple physiological traits influencing crop yield, and several studies have found ncRNAs and their target genes are involved in those traits. Some physiological traits which influence crop yield are; plant manipulation, abiotic stress, biotic stress, and fruit improvement.

2.1. PLANT ARCHITECTURE

2.1.1. Biomass

RNA interferences are one application that can improve the yield, quality, quantity, and nutritional contents of the crops and fruits to manipulation of its traits. The RNA interferences can increase the biomass of the crops and fruits by manipulation of plant height, inflorescences, branching, and size of the crops.

RNA interference knockdown to the OsDWARF4 gene in rice to decrease the plants erect leaf and increasing the photosynthesis by lower leaf. The yield of such plants is improved or increased by dense planting conditions (Feldmann, 2006). Over-expression of the maize MIR156, Corngrass1 (Cg1) (Chuck *et al*., 2011), and Red clover (*Trifolium pratense* L.) (Zheng *et al*., 2016) cause to prolong the vegetative parts of maize and delayed the flowering. That means at the reproductive stage is delayed, the plant's vegetative parts are to be increasing and at the same time biomass of plants becomes increasing.

Moderate and low levels of miR156 expression had 58–101% more biomass production than wild-type controls as a result of increases in tiller numbers in switchgrass plants. Over-expression of rice miR156 could improve the yield of solubilized sugar as well as forage digestibility (Xie *et al*., 2012, Johnson, 2017), suspending the flowering time in the *Arabidopsis* (Roussin *et al*., 2020), and it can manipulate the flowering colors (Yamagishi and Sakai, 2020).

2.1.2. Grain Yield

The improvements of the crops by different technology or methods are mainly for increasing the yield of the crops. RNA interferences are one of the technologies which are used to improve the crop's yield by manipulating the crop's traits. One of the applications of RNA interferences is the manipulation of the gene, which affects the characteristics of the crops whether to increasing or decreasing the crop yield.

Regulatory ncRNAs are also influenced in the reproductive stage, it is a very important key factor that affects crop yield and it's used for genomic association. According to Ding *et al.* (2012), lncRNA, called LDMAR (for LONG-DAYSPECIFIC MALE-FERTILITY-ASSOCIATED RNA), regulates the photoperiod sensitive male sterility of the rice variety whose pollen is completely sterile in a long-day condition. Genome-wide investigation on rice also identified a set of lncRNAs that are specifically expressed during the reproduction stage (Zhang *et al*., 2014).

The RNA mediates suppression of GA 20-oxidase (OsGA20ox2) gene resulted in semi-dwarf plants from a taller rice variety QX1. It is increasing in panicle length, the number of seeds per panicle, and higher test weight. OsSPL14 (Souamosa promoter binding protein-like14) was reported to be the target of Osa-miR156 in rice to increase the yield of the rice grain, decreased tiller number, and increased grain yield (Wang *et al*., 2012; Jiao *et al*., 2010). Overexpression of *Osa-miR1873* also resulted in some defects in yield traits, including grain numbers and seed setting rate in rice (Zhou *et al*., 2020). Overexpression of *the OsAGO17* gene was furthermore improved grain size and weight and promote stem development in rice (Zhong *et al*., 2020).

2.2. FRUIT IMPROVEMENT

The application of transgenic protects crops from different pests which can affect the plant products. Genetic engineering was also applied to improve the composition and quality of the harvested organs to reduce post-harvest deterioration of fruits or increase agronomic quality and nutritional value. Fruits are consumed when fresh because they are very sensitive to pests and are perishable. RNA interference is the technology that can overcome this problem (Meli *et al*., 2010).

2.2.1. Enhanced Nutritional Value and Edibility

RNA silencing-based technology has also enjoyed success in improving the nutritional value of crops. By down-regulating key genes in plant metabolic pathways using RNAi constructs, transgenic crops may accumulate more favorable metabolites or produce fewer unwanted ingredients. Crops have different nutritional values, some of them are allergens to a human being, some of them are pollution to environments, and some of them are allergens to the other crops, and so on. But there are crops which have very important nutritional values that are consumed by human or animals or others. RNA interference is to manipulation of all these nutrients by adding the important one and removing the unwanted.

Tomato (*Lycopersicon esculentum*) is one of the most economically important fruit crops across the world rich in antioxidants, minerals, fibers, and vitamins. RNAi has been utilized in the development of tomato fruit with an enhanced level of carotenoids and flavonoids which are highly beneficial for human health (from Kamthan *et al*., 2015).

RNAi approach has also been used in apple to improve the fruit quality by enhanced self-life reducing the amount of a major apple allergen and metabolisms and accumulation of sugar in the fruits were affect the sorbitol synthesis which affects fruit quality such as starch accumulation (Romer *et al*., 2020) and sugar-acid balance (Teo *et al*., 2006)

Some examples of nutrition adding crops by RNAi are; high lysine maize to the expression of zein proteins (Li & Song, 2020; Choudhary *et al*., 2021), Silencing of carotenoid β-hydroxylases increases the β-carotene content of maize (Berman *et al*., 2017).

RNAi has also been used to down-regulate the starch-branching enzyme resulting in high-amylose wheat, which has a great potential to improve human health (Man and Hong, 2013). Corn with increased essential amino acids (Hasan and Rima, 2021), improve soybean by oil quality (Yang *et al*., 2018), cotton with improved fatty acid composition and Overexpression of *GmPDAT* genes were increased seed size and oil content (Gao *et al*., 2020), and whereas RNAi lines had decreased seed size and oil content of soybean (Liu *et al*., 2020)were some reported. Suppression of three carotenoid-cleavage dioxygenase genes, OsCCD1, 4a, and 4b, increases carotenoid content in rice (Ko *et al*., 2018).

2.2.2. Enhanced Shelf Life

The major problems of fruits are post-harvest deterioration and spoilage of vegetables and fruits. It is a major economic loss. This may be because of some problems like; method of harvesting, transports, storage, and other problems. Therefore, an increase in the shelf life of vegetables and fruits by delayed ripening is another essential agronomic trait. That is being addressed through non-coding RNA technology. Initiation of ripening in climacteric fruits like tomato is characterized by a climacteric burst of ethylene, resulting in the regulation of the expression of ripening-specific genes. Manipulating the gene which is responsible to produce ethylene and reduce the production of ethylene (Osorio *et al*., 2011). RNA interference is the decreasing the expression of 1-aminocyclopropane-1-carboxylate (ACC) oxidase, a gene of ethylene biosynthesis pathway in tomato and inhibited the ethylene production at the ripening time then the fruit can survive a long time.

RNA silencing was first genetically modified in the Flavr Savr tomato, introduced the antisense of transcript polygalacturonase (PG) which is suppressed to PG expression. The polygalacturonase is responsible for cell wall degradation during tomato ripening; suppression of polygalacturonase delayed the natural softening of tomatoes and allowed tomatoes to ripen on the vine longer resulting in a more flavorful fruit (Renyi and Jian, 2014). The increasing shelf lives of tomatoes were observed after increasing abscisic acid (ABA) contents are increasing. Manipulation of β-carotene levels results in an improvement not only in the shelf life of tomato fruits but also in their nutritional value (Diretto *et al*., 2020).

However, overexpression of the mi156 gene in tomato resulted in small fruits, increased leaf numbers, and long shelf life, but if low expression high yielding and short shelf life were observed (Zhang *et al*., 2011a).

Overexpression of different genes was also increasing the shelf life of tomato were informed in different reports, like overexpression of Pti4, Pti5, and Pti6 genes (Wang *et al*., 2021), *SlGRAS4* gene (Liu *et al*., 2021) in tomato are very significant to accelerated fruit ripening, increased the total carotenoid content.

Non-coding RNA is very essential in an increase in the shelf life of vegetables and fruits by delayed ripening is another essential agronomic trait. RNAi application in crops shelf life increasing were including; tobacco shelf life increasing (Moreno *et al*., 2020), Tomatoes (Arefin *et al*., 2020), superoxide dismutase (SOD) genes that expressed during ripening of apple fruit were identified from the apple genome (Lv *et al*., 2020), cold storage responses genes in the peach were reported (Antonella *et al*., 2020), Blueberry cultivars with higher fruit firmness and longer shelf life were identified (Liu *et al*., 2021), preserved post-harvest shelf life and quality of banana fruit (Yumbya *et al*., 2021). RNAi strategy targeting suppression of more than one homolog would be certainly much effective than the knockdown of a single homolog (Gupta *et al*., 2013).

2.2.3. Seedless Fruit Development (Parthenocarpy)

Parthenocarpy or seedless fruit development is a method of fruit production from seedless crops by ovaries without pollination and fertilization. Parthenocarpy or seedless is highly appreciated under agronomic traits, especially in edible fruit crops, it produces high yielding under harsh environments because of no need for pollination and fertilization. Customers always need fresh fruit, while through all the seasons it is possible to produce the fruit. Production of high quality and marketable fruit through the season, also it is very important for a producer to gain income (Molesini *et al*., 2012)

In some cases, fruits produce hard seed which is problematic to overcome the dormancy fruits, some others produce seeds with bad tests, the absence of seeds can be also an advantageous trait for both direct fresh consumption (e.g. grape, citrus, and banana) and industrial processes (e.g. frozen eggplants, and tomato sauce) (Meli *et al*., 2010).

RNA interferers are one of the foremost fundamental technologies to overcome the issues of parthenocarpy plants. A few crops which can move forward by RNAi innovation are; Manipulation of auxin response factors8 (ARF8), it is the target of miR167 result produce of parthenocarpic fruit in both *Arabidopsis* and tomato (Molesini, *et al*., 2012), suppression of the orthologous genes of *Pad-1* induced parthenocarpic fruit development in Solanaceae plants are very powerful tools to improve Solanaceae fruit production (Matsuo *et al*., 2020), Overexpression of *PbGA20ox2* gene was altered the GA biosynthetic pathway and enhanced GA_4 synthesis, thereby promoting fruit set and parthenocarpic fruit development in pear (*Pyrus Bretschneider* Rehd.) fruit (Wang *et al*., 2020). Gene of PpIAA19 was involved in regulating tomato lateral root numbers, stem elongation, parthenocarpy, and fruit shape (Ding *et al*., 2019), Grapevine (Wang *et al*., 2020), *MaTPD1A* gene overexpression in banana plants produce seedless fruit compared to wild-type plants (Hu *et al*., 2020).

2.3. BIOTIC STRESS RESISTANCE

Phyto-pathogens are diseases that can affect crops and crop products. The disease may affect the crops at different stages; seedling, vegetative, and also at productive stages. RNAi strategies have been employed to improve the Small RNA-mediated crop improvement defense mechanism in crop plants against various biotic stresses including an attack by viruses, bacteria, fungi, nematodes, and insects (Park and Shin, 2015).

2.3.1. Virus Resistance

Viruses are pathogens that can affect crop products. RNA interference is one of the technologies which can overcome the effects. The functions of RNA interferences are defense mechanism of virus that invades nucleic acid molecules. The transgenic plant technology was to express genes encoding viral coat proteins in transgenic plants and it can be resistant to viruses containing the coat proteins. The viral resistance was due to the recognition of coat proteins by the transgenic plant cells and because of that the plant immune response against the coat proteins. But, know the application of genetic transformation technology, the plants can express the coat protein gene transcript, degraded the protein coat but not produce the protein which against the protein coat itself. Transgenic plants which are resistant to viruses were produced by sense transgene triggered RNAi against the viral RNAs (Ding, 2010, Renyi and Jian-kang, 2014, Yogindran & Rajam, 2015, Uslu et al., 2020).

RNAi has revealed a way of obtaining virus-resistant traits in many crop plants. The transgenic plant can produce the viral sequences that match coat proteins, replication-associated proteins, ATPases, or promoter regions in the viral genomes which can derive transgene siRNAs to target viral RNAs for degradation upon infection. The reported crops which including viral resistance were including; papaya (Kertbundit *et al*., 2007), cassava (Vanitharani *et al*., 2004; Vanderschuren *et al*., 2012), and Potato (Sajid et al., 2019), were some reported crops. Some examples are Potato Resistance to Spindle Tuber Viroid (PSTVd) and Cassava resistance to African Cassava Mosaic Virus (ACMV) (Renyi and Jian, 2014). In the virus prevention strategies, the ecological implication is underlying, raised Carbon Dioxide Levels Decrease Cucumber Mosaic Virus Accumulation, in the *Nicotiana tabacum* by viral suppressor of RNAi (VSR) 2b protein of CMV (Guo *et al*., 2021).

2.3.2. Bacterial Resistance

Bacterial diseases are extremely difficult to control due to the high production rate of spreading. The reproduction rates of bacteria are very high as compared to plant production; because of this, it can simply control plant development.

RNA interference application technologies are applied in different plants to resist bacterial diseases. Some crops that can RNA interference are; In *Arabidopsis*, miR393 was reported to be induced by a bacterial pattern associated molecular pattern (PAMP) peptide flg22 and negatively regulate the F-box auxin receptors TIR1, AFB2, and AFB3. This repression of auxin signaling positively contributed to the restriction of bacterial (*Pseudomonas syringae*) infection (Li *et al*., 2010a; Zhang *et al.,* 2011a). The other application RNAi has mediated suppression of two genes of *Agrobacterium tumefaciens* involved in crown gall tumor formation (iaaM and ipt) could significantly reduce the production of tumors in *Arabidopsis* (from Kamthan *et al*., 2015). Overexpression of the rice chorismate mutase (OsCM) gene was altered the downstream pathway of aromatic amino acids, mitigating bacterial leaf blight (BLB) stress by altering stress-responsive genes and hormonal accumulation (Jan et al., 2020). Some of the detailed reported crops which can resist bacteria by manipulation RNAi genes were including; Tomato (Bento et al., 2020), Citrus (Yu et al., 2020), Soybean (Tian, *et al*., 2020), and Arabidopsis (Guo, *et al*., 2020) were recently reported.

2.3.3. Fungal Resistance

RNA silencing-based genetic engineering has contributed significantly to crop improvement; resistance to pathogens. Fungus is a pathogen that can affect crop production and quality. RNAi has proved to be an important strategy to generate tolerance in various crop plants (Kamthan *et al*., 2015).

RNA interference involved in response to attack by the fungus in wheat *Blumeria graminis* f. sp. *tritici* (Bgt) by using 24 miRNAs which cause devastating diseases of wheat powdery mildew (Xin *et al*., 2010). Transgenic of the Rice plants overexpression of OSA-miR7695 (Campo *et al*., 2013), *miR160a* or *miR398b* (Li et al., 2014), miR169 (Li et al., 2017), and Osa-miR167d (Zhao *et al*., 2020) was found to negatively regulate in response to the blast fungus *Magnaporthe oryzae* and improved resistance to rice blast infection was achieved by overexpression of those genes.

Treating the *Fusarium* spec. with various dsRNAs targeting the genes was destructive to the fungus and resulted in growth retardation *in vitro* cultures was observed (Koch *et al*., 2018). In the *Arabidopsis thaliana* line reduction, the activity of miR396 using artificial miRNA target mimics was found to confer resistance to necrotrophic and hemibiotrophic fungal pathogens (Soto-Suárez *et al*., 2017). Overexpression of the BnaNPR1 gene within the oilseed rape played a positive role in the resistance of *Brassica napus* against *Sclerotinia sclerotiorum*, which gives resistance to this pathogen (Wang *et al*., 2020).

2.3.4. Insect and Resistance

Defoliating plants or sucking out their sap insects can cause considerable damage to crop plants, by slow down their growth, weakening, and sometimes killing them (Yogindran and Rajam, 2015). The use of RNAi for insect control seems to be the consequence of the success of Cry toxins from Bt as an insecticide. Insect resistance crops are producing the dsRNA which affects the insects and in insects use as diet, the gene expresses itself (Huvenne and Smagghe, 2010).

This technology is to protect insects from different crop production; western corn rootworm *Diabrotica virgifera*, the cotton ball worm *Helicoverpa armigera*, and the tobacco hornworm *Manduca sexta*, among others (Renyi and Jian-kang, 2014). Transgenic tobacco lines expressing dsRNA of v-ATPase, leading to the mortality rate of whiteflies (Thakur *et al*., 2014) transcript level of the target genes in *Bemisia tabaci* (B. tabaci) more than 70% mortality was observed (Raza *et al*., 2016, Shelby *et al*., 2020). RNA interference-mediated knockdown of CsKr-h1 considerably reduced the transcription of vitellogenesis (Vg) within the C. suppressalis, its exceptionally critical within suppressing rice pests (Tang *et al*., 2020). For pest management in the field through topical application or spraying dsRNA shows very great potential, up to 81.67% mortality was recorded for soybean aphids Aphis *glycines*(Yan *et al*., 2020), and up to 90% mortality was recorded for soybean *Nezara viridula* (Sharma *et al*., 2021).

2.4. ABIOTIC STRESS TOLERANCE

Plant growth on the field may face multiple abiotic stresses like; drought, salt, heat, and cold stresses. RNA silencing coding is one of the technologies which can overcome this problem, the abiotic stress of plant products. By regulating the endogenous level of regulatory ncRNAs in response to abiotic stresses, they fine-tune the expression of their target genes that are closely involved in specific or multiple stresses.

2.4.1. Drought and Salinity Tolerance

Water deficit or drought and salinity are the abiotic stress which is major environmental stress limiting crop productivity. RNA silencing coding has been applied successfully to develop drought tolerance and salinity tolerance crops. RNA interferences are using in the canola to transfer the AtHPR1 promoter which is resistant to seed abortion during flowering induced by water deficiency without affecting yield during drought stress.

Transgenic rice plants tolerant to drought stress were developed including; receptor for activated C-kinase1 (RACK1) (Li *et al.,* 2009), knockdown of a RING finger E3 ligase gene-OsDSG1 and also silencing of OsDIS1 for *Oryza sativa* drought-induced SINA protein (Park *et al*., 2010).

A few miRNAs have been recognized that respond to drought, high salt, and high-temperature stress in Brassica. Of 126 novel miRNAs identified, miR164, miR160, and miR156 were experimentally approved to target NAC-domain-containing protein, ARF17-like, and SPL2-like proteins, respectively and for control different stress condition conserved miRNA in Brassica were identified (Bhardwaj *et al*. 2014)

However, RNA interference has also regulated gene to respond the salinity tolerance crops just like; overexpression of GmNFYA3 in *Arabidopsis* resulted in increased sensitivity to salinity stress and exogenous ABA (Ni *et al.,* 2013) and also transgenic creeping bentgrass (*Agrostis stolonifera*) plants overexpressing a rice miR319 gene (OsamiR319) exhibited enhanced tolerance to drought and salinity that was associated with increased leaf wax content and water retention but reduced sodium uptake (Zhou *et al*., 2013). Tomato, overexpression of miR169c gene led to reducing stomatal openings, transpiration rate, and leaf water loss, thus enhanced drought tolerance in transgenic plants in comparison to wild-type controls (Zhang *et al.,* 2011a). In the rice correspondingly, knockdown of *the OsTBP2.2* gene was generated to increase the rice sensitivity to drought stress (Zhang *et al*., 2020).

2.4.2. Cold and Heat Stress Tolerance

Cold and heat are the abiotic stress types that affect the production of the crops, at the variable condition the crops are shocking then the yield and quality of crop products decrease and also affect the economic loss. Transgenic crops using RNA silencing coding can overcome the problem of this stress. The expression of miR319 was reported to change in response to cold stress in *Arabidopsis*, Rice, and sugarcane (Lv *et al*., 2010; Thiebaut *et al*., 2011).

The overexpression of the Osa-miR319 gene led to increased cold stress tolerance (4◦C) after chilling acclimation (12◦C) of plants (Yang *et al*., 2013). In rice, the over-expression of OsPCF5 and OsTCP21 resulted in the production of the cold-resistant transgenic plant (Yang *et al*., 2013).

Guan *et al*. (2013) discovered a novel plant thermo-tolerance mechanism, especially for the protection of reproductive organs. It involves induction of miR398 to down-regulate its targets CSD (copper/zinc superoxide dismutase) genes, CSD1 and CSD2 as well as CCS (a gene encoding copper chaperone for both CSD1 and CSD2). They found that csd1, csd2, and ccs mutants displayed higher heat stress tolerance than wild type plants associated with increased accumulation of heat stress transcription factors and heat shock proteins and reduced damage to flowers (Guan *et al*., 2013) and Many lncRNAs were identified in maize (Yuanda, *et al*, 2019) and Cassava (Suksamran, *et al*., 2020) that respond to abiotic stresses like heat, cold, salt, and drought.

3. CONCLUSION

One of the most basic requirements of a human being is food. And, we (human beings) have moved countable steps on the road of maintaining sustainable food production. But, food insecurity and malnutrition are currently among the most serious concerns for human health, causing the loss of countless lives in developing countries. Therefore, we need innovative technologies to improve our crop production methods and practices. RNA interference applications are an innovative technology that can contribute to the solution to these problems. In crop improvement, it has a wider application in the production of transgenic plants which have improved yield, increased nutritional qualities with improved taste, texture or appearance, and health benefits. It also enables us to produce plants that have reduced dependence on fertilizers, pesticides, and other agrochemicals, and plants which have reduced the vulnerability of crops to Environmental Stresses. RNA silencing gene is an advanced application that can solve the agricultural problem in a short period. It has also some limitations like; identification of appropriate target genes, off-target effects, and the application of RNAi is more sophisticated and needs highly skilled human power.

REFERENCES

Anita Quintal Gomes, Sofia Nolasco and Helena Soares, (2013). Non-Coding RNAs: Multi-Tasking Molecules in the Cell. *Int. J. Mol. Sci*., **14**: 16010-16039

Antonella, M., Müller, C. T., Bruno, L., McGregor, L., Ferrante, A., Ceverista, C. A. A., ... & Spadafora, N. D. (2020). Fruit volatile profiling through GC× GC-ToF-MS and gene expression analyses reveal differences amongst peach cultivars in their response to cold storage. *Scientific Reports (Nature Publisher Group)*, *10*(1).

Arefin, P., Amin, R., Sadiq, M. Z. A., Dey, S. S., Boby, F., & Parvaz, M. S. (2020). A Study of Effectiveness of Natural Coatings on the Shelf Life Extension of Tomatoes by the Observation of TomloxC Gene Expression. *Biosciences Biotechnology Research Asia*, *17*(3), 459-465.

Bader, A. S., Hawley, B. R., Wilczynska, A., & Bushell, M. (2020). The roles of RNA in DNA double-strand break repair. *British journal of cancer*, *122*(5), 613-623.

Bento, F. M., Marques, R. N., Campana, F. B., Demétrio, C. G., Leandro, R. A., Parra, J. R. P., & Figueira, A. (2020). Gene silencing by RNAi via oral delivery of dsRNA by bacteria in the South American tomato pinworm, Tuta absoluta. *Pest management science*, *76*(1), 287-295.

Berman, J., Zorrilla-López, U., Sandmann, G., Capell, T., Christou, P., & Zhu, C. (2017). The silencing of carotenoid β-hydroxylases by RNA interference in different maize genetic backgrounds increases the β-carotene content of the endosperm. *International journal of molecular sciences*, *18*(12), 2515.

Bhardwaj, A. R., Joshi, G., Pandey, R., Kukreja, B., Goel, S., Jagannath, A., & Agarwal, M. (2014). A genome-wide perspective of miRNA ome in response to high temperature, salinity, and drought stresses in Brassica juncea (Czern) L. *PloS one*, ***9*****(3),** e92456.

Bologna, N. G., Voinnet, O. (2014). The diversity, biogenesis, and activities of endogenous silencing small RNAs in Arabidopsis. *Annu Rev Plant Biol.* **65**: 473–503

Brant, E. J., & Budak, H. (2018). Plant small non-coding RNAs and their roles in biotic stresses. *Frontiers in plant science*, ***9***, 1038

Campo, S., Peris-Peris, C., Sire, C., Moreno, A. B., Donaire, L., Zytnicki, M., (2013). Identification of a novel microRNA (miRNA) from rice that targets an alternatively spliced transcript of the Nramp6 (Natural resistance-associated macrophage protein6) gene involved in pathogen resistance. *New Phytol*. **199**: 212–227.

Choudhary, M., Grover, K., & Singh, M. (2021). Maize's significance in the Indian food situation to mitigate malnutrition. *Cereal Chemistry*, *98*(2), 212-221.

Chuck, G. S., Tobias, C., Sun, L., Kraemer, F., Li, C., Dibble, D., (2011). Overexpression of the maize corn grass1 microRNA prevents flowering, improves digestibility, and increases the starch content of switchgrass. *Proc. Natl. Acad. Sci. USA*. **108**: 17550–17555.

Crick, F. (1970). Central dogma of molecular biology. *Nature*, **227**: 561-563.

Ding, S. W. (2010). RNA based antiviral immunity. *Nat. Rev. Immunol.* **10**: 632–644.

Ding, Y., Zeng, W., Wang, X., Wang, Y., Niu, L., Pan, L., ... & Wang, Z. (2019). Over-expression of Peach PpIAA19 in Tomato Alters Plant Growth, Parthenocarpy, and Fruit Shape. *Journal of Plant Growth Regulation*, *38*(1), 103-112.

Diretto, G., Frusciante, S., Fabbri, C., Schauer, N., Busta, L., Wang, Z., ... & Giuliano, G. (2020). Manipulation of β-carotene levels in tomato fruits results in increased ABA content and extended shelf life. *Plant biotechnology journal*, *18*(5), 1185-1199.

Fatica A, Bozzoni I., (2014). Long non-coding RNAs: new players in cell differentiation and development. *Nat Rev Genet*, **15**: 7–21.

Feldmann, K. A. (2006). Steroid regulation improves crop yield. *Nat. Biotechnol*. **24**: 46–47.

Gao, L., Chen, W., Xu, X., Zhang, J., Singh, T. K., Liu, S., ... & Liu, Q. (2020). Engineering Trienoic Fatty Acids into Cottonseed Oil Improves Low-Temperature Seed Germination, Plant Photosynthesis and Cotton Fiber Quality. *Plant and Cell Physiology*, *61*(7), 1335-1347.

Guan, Q., Lu, X., Zeng, H., Zhang, Y., and Zhu, J. (2013). Heat stress induction of miR398 triggers a regulatory loop that is critical for thermo-tolerance in *Arabidopsis*. *Plant J*. **74**: 840–851.

Guo, H., Ge, P., Tong, J., Zhang, Y., Peng, X., Zhao, Z., ... & Sun, Y. (2021). Elevated Carbon Dioxide Levels Decreases Cucumber Mosaic Virus Accumulation in Correlation with Greater Accumulation of rgs-CaM, an Inhibitor of a Viral Suppressor of RNAi. *Plants*, *10*(1), 59.

Guo, Q., Liu, Q., A Smith, N., Liang, G., & Wang, M. B. (2016). RNA silencing in plants: mechanisms, technologies, and applications in horticultural crops. *Current Genomics*, *17*(6), 476-489.

Guo, W., Chen, W., Zhang, Z., Guo, N., Liu, L., Ma, Y., & Dai, H. (2020). The hawthorn CpLRR-RLK1 gene targeted by ACLSV-derived vsiRNA positively regulates resistance to bacteria disease. *Plant Science*, *300*, 110641.

Gupta, A., Pal, R. K., and Rajama, M. V. (2013). Delayed ripening and improved fruit processing quality in tomatoes by RNAi-mediated silencing of three homologs of 1-aminopropane-1-carboxylate synthase gene. *J. Plant Physiol*. **170**: 987–995.

Hangauer, M. J., Vaughn, I. W., McManus, M. T., (2013). Pervasive transcription of the human genome produces thousands of previously unidentified long intergenic noncoding RNAs. *PLoS Genet*. **9**

Hasan, M. M., & Rima, R. (2021). Genetic engineering to improve essential and conditionally essential amino acids in maize: transporter engineering as a reference. *Transgenic Research*, 1-14. http://www.fao.org/publications/sofi/en/

Hu, C., Sheng, O., Dong, T., Yang, Q., Dou, T., Li, C., ... & Bi, F. (2020). Overexpression of MaTPD1A impairs fruit and pollen development by modulating some regulators in Musa itinerant. *BMC Plant Biology*, *20*(1), 1-12.

Huvenne, H., and Smagghe, G. (2010). Mechanisms of dsRNA uptake in insects and potential of RNAi for pest control: a review. *J. Insect. Physiol.* **56**: 227–235.

Jan, R., Khan, M. A., Asaf, S., Lee, I. J., Bae, J. S., & Kim, K. M. (2020). Overexpression of OsCM alleviates BLB stress via phytohormonal accumulation and transcriptional modulation of defense-related genes in Oryza sativa. *Scientific reports*, *10*(1), 1-15.

Jiao Y., Wang Y., Xue D., Wang J., Yan M., Liu G., Dong G., Zeng D., Lu Z., Zhu X., Qian Q., Li J. (2010). The regulation of OsSPL14 by OsmiR156 defines the ideal plant architecture in rice. *Nat Genet.* **42**: 541–544

Johnson, C. R. (2017). Reproduction and confinement of miR156 transgenic switchgrass (Panicum virgatum L.).

Kamthan, A., Chaudhuri, A., Kamthan, M. and Datta, A. (2015). Small RNAs in plants: recent development and application for crop improvement. *Front. Plant Sci*. **6**: 208-210.

Kertbundit, S., Pongtanom, N., Ruanjan, P., Chantasingh, D., Tanwanchai, A., Panyim, S., & Juříček, M. (2007). Resistance of transgenic papaya plants to Papaya ringspot virus. *Biologia Plantarum*, *51*(2), 333-339.

Khraiwesh, B., Zhu, J. K., and Zhu, J. (2012). Role of miRNAs and siRNAs in biotic And abiotic stress responses of plants. *Biochim. Biophys. Acta*, **1819**: 137–148.

Ko, M. R., Song, M. H., Kim, J. K., Baek, S. A., You, M. K., Lim, S. H., & Ha, S. H. (2018). RNAi-mediated suppression of three carotenoid-cleavage dioxygenase genes, OsCCD1, 4a, and 4b, increases carotenoid content in rice. *Journal of experimental botany*, *69*(21), 5105-5116.

Koch, A., Stein, E., & Kogel, K. H. (2018). RNA-based disease control as a complementary measure to fight Fusarium fungi through silencing of the azole target Cytochrome P450 Lanosterol C-14 α-Demethylase. *European journal of plant pathology*, *152*(4), 1003-1010.

Li Y., Zhang Q., Zhang J., Wu L., Qi Y., Zhou J.M. (2010a). Identification of microRNAs involved in pathogen-associated molecular pattern-triggered plant innate immunity. *Plant Physiol.* **152**:2222–2231

Li, D. H., Hui, L. I. U., Yang, Y. L., Zhen, P. P., & Liang, J. S. (2009). Down-regulated expression of RACK1 gene by RNA interference enhances drought tolerance in rice. *Rice Science*, *16*(1), 14-20.

Li, H., Guan, R., Guo, H., & Miao, X. (2015). New insights into an RNAi approach for plant defense against piercing-sucking and stem-borer insect pests. *Plant, cell & environment*, *38*(11), 2277-2285.

Li, Y., Lu, Y. G., Shi, Y., Wu, L., Xu, Y. J., Huang, F., ... & Wang, W. M. (2014). Multiple rice microRNAs are involved in immunity against the blast fungus Magnaporthe oryzae. *Plant Physiology*, *164*(2), 1077-1092.

Li, Y., Zhao, S. L., Li, J. L., Hu, X. H., Wang, H., Cao, X. L., ... & Wang, W. M. (2017). Osa-miR169 negatively regulates rice immunity against the blast fungus Magnaporthe oryzae. *Frontiers in plant science*, *8*, 2.

Liu, J. Y., Zhang, Y. W., Han, X., Zuo, J. F., Zhang, Z., Shang, H., ... & Zhang, Y. M. (2020). An evolutionary population structure model reveals pleiotropic effects of GmPDAT for traits related to seed size and oil content in soybean. *Journal of Experimental Botany*, *71*(22), 6988-7002.

Liu, Y., Shi, Y., Su, D., Lu, W., & Li, Z. (2021). SlGRAS4 accelerates fruit ripening by regulating ethylene biosynthesis genes and SlMADS1 in tomatoes. *Horticulture Research*, *8*(1), 1-11.

Liu, Y., Wang, Y., Pei, J., Li, Y., & Sun, H. (2021). Genome-wide identification and characterization of the COMT gene family during the development of blueberry fruit. *BMC Plant Biology*, *21*(1), 1-16.

Lv, J., Zhang, J., Han, X., Bai, L., Xu, D., Ding, S., ... & Li, J. (2020). Genome-wide identification of superoxide dismutase (SOD) genes and their expression profiles under 1-methyl cyclopropane (1-MCP) treatment during the ripening of apple fruit. *Scientia Horticulturae*, *271*, 109471.

Lv, Y., Hu, F., Zhou, Y. *et al* (2019). Maize transposable elements contribute to long non-coding RNAs that are regulatory hubs for abiotic stress response. *BMC Genomics* 20, **864**, https://doi.org/10.1186/s12864-019-6245-5

Ma, L., Bajic, V. B., Zhang, Z., (2013). On the classification of long non-coding RNAs. *RNA Biol*., **10**: 925–933.

Man Zhou and Hong Luo, (2013). MicroRNA-mediated gene regulation: potential applications for plant genetic engineering. *Plant Mol Biol.,* **83**:59–75

Måns Ehrenberg, (2009). Structure and function of the ribosome. *Uppsala*

Matsuo, S., Miyatake, K., Endo, M., Urashimo, S., Kawanishi, T., Negoro, S., ... & Fukuoka, H. (2020). Loss of function of the Pad-1 aminotransferase gene, which is involved in auxin homeostasis, induces parthenocarpy in Solanaceae plants. *Proceedings of the National Academy of Sciences*, *117*(23), 12784-12790.

Meli, V. S., Ghosh, S., Prabha, T. N., Chakraborty, N., Chakraborty, S., and Datta, A. (2010). Enhancement of fruit shelf life by suppressing N-glycan processing enzymes. *Proc.Natl.Acad.Sci.USA*. **107**: 2413–2418.

Mezzetti, B., Smagghe, G., Arpaia, S., Christiaens, O., Dietz-Pfeilstetter, A., Jones, H., ... & Sweet, J. (2020). RNAi: What is its position in agriculture?. *Journal of Pest Science*, *93*, 1125-1130.

Minna, U. Kaikkonen, Michael, T. Y., Lam, and Christopher, K. Glass, (2011). Non-coding RNAs as regulators of gene expression and epigenetics. *Cardiovascular Research*, **90:** 430–440

Molesini Barbara, Youry Pii and Tiziana Pandolfini, (2012). Fruit improvement using intragenesis and artificial microRNA. *Trends in Biotechnology*. **30**: No. 2

Moore, K.J., Sheedy, F.J., Fisher, E.A., 2013. Macrophages in atherosclerosis: a dynamic balance. *Nat. Rev. Immunol*., **13**: 709–721.

Moreno, J. C., Mi, J., Agrawal, S., Kössler, S., Turečková, V., Tarkowská, D., ... & Schöttler, M. A. (2020). Expression of a carotenogenic gene allows faster biomass production by redesigning plant architecture and improving photosynthetic efficiency in tobacco. *The Plant Journal*, *103*(6), 1967-1984.

Ni, Z., Hu, Z., Jiang, Q., and Zhang, H., (2013). GmNFYA3, a target gene of miR169, is a positive regulator of plant tolerance to drought stress. *Plant Mol. Biol.* **82**: 113–129.

Osorio, S., Alba, R., Damasceno, C. M., Lopez-Casado, G., Lohse, M., Zanor, M. I., *et al*. (2011). Systems biology of tomato fruit development: combined tran- script, protein, and metabolite analysis of tomato transcription factor (nor,rin) and ethylene receptor (Nr) mutants reveals novel regulatory interactions. *Plant Physiol*. **157**: 405–425.

Park, G. G., Park, J. J., Yoon, J., Yu, S. N., & An, G. (2010). A RING finger E3 ligase gene, Oryza sativa Delayed Seed Germination 1 (OsDSG1), controls seed germination and stress responses in rice. *Plant molecular biology*, *74*(4-5), 467-478.

Park, J. H., Shin, C., (2015). The role of plant small RNAs in NB-LRR regulation. *Briefings in functional genomics,* **14**: 268–274

Raza A, Malik HJ, Shafiq M, Amin I, Scheffler JA, Scheffler BE, et al. (2016) RNA Interference based Approach to Down Regulate Osmoregulators of Whitefly (*Bemisia tabaci*): Potential Technology for the Control of Whitefly. PLoS ONE 11(4): e0153883.

Renyi, Liu and Jiankang, Zhu. (2014). Non-coding RNAs as potent tools for crop improvement. *National Science Review*, **2**: 186-189

Rodnina, M. V. and Wintermeyer, W., (2011). The ribosome as a molecular machine: the mechanism of tRNA–mRNA movement in translocation. *Biochem. Soc. Trans.* **39**, 658–662.

Romer, E., Chebib, S., Bergmann, K. C., Plate, K., Becker, S., Ludwig, C., ... & Schwab, W. (2020). Tiered approach for the identification of Mal d 1 reduced, well tolerated apple genotypes. *Scientific Reports*, *10*(1), 1-13.

Roussin-Léveillée, C., Silva-Martins, G., & Moffett, P. (2020). ARGONAUTE5 represses age-dependent induction of flowering through physical and functional interaction with miR156 in Arabidopsis. *Plant and Cell Physiology*, *61*(5), 957-966.

Smit S., Widmann J. and Knight R. (2007) Evolutionary rates vary among rRNA structural elements; *Nucleic Acids Research,* **35**:3339–3354

Sajid, I. A., Tabassum, B., Yousaf, I., Khan, A., Adeyinka, O. S., Shahid, N., ... & Husnain, T. (2019). In vivo gene silencing of potato virus X by small interference RNAs in transgenic potato. *Potato Research*, 1-13.

San Miguel, K., & Scott, J. G. (2016). The next generation of insecticides: dsRNA is stable as a foliar-applied insecticide. *Pest management science*, *72*(4), 801-809.

Sang-Yoon Shin and Chanseok Shin, (2016). Regulatory non-coding RNAs in plants: potential gene resources for the improvement of agricultural traits. *Plant Biotechnol. Rep*. **10**:35–47

Santosh Baby, Akhil Varshney and Pramod Kumar Yadava, (2014). Non-coding RNAs: biological functions and applications. *Cell Biochem Funct.* **33**: 14–22.

Sebastian Kirchner and Zoya Ignatova, (2014). Emerging roles of tRNA in adaptive translation, signalling dynamics and disease. *Macmillan Publishers Limited,* **16**: 25-34

Sharma, R., Christiaens, O., Taning, C. N., & Smagghe, G. (2021). RNAi-mediated mortality in southern green stinkbug Nezara viridula by oral delivery of dsRNA. *Pest Management Science*, *77*(1), 77-84.

Sharp, Stephen J; Schaack, Jerome; Cooley, Lynn; Burke, Deborah J; Soll, Dieter (1985). "Structure and Transcription of Eukaryotic tRNA Genes". *CRC Critical Reviews in Biochemistry*. **19** (2): 107–144.

Shelby, E. A., Moss, J. B., Andreason, S. A., Simmons, A. M., Moore, A. J., & Moore, P. J. (2020). Debugging: Strategies and Considerations for Efficient RNAi-Mediated Control of the Whitefly Bemisia tabaci. *Insects*, *11*(11), 723.

Soto-Suárez, M., Baldrich, P., Weigel, D., Rubio-Somoza, I., & San Segundo, B. (2017). The Arabidopsis miR396 mediates pathogen-associated molecular pattern-triggered immune responses against fungal pathogens. *Scientific reports*, *7*(1), 1-14.

Suksamran, R., Saithong, T., Thammarongtham, C., & Kalapanulak, S. (2020). Genomic and transcriptomic analysis identified novel putative cassava lncRNAs involved in cold and drought stress. *Genes*, ***11*****(4),** 366

Tang, Y., He, H., Qu, X., Cai, Y., Ding, W., Qiu, L., & Li, Y. (2020). RNA interference-mediated knockdown of the transcription factor Krüppel homologue 1 suppresses vitellogenesis in Chilo suppressalis. *Insect molecular biology*, *29*(2), 183-192.

Teo, G., Suzuki, Y., Uratsu, S. L., Lampinen, B., Ormonde, N., Hu, W. K., ... & Dandekar, A. M. (2006). Silencing leaf sorbitol synthesis alters long-distance partitioning and apple fruit quality. *Proceedings of the National Academy of Sciences*, *103*(49), 18842-18847.

Thakur N, Upadhyay SK, Verma PC, Chandrashekar K, Tuli R, Singh PK (2014) Enhanced Whitefly Resistance in Transgenic Tobacco Plants Expressing Double Stranded RNA of *v-ATPase A* Gene. PLoS ONE 9(3): e87235.

Tian, S. N., Liu, D. D., Zhong, C. L., Xu, H. Y., Yang, S., Fang, Y., ... & Liu, J. Z. (2020). Silencing GmFLS2 enhances the susceptibility of soybean to bacterial pathogen through attenuating the activation of GmMAPK signaling pathway. *Plant Science*, *292*, 110386.

Uslu, V. V., & Wassenegger, M. (2020). Critical view on RNA silencing-mediated virus resistance using exogenously applied RNA. *Current opinion in virology*, *42*, 18-24.

Valle, M., Zavialov, A., Li, W., Stagg, S.M., Sengupta, J., Nielsen, R.C., Nissen, P., Harvey, S.C., Ehrenberg, M., and Frank, J. (2003). Incorporation of aminoacyl-tRNA into the ribosome as seen by cryo-electron microscopy. *Nature structural biology,* **10**: 899-906.

Vanderschuren, H., Moreno, I., Anjanappa, R. B., Zainuddin, I. M., & Gruissem, W. (2012). Exploiting the combination of natural and genetically engineered resistance to cassava mosaic and cassava brown streak viruses impacting cassava production in Africa. *PloS one*, *7*(9), e45277.

Vanitharani, R., Chellappan, P., Pita, J. S., & Fauquet, C. M. (2004). Differential roles of AC2 and AC4 of cassava geminiviruses in mediating synergism and suppression of posttranscriptional gene silencing. *Journal of virology*, *78*(17), 9487-9498.

Wang, C., Wang, W., Abdelrahman, M., Jiu, S., Zheng, T., Gong, P., ... & Fang, J. (2020). Study on miRNAs-Mediated Seed and Stone-Hardening Regulatory Networks and the Mechanism of miRNAs' Manipulating Gibberellin-Induced Seedless Berries in Grapevine (Vitis vinifera L.).

Wang, S.,Wu, K., Yuan, Q., Liu, X., Liu, Z., Lin, X., *et. al*. (2012). Control of grain size, shape and quality by OsSPL16 in rice. *Nat.Genet*. **44**: 950–954.

Wang, Y., Feng, G., Zhang, Z., Liu, Y., Ma, Y., Wang, Y., ... & Niu, X. (2021). Overexpression of Pti4, Pti5, and Pti6 in tomato promote plant defense and fruit ripening. *Plant Science*, *302*, 110702.

Wang, Z., Zhang, W. H., Ma, L. Y., Li, X., Zhao, F. Y., & Tan, X. L. (2020). Overexpression of Brassica napus NPR1 enhances resistance to Sclerotinia sclerotiorum in oilseed rape. *Physiological and Molecular Plant Pathology*, *110*, 101460.

Wheeler T, von Braun J (2013). Climate change impacts on global food security. *Science* 341:508–513

Wilson, R. C., and Doudna, J. A. (2013). Molecular mechanisms of RNA interference. *Annu. Rev. Biophys.* **42**: 217–239.

Xie K., Shen J., Hou X., Yao J., Li X., Xiao J., Xiong L., (2012). Gradual increase of miR156 regulates temporal expression changes of numerous genes during leaf development in rice. *Plant Physiol*., **158**:1382–1394

Xin, M., Wang, Y., Yao, Y., Xie, C., Peng, H., Ni, Z., *et al.* (2010). Diverse set of microRNAs are responsive to powdery mildew infection and heat stress in wheat (*Triticum aestivum* L.). *BMC Plant Biol*. **10**:123–134.

Yamagishi, M., & Sakai, M. (2020). The MicroRNA828/MYB12 Module Mediates Bicolor Pattern Development in Asiatic Hybrid Lily (Lilium spp.) Flowers. *Frontiers in plant science*, *11*.

Yan, S., Qian, J., Cai, C., Ma, Z., Li, J., Yin, M., ... & Shen, J. (2020). Spray method application of transdermal dsRNA delivery system for efficient gene silencing and pest control on soybean aphid Aphis glycines. *Journal of Pest Science*, *93*(1), 449-459.

Yan, S., Ren, B. Y., & Shen, J. (2021). Nanoparticle-mediated double-stranded RNA delivery system: A promising approach for sustainable pest management. *Insect Science*, *28*(1), 21-34.

Yan, S., Ren, B., Zeng, B., & Shen, J. (2020). Improving RNAi efficiency for pest control in crop species. *BioTechniques*, *68*(5), 283-290.

Yang, C., Li, D., Mao, D., Liu, X., Li, C., Li, X., (2013). Over expression of microRNA319 impacts leaf morphogenesis and leads to enhanced cold tolerance in rice (*Oryza sativa* L.). *Plant Cell Environ*. **36**: 2207–2218.

Yang, J., Xing, G., Niu, L., He, H., Guo, D., Du, Q., ... & Yang, X. (2018). Improved oil quality in transgenic soybean seeds by RNAi-mediated knockdown of GmFAD2-1B. *Transgenic research*, *27*(2), 155-166.

Yogindran, S., & Rajam, M. V. (2015). RNAi for Crop Improvement. *Plant Biology and Biotechnology, 623–637.* doi:10.1007/978-81-322-2283-5_31

Yu, X., & Killiny, N. (2020). RNA interference-mediated control of Asian citrus psyllid, the vector of the huanglongbing bacterial pathogen. *Tropical Plant Pathology*, *45*, 298-305.

Yumbya, P., Ambuko, J., Hutchinson, M., Owino, W., Juma, J., Machuka, E., & Mutuku, J. M. (2021). Transcriptome analysis to elucidate hexanal's mode of action in preserving the post-harvest shelf life and quality of banana fruits (Musa acuminata). *Journal of Agriculture and Food Research*, 100114.

Zhang W., Gao S., Zhou X., Chellappan P., Chen Z., Zhou X., Zhang X., Fromuth N., Coutino G., Coffey M., Jin H. (2011a). Bacteria responsive microRNAs regulate plant innate immunity by modulating plant hormone networks. *Plant Mol Biol,* **75**:93–105.

Zhang, X., Zou, Z., Zhang, J., Zhang, Y., Han, Q., Hu, T., *et al*. (2011a). Over expression of sly-miR156a in tomato results in multiple vegetative and reproductive trait alterations and partial phenocopy of the sft mutant. *FEBS Lett*. **585**: 435–439.

Zhang, Y. C., Liao, J. Y., Li, Z. Y., Yu, Y., Zhang, J. P., Li, Q. F., Qu, L. H., Shu, W., Chen, Y. Q., (2014). Genome-wide screening and functional analysis identify a large number of long noncoding RNAs involved in the sexual reproduction of rice. *Genome Biol.* **15**:5-12

Zhang, Y., Zhao, L., Xiao, H., Chew, J., Xiang, J., Qian, K., & Fan, X. (2020). Knockdown of a Novel Gene OsTBP2. 2 Increases Sensitivity to Drought Stress in Rice. *Genes*, *11*(6), 629.

Zhao, Z. X., Feng, Q., Cao, X. L., Zhu, Y., Wang, H., Chandran, V., ... & Wang, W. M. (2020). Osa-miR167d facilitates infection of Magnaporthe oryzae in rice. *Journal of integrative plant biology*, *62*(5), 702-715.

Zheng L L, Qu L H. (2015) Application of microRNA gene resources in the improvement of agronomic traits in rice. *Plant Biotechnol. J.* **13**:329–336

Zheng, Q., Liu, J., Goff, B. M., Dinkins, R. D., & Zhu, H. (2016). Genetic manipulation of miR156 for improvement of biomass production and forage quality in red clover. *Crop Science*, *56*(3), 1199-1205

Zhong, J., He, W., Peng, Z., Zhang, H., Li, F., & Yao, J. (2020). A putative AGO protein, OsAGO17, positively regulates grain size and grain weight through OsmiR397b in rice. *Plant biotechnology journal*, *18*(4), 916-928.

Zhou, M, Luo H (2013). MicroRNA-mediated gene regulation: potential applications for plant genetic engineering. *Plant Mol Biol.* **83**:59–75

Zhou, S. X., Zhu, Y., Wang, L. F., Zheng, Y. P., Chen, J. F., Li, T. T., ... & Wang, W. M. (2020). Osa-miR1873 fine-tunes rice immunity against Magnaporthe oryzae and yield traits. *Journal of integrative plant biology*, *62*(8), 1213-1226.