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Review

Advances on the application of non-coding RNA in crop improvement

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Thanks to committed plant breeding researchers over the few decades, many problems associated with food supply and qualities have been improved. Food security is an exceptionally serious worldwide issue via world climate change, the increase in human population, and the use of plants for bioethanol production in current years. Improved tolerance to abiotic and biotic stress, resistance to herbicides, improved yield, and plants with wonderful nutritional value are essential goals of crop improvement. RNAi applications are the modern innovation that can assist in the solution for these issues. A natural protection mechanism against invading viruses, nucleic acids, and transposons non-coding RNAs (ncRNAs), are identified as effector molecules in RNA-mediated gene silencing and used in the genetic modification of crops. These ncRNAs are concerned with the regulation of growth, development, and response to stress at the transcriptional and translational levels. Improving crop yields is the final purpose of molecular plant breeding. ncRNAs, along with transfer RNAs (tRNAs) and ribosomal RNAs (rRNAs), as well as small non-coding RNAs (sncRNAs) and the long non-coding RNAs (lncRNAs), have been recognized as essential regulators of gene expression in plants in plant immunity and adaptation to abiotic and abiotic stages biotic stress.

Key words: Crop improvement, long RNA, ncRNA, RNA interference, small RNA.

INTRODUCTION

The world population is growing rapidly; however, world food security is still threatened in recent years via climate change, the increase in human population and the use of plants for bioethanol production in recent years (FAO, 2020). In addition, the future capacity to meet the world's food security needs has come to be unstable as the area of arable and cultivated land continues to decrease. These issues and threats have led scientists to look for options to increase crop productivity. Proponents of new technologies, which include recombinant DNA, have a promise in current green transformation, with genetically engineered plants which include transgenes achieving focused traits (Wheeler and von Braun, 2013; Sang and Chanseok, 2016; Bader et al., 2020; Mezzetti et al., 2020).

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Non-coding RNAs refer to transcripts that no longer code for proteins; however play vital regulatory roles within the cell, which precludes the possibility of the production of exogenous protein products. Non-coding RNA can be a gene silencing phenomenon that involves sequence-specific gene legislation encapsulated via double-stranded RNA, resulting in inhibition of protein production (Zheng and Qu, 2015; Brant and Budak, 2018; Yan et al., 2020, 2021).

Some classes of regulatory ncRNAs especially regulate a single goal gene, while others modulate more than one gene at the genome-wide level via various molecular mechanisms. These cumulative consequences advise that regulatory ncRNAs may want to be possible goals for molecular plant breeding (Zhou and Luo, 2013).

Non-coding RNAs are divided into functionally vital RNAs such as transfer RNA (tRNA), ribosomal RNA (rRNA), small non-coding RNAs, including Micro-RNAs (miRNAs), small interfering RNAs (siRNAs), piwi-interacting RNAs (piRNAs), and the long non-coding RNAs (lncRNAs). Two lncRNAs that have been substantially studied are the X-inactivation-specific transcript (XIST) and the HOX-antisense-intergenic RNA (HOTAIR) (Renyi and Jiankang, 2014; Santosh et al., 2014; Brant and Budak, 2018).

Post-transcriptional gene silencing (PTGS) is mentioned in many organisms; fungi, animals, and plants (Zhou and Luo, 2013; Brant and Budak, 2018). These products are the result of non-coding dsRNA, the DICER or Dicer-like enzyme, which performs this cleavage. Small non-coding RNA consists of 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, while Argonauts are RNase H enzymes (Wilson and Doundna, 2013; Kamthan et al., 2015; Brant and Budak, 2018).

The goals of this review had been to discuss non-coding RNA and its use in plant improvement. The RNA interferences in the adaptation of plants to the environment serve the tolerance to abiotic stress and the plant protection towards biotic stress. The RNA interference increases the yield potential of plants, such as manipulating plant improvement and improving nutrition. They modulate a broad range of gene regulatory networks via regulating a specific subset of downstream genes that are closely associated to agricultural traits such as seed maturation, flower development, pathogen resistance, and other abiotic stress resistance.

Natures of non-coding RNAs (ncRNAs)

Ribosomal RNA (rRNA)

Ribosomal RNA is the most abundant small non-coding RNA molecule that is made up of 4-10% cellular RNA. They are translation machines in so far as they translate the genetic information into a corresponding polypeptide chain in a mRNA template-directed manner (Rodnina and Winter Meyer, 2011). The mRNA sequence starts with the begin codon of AUG and is followed by termination codon signaling for subsequent folding into its functional state (Crick, 1970). The ribosomes in eukaryotes are more complex than in prokaryotic ones; in general, ribosomes have three exclusive binding sites; A (aminoacyl) site, the P (peptidyl) site and the E (parent) site in the interface between the subunits. The mRNA that binds to the 30S subunit can regularly move one codon at a time during peptide elongation (Crick, 1970).

The ribosomal RNA is about 60% by weight rRNA and 40% by weight protein and additionally includes two most important rRNAs and 50 or more proteins. The 60S subunit incorporates (three rRNAs 5S, 5.8S, 28S and about forty proteins) and the 40S subunit contains (an18S rRNA and about 30 proteins). The LSU rRNA acts as a ribozyme and catalyzes the formation of peptide bonds (Anita et al., 2013).

Transfer RNA (tRNA)

Transfer RNA is the link between mRNA and the peptide sequence in transfer RNA (tRNA). There is an enzyme that can understand both tRNA and an anticodon, which is complementary to the mRNA codon associated to this amino acid, and couples it to a highly standardized free energy complex known as aminoacyl-tRNA at the rate of ATP hydrolysis (Sharp at el., 1985). The mRNA binds to the 40s ribosomal subunit, followed by using the binding of the initiator tRNA loaded with formylated methionine to the P site in a reaction step that is considerably accelerated by the three initiation factors. As soon as the mRNA and the initiator tRNA are successfully bound, the large 60S subunit binds to form an 80s initiation complex, releasing the eIF factors. With the mRNA in the correct reading frame, the initiator tRNA in the P-site and the empty A-site programmed with the first interior codon of the protein to be synthesized. The ribosome has now left the initiation phase and has entered the peptide elongation phase (Valle et al., 2003).

Short non-coding RNAs

Micro RNA (miRNA)

Micro-RNA is the party of the small non-coding RNA (20-22nt) with partially double-stranded stem loop structures, which regulates negative gene expression in both animals and plants. It is transcribed by the RNA polymerase II enzyme and, after transcription, cleaved by the Dicer-like 1

(DCL1) enzyme and transported into the cytoplasm and incorporated into the Argonaut (AGO) protein. Mature miRNA is produced in various processes and incorporated into the protein RISC (RNA-induced Stille complex). Mature single miRNA that incorporates the RISC protein binds with other complementary mRNA sequences to produce the protein (Khraiwesh et al., 2012).

Small interfering RNA (siRNA)

Small interfering RNAs are the parts of small non-coding RNA that have the function of facilitating post-transcriptional gene silencing (PTGS) by breaking down mRNA. Small interfering RNA is generated from RNA double strands that are nearly perfectly complementary and cleaved by DCL2, DCL3 and DCL4 to produce siRNA duplexes 22nt, 24nt and 21nt, respectively (Sang and Chanseok, 2016). The other cofactors of siRNA are the RNA-dependent RNA polymerases 2 and 6 (RDR2, RDR6), SUPPRESSOR OF GENE SILENCING 3 (SGS3) and the plant-specific DNA-dependent RNA polymerases IV and V (Bologna and Voinnet, 2014). After the siRNAs have been cleaved by DCL, they are loaded into AGRONAUTS (AGOs) and companies with a complexing gene silence machinery 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 a cleavage or transcription gene silencing (TGS) mechanism. Small, interfering RNA is naturally very important in the organism to protect it from various natural enemies. For example, RNA interference (RNAi) was seen as a natural defense mechanism that used exogenous siRNAs to protect organisms from viruses, but it soon became clear that endogenous siRNAs (endo-siRNAs) also play a role in regulating genomic functions (Kamthan et al., 2015).

Piwi-interacting RNAs (piRNA)

Piwi-interacting RNAs are small non-coding RNAs that have 20-31nt. The name, piRNA (Piwi-interacting RNA), reflects the fact that piRNAs bind to Piwi proteins under physiological conditions. 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 (Lindsay et al., 2013).

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 is the understanding of the functional role for these transcripts (Hangauer et al., 2013).

The lncRNA regulates 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).

APPLICATION OF RNAi IN CROP IMPROVEMENT

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

Plant architecture

Biomass

RNA interferences are one application that can improve the yield of crops and fruit vegetation via the manipulation of primary agronomic traits. It can increase the biomass of the crops and fruits by manipulation of plant height, prolonged vegetative segment and delayed flowering time, a number of branches or branching type, and measurement of the plants.

RNA interference knocks down the OsDWARF4 gene in rice to limit the plant's erect leaf and growing the photosynthesis through reducing leaves. The yield of such plants is improved or increased via dense planting conditions (Feldmann, 2006). Over-expression of the maize MIR156, Corngrass1 (Cg1) gene (Chuck et al., 2011), and red clover (*Trifolium pratense* L.) (Zheng et al., 2016) motives prolong the vegetative parts of maize and delayed flowering. This means that the reproductive stage is delayed, the vegetative parts are increased in size, and at the identical time, biomass of plants become increasing.

Moderate and low ranges of miR156 expression had 58–101% more biomass production than wild-type controls because of increases in tiller numbers in change grass plants. Over-expression of rice miR156 should improve the yield of solubilized sugar as properly 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 white and lily flowering colors (Yamagishi and Sakai, 2020).

Grain yield

Improvements of the plants by way of special technology or techniques 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 using manipulating traits. One of the functions of RNA interferences is the manipulation of the gene, which affects the characteristics of the crop whether to be increasing or reducing the crop yield.

Regulatory ncRNAs influence the reproductive stage. It is a very important aspect that affects crop yield and is used for genomic association. According to Ding et al. (2019), lncRNA, called LDMAR (for LONG-DAYSPECIFIC MALE-FERTILITY-ASSOCIATED RNA), regulates the photoperiod sensitive male sterility of rice with absolutely sterile pollen in a long-day condition. Genome-wide investigation on rice additionally identified a set of lncRNAs that are particularly expressed during the reproduction stage (Zhang et al., 2014).

The RNA mediates suppression of GA 20-oxidase (OsGA20ox2) gene which resulted in semi-dwarf plant life from a taller rice variety QX1. It increases panicle length, the quantity of seeds per panicle, and greater seed weight. OsSPL14 (Souamosa promoter binding protein-like14) is the goal of Osa-miR156 in rice to increase the yield of the rice grain, reduced tiller number, and accelerated grain yield (Wang et al., 2012; Jiao et al., 2010). Overexpression of Osa-miR1873 also resulted in some defects in yield traits, which include grain numbers and seed putting rate in rice (Zhou et al., 2020). Overexpression of the OsAGO17 gene was once also found to be involved in increased grain size, weight, and promote stem development in rice (Zhong et al., 2020).

Fruit improvement

The application of transgenic protects crops from different pests that can affect the plant products. Genetic engineering can be 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).

Enhanced nutritional value and edibility

RNA silencing-based technology has been used in improving the nutritional value of crops. By down-regulating key genes in plant metabolic pathways usage of RNAi constructs, transgenic plants may also accumulate more favorable metabolites or produce fewer undesirable ingredients. Crops have exclusive nutritional values; some of them are allergens to human being, some pollutants to the environments, and some allergens to the different crops. But there are plants which have very essential nutritional values that are consumed by means of human beings or animals. RNA interference is to manipulate all these nutrients through adding the essential one and removing the unwanted ones.

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

RNAi approach has additionally been used in apple to improve the fruit quality via enhancing self-life and reducing the quantity of a major apple allergen, metabolites, and accumulation of sugars in the fruits through sorbitol synthesis, which impacts fruit starch accumulation (Romer et al., 2020) and sugar-acid stability (Teo et al., 2006).

Some examples of nutrition adding plants through RNAi are high lysine maize for the expression of zein proteins (Li and Song, 2020; Choudhary et al., 2021), and silencing of carotenoid β-hydroxylases which 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 an amazing potential to improve human health (Man and Hong, 2013). Corn with increased essential amino acids (Hasan and Rima, 2021), improved soybean by using oil quality (Yang et al., 2018), and cotton with improved fatty acid composition have been developed with the use of RNAi. Overexpression of GmPDAT genes elevated seed size and oil content (Gao et al., 2020), whereas RNAi strains had reduced seed size and oil content of soybean (Liu et al., 2020). Suppression of three carotenoid-cleavage dioxygenase genes, OsCCD1, 4a, and 4b, increases carotenoid content in rice (Ko et al., 2018).

Enhanced shelf life

The fundamental issues of fruits are post-harvest deterioration and spoilage. It is a main financial loss. This may be because of some issues like; technique of harvesting, transports, and storage. Therefore, an increase in the shelf life of vegetables and fruits through delayed ripening involves any other critical agronomic trait that is being addressed through non-coding RNA technology. Initiation of ripening in climacteric fruits like tomato is characterized through a climacteric burst of ethylene, resulting in the regulation of the expression of ripening-specific genes. Manipulating the gene which is accountable to produce ethylene and decrease the production of ethylene was once increasing the shelf life of tomato (Osorio et al., 2011). RNA interference decreases the expression of 1-aminocyclopropane-1-carboxylate (ACC) oxidase, a gene of the ethylene biosynthesis pathway in tomato, and inhibited the ethylene production at the ripening time so that the fruit can survive a long time.

RNA silencing used to be first genetically modified in the Flavr Savr tomato, bringing about the antisense of transcript polygalacturonase (PG) which is suppressed to PG expression. The polygalacturonase is accountable for cell wall degradation during tomato ripening. Suppression of polygalacturonase delayed the natural softening of tomatoes and allowed tomatoes to ripen on the vine for long and resulting in a greater flavorful fruit (Renyi and Jiankang, 2014). The increase in shelf life of tomatoes used to be observed after increasing abscisic acid (ABA) content. Manipulation of β-carotene stages results in an enchantment that is no longer only limited to the shelf life of tomatoes, but also their nutritional value (Diretto et al., 2020).

Tomato fruits are considered to be climacteric and require the gaseous hormone ethylene to ripen. In every case, the change of the expression of the mi156 gene in tomatoes led to an increase in yield and shelf life (Zhang et al., 2011). Definitely, tomato has emerged as the pre-eminent experimental model for studying fleshy fruit, which includes the developmental control of ripening, ethylene synthesis and perception. Overexpression of Pti4, Pti5, and Pti6 genes (Wang et al., 2021), and SlGRAS4 gene (Liu et al., 2021a) in tomato are very crucial to accelerated fruit ripening and elevated the complete carotenoid content.

Non-coding RNA is especially primary within the increased shelf life of vegetables and fruits. Tobacco (Moreno et al., 2020), and tomatoes (Arefin et al., 2020), superoxide dismutase (SOD) genes expressed at some stage in the ripening of apple fruit (Lv et al., 2020), cold storage responses genes in the peach (Antonella et al., 2020), blueberry cultivars with higher fruit firmness and longer shelf lifestyles (Liu et al., 2021), preserved post-harvest shelf life and quality of banana fruit (Yumbya et al., 2021) have been developed using RNAi. RNAi method concentrated on suppression of more than one homolog would be much effective than the knockdown of a single homolog (Gupta et al., 2013).

Seedless fruit development (Parthenocarpy)

Parthenocarpy or seedless fruit improvement is a technique of fruit production from seedless crops by way of ovaries without pollination and fertilization. Parthenocarpy or seedlessness is a highly preferred agronomic trait, particularly in safe to eat fruit crops. It produces high yields in harsh environmental conditions, as no pollination or fertilization is required. Customers constantly want fresh fruit, while it is possible to produce the fruit at any time of the year. It produces excessive yields in harsh environmental conditions, as no pollination or fertilization is required. Customers continually want clean fruit, while it is possible to produce the fruit at any time of the year.

In some cases, fruits produce hard seed which makes it difficult to overcome the dormancy of the seed, whereas others produce seeds with the difficult tests, as the absence of seeds can also be a positive trait for both direct clean consumption (e.g. grape, citrus, and banana) and industrial approaches (e.g. frozen eggplants, and tomato sauce) (Meli et al., 2010).

RNA interferers are one of the most essential primary technologies for overcoming the issues of vegetation with parthenocarpy. Some plants can move forward through RNAi innovation like; manipulation of auxin response factors8 (ARF8), which is the goal of miR167 to affect product from parthenocarpic fruits in each arabidopsis and tomatoes (Molesini, et al., 2012). Suppression of the orthologous genes of Pad-1 caused parthenocarpic fruit improvement in Solanaceae plants; hence, it is a very powerful tool in improving Solanaceae fruit production (Matsuo et al., 2020).

The overexpression of the PbGA20ox2 gene modified the GA biosynthetic pathway and improved GA4 synthesis, which promoted fruit set and parthenocarpic fruit improvement in pear fruits (Pyrus Bretschneider Rehd.) (Wang et al., 2020a). The PpIAA19 gene used to be concerned in the regulation of lateral root number, stem elongation, parthenocarpy, and fruit structure of tomatoes (Ding et al., 2019) and grapevines (Wang et al., 2020a). The overexpression of the MaTPD1A gene in banana plants produces seedless fruits in contrast to wild-type plants (Hu et al., 2020).

Biotic stress resistance

Phyto-pathogens are disease causing agents that can affect crops and crop products. The disease may affect the crop at different stages, seedling, vegetative, and 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).

Virus resistance

Viruses are pathogens that can influence crop products. RNA interference is one of the technologies which can overcome their effect. The functions of RNA interferences are protection mechanism of virus that invades nucleic acid molecules. Transgenic plant technology has been used to express genes encoding viral coat proteins in transgenic plants and can be resistant to viruses containing the coat proteins. The virus resistance was primarily based on the recognition of coat proteins through the transgenic plant cells and therefore on the plant immune response against the coat proteins. However, for understanding the application of genetic transformation technology, the plants can express the coat protein gene transcript and damage the protein coat; however, it cannot produce the protein that acts against the protein coat itself. Transgenic plants that are resistant to viruses have been produced by way of sense transgene-triggered RNAi against the viral RNAs (Ding, 2010; Renyi and Jian-Kang, 2014; Bahadur et al., 2015; Uslu and Wassenegger, 2020).

RNAi has shown a way to keep virus-resistant traits in many crops. The transgenic plant can produce the viral sequences that match coat proteins, replication-associated proteins, ATPases, or promoter areas in the viral genomes that can derive transgenic siRNAs to goal viral RNAs for degradation upon infection. Plants reportedly consisting of virus resistance included some suggested crops like papaya (Kertbundit et al., 2007), cassava (Vanitharani et al., 2004; Vanderschuren et al., 2012) and potato (Sajid et al., 2019). Some examples are potato resistance to spindle tuber viroid (PSTVd) and cassava resistance to African Cassava Mosaic Virus (ACMV) (Renyi and Jiankang, 2014). The virus prevention strategies are based totally on the ecological implication that increased carbon dioxide concentrations minimize the accumulation of the cucumber mosaic virus in *Nicotiana tabacum* through the viral suppressor of the RNAi (VSR) 2b protein of CMV (Guo et al., 2021).

Bacterial resistance

Bacterial diseases are extremely hard to manage due to the high rate of production of the spread. The multiplication rates of microorganism are very excessive in contrast to plant production, due to which, it can effortlessly manage plant development. RNA interference application technologies are used in various facilities to resist bacterial diseases. Some plants that can withstand RNA interference are; In Arabidopsis, it was reported that miR393 is caused through a bacterial pattern-associated molecular pattern (PAMP) peptide flg22 and negatively regulates the F-box auxin receptors TIR1, AFB2, and AFB3. This suppression of auxin signaling contributed positively to the predicament of bacterial infection (*Pseudomonas syringae*) (Li et al., 2010; Zhang et al., 2011a). The other application RNAi mediated the suppression of two genes from *Agrobacterium tumefaciens* that are involved in the formation of crown gall tumors (iaaM and ipt), which could appreciably minimize tumor production in Arabidopsis (Kamthan et al., 2015).

Overexpression of the rice chorismate mutase (OsCM) gene modified the downstream pathway of the aromatic amino acids, whereby the stress of bacterial leaf rot (BLB) was weakened through changing stress-sensitive genes and hormonal accumulation (Jan et al., 2020). Some of the well-reported plants that showed bacteria can be resisted through manipulating RNAi genes include tomatoes (Bento et al., 2020), citrus (Yu and Killiny, 2020), soybean (Tian et al., 2020), and Arabidopsis (Guo et al., 2020).

Fungal resistance

Genetic engineering based on RNA silencing has made a primary contribution to improving crops, that is, resistance to pathogens. Fungus is a pathogen that can affect plant production and quality. RNAi has been confirmed to be an essential strategy for producing tolerance in various crops (Kamthan et al., 2015).

RNA interference is done in response to fungal attack in wheat *Blumeria graminis* f. Sp. tritici (Bgt) through the use of the 24 miRNAs gene that causes devastating diseases of wheat powdery mildew (Xin et al., 2010). Transgenic overexpression of rice plants through OSA-miR7695 (Campo et al., 2013), miR160a or miR398b (Li et al., 2014), miR169 (Li et al., 2017) and OsamiR167d (Zhao et al., 2020) has been negatively regulated as a response to the blast fungus Magnaportheoryzae, and through overexpression of these genes, an improved resistance against rice blast infections was achieved.

Treating of the *Fusarium* species with various dsRNAs that concentrated on the genes was destructive to the fungus and leads to determination of an extended growth in *in vitro* cultures (Koch et al., 2018). In the reduction of the *Arabidopsis thaliana* line, it was observed that the activity of miR396 confers resistance to necrotrophic and hemibiotrophic fungal pathogens using artificial miRNA goal mimetics (Soto-Suárez et al., 2017). Overexpression of the BnaNPR1 gene in oilseed rape played a high-quality role in the resistance of *Brassica napus* to *Sclerotinia sclerotiorum*, which confers resistance to this pathogen (Wang et al., 2020b).

Insect and resistance

Defoliation of plants or sucking out their sap insects can cause great damage to plants by slowing, weakening, and sometimes killing their increase (Bahadur et al., 2015). The use of RNAi has been confirmed to be an essential approach for developing tolerance in various plants. Insect-resistant plants produce the dsRNA that attacks the insects, and when insects ingest, the gene expresses itself (Huvenne and Smagghe, 2010).

This technology is intended to protect insects from various kinds of plant production; among others, the western corn rootworm *Diabrotica virgifera*, the cotton bollworm *Helicoverpa armigera*, and the tobacco hawk *Manduca sexta* (Renyi and Jian-kang, 2014). Transgenic tobacco lines expressing dsRNA of v-ATPase and leading to whitefly mortality rate (Thakur et al., 2014) transcript level of goal genes in *Bemisia tabaci* at more than 70% mortality was observed (Raza et al., 2016; Shelby et al., 2020).

The knockdown of CsKrh1 mediated through RNA interference substantially decreased the transcription of vitellogenesis (Vg) within *C. suppressalis*, which is extremely essential in the suppression of rice pests (Tang et al., 2020). For pest management in the field through topical application or spraying, dsRNA shows a very high potential, with soybean aphids *Aphis glycines* (Yan et al., 2020a) mortality of up to 81.67% and with soybean *Nezara viridula* mortality of up to 90% recorded (Sharma et al., 2021).

Abiotic stress tolerance

Plant growth in the field can be exposed to several abiotic stresses, such as: drought, salt, heat and cold. RNA silencing coding is one of the technologies that can overcome this problem, the abiotic stress of plant products. By regulating the endogenous level of regulatory ncRNAs in response to abiotic stress, they regulate the expression of their target genes, which are closely involved in specific or multiple stresses.

Drought and salinity tolerance

Lack of water or drought and salinity are the abiotic stresses that are the main environmental stresses that restricts crop productivity. RNA silencing coding has been used successfully to enhance drought and salt tolerance cultures. RNA interference is used in oilseed rape to supply the AtHPR1 promoter, which is resistant to seed break-off during drought-induced flowering, besides affecting yield in drought stress. Transgenic rice plants that are drought stress-tolerant have been developed such as the receptor for activated C-kinase1 (RACK1) (Li et al., 2009), knockdown of a RING finger E3 ligase gene OsDSG1, and also silencing OsDIS1 for the drought-induced SINA protein through *Oryza sativa* (Park et al., 2010).

A wide variety of miRNAs has been recognized in *Brassica* that responds to drought, high salinity, and stress at high temperatures. Of 126 newly recognized miRNAs, miR164, miR160, and miR156 were experimentally approved to goal NAC domain-containing proteins, ARF17-like, and SPL2-like proteins, respectively (Bhardwaj et al., 2014).

However, the RNA interference has also regulated the gene to respond in an identical way to the salinity tolerance plants. The overexpression of GmNFYA3 in Arabidopsis led to an increased sensitivity to salinity stress, and exogenous ABA (Ni et al., 2013) as well as transgenic creeping bentgrass (*Agrostis stolonifera*) plants that overexpressed a rice miR319 gene (OsamiR319) also confirmed an increased tolerance to drought and salinity related with increased leaf wax content and water retention; however, there was decreased sodium intake (Zhou and Luo, 2013). In tomato, overexpression of the miR169c gene led to a reduction in stoma openings, the transpiration rate, and the loss of leaf water, which improved the drought tolerance in transgenic plants compared to wild-type controls (Zhang et al., 2011a). Accordingly, a knockdown of the OsTBP2.2 gene was generated in rice in order to increase rice sensitivity to drought stress (Zhang et al., 2020).

Cold and heat stress tolerance

Cold and heat are the abiotic types of stress that can influence plant production. With the variable conditions, the plants are pale; also, the yield and quality of plant products decrease and additionally affects the financial loss. Transgenic plants that use RNA silencing coding can overcome the problem of this stress. It has been suggested that miR319 expression changes in response to cold stress in Arabidopsis, rice, and sugar cane (Lv et al., 2020).

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

Guan et al. (2013) observed a new kind of plant thermotolerance mechanism, in particular to protect the reproductive organs. It includes the induction of miR398 to downregulate its goal genes CSD (copper / zinc superoxide dismutase), CSD1 and CSD2, and CCS (a gene that codes for copper chaperones for CSD1 and CSD2). They observed that csd1, csd2 and ccsmutanten showed a higher heat stress tolerance than wild-type plants, combined with an increased accumulation of heat stress transcription factors and heat shock proteins as well as much less damage to plants (Guan et al., 2013). Corn (Yu et al., 2018) and cassava (Suksamran et al., 2020) responds to abiotic stress such as heat, cold, salt, and drought.

CONCLUSION

One of the basic requirements of a human being is food. However, food insecurity and malnutrition are currently among the most serious concerns for human health, causing the loss of countless lives in developing countries. Therefore, there is need for an innovative technology to improve upon our crop production methods and practices. RNA silencing gene is an advanced application that can solve the agricultural problem in a short period. 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 interference applications are innovative technologies that can contribute to the solution to these problems. It describes several mechanistically related pathways which are involved in controlling and regulating gene expression. RNA silencing pathways are associated with the regulatory activity of non-coding RNAs that function as factors involved in inactivating homologous sequences, promoting endonuclease activity, translational arrest, and/or chromatic or DNA modification. It also has some limitations which include identification of appropriate target genes, off-target effects, and the application of RNAi is more sophisticated and needs highly skilled human power.

CONFLICT OF INTERESTS

The author has not declared any conflict of interests.

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