The application of antisense technology for crop improvement: A review

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Abstract: The global population is increasing alarmingly, which calls for efficient methods of food production. Since domestication, people have been working on improving food crops for production and productivity. Genetic modification is one of the methods under use. Antisense technology, which includes antisense RNA (asRNA) and RNA interference (RNAi), is a gene regulatory strategy that is widely used for crop improvement. asRNA and RNAi mediated gene silencing is effective methods for regulating undesirable organic phenomena with no genomic alteration of the target gene by base pairing between asRNA and mRNA targets. Crops modified by engineering novel RNA interference and asRNA pathways allow small RNA molecules to alter organic phenomenon. Antisense technology plays an important role in generating high yielding, disease and pest resistant, high nutritional value, and stress-tolerant crop varieties. This review encompasses research articles on the two important antisense technologies to which RNAi (93%) was frequently used for crop improvement as compared to the asRNA (7%) technology. Regarding the crops improved using antisense technology, vegetables (41%) took the first attention. Most of the research articles reviewed were on improving crops for biotic stress resistance (29.6%), followed by fruit improvement and enhancing

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PUBLIC INTEREST STATEMENT
Nowadays, the major problem in the world is to sustain food security and feed the alarmingly increasing world human population. Improving crop production and productivity using scientific research findings and technological packages could contribute to reduced food insecurity. Crop improvement methods include molecular breeding, antisense RNA technology, mutational breeding, and plant tissue culture. Recently, antisense RNA technology has been playing a key role in producing biotic and abiotic stress-tolerant crops. This review work addresses the application of antisense RNA technology in producing high-yielding, and high nutritional value crops through the development of biotic and abiotic resistance and enhancing nutritional value. Most of the reviewed articles used RNA interference (93%), to improving crops for biotic stress resistance (29.6%). Most of the reviewed articles were on vegetables (41%). This review paper provides a comprehensive overview of the recent developments in antisense technology and its contribution to crop improvement.
nutritional values (18.5%). This review provides an overview of the recent developments in antisense technology and its contribution to crop improvement.

Subjects: Crop Science; Plant Biology; Plant Biotechnology; Genetics

Keywords: Antisense technology; genomics; micro RNA; RNA interference; small interfering RNA

1. Introduction
The world population has reached around 7.7 billion and it is projected to reach 9.7 billion by 2050 (Rajam, 2020). To satisfy the world food demand, improving the production and productivity of food crops is required (Saurabh et al., 2014). Since domestication, people have been selecting the best germplasm over the other to improve productivity, environmental adaptation, and quality. The foremost important crops that we cultivate and consume nowadays are the invention of centuries, before Mendel’s work of genetics of plant breeding (Miflin, 2000). The current long-standing dream of crop improvement often combines traditional plant breeding with the inventions made possible by biotechnology (Auer & Frederick, 2009). This effort has begun with conventional methods, the subliminal selection by early agricultural societies of genotypes with high yields and good agronomic properties, followed by molecular methods, and thus the growth of scientific plant breeding over the past century (Shewry et al., 2001). On the other side, climate change and environmental stresses have major implications on worldwide crop production which calls for the development of crops that can resist a range of climate changes and environmental stresses such as irregular water-supplies leading to drought or water-logging, hyper soil-salinity, extreme and variable temperatures, ultraviolet radiations, and metal stress (Jin Xu et al., 2019).

Nowadays, the foremost convenient methods of crop improvement are molecular breeding, antisense RNA technology, crop gene editing, and crop genetic transformation (Afzal et al., 2020; Jawhar, 2006; Lubna et al., 2016; Zaidi et al., 2019). Crop genome editing is a recently developed, accurate method and can alter gene function rapidly. This involves using the application of sequence-specific nucleases like zinc-finger nucleases (ZFN), transcription activator-like effector nuclease (TALENs), and clustered regularly interspersed short palindromic repeats (CRISPR/Cas9). This technique has been applied for the enhancement of disease resistance production in different crops (Bezie et al., 2020).

The molecular methods of crop improvement currently under use include hybridization, mutation, tissue culture, and antisense technology (Kim et al., 2007; Ismail & Horie, 2017; Bailey-Serres et al., 2019; Pandey et al., 2019). Antisense technology is the most convenient and novel technology employed by crop breeders for the development of various crop species/varieties. Antisense technology is a comprehensive term, which includes antisense RNA (asRNA), RNA interference (RNAi), long non-coding RNA (IncRNA), and several other enzymes and molecules. The types and mechanisms of antisense technology have discussed in the literature (Basso et al., 2019; Brant & Budak, 2018; Budak et al., 2020; Crooke, 2004; Das & Sherif, 2020; Du & Zamore, 2005; Gaffar & Koch, 2019; Guo et al., 2016; Heliwell & Waterhouse, 2005; J-Z. Xu et al., 2018; Koller et al., 2000; Lam et al., 2015; Lee & Roth, 2003; Lin et al., 2020; Mattick & Lee, 2009; Nejat & Mantri, 2018; Pathak & Gogoi, 2016; Rajam, 2020; Saurabh et al., 2014; Stuti Gupta et al., 2011; Summanwar et al., 2020; Tuschi et al., 1999; Villegas & Zaphiropoulos, 2015; Yang et al., 2019). The enzymes and molecules participating in antisense technology are presented in Table 1.

The rule of using antisense technology is that antisense macromolecule sequence base pairs with its complementary sense RNA strand and prevents it from being translated into a protein (Stuti Gupta et al., 2011). Using antisense RNAs complementary for the mRNA changes the organic phenomenon in crops. The non-coding RNAs play a major role in transcriptional levels instead of playing a biological role at post-transcriptional and translational levels that do not produce any functional peptides or proteins, which makes it different from other antisense technologies (Shin &
Table 1. Enzymes and sophisticated molecules participating in antisense technology

| Enzyme                                      | Function                                                                 |
|---------------------------------------------|--------------------------------------------------------------------------|
| DICER (DCL)                                 | Cleave the dsRNA for biogenesis of siRNAs and miRNAs                    |
| Argonautes (AGO)                            | Specialized proteins that function binding modules of small RNAs component and coordinate gene silencing together with RISC |
| RNA-induced silencing complex (RISC)        | Unwind the double-stranded siRNA produced by dicer and guide it to cleave the target mRNA in RNA interference methods |
| Ribonuclease H (RNase H)                    | Cleave the target mRNA in the case of antisense RNA methods              |

Shin, 2016). Different current studies have revealed that LncRNAs play a vital role in plant stress responses and thereby create new opportunities for genetic plant breeding (Fei et al., 2020).

The asRNA and RNAi are used to generate crops with new crop quality traits or to protect against insects, nematodes, and pathogens (Ali, Datta and Datta 2010; Auer & Frederick, 2009). The small RNA technology has relatively supported a serious paradigm shift from “one gene, one protein” to the concept that non-coding DNA can have profound effects in cells and organisms (Auer & Frederick, 2009). One breakthrough was the advent of transgenic maize engineered from a precise double-stranded RNA (dsRNA) of an important insect gene to guard maize roots against damage (Zhu & Palli, 2019). Long non-coding RNAs (lncRNAs) have been developed as vital regulators of gene expression in different biological processes and in many species. In plants, they are transcribed with the aid of various RNA polymerases and show diverse structural features. With the help of next-generation sequencing technologies, a huge number of lncRNAs have been recognized in model plants as well as in crops (Bazin & Bailey-Serres, 2015). Analogous to the huge potential and applications, this technology has encountered some challenges, like the off-target effects, delivery of optimum, and sufficient small RNAs at the targeted cells, its measure for persistence within the environment, and several other ethical issues (Ravi K Singh et al., 2020).

Different review papers have been done so far on gene regulation in crop improvement using RNAi, asRNA, and lncRNA, independently (Basso et al., 2019; Das & Sherif, 2020; Hong Zhang et al., 2020; Jin Xu et al., 2019; Liu et al., 2020; Rajam, 2020; Shin & Shin, 2016). This review article provides comprehensive information on the antisense technologies and shows the focus of the technologies on crop improvement. The manuscript addresses an overview of the antisense technologies, their crop improvement successes, and the major challenges facing the technology during crop improvements.

2. Applications of antisense technology for crop improvement

There are various types of antisense technology employed for crop improvement including lncRNA; however, due to the different mechanisms they used for improvement, we did not include their application in this review. This review mainly focuses on the asRNA and RNAi applications. Table 2 summarizes the major applications and achievements of the antisense technology in crop improvement.

RNA silencing technology is playing an important role in generating high yielding, disease-resistant, insect-resistant, high nutritional value, produce male sterility and fertility, and stress-tolerant crops (Williams et al., 2004). In RNA silencing, the metabolite enzymes are regulated and used for the accumulation of beneficial plant metabolites; hence, the end product is enhanced by removing poor proteins and allergenic metabolites (Meena et al., 2017). Antisense technologies play an important role in the improvement of crops by suppression or elimination of the expression
Table 2. The major applications and achievements of the antisense technology in crop improvement

| Improved traits                     | RNA tools | Targeted gene   | Crops          | References                          |
|-------------------------------------|-----------|-----------------|----------------|-------------------------------------|
| Removing toxic compounds            |           |                 |                |                                     |
| Removing linamarin                  | RNAi      | CYP79D1/D2      | Cassava        | Meena et al. (2017)                 |
| Removing ODAP                       | asRNA     | CoA synthase    | Khesari        | Shiv Kumar et al. (2011)            |
| Decaffeinating                      | RNAi      | CaMXMT1         | Coffee         | Pathak and Gogol (2016)             |
| Enhance nutrition value             |           |                 |                |                                     |
| Lysine                              | RNAi      | 22-KD           | Maize          | Song et al. (2001)                  |
| Amylose                             | asRNA     | Sbe2a           | Wheat          | Sestili et al. (2010)               |
| Reduce glutinin                     | RNAi      | γ-gliadins      | Wheat          | Gil-Humanes et al. (2008)           |
| Reduced cadmium                     | RNAi      | OsPCS1          | Rice           | Li et al. (2007)                    |
| Reduced erucic acid                 | RNAi      | BnFAE1          | Brassica       | Shi et al. (2015)                   |
| Fruit improvement                   |           |                 |                |                                     |
| Beta-caroteneis                     | RNAi      | BCH             | Potato         | Van Eck et al. (2007)               |
| Carotenoids and flavonoids          | RNAi      | DEF1            | Tomatoes       | Davuluri et al. (2005)              |
| Seedless fruit improvement          | RNAi      | CHS             | Tomato         | Schijlen et al. (2007)              |
| Enhanced shelf life                 | RNAi      | MaMADS1/S2      | Banana         | Elitzur et al. (2016)               |
| Reduce ethylene                     | RNAi      | ACC synthase    | Tomato         | Aarti Gupta et al. (2013)           |
| Biotic stress resistance            |           |                 |                |                                     |
| Bacteria resistance                 |           |                 |                |                                     |
| Leaf blight                         | RNAi      | OsSSI2          | Rice           | Younis et al. (2014)                |
| Fungal resistance                   |           |                 |                |                                     |
| Sheath blight pathogen              | RNAi      | RPMK1-1/-2      | Rice           | Ila Mukul Tiwari et al. (2017)      |
| Apple scab fungus                   | RNAi      | GFP & THN       | Apple          | Fitzgerald et al. (2004)            |
| Virus resistance                    |           |                 |                |                                     |
| Tobacco mosaic virus                 | asRNA     | CP              | Tobacco        | Powell et al. (1989)                |
| PMMaV                               | RNAi      | PMMoV replicase | Pepper         | Dalakouras et al. (2020)            |
| Insect resistance                   |           |                 |                |                                     |
| Helicooverpa armigera               | RNAi      | CYP6AE14        | Cotton         | Younis et al. (2014)                |
| Nematode                            | RNAi      | Mi-msp2         | Arabidopsis    | Joshi et al. (2019)                 |
| Whitefly                            | RNAi      | γ-ATPase        | Lettuce        | Ibrahim et al. (2017)               |
| Abiotic stress tolerance            |           |                 |                |                                     |
| Salt tolerance                      | RNAi      | TaPUB1          | Wheat          | Wenlong Wang et al. (2020)          |

(Continued)
of genes involved in the synthesis of harmful substances in food (J-Z. Xu et al., 2018). Here, we present and discuss the application of these techniques in food and cash crop production.

3. Applications in food crops

3.1. Enhancing nutritional values and quality

It is well known that antisense technology is capable of increasing the nutritive value of crops (e.g., amino acids, fatty acids, fiber), removing/decreasing undesired toxic compounds, creating male sterility for crop breeding, enhancing shelf life, and modifying many other traits (Auer & Frederick, 2009).

Improving protein quality has been reported using antisense RNA technology. Zeins are proteins that are specifically expressed during seed development and act as a reservoir of free amino acids. In maize, RNA silencing has been successfully applied to produce high lysine maize variants by knocking out the expression of 22-kD maize zein storage proteins (Song et al., 2001). Corn proteins are largely (60%) made up of zein, storage proteins, that barren essential amino acids like lysine and tryptophan. Reducing the zein content by RNA silencing technology results in the production of high lysine and tryptophan content maize (Frizzi & Huang, 2010).

Glutenin is a major protein found in most food crops including rice, wheat, and maize. Glutenin is essentially liable for the functional properties of dough that increase the viscoelasticity of the dough from these crops (Zhaojun Wang et al., 2017). Gliadins contribute mainly to the extensibility and viscosity of gluten and dough, with the polymeric glutenins being liable for elasticity. The extent of glutenin could be reduced using RNA silencing technology producing a spread of crops called LGC (low glutenin content). Silencing the expression of specific γ-gamma-gliadins by RNAi has demonstrated the feasibility of systematically silencing specific groups of gluten proteins (Gil-Humanes et al., 2008).

In wheat, SBEIIa genes have been characterized and located to be on the long arm of the homologous group 2 chromosomes which determine the amylase content of durum. Amylose content was markedly improved within the durum wheat transgenic lines exhibiting SBEIIa gene silencing (Sestili et al., 2010).

Reduction of the saturated fatty acid content of Camelina sativa, a re-emerging oil crop, is crucial to meet different application requirements. In a study by Ozseyhan et al. (2018), a reduction in saturated fatty acids was made possible by down-regulating genes encoding fatty acyl-ACP thioesterases (FATB). In the study, seeds with a specific expression of camFATB caused an increase in oleic acid content while reducing palmitic acid (16:0) by 54% and stearic acid (18:0) by 38% as compared to the wild type. This finding demonstrates that FATB genes in camelina can be effectively knocked down by an artificial microRNA targeting gene-specific sequences (Ozseyhan et al., 2018).

| Improved traits     | RNA tools | Targeted gene | Crops | References               |
|---------------------|-----------|--------------|-------|-------------------------|
| Phytate accumulation| RNAi      | GmMIPS1      | Soybean | Kumar et al. (2019)     |
| Drought tolerance   | RNAi      | GhSnRK2      | Cotton | Bello et al. (2014)     |
| Drought tolerance   | RNAi      | GbMYB5       | Cotton | Chen et al. (2015)      |
| Male sterility      | RNAi      | TA29         | Tobacco | Nawaz-ul-Rehman et al. (2007) |
| Male sterility      | RNAi      | SmTAF10/13   | Tomato | Toppino et al. (2011)   |
Fragrance rice development is a crucial process in the agricultural sector to increase the marketability of rice. 2-Acetyl-1-pyrroline (2AP) is an aroma that could give fragrance to the rice. However, naturally, the 2AP production is inhibited by OSBADH2 gene output (betaine aldehyde dehydrogenase) and avoids aroma production in rice. By introducing RNAi to IR-64 rice variety using binary vectors agrobacterium-mediated gene delivery method, the transformed rice seed increased 2AP production by about 30–40% (Khandagale et al., 2020).

Tomatoes are the source of carotenoids and flavonoids, which are highly beneficial for human health. The suppression of genes (DET1) encoding biosynthetic enzymes has led to tomatoes with improved carotenoids or flavonoids. Fruit specific RNAi-mediated suppression of DET1 enhances carotenoid and flavonoid content in tomatoes (Davuluri et al., 2005).

Carotenoids are lipid-soluble pigments which play a key role in numerous plant functions. Carotenoids also play a big role in the human diet by serving as a precursor for vitamin A synthesis and reducing the occurrence of certain diseases (Wenefrida et al., 2009). The assembly of carotenoids in potatoes is inhibited by the beta-carotene hydrogenase gene. The beta-carotene hydroxylase gene (bch), which converts beta-carotene to zeaxanthin, needs to be silenced. In this regard, *Agrobacterium tumefaciens*-mediated transformation was employed to introduce RNAi constructs into potato lines (Van Eck et al., 2007).

Parthenocarpy is the formation of seedless fruits in the absence of useful fertilization which is a desirable trait for several important crop plants (George et al., 1984). The absence of seeds is usually appreciated by consumers and producers for it increases fruit quality and fruit shelf-life. It is potentially helpful for producing vegetables and fruits when pollination is suffering from heat or unfavorable conditions (Sourabh et al., 2014). Parthenocarpic tomatoes were obtained by down-regulation of the flavonoid biosynthesis pathway using RNAi-mediated suppression of chalcone synthase (CHS), the primary gene within the flavonoid pathway (Schijlen et al., 2007).

Food spoilage is a metabolism process that changes sensory characteristics that causes food unacceptable for human consumption. Most fruits and vegetables are sensitive to spoilage than cereals due to their nature and composition (Meli et al., 2010). Post-harvest deterioration and spoilage of vegetables and fruits are the main explanations for economic loss. Delayed ripening is an essential agronomic trait being addressed through microRNA technology (Kamthan et al., 2015).

In bananas, the MaMADS box genes, *MaMADS1* and *MaMADS2*, are necessary genetic components of the ripening gene. Transgenic banana plants blocking either of the genes through asRNA were created and exhibited specific ripening delay and extended shelf-life phenotypes, including delayed color development and softening (Elitzur et al., 2016). The well-known ripening hormone ethylene is liable for initiating, modulating, and coordinating the expression of various genes involved within the ripening process. The burst in ethylene production is the main event for the start of ripening in climacteric fruits, including tomatoes. Therefore, hindered ripening tomatoes were formed by silencing three homologs of 1-aminocyclopropane-1-carboxylate (ACC) synthase (ACS) gene during ripening using RNAi technology. The chimeric RNAi-ACS construct designed to focus on ACS homologs effectively repressed the ethylene production in tomato fruits. Fruits from such lines exhibited delayed ripening and extended the time period of one month and 15 days, with improved juice quality (Aarti Gupta et al., 2013).

Other efforts were to decrease fruit softening in transgenic tomatoes through the suppression of genes encoding cell wall-degrading proteins. N-Glycans are reported to play a crucial role during fruit ripening by degrading the cell-wall in tomatoes. The two identified ripening-specific N-glycoprotein-modifying enzymes are α-mannosidase (α-Man) and β-α-N-acetyl hexosaminidase (β-Hex). The suppression of these enzymes enhanced the fruit period by almost one month, due to the reduced rate of softening (Meli et al., 2010).
Male sterility is an important trait in hybrid seed production (Saurabh et al., 2014). Male sterility is produced using RNA silencing techniques. Scientists have developed male sterile tobacco lines by inhibiting the expression of TA29 gene which is liable for pollen production. RNA silencing is also important to revive the fertility of male-sterile plants (Meena et al., 2017). Male-sterile traits are due to the rearrangement of the mitochondrial genome and demonstrate maternal inheritance patterns. From the agricultural point of view, this sort of male sterility is effective to the hybrid seed industry as a way of generating cross-pollinated seed without the necessity of intensive labors (Sandhu et al., 2007). TA29 is articulated exclusively within the anthers at the time of microspore development. In a research by Nawaz-ul-Rehman et al. (2007), about 10 out of 13 tobacco lines transformed with a hairpin RNAi construct containing TA29 sequences were male sterile. The designed microRNAs suppress or inactive towards the transcripts of the SITAF10 and SITAF13 genes of Solanum lycopersicum (tomato). Inducing the expression of those tomato, TAF genes are predicted to enrich the loss of SmTAF10 and SmTAF13 activity and can therefore restore male fertility (Toppino et al., 2011).

3.2. Biotic stress resistance

Phytopathogens are the source of various plant infections that result in a significant loss to crop production and thus lead to high economic loss. Numerous RNAi, asRNA, and IncRNA approaches have been developed to improve the protection mechanism in crop plants against several biotic stresses such as viruses, bacteria, fungi, nematodes, and insects.

Micro RNA plays an important role in responding to crop pathogens and thereby enhancing productivity. Rice blast disease was inhibited by miRNA overexpression against the Guy11 fungal strain. Transgenic rice by MiR319b (OE) silent multiple jasmonic acid synthesis and signaling components, and the rice becomes resistant when exposed to the Guy11 strain (Xin Zhang et al., 2018).

Fusarium wilt is caused by Fusarium oxysporum f.sp. filamentous fungal pathogen which affects many crop species among which tomato is the one. According to a recent research report, by using RNAi, control of fusarium wilt became possible by inhibiting a key polyamine (PA) biosynthesis gene, ornithine decarboxylase (ODC) of the pathogen as PAs (putrescine, spermidine, and spermine) are essential for normal development of the pathogen. The target ODC gene fragment was cloned in the hairpin RNA construct and used to develop transgenic tomatoes. The RNAi transgene lines expressed small interfering RNAs and exhibited moderate to high resistance to fusarium wilt from transgenic tomatoes (Neeru Singh et al., 2020).

RNA silencing also plays a crucial role as an antivirus defense reaction in plants (Pathak & Gogoi, 2016). Resistance to RNA viruses occurs through a self-perpetuating (RNA-dependent RNA polymerase) sequence-specific degradation of targeted viral mRNA (Auer & Frederick, 2009). Most plant viruses have single-stranded RNA genomes that replicate using dsRNA intermediates. The dsRNA processed into virus-derived miRNAs leading to degradation of any homologous RNA because the virus has single-stranded RNA genomes (Dalakouras et al., 2020). The experiments conducted with tobacco showed that the host-delivered RNA interference (HD-RNAi) might be achieved by using short interference RNA or double-stranded RNA (dsRNA) molecule that is complementary to viral coat protein (Fairbairn et al., 2007). The main antiviral method in plants mediated by RNA silencing technology relies on the cleavage of viral dsRNA into virus-derived small interfering RNAs (siRNAs) by Dicer-like enzymes (Meena et al., 2017).

In rice, RNAi can knockdown the OsSSI2 (OsSSI2-kd) gene which is liable for carboxylic acid desaturase activity that results in increased resistance against the bacterial pathogen Xanthomonas oryzae pv. Oryzae) of blight and blast fungus (Magnaporthe grisea) (Younis et al., 2014). Host-delivered RNAi is an efficient approach to extend rice resistance to sheath blight pathogen (Rhizoctonia solani). The HD-RNAi technology is used to aim at two pathogenicity map kinase 1 (PMK1) homologs, RPMK1-1, and RPMK1-2, from R. solani employing a hybrid RNAi
construct. Evaluation of transgenic rice lines revealed a large decrease in mycosis levels compared to non-transformed controls (Ila Mukul Tiwari et al., 2017).

When pepper mild mottle virus (PMMoV) is inoculated in N. benthamiana leaf with in vitro transcribed 997 bp dsRNAs targeting the PMMoV replicase viral infections were reduced (Dalakouras et al., 2020). Mechanical inoculation of RNA extract from E. coli M-JM109lacY expressing 480 bp dsRNA that focus on the mosaic virus (TMV) coat protein (CP) and RNA extract from E. coli HT115 (DE3) expressing 480 bpdsRNA targeting the TMV movement protein (MP) both resulted in viral resistance in tobacco (Nicotiana tabacum). These results show that sequences complementary to the terminal 117 nucleotides of TMV, are liable for the protection (Powell et al., 1989).

The present biotechnology approaches in controlling pest resistance in crops depend mostly on the expression of Bacillus thuringiensis (Bt) insecticidal proteins (Meena et al., 2017). Combining Bt technology with a second, independent mode of insect control through RNAi would enhance product performance and act as an extraprotector against the event of resistance to Bt proteins (Pathak & Gogoi, 2016). RNA silencing technology is applied to produce plants that are immune to plant insect pests. Insects do not have genes for the RNA-dependent RNA polymerase (RdRp) enzyme to duplicate siRNA molecule and make complete RNAi action; therefore, the development of HD-RNAi is required to stop plant insect pests. Consistent with this, studies on RNA silencing technology reported that it provides resistance to nematodes, fungus, bacteria, and mites in transgenic Arabidopsis and tobacco plants (Mansoor et al., 2006). A “CYP6AE14” gene was recognized in Helicoverpa armigera. This identified gene expressed within the insect midgut was related to larval growth when the food contained gossypol. Therefore, later, sucking on plant material exhibiting dsRNA specific to gene “CYP6AE14,” the result of the transcript decreased in midgut and larva growth also retarded (Younis et al., 2014).

Whitefly (Bemisia tabaci Gennadius), an important agricultural insect pest in tropical and sub-tropical regions, is a highly polyphagous insect pest that causes great damage in several crops. There have been successful developments in RNAi-based plasmids having an interfering cassette designed to generate dsRNAs that target a novel v-ATPase transcript in whitefly (Bemisia tabaci). Quantitative reverse transcription PCR showed a decreased expression level of the endogenous v-ATPase gene in whiteflies feeding on transgenic plants (Ibrahim et al., 2017).

The advance of nematode resistance in Arabidopsis by HD-RNAi-mediated silencing of the effector gene Mi-msp2. Root-knot nematodes (RKNs) are irresistible parasites that attack thousands of plants. Since RKN infection is facilitated by oesophageal gland effector genes, one among such effector genes is Mi-msp2, supported by domain analysis, the Mi-MSP2 protein contains a ShKT domain, which is probably going to be concerned in blocking K+ channels and may help in evading the plant defense response. Blocking the expression of Mi-MSP2 effectors gene using RNAi results in an increase in plant resistance to nematodes (Joshi et al., 2019). In another way, two marker genes, the green fluorescent protein (GFP) transgene, and therefore the endogenous gene trihydroxynaphthalene reductase (THN), were wont to develop a gene silencing protocol for apple scab fungus V. inaequalis. High-frequency gene silencing was achieved using hairpin constructs for GFP or the THN genes transferred by Agrobacterium tumefaciens (Fitzgerald et al., 2004).

3.3. Abiotic stress tolerance
TaPUB1-overexpressing wheat (Triticum aestivum L.) has been produced to evaluate its function in salt tolerance. These plants were more salt stress-tolerant at the time of seedling and flowering stages, whereas the TaPUB1-RNAi-mediated knockdown transgenic wheat showed more salt stress sensitivity than the wild type (WT) (Wenlong Wang et al., 2020).

Phytic acid (PA), the main phosphorus reserve in soybean seeds (60–80%), is a potent ion chelator resulting in deficiencies that leads to malnutrition. Therefore, seed targeted RNAi-mediated silencing of GmMIPS1 gene limits phytate accumulation and improves mineral bioavailability in soybeans (Jin Xu
et al., 2019). In rice, phytochelatins (PCs) play a crucial role in heavy metal resistance and accumulation. To scale back the buildup of cadmium (Cd) in rice seeds, the expression of phytochelatin synthase (PCS) gene OsPCS1 was suppressed by RNAi (Li et al., 2007).

4. Applications in cash crops

4.1. Enhancing nutritional values

RNAi-based downregulation of the three main lignin genes in sugarcane increases glucose release in the absence of a reduction in sugar production. These genes are caffeoyl-CoA O-methyltransferase (CCoAOMT), ferulate 5-hydroxylase (F5H), and caffeic acid O-methyltransferase (COMT), which have an influence on lignin content and/or composition (Bewg et al., 2016).

This research has been conducted to genetically improve the nutritional quality of *Brassica napus* cultivar CY2, the oil of which is increased in erucic acid (about 40%) and decreased in oleic acid (about 20%). Therefore, they use a seed-specific napin A promoter to drive the knockdown of BnFAE1 in transgenic CY2. The RT-PCR analysis revealed that the levels of BnFAE1 were highly reduced in BnFAE1-Ri lines compared with the CY2 cultivar. Knockdown of BnFAE1 sharply reduced the levels of erucic acid (less than 3%), highly increased the contents of oleic acid (more than 60%), and slowly increased the polyunsaturated chain fatty acids (Shi et al., 2015).

4.2. Removing undesired toxic compounds

Gossypol (C30H30O8) is a polyphenolic complex derived from cottonseed. The presence of six phenolic hydroxyl groups and two aldehydic groups makes gossypol chemically reactive (Xi Wang et al., 2009). Cottonseed could be a source of protein for humans if the toxic compound, gossypol, is down-regulated in seeds (Mansoor et al., 2006). Otherwise, it is going to cause muscle weakness, extreme paralysis, and low potassium blood level. In a research report by Palle et al. (2013), cotton plants were transformed with an antisense construct of cdn1-C1, a member of the posh gene family of delta-cadinene (CDN) synthase, which reinforces the assembly of gossypol. The incorporation of the antisense cdn1-C1 cDNA into the cotton genome altered the activity of CDN synthase and suppressed the biosynthesis of cadinane sesquiterpenoids in cottonseed and cadinane sesquiterpenoids and helicoids in cotton plants (Martin et al., 2003).

Cassava contains potentially toxic levels of cyanogenic glycosides (linamarin) which protect the plant from herbivory and theft (Siritunga & Sayre, 2004). Linamarin is a cyanogenic glucoside found within the leaves and roots of cassava plants. Upon exposure to enzymes and gut flora within the human intestine, it decomposes into a toxic chemical compound (Mansoor et al., 2006). Linamarin is synthesized through two cytochrome P450 enzymes, CYP79D1 and CYP79D2, in leaves and transported to roots (A K. Pandey et al., 2019). The leaf-specific inhibition of these enzymes lowered the linamarin content of the roots in transgenic plants by 99% (Meena et al., 2017). To alleviate the issues caused by linamarin, it is important to get transgenic cassava during which cyanogenic glycoside synthesis has been selectively inhibited in leaves and roots by antisense expression of CYP79D1/D2 gene fragments (Siritunga & Sayre, 2004).

A major toxic compound present in dry seeds and seedlings of grass pea is neurotoxin 3-N-oxalyl-L-2,3-diaminopropionic acid (beta-ODAP). ODAP is taken into account as an explanation for disease neurolathyrism which results in paralysis of lower limbs, muscular rigidity, and weakness in humans and livestock (Lambein et al., 1993). This irreversible nervous disorder in humans and animals will occur due to the presence of neurotoxin, β-N-oxalyl-L-α,β-diaminopropionic acid (β-ODAP), in its seedlings and seeds (Shiv Kumar et al., 2011). ODAP is synthesized by a two-step reaction using two-terminal enzymes, namely, oxalyl-CoA synthetase and ODAP synthase. Biosynthesis pathway manufacturing by silencing any of the two enzymes by antisense RNA technology or co-suppression technology can block ODAP synthesis (Shiv Kumar et al., 2011).
Decaffeination of coffee is the removal of caffeine from coffee. Caffeine is a natural stimulant or chemical found in coffee, tea, Coca-Cola, and other products (Berkowitz et al., 1971). The demand for decaffeinated coffee is increasing because the stimulatory consequences of caffeine can harmfully affect susceptible individuals by triggering palpitations, increasing blood pressure, and causing insomnia (Ogita et al., 2003). The expression of the gene encoding theobromine synthase (CaMXMT1) which is liable for caffeine production is repressed by RNAi. In Coffee, RNA silencing technology has enabled the manufacture of sorts with decaffeinated coffee (DECAF) that produces natural coffee with low or very low caffeine content (Pathak & Gogoi, 2016).

4.3. Biotic stress resistance
Functional analysis revealed that two core IncRNAs, GhIncNAT-ANX2- and GhIncNAT-RLP7-silenced seedlings, displayed an enhanced resistance towards V. dahliae and Botrytis cinerea, possibly associated with the increased expression of LOX1 and LOX2 genes (Lin et al., 2018).

One of the important cash crop, cotton, is affected by a cotton leaf curl virus which has a sense transcript BC1 that could induce pathogenicity symptom. Antisense construct to BC1 gene was introduced to Nicotianatabacum plant and after expression the BC1 anti-transgenic tobacco showed resistance to Viruliferous whitefly (Bemisia tabaci) which is the group of Begomovirus (Abhinav Kumar et al., 2020).

4.4. Abiotic stress tolerance
Understanding the tolerance methods of the abiotic stress and their molecular origin has been the main goals of plant research municipal. Hence, they are vigorous to the development of stress tolerance, and environmentally adapted crop plants, including cotton. RNAi approach is most productive in describing the biological function of many cotton genes in abiotic stress tolerance. For example, virus-induced gene silencing facilitated sucrose non-fermenting 1-related protein kinase 2 (GhSnRK2) gene silencing that alleviated drought tolerance in cotton plants. This implies that GhSnRK2 positively influences drought stress and enhances low-temperature tolerance (Bello et al., 2014). A similar study of VIGS-mediated RNAi recommended that GbMYB5 is a significant positive factor contributing to the environmental adaptation of plants during drought stress conditions (Chen et al., 2015).

In addition, we have mentioned in previous RNAi of cotton PHYA1 genes produced a higher level of improved drought, salt, and heat tolerance relative to wild-type plants. This high tolerance capability might be due to increased photosynthesis, regulation of plant salt tolerance genes, and longer and better-developed root systems of PHYA1 RNAi cotton plants (Abdurakhmonov et al., 2014).

Starting from the discovery of asRNA in 1983, and RNAi in 1998, all are involved in gene regulation mechanisms in both plants and animals. In plants, asRNAs and RNAi are mainly used in the inhibition of fruit maturation, removing undesired toxic compounds, disease resistance, enhancing nutritional values, male sterility, and fertility (Manish Tiwari et al., 2014). However, the frequency of using the antisense technology for crop improvement is different through the development of biotechnological tools. In Figure 1, the RNAi (93%) was the widely used method of antisense technology compared to asRNA (7%). Even if asRNA and RNAi are used for crop improvement by knockdown the expression of target genes, RNAi is more specific, efficient, reliable, and better than asRNA technology.

Antisense technology has been employed in all types of crops to improve their nutritional quality, to increase yield, and to produce tolerant and resistant crops for different stresses. The application is greater in vegetable crops (41%) followed by cereals (33%). As we know most vegetables and fruits are perishable that are sensitive to spoilage and diseases. According to Meli et al. (2010), most fruits and vegetables are sensitive to spoilage than cereals due to their nature and composition. Therefore, various researches (41%) of the reviewed paper have been conducted to alleviate these problems. Cereals are the main sources of food throughout the world. Therefore, enhancement of products, quality, nutritional values, and their ability to resist harsh

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environmental conditions and disease is needed to feed the alarmingly growing population. Hence, because of their necessities, several pieces of research (33%) have been conducted to make it more fruitful. In cash crops, 26% of the research has been conducted, which is the minimum from other crops. Cash crops are essential for a country because they can be a source of foreign exchange and can serve as a raw material for other sectors of the economy, but do not serve as direct food consumption for human beings like other food crops. Therefore, priority must be given to the improvement of food crops than cash crops. The results of the review in Figure 2 confirmed the general logic that vegetables take the first attention.

All the applications of antisense technology are applied using the production of male sterility, abiotic tolerance crops, biotic resistance crops, improved fruits, high nutritional values, and by removing undesired toxic compounds. According to the reviewed researches (Figure 3), most (29.6%) of applications are done on the production of biotic resistance crops (fungus, bacteria, virus, and insects and pests). Most of the yield loss of crops and reduction of their nutrition is due
to biotic stresses. From 1.3 USD trillion yearly food production capability in the world, the biotic stresses caused by insects, diseases, and weeds range from 31% to 42% loss ($500 billion (Dhlamini et al., 2005)). Crop damages due to pathogens are frequently more severe in developing countries (e.g., cereals, 22%) when compared to crop losses in developed countries (e.g., cereals, 6%) (Oerke, 1994). Therefore, more attention is given to the production of biotic resistant crops. Following biotic resistant crop development antisense technology working on traits related to fruit improvement and enhancing nutritional values (18.5%). Producing crops with higher yield and nutritional quality is of indispensable importance to alleviate food security problems in the world population. Abiotic stress tolerance (14.8%), removing undesired toxic compounds (11.1%), and male sterility for breeding (7.4%), have a lower rank due to their effect on food security.

5. Challenges of antisense technology
Small RNA-mediated gene silencing has been proven to be a talented and influential genetic tool for the development of improved crops. Equivalent with the major potential applications, this technology has encountered several new challenges, including the off-target effects, the delivery of finest and sufficient small RNAs in the targeted cells, the measurement of persistence in the environment, and several ethical issues (Ravi K Singh et al., 2020).

The nature of RNA-based gene silencing mechanisms was believed to require specific sequence homology between siRNA, miRNA, and asRNA and target mRNA. Antisense oligonucleotides that are adequately long enough to specify unique species of mRNA may direct ribonuclease H (RNase H) to cleave non-targeted mRNAs at sites of partial complementarities (Tidd, 1996). Due to this reason, the undesired side effects may happen when using asRNA technology. This undesired side effect of asRNA is due to the unexpected hybridization and cleavage of RNAs other than the intended target RNA by ribonuclease H (RNase H) enzyme (Woolf et al., 1992). The specificity of antisense action against selected gene expression can be achieved by increasing the inflexibility of hybridization under stable physiological conditions through the assimilation of a limited number of helix-distabilizing methyl-phosphate analog backbone modifications in the molecules (Fisher et al., 2002). This unintentional gene silencing can lead us to false conclusions in RNA silencing experiments which are aimed to study the functional role of a particular target gene in plants (Senthil-Kumar & Mysore, 2011). The off-target activity can make difficult the interpretation of the phenotypic effect in gene silencing experiments and potentially lead to unwanted toxicities (Jackson & Linsley, 2010). To overcome the unintended effect of antisense technology, the following criteria must be fulfilled: choose a highly specific trigger sequence for RNAi vectors, use RNAi vectors with tissue-specific and inducible promoters, and maintain the lowest effective number of dsRNA and siRNA (Senthil-Kumar & Mysore, 2011).
6. Conclusion
To respond to the ever-increasing worldwide food demand and dwindling cultivable land, improving efficiency and productivity of crops, especially cereals, is required. The antisense technology is one of the novel approaches that is gaining more acceptance in agricultural sciences. This technology performs with the use of asRNA, IncRNA, and RNAi. In this review, the RNAi-based antisense technology was found to be frequently applied (93%). The technique is applied on vegetables (41%), cereals (33%), and cash crops (26%) to improve biotic resistance (29.6%), and fruit and enhance nutritional values (18.5%) of crops. Despite its numerous applications, the technology has met various new challenges including the off-target effects, the delivery of finest and sufficient small RNAs in the targeted cells, the measurement of persistence in the environment, and several ethical issues.

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