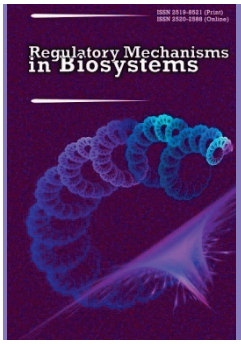

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Improvement of economic and useful characters of wheat using RNA interference technology

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Wheat is a strategic cereal crop of global importance and plays a leading role in the food supply of mankind. Despite the general trend to increase in its production, climatic changes leading to significant temperature changes, unpredictable precipitation or droughts and the appearance of new races of pathogens and pests significantly affect its yield. In order to prevent the negative impact of changes in climatic conditions on the productivity of this crop, it is necessary to develop innovative technologies for improving the resistance of wheat to environmental stresses. RNA interference (RNAi) represents a new potential tool for wheat breeding by introducing small non-coding RNA sequences with the ability to silence gene expression in a sequence-specific manner. A decrease in the expression of a certain gene determines the acquisition of a new characteristic through the elimination or accumulation of certain plant traits, which leads to biochemical or phenotypic changes that the original plants do not have. This literature review describes the progress achieved over the past decades in the application of RNAi to create wheat plants with improved economic and valuable traits. The main stages of the gene silencing mechanism mediated by short interfering RNA (siRNA) and microRNA (miRNA), features of their biogenesis, modes of action and distribution are presented. Examples of the use of various biotechnological approaches to wheat improvement using gene transformation, endogenous and exogenous double-stranded RNA molecules (dsRNA) are given. The possibility of using RNAi technology to increase the nutritional value and quality of grain, remove toxic compounds and allergens is highlighted. Considerable attention is paid to the practical results of various applications of RNAi to increase the resistance of wheat to biotic stress factors, in particular, viruses, bacteria, fungi, insect pests, and nematodes. Examples of the use of siRNA-mediated RNAi and the role of miRNA in improving wheat tolerance to abiotic stresses are summarized.

Keywords: wheat; RNA interference; transgenic plants; grain quality; resistance to stresses.

Introduction

Wheat (*Triticum aestivum* L., AABBDD, 2n = 42) is one of the world's major food crops, grown on more than 17% of arable land and consumed by ~40% of the world's population (Shewry, 2009). The grain of this crop contains proteins, carbohydrates, vitamins, fats, trace elements, is well stored and relatively easily processed into food and feed products, provides more than 20% of the total caloric content of the diet and proteins for people in the world (Shiferaw et al., 2013). It is now the most widely cultivated cereal crop with more than 220 million ha planted annually in a wide range of climates and in many geographic regions. Depending on agro-climatic conditions, more than 700 million tons of products are produced annually (http://faostat.fao.org). Despite advances in agricultural technologies and the associated increase in yields, a significant amount of wheat grain is lost due to unfavorable growing conditions (Budak et al., 2015a). In particular, climate changes, which have accelerated in recent decades, have a direct negative impact on the yield and grain quality of this crop. Abiotic and biotic stresses are also important limiting factors in its production (Nowsherwan et al., 2018). In recent years, the negative impact of stressors on wheat production has been growing at an alarming rate, and further deterioration is predicted due to climate change, land degradation, and reduced water supply (Kapoor et al., 2020). To overcome negative climate changes and environmental stressors, innovative technologies are being developed, in particular, RNA interference, which provides an important tool with significant potential for increasing wheat resistance to stress factors and other economically useful traits (Liu et al., 2021). RNA interference (RNAi) is a biological mechanism of gene activity control using short double-stranded RNAs and the synthesis of special ribonucleases (RNase), which induce selective degradation of target RNAs or inhibition of their translation or replication (Bharathi et al., 2023). RNAi involves the regulation of gene expression in several ways: efficient post-transcriptional gene silencing (PTGS), translational inhibition, RNA destabilization, and/or transcriptional gene silencing (TGS) by targeted methylation (Liu et al., 2021). Key molecules in RNAi are small interfering RNAs (siRNAs and miRNAs), which can interact with complementary sequences in other RNA molecules, such as matrix RNAs, and inhibit their activity.

The phenomenon of RNAi is found in the cells of most eukaryotes (humans, animals, plants, fungi, insects, nematodes, etc.) and is involved in many biological processes – regulation of growth, development, reproduction and protective reactions of organisms (Kumar et al., 2020; Hernández-Soto & Chacón-Cerdas, 2021; Bilir et al., 2022). The mechanism of RNAi was confirmed in 1998 by American scientists Andrew Fire and Craig Mello using the nematode *Caenorhabditis elegans* as a research object (Fire et al., 1998). For this discovery in 2006, the researchers received the Nobel Prize in Physiology and Medicine.

Induction of RNAi in plants using transgenesis, which includes sequence-specific gene regulation using small non-coding RNAs, has become one of the most powerful approaches in improving crops, their development and protection against various pathogens and pests by manipulating the expression of target genes (Abdellatef et al., 2021; Akbar et al., 2022; Bharathi et al., 2023). It is believed that transgenic plants created on the basis of RNAi are profitable and more environmentally friendly, since they do not produce any functionally foreign proteins and biocidal sub-

stances, and also do not pollute the environment (Rajam, 2020; Rodrigues & Petrick, 2020; Kaur et al., 2021). In addition, RNAi generates target gene knockdown instead of knockout, making it more advantageous compared to recently developed genome editing tools (Mezzetti et al., 2020). RNAi technologies can be used to reduce the expression of any genes without disrupting the expression of other genes. These unique features of RNAi make it a popular and effective strategy for the improvement and protection of agricultural plants (Mezzetti et al., 2020; Rajam, 2020).

The role of RNAi technology in wheat improvement has been shown to improve grain quality by enriching it with essential amino acids, antioxidants and other nutrients beneficial for human health or by reducing the number of allergens or antinutrients (Barro et al., 2016; Gasparis et al., 2017), increasing plant tolerance to various biotic (viruses, bacteria, fungi, nematodes, insects) and abiotic stresses (drought, salinity, extreme temperatures, etc.) (Qi et al., 2019; Wang et al., 2020; Zeeshan et al., 2021). This review of the literature highlights the achievements of the practical application of RNA interference technology in the improvement of economically useful traits of common wheat.

Mechanism of RNA interference

The mechanism of RNAi consists in induction by double-stranded RNA (dsRNA) of processes of recognition and degradation of cellular mRNA. An important aspect of the RNAi mechanism is that it does not change the primary chromosomal structure of the target genes, but is able to significantly weaken their expression and lead to certain changes in the phenotype of cells and whole organisms (Baulcombe, 2019). The RNAi system is conservative and highly selective: each recognizes and silences only its target RNA. In plants, RNAi triggers dsRNA, which can have different origins, ranging from viral replication intermediates, transcription of inverted repeats, stress-induced splicing of antisense transcripts, and RNA-dependent RNA polymerase transcription of aberrant transcripts (Dalakouras et al., 2020).

Mechanism of RNA interference mediated by siRNA

In transgenic plants, the RNAi technology is based on silencing mRNA of target genes using the genetic constructions containing inverted repetitive sequences of the fragments of these genes. mRNA transcribed from such constructions forms a hairpin structure (Younis et al., 2014). The initial stage of RNAi is associated with the delivery of dsRNA (through the transgene introduction) into the cell, which is absolutely homologous (by the sequence) to the target gene (Ali et al., 2010). At the first stage, a degradation of long dsRNA/shRNA (hairpin loop RNA from alien genes) occurs as a result of the induction of the enzymatic activity of Dicer-like dsRNAses (DCL-like ribonucleases) typical for higher plants. DCL-catalyzed hydrolytic cleavage of dsRNA ultimately leads to the formation of short double-stranded fragments (duplexes) called short interfering RNAs, 21–24 nucleotides (nt) in length, with a 5'-terminal phosphate group and symmetrical ledges of two nucleotides at the 3'-ends that are stabilized by methylation of the 3'-ends by methyltransferase HUA Enhancer 1 (HEN1) (Kaur et al., 2021). Double-stranded duplexes bind (with the involvement of auxiliary proteins Aux) to the effector protein Argonaute (AGO) in order to form the core of RNA-induced silencing complex (RISC). At the next stage of RNAi, both strands of previously formed duplexes are separated. At the same time, the sense chain (passenger) is degraded, and the antisense (directional, which is characterized by a lower thermodynamic stability of the 5'-end) is included in the AGO for the formation of activated RISC and is used as a "navigator," due to which the AGO protein cleaves the molecule, which will be complementary to the antisense chain (Rajam, 2020). After loading, RISC tracks its related mRNA and provides downregulation of the desired gene(s) either by degradation of the target mRNA or by the repression of translation (Kaur et al., 2021). The target mRNA, complementary to the guide sequence of the siRNA with the length of 21nt, in the mature RISC is cleaved between the tenth and 11th nucleotides (from the 5'-end) by a PIWI domain of the AGO protein, forming the products containing 5'-monophosphate and 3'- hydroxyl ends (Halder et al., 2023). These products of cleavage are rapidly degraded by endogenous exonuclease due to the lack of 5'-capping or 3'poly(A) tail. After completion of the cleavage, the RISC leaves, and the siRNA can be used in a new cycle of mRNA recognition and cleavage. A generalized scheme of the RNAi mechanism mediated by siRNA is presented in Figure 1.

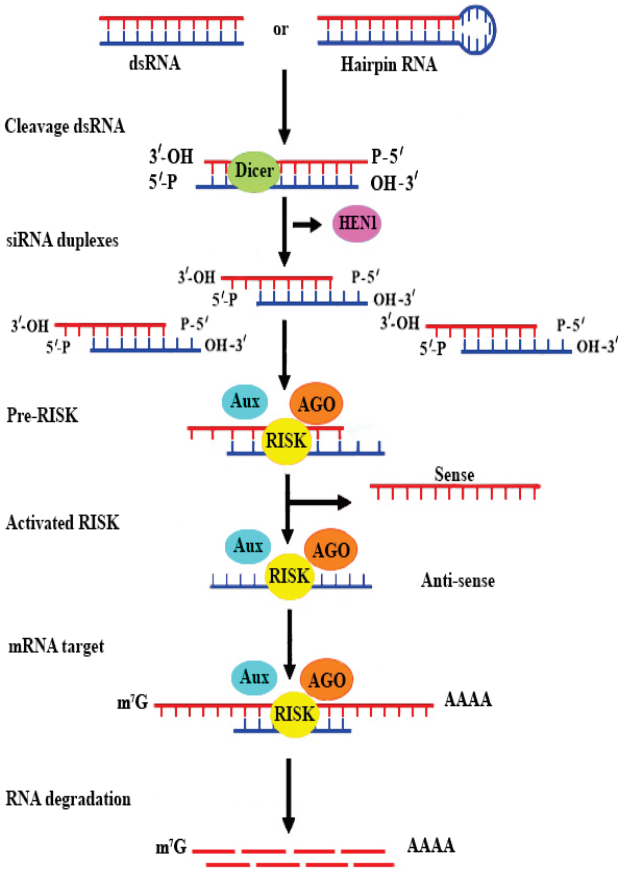

Fig. 1. Mechanism of gene silencing in plants mediated by siRNA (adapted from Kaur et al., 2021)

Single-stranded siRNA fragments complementary to the target mRNA regions can be used as primers for the plant RNA-dependent RNA polymerase (RdRP), which completes the second strand using the target RNA as a matrix (the effect is limited to a distance of ~300–500 nucleotides in the 5'-direction from the cleavage initiation site). During the degradation of de novo synthesized dsRNA by DCL ribonuclease, new siRNAs are formed that are called secondary. Thus, signal amplification is carried out. In the future, secondary siRNAs can not only be involved in the direct degradation of mRNA targets as a part of RISC but also be transported between the cells by plasmodesmata as signaling molecules.

Mechanism of RNA interference mediated by miRNA

miRNAs are of endogenous origin because they are transcribed from MIR genes, which are mainly located in intergenic regions, although several miRNAs originate from intronic or exonic sequences of protein-coding genes and can also be localized within transposons (Song et al., 2019). MiRNA biogenesis is a multistep process that involves transcription, processing, modification, and RISC assembly (Yu et al., 2019). The majority of plant miRNA genes are transcribed by RNA polymerase II (Pol II) with the formation of long primary transcripts that have partially double-stranded stem-loop structures – pri-miRNA (primary precursor miRNA) (Halder et al., 2023). In plants, the structure of pri-miRNA is relatively variable in terms of length, which starts at 60 nt and can extend up to 500 nt. Pri-miRNAs are recognized and processed to the direct precursor of miRNAs (pre-miRNAs) by the Dicer protein. At the first stage of maturation, the enzyme Dicer-like 1 (DCL1) with the participation of the double-stranded RNA-binding protein Hyponastic leaves 1 (HYL1) and Serrate (SE), which is a zinc finger protein, sequentially processes pri-miRNA first into

pre-miRNA (Kaur et al., 2021). Together, these enzymes form a cubic complex visualized as a D-body in the nucleus, which can be activated by specific kinases and deactivated by phosphatases (Kaur et al., 2021). As a result, pre-miRNA is formed, which is able to fold into a characteristic pin-shaped secondary structure, and its formation occurs in subnuclear D-bodies. The DCL1 enzyme further processes the pre-miRNA and forms an unstable miRNA/miRNA* duplex. With the help of the small RNA methyltransferase HUA Enhancer 1 (HEN1), the duplex is methylated from the 3'-end, after which it is released into the cytoplasm by the exportin-like protein Hasty (HST1) (Kaur et al., 2021). In the cytoplasm, one strand of the miRNA duplex (guide) is included in the RISC complex, while the other strand (passenger), designated as miRNA*, is normally degraded. The choice of the guide chain is determined by the degree of stability of the ends of the duplex: the guide chain will be the chain whose 5'-end is in the composition of the less stable part of the duplex. Mature single-stranded miRNA activates the catalytic part of the RISC complex – the AGO1 protein (ARGONAUTE) and interacts with its complementary target mRNA, which leads to the inhibition of translation or cleavage of the target mRNA (Kaur et al., 2021). A generalized diagram of the RNAi mechanism mediated by miRNA is presented in Figure 2.

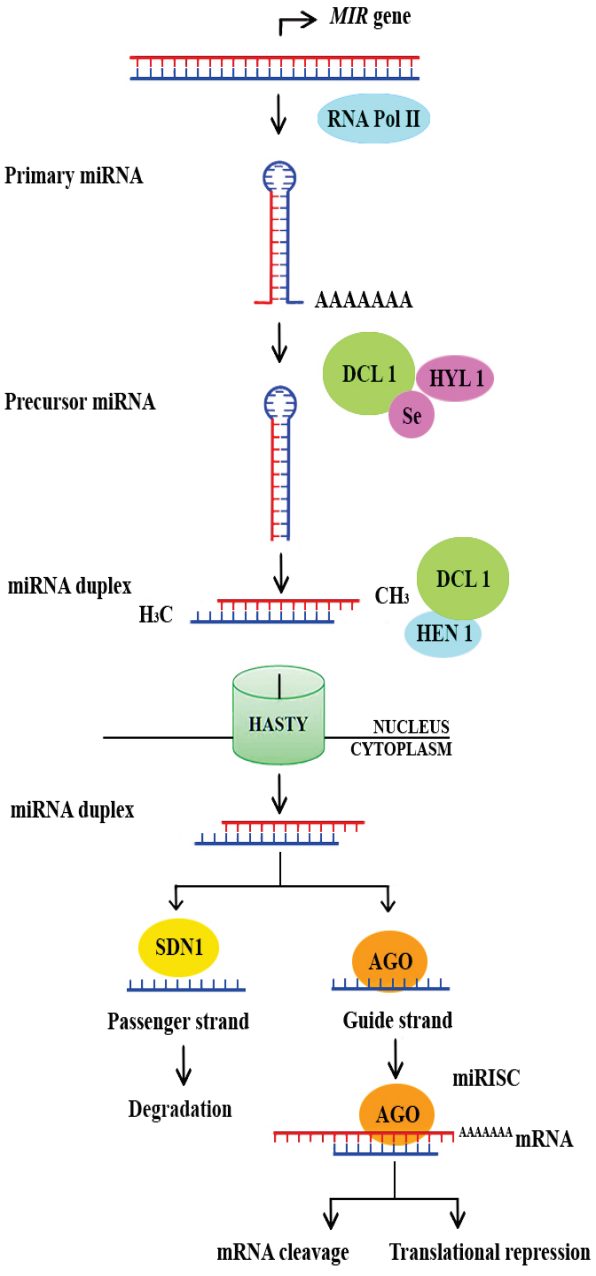

Fig. 2. Mechanism of gene silencing in plants, mediated by miRNA (adapted from Kaur et al., 2021)

Small non-coding regulatory RNAs

Small non-coding regulatory RNAs include two major classes – short interfering RNAs (siRNAs) and microRNAs (miRNAs). siRNA and miRNA are non-coding molecules, 20–25 bp in size and are important biologically active molecules that significantly participate in the creation of various genotypes and phenotypes (Halder et al., 2023). The difference between miRNAs and siRNAs is that the latter are formed by cutting a long dsRNA molecule, while miRNAs have a hairpin structure formed from single-stranded RNA under the influence of ribonucleases of the RNase III class. This category of RNA is transcribed from DNA, but they do not carry out the process of translation and do not produce proteins (Brosnan & Voinnet, 2009). They specialize in performing important functions related to plant growth and development and, most importantly, induce responses to various abiotic and biotic stress factors transcriptionally and posttranscriptionally (D'Ario et al., 2017; Song et al., 2019; Bharathi et al., 2023).

Short interfering RNAs (siRNAs)

siRNAs, which were discovered in 1999 (Hamilton & Baulcombe, 1999), are noncoding molecules (20–24 nt in size) found in different organisms and involved in many regulatory processes. They are key components for the cellular functions during plant development, hormone signaling, and stress responses. Long dsRNA is their precursor; unlike miRNA, they are completely complementary to the sequence of the target gene. The mechanism of gene regulation is transcriptional and posttranscriptional, and the mode of action is histone modification, DNA methylation, and mRNA degradation (Halder et al., 2023). siRNAs in plants are distinguished by the AGO family proteins (AGO1, AGO4, AGO6, AGO7), the catalytic component of the silencing complex, largely based on the size, and identity at the 5'-nucleotide level to form RISC, which mediates posttranscriptional gene silencing through mRNA cleavage or repression of translation (Halder et al., 2023). They play a major role in the regulation of the mechanism of plant protection against potential pathogens, such as bacteria, viruses, fungi, oomycetes, parasitic nematodes, and parasitic plants.

siRNAs are well known for their silencing function in the case of viral RNAs (Akbar et al., 2022). At the same time, 21–22 nt siRNAs are involved in the direct degradation of viral RNA and some endogenous mRNAs important in the system of protection against viruses. 21-nucleotide siRNAs with 5'-U are loaded onto the AGO1 and scan the cytoplasm for the complementary transcripts for the cleavage and degradation in the process of posttranscriptional gene silencing. 22-nucleotide siRNAs are also loaded onto the AGO1, but they apparently change the AGO1 conformation and recruit RdRR6 to the 3'-sequence, transcribing the target transcript into dsRNA and, thus, generating the additional (secondary) siRNAs by a mechanism called "transient silencing" (Dalakouras et al., 2020). Recent studies provided evidence for the ability of siRNAs to inhibit the development of fungi and oomycetes by silencing pathogen-specific genes associated with the pathogenesis (Canto-Pastor et al., 2019; Sang & Kim, 2020). Scientists concluded that siRNA is a potential mixture of different gene sequences and is used as a "shotgun," which affects certain pathogen genes with a high efficiency (Fletcher et al., 2020).

Noncoding siRNAs are highly mobile and are able to move both within and between interacting organisms (for example, from a host plant to a pathogen and vice versa). In plants, they usually move through the phloem from the areas of high concentration to places with a deficiency of these molecules (Kong et al., 2022). The movement of siRNA in the plant is divided into intercellular (proximal) and systemic (distant) transport. This process is carried out from the place of initiation to neighboring cells through the intercellular channels of the plasmodesma and spreads systemically over considerable distances through the conducting tissue of the phloem. Within a few days of initiation, there is systemic movement of the silencing signal, which is usually directed from photosynthetic sources (i.e., leaves) to roots and growing points. A family of related genes can be silenced by only one RNAi construction due to heterogeneity of siRNA sequences. However, the use of RNAi strategy based on siRNA can also cause unpredictable effects, leading to the unintentional silencing of non-

target genes with the regions homologous to target gene sequences. In addition, since posttranscriptional gene silencing in plants is mobile and can be induced locally and then spread throughout the plant, siRNA-mediated RNAi technologies are not suitable for some applications that require a tissue-specific gene silencing.

Plant miRNAs (miRNAs)

Discovered in 1993 (Lee et al., 1993), miRNAs are endogenous non-coding molecules, 20–22 nt in size, which act as regulators of gene expression at the post-transcriptional level and affect many molecular and biochemical processes in plants (Halder et al., 2023). In particular, they coordinate various aspects of ontogenesis programs, including the formation, separation and development of vegetative and generative organs, the transition to different phases of vegetation, reproduction, and also regulate adaptation to environmental conditions and plant responses to biotic and abiotic stresses (Yu et al., 2019). Their precursor is a long single-stranded RNA that contains a local hairpin structure, they, unlike siRNA, may not be completely complementary to the sequence of the target gene. The mechanism of gene regulation is only post-transcriptional, and the mode of action is repression of translation and degradation of mRNA (Halder et al., 2023). miRNAs in plants are also sorted by AGO family proteins (AGO1, AGO10), mostly based on size as well as identity at the 5'-nucleotide level (Halder et al., 2023). miRNAs have been classified based on their position within the genome and are "intronic" or "intergenic". Intronic miRNAs are formed from introns present in the host transcript (Budak & Akpinar, 2015). Intergenic miRNAs connect two protein-coding genes, the transcription of which occurs in separate independent units with the help of RNA polymerase II (Pol II).

In plants, miRNAs can be conventionally divided into two groups: conservative miRNAs found in different families (there are few such miRNAs, they are characterized by a high level of expression), and species-specific miRNAs (there are many such miRNAs, they are characterized by a low level of expression) (Zhang & Wang, 2016). Conservative miRNAs regulate the expression of key transcription factors that determine the transition of a plant to a new ontogenetic state, the formation of meristems, the differentiation of cells and organs, their polarity, as well as resistance to environmental stress factors. Species-specific miRNAs make up a significant proportion of plant miRNAs and perform certain functions in a specific species.

It is believed that miRNAs form a regulatory network, with the help of which the spatial and temporal regulation of the gene complex is carried out at various stages of development. Basically, miRNA targets include genes encoding transcription factors, as well as other regulatory proteins related to plant development (Li & Zhang, 2016). In many plants, it has been clearly demonstrated that the expression of a wide range of miRNAs occurs to combat various stress factors. To date, a large amount of experimental material has been accumulated regarding their possible role in the formation of the response to viral and bacterial infections, the transmission of signals in the event of a lack or excess of mineral nutrients, in the regulation of the genes of the antioxidant system under conditions of abiotic stress (Alptekin et al., 2017; Song et al., 2019; Bhogireddy et al., 2021). miRNAs interact with transcription factors to regulate the signaling of stress-related hormones such as auxin, ethylene, abscisic and gibberellic acids under stress conditions (Halder et al., 2023).

The molecular mechanism of action under stress is known only for some miRNAs, while for others only the nature of the change in their expression level has been described. First of all, it is possible to distinguish a group of miRNAs whose expression changes under almost all types of stress (for example, miR160, miR167, miR393), as well as a group of stress-specific miRNAs that respond only to certain types of stress (miR392, miR395, etc.). Thus, an increase in the expression of miR393, miR160 and miR167 under the effects of drought or salt stress was found in most plant species, which is accompanied by a slowdown in plant growth and development (Sunkar et al., 2012). As an example of miRNA, the expression of which varies in different directions in different species, we can point out miR169, which is involved in the response to salt stress and drought (Bhogireddy et al., 2021). miRNAs move locally from cell to cell over short distances, mainly through plasmodesmata, but some miRNAs are distributed over long distances through the phloem (Skopelitis et al., 2018). Through extensive research, it has been established that some miRNAs (miR390, miR173 and miR845) have the potential to initiate mass production of secondary siRNAs called phasiRNAs (Deng et al., 2018).

A miRNA strategy that is effective in one plant species may not be successful in another, and this phenomenon is explained by the different types of regulation of evolutionarily conserved miRNAs in different species (Halder et al., 2023). It has also been established that different existing isoforms of the same miRNA family can play an active role in regulating different physiological functions through the same or other genes (Alptekin et al., 2017). It should be noted that the possibility of obtaining an off-target effect is significantly lower with miRNA-based RNAi compared to kiRNA, as they require a smaller number of nucleotides (one sequence of 21/22 nt) to identify the target sequence (Halder et al., 2023).

Improvement of economic and valuable traits of wheat based on RNAi

RNAi has great potential for changing gene expression in wheat to improve its quality characteristics and nutritional status, increase resistance to abiotic and biotic stresses. This approach facilitates target gene identification and development of RNAi vector constructs for transformation. For stable gene silencing, RNAi hairpin constructs are used, containing a partial sequence of the target gene (200–300 bp) in sense and antisense orientation with a small intron between them under the control of a suitable promoter designed for dsRNA expression in transgenic plants (Rajam, 2020). It is also common to use the miRNA overexpression method along with the process of introducing artificially synthesized miRNA targeting the gene of interest. Artificial synthetic siRNAs have enormous applications as they can overcome the potential drawbacks and limitations associated with siRNAs, given that they induce gene silencing with maximum precision (Halder et al., 2023). The summarized practical results of the application of RNA interference for wheat improvement are presented in Table 1.

Table 1
Improvement of economic and valuable traits of wheat by means of RNA interference

Trait for improvement	Target gene	A type of small RNA	References
Nutritional value and grain quality			
Improving the baking quality of flour and dough	γ-gliadin	siRNA	Gil-Humanes et al. (2008, 2012); Pistón et al. (2011)
	α- gliadin		Wieser et al. (2006)
	ω-5- gliadin		Altenbach et al. (2014b)
	α-, γ-, ω-gliadins		Gil-Humanes et al. (2010)
Reduction in the content of immunogenic epitopes	ω-5- gliadin	siRNA	Altenbach et al. (2014a)
	α-, γ-, ω-gliadins		Gil-Humanes et al. (2010); Barro et al.(2016)
	DME	siRNA	Wen et al. (2012)
Increase in grain hardness	*Pina, Pinb*	siRNA	Gasparis et al. (2011)
		амиРНК	Gasparis et al. (2017)
Increased content of amylose	*SbeIIa, SbeIIb*	siRNA	Regina et al. (2006)
Biotic stresses			
Resistance to viruses			
Wheat streak mosaic virus (WSMV)	*Nla*	siRNA	Fahim et al. (2010)
	CP	siRNA	Sivamani et al. (2002); Li et al. (2005); Cruz et al. (2014)
	Nlb	siRNA	Sivamani et al. (2000)
	5' UTR region,	amiRNA-1	Fahim et al. (2012)
	ORF pipo region,	amiRNA -2	
	P3 cistron,	amiRNA -3	
	P1,	amiRNA -4	
	P3 cistron, HCpro	amiRNA -5	
Triticum mosaic virus (TriMV)	*CP*	siRNA	Shoup Rupp et al. (2016)
Wheat yellow mosaic virus (WYMV)	*TaAAED1*	amiRNA-1	Liu et al. (2021)
Resistance to fungi			
Fusarium	*Chs3b*	siRNA	Cheng et al. (2015)

Trait for improvement	Target gene	A type of small RNA	References
graminearum	*SGE1, STE12, PP1*		Wang et al. (2020)
	FGSG_03101	miRNA	Jiao & Peng (2018)
Fusarium culmorum	*Gls1, Fmk1, Fgl1, ChsV*	siRNA	Chen et al. (2016)
Puccinia triticina Erikss	*MAPK1, CYC1, CNB*	siRNA	Panwar et al. (2013, 2018)
	α- gliadin, GST, LRR	tasiRNA	Dutta et al. (2017)
Puccinia striiformis f. sp. *tritici*	*GSRE1*	siRNA	Qi et al. (2019)
	CPK1	siRNA	Qi et al. (2018)
	Fuz7	siRNA	Zhu et al. (2017)
	TaCSN5	siRNA	Bai et al. (2021)
	RabGAP/TBC domain, zinc finger protein, receptor-like protein kinase 41	miRNA	Feng et al. (2015)
	TaCLP1	miRNA	Feng et al. (2013)
	TaNAC21/22	miRNA	Feng et al. (2014)
	PR2	milRNA	Wang et al. (2017)
Blumeria graminis f.sp. *tritici*	*Avra10,GTF1, GTF2*	siRNA	Nowara et al. (2010)
	MLO		Riechen (2007); Varallyay et al. (2012)
	SvrPm3a1/f1	siRNA	
	Bgt_Bcg-6		Schaefer et al. (2020)
	Bgt_Bcg-7		
Rhizoctonia cerealis	*TaCRK1*	siRNA	Yang et al. (2013)
Resistance to insects			
Sitobion avenae F.	*ZFP*	siRNA	Sun et al. (2019)
	lmf2-like	siRNA	Xu et al. (2017)
	CHS1	siRNA	Zhao et al. (2018)
	CbE E4	siRNA	Xu et al. (2014)
Resistance to nematodes			
Heterodera avenae	galexin, cathepsin L, *vap1*, serpin, *flp12, RanBPM*	siRNA	Dutta et al. (2020)
	Nlw, Pbp,Ibp,Eps	siRNA	Gantasala et al. (2015)
	Ha-annexin	siRNA	Chen et al. (2015)
	Ha18764	siRNA	Yang et al. (2019)
Meloidogyne incognita	HSP90, *ICL, Mi-cpl-1*	siRNA	Lilley et al. (2007)
Pratilenchus thornei	*pat-10, unc-87*	siRNA	Tan et al. (2013)
Resistance to abiotic stresses			
Drought	*ProDH*	siRNA	Dubrovna et al. (2020; 2022)
	NRX1	siRNA	Zhang et al. (2021)
	WZY2	siRNA	Yu et al. (2019)
	PRM1, RPP13, PFT1	miR08, miR15	Hua et al. (2019)
	SPL, SBP	miR156	Pandey et al. (2014); Ma et al. (2015)
	MYB3	miR159a	Ma et al. (2015)
	ARF	miR160a	Ma et al. (2015)
	NAC	miR164b	Ma et al. (2015)
	HD-ZIP4	miR166h	Ma et al. (2015)
	CCAAT-box	miR169d	Ma et al. (2015)
	TIR1	miR393	Ma et al. (2015)
	GRF	miR396	Ma et al. (2015)
	CSD1,CSD2	miR398	Ma et al. (2015)
	MADS-box	miR444c1	Ma et al. (2015)
	IF3	miR444d3	Ma et al. (2015)
	AFH	miR628	Ma et al. (2015)
	6-PGDH	miR2111c	Hua et al. (2019)
	EG	miR5054	Hua et al. (2019)
Salinization	CBL7	miR59	Zeeshan et al. (2021)
	SPL3	miR156	Zeeshan et al. (2021)
	R2R3-MYB	miR159	Zeeshan et al. (2021)
	ARF8	miR160	Zeeshan et al. (2021)
	ARF	miR167	Lu et al. (2011)
	NF-YA	miR169	Zhao et al. (2009)
	MYB, SCL6	miR171	Wang et al. (2014); Zeeshan et al. (2021)
	PCF5	miR319	Zeeshan et al. (2021)
	ATP-dependent RNA helicase	miR399	Lu et al. (2011)
	SnRK2	miR408	Bai et al. (2018)
High and low temperatures	HSP90	miR156	Xin et al. (2010); Kumar et al. (2015)
	GAMYB1	miR159	Wang et al. (2012)
	HSP70, ARF, TPR	miR160	Goswami et al. (2014)
	HSP17	miR164	Kumar et al. (2015)
	HSP, ARF, Dnaj (HSP40)	miR167	Goswami et al. (2014); Kumar et al. (2015); Ragupathy et al. (2016)
	NF-Y	miR169	Ravichandran et al. (2019)
	TCP, HSFA4a MYB3	miR319	Goswami et al. (2014); Kumar et al. (2015)
	HSP70, *CSD1*	miR398	Goswami et al. (2014); Kumar et al. (2015)

Improvement of grain quality

In recent years, RNAi technology has been actively used to increase the nutritional value of wheat, as well as for elucidation of the mechanisms of endosperm structure formation and the role of different classes of proteins in determining the technological properties of flour and dough (Gil-Humanes et al., 2012; Altenbach et al., 2014b). It is known that in wheat grain, reserve proteins make up to 80% of the total protein content of the mature seed (Godwin et al., 2009). Gluten, which is largely responsible for the functional properties of the dough, is the main protein part of the wheat grain. Gliadins mainly contribute to the extensibility and viscosity of gluten and dough, while polymeric glutenins are responsible for its elasticity. In wheat of the Bobwhite variety, seven transgenic lines with silencing of the γ-gliadin gene were obtained (Gil-Humanes et al., 2008), in which their portion was reduced by 33–80%, which contributed to the formation of a stronger dough with improved resistance to overmixing. Subsequently, the same researchers used interfering RNAs capable of repressing the genes of all three groups of α-, γ-, and ω-gliadins and obtained the wheat lines with a significant reduction (in some cases up to 90%) of gliadin content (Gil-Humanes et al., 2010). Subsequently, the construction for RNA silencing was transferred to three other common wheat varieties by crossing, which resulted in an increase in the amount of high and low molecular weight glutenins and SDS sedimentation index (Gil-Humanes et al., 2012). It was demonstrated that RNAi silencing of gamma gliadins in wheat lines leads to an increase in the content of all other gluten proteins but has a little effect on SDS test parameters (Pistón et al., 2011). Transgenic wheat with silencing of the α-gliadin gene was also reported, which was characterized by increased strength of flour and increased volume of bakery products (Wieser et al., 2006).

WDEIA (wheat-dependent exercise-induced anaphylaxis) is a rare but potentially severe food allergy, which is characterized by anaphylactic reactions that occur 1–4 h after eating wheat with subsequent physical activity. Namely the presence of ω-5 gliadin is associated with this food allergy. Using RNAi technology, the transgenic lines of wheat with a decreased level of omega-5 gliadin were obtained (Altenbach et al., 2014a). It was established that ω-5 gliadins have a negative effect on the flour quality; therefore, the transgenic lines with inhibited synthesis are distinguished by better quality (Altenbach et al., 2014b).

It should be noted that the flour in the lines of transgenic wheat with suppressed synthesis of gliadins is less toxic for individuals who suffer from celiac disease and who adhere to a gluten-free diet. It was demonstrated that gluten proteins in the transgenic wheat lines with silencing of α-, γ-, and ω-gliadins decrease the formation of celiac disease-related specific epitopes DQ2 and DQ8 that are recognized by T leukocytes (Gil-Humanes et al., 2010). Therefore, such wheat can be used to feed individuals with celiac disease who cannot consume the products made from ordinary wheat, rye, and barley flour. An attempt to develop a natural dietary therapy for this disorder by inhibiting the transcription of wheat DEMETER (DME) homeologs using RNAi was described. DME encodes a 5-methylcytosine DNA glycosylase responsible for a derepression of the transcription of gliadins and low molecular weight glutenins by active demethylation of their promoters in the wheat endosperm. Previous studies detected that these proteins are a major source of immunogenic epitopes. The wheat transformants expressing hairpin RNA in their endosperm were obtained and demonstrated up to 85.6% suppression of DME transcripts and up to 76.4% decrease in the number of immunogenic prolamins, demonstrating the possibility of creating the wheat varieties compatible for celiac patients (Wen et al., 2012).

The plasmids containing RNA fragments from α-, γ-, and ω-gliadins and low-molecular-weight glutenin subunits were developed to silence the expression of different prolamin fractions (Barro et al., 2016). In transgenic wheat plants that carried certain combinations of them in these plas-

mids, there was a strong decrease in the content of gluten (above 90%) as compared with the controls. Some of the modified lines were completely devoid of celiac disease epitopes from highly immunogenic α- and ω-gliadins. These results raise the prospect of creating nontoxic wheat varieties with a low level of harmful gluten.

An important agrotechnical feature is the grain hardness of cereals, which affects the quality of flour and the properties of the final products. The soft grain phenotype in wheat is determined by alleles of the Pina and Pinb genes, and any mutations in one or both of the Pin genes result in more or less hard grains. An increase in grain hardness was reported after siRNA-mediated silencing of the Pina and Pinb genes in wheat (Gasparis et al., 2011). Gene transcript levels were reduced by more than 80% in T1 and more than 90% in T2–T4 generations and remained stable. In addition, RNA-mediated silencing of one of the Pin genes simultaneously reduced the expression of the second gene. Decreased transcript levels of both genes resulted in a significant reduction or absence of puroindoline proteins and an increase in wheat grain hardness. Subsequently, this group of researchers (Gasparis et al., 2017) used artificial miRNAs (amiRNAs) of the same target genes to compare the efficiency of both gene silencing pathways. Each of the two amiRNA cassettes contained a 21 nt miRNA precursor derived from the conserved region of the Pina or Pinb genes. Pinb gene silencing in wheat was highly effective in the T1 generation – its transcription decreased to 92%, which was associated with strong expression of Pinb-derived amiRNA. Silencing of target genes correlated with increased grain hardness.

Foods high in resistant starch can improve human health and reduce the risk of serious non-communicable diseases. RNAi was used to down-regulate two different isoforms of the starch branching enzyme SBEII (SBEIIa and SBEIIb) in wheat grain endosperm to increase amylose content. Suppression of both SBEIIa and SBEIIb gene expression increased starch amylose to >70% (Regina et al., 2006). These results indicate that high-amylose wheat has significant potential to improve human health through its resistant starch content.

Resistance to biotic stresses

Pests such as viruses, bacteria, fungi, nematodes and insects pose a serious threat to wheat (Figueroa et al., 2018). Globally, about 20% of the harvest of this crop is lost due to damage by pests, and the quality of products obtained from the grain of affected plants also deteriorates. RNAi is used to obtain pest-resistant cultures by means of host-induced gene silencing (HIGS). HIGS is a biotechnology that is mainly based on the creation of transgenic plants, and allows silencing of specific genes of pests or pathogens that attack them by expressing homologous dsRNAs in the host plant. The modified plant transcribes dsRNAs, which are processed into siRNAs, which in turn are transferred to plant pathogens (Sang & Kim, 2020). A crucial step for a successful HIGS strategy is the identification of appropriate target genes in the pathogen (Koch & Kogel, 2014). The most interesting technologies also include virus-induced gene silencing (VIGS), based on the inclusion of a segment of the host plant gene in the viral genome to induce RNAi and silence the expression of the target gene at the post-transcriptional level (Akbar et al., 2022).

Resistance to viruses

Plant viruses pose a significant threat to wheat and cause approximately 10–15% of crop losses (Yu et al., 2022). Methods aimed at activating the key components of the plant's RNAi mechanism and directing them against the nucleotide sequences of invasive nucleic acids of pathogens can increase the level of antiviral protection. One of the most common approaches in this way is the genetic transformation of plants with constructs containing sense or antisense nucleotide sequences that copy the nucleotide sequences of the viral genome (Abdellatef et al., 2021; Akbar et al., 2022; Yu et al., 2022). In addition, chimeric hairpin constructs carrying sequences from different viruses have been successfully used to induce multiple resistance against target viruses in transgenic plants (Halder et al., 2023). Similar to chimeric hairpin constructs acting on different viruses, chimeric constructs carrying sequences of different genes of the same virus have been used to maximize resistance efficacy (Lacombe et al., 2021; Akbar et al., 2022). As a rule, to provide resistance to certain strains of viruses, nucleotide sequences encoding viral suppressor proteins or proteins responsible for cell entry, division or spread of the virus are selected as targets (Yu et al., 2022).

Another effective way to provide resistance to viruses is the transformation of plants with constructs that contain the precursor of artificial miRNA, the process of creating which is described in detail in the work of Tiwari et al. (2014). Unlike siRNA-producing constructs, miRNA constructs produce a single 21nt small RNA with a well-defined nucleotide sequence that provides ultraspecific degradation of the target transcript in a conserved region. The miRNA constructs are also less prone to the effect of nonspecific degradation, potentially making them more biologically safe. As with transformation by a dsRNA construct, miRNA-based constructs can be used to confer resistance to one or more viruses.

Quite often, to acquire resistance to viruses, wheat has been transformed with a target virus sequence, mainly from the gene encoding the coat protein (CP) (Li et al., 2005; Cruz et al., 2014; Shoup Rupp et al., 2016). The common wheat plants of the ROC22 variety were stably transformed with the CP gene of the wheat streak mosaic virus (WSMV), and 11 transgenic lines were obtained. Out of five analyzed ones, one demonstrated a high resistance to inoculation with two WSMV strains (Sivamani et al., 2002). In similar studies, all transgenic wheat plants of 566B genotype of T1 generation carrying the WSMV-CP demonstrated a strong resistance to WSMV. However, all modified plants in T2–T3 generations demonstrated a transgenic silencing (Li et al., 2005). In further studies, using the transgenes of the WSMV virus CP protein, it was possible to reach an efficient resistance in plants of this crop, which was evidenced by the absence of viral RNA in the tissue. A resistance to the virus was stably inherited up to the T5 generation (Cruz et al., 2014). To increase resistance to a Triticum mosaic virus (TriMV), the wheat of Bobwhite variety was transformed with a construction containing TriMV CP gene sequences (Shoup Rupp et al., 2016). Several lines were obtained that were resistant and had little or no viral RNA. According to the results of realtime PCR and ELISA, resistant lines of the T6 generation demonstrated a high level of resistance when infected with a virus.

The common wheat of the Hi-Line variety was transformed with a construction for silencing the replicase gene (Nib) of wheat streak mosaic virus (WSMV). Six independent transgenic plant lines were analyzed for resistance to mechanical inoculation of WSMV in the T3 and T4 generations. Four lines demonstrated a different degree of resistance to WSMV, ranging from milder symptoms, significantly delayed symptoms, or no symptoms. Two lines showed higher resistance with very weak viral symptoms after inoculation. In 18 of 25 plants (72%) of line No. 4.4 and in 9 of 28 plants (32%) of line No. 7.21, no virus or any disease symptoms were detected during the growing season (Sivamani et al., 2000). The RNAi construction was developed to target the nuclear inclusion protein (Nia) gene of WSMV (Fahim et al., 2010). Ten of sixteen T1 lines demonstrated a complete resistance to WSMV, which was classified as immunity. In the transgenic plants, the accumulation of viral RNA was decreased by more than 105 times as compared with sensitive control groups. These Australian researchers further developed an artificial miRNA strategy against wheat stripe mosaic virus and transformed wheat with a construct containing five artificial miRNA precursors targeting conserved domains of WSMV (Fahim et al., 2012). These amiRNAs replaced the native miRNA in each of the five arms of the polycistronic miR395, producing the precursor amiRNA FanGuard (FGmiR395). All 5 amiRNAs were expressed in transgenic plants, whose resistance to virus damage was evaluated in two generations. Three types of responses were observed in T1 plants: complete immunity; gradual decrease in resistance; initially susceptible with subsequent recovery of the plant. Analysis in the T2 generation confirmed the inheritance of immunity – stable resistant lines did not develop any symptoms of the virus according to the ELISA test. This study demonstrates the utility of a polycistronic amiRNA strategy in wheat against WSMV.

Chinese researchers (Liu et al., 2021) created several transgenic lines of wheat using 4 artificial miRNA expression vectors that carried viral siRNAs (vsiRNAs) from RNA1 of wheat yellow mosaic virus (WYMV). Laboratory and field tests showed that two transgenic lines expressing amiRNA1 were highly resistant to WYMV infection. Further analyses

showed that vsiRNA1 can modulate the expression of the wheat thioredoxin-like gene TaAAED1, which encodes a negative regulator of reactive oxygen species (ROS) production in chloroplasts. In addition, the expression of amiRNA1 in transgenic wheat gave it resistance to a wide range of diseases: Chinese wheat mosaic virus, barley stripe mosaic virus, and yellow stripe rust of wheat, indicating that amiRNA1 participates in the immunity of this crop through ROS signaling.

Resistance to fungi

Fungal pathogens cause significant wheat crop losses worldwide and RNAi technology is widely used to produce plants resistant to them (Qi et al., 2019). RNAi-based HIGS provides a novel, innovative approach to combating fungal plant diseases since it downregulates the expression of key pathogen genes that are required for the development of the disease in the host (Bilir et al., 2022). In addition, HIGS can be used to confer resistance to multiple crop diseases since it is possible to design the constructions containing multiple ("stacked") RNAi transgenes directed against different pathogens (Nowara et al., 2010). Another advantage consists in the fact that the transgenes can be designed as "racespecific" or broad-spectrum transgenes based on the degree of sequence conservation within the target region. Machado et al. (2018) highlighted recent advances in RNAi-based methods to control fungal diseases of cereal plants and emphasized that, despite some disadvantages, HIGS became a promising new approach to control these diseases.

Wheat scab (also called fusarium wilt) is a devastating disease that is mainly caused by *Fusarium graminearum*. RNAi of the chitin synthase (Chs3b) gene, which controls the chitin biosynthesis of *Fusarium graminearum*, was used as a method of increasing resistance to fusarium head blight and the development of the fungus on wheat seedlings (Cheng et al., 2015). It was detected that three hairpin RNAi constructions corresponding to different Chs3b regions repress this gene in fungi colonizing wheat seedlings and spikelets. Coexpression of these constructions in two independent transgenic lines of elite wheat varieties provided high levels of stable resistance both to fusarium head blight and seedlings during T3–T5 generations. In the review work (Sang & Kim, 2020) there are at least 11 variants of effective plant protection technologies using RNAi, in particular wheat, from *Fusarium graminearum* with suppression of CYP 51 and Chs3b gene expression.

Deoxynivalenol (DON) is the most common *Fusarium* mycotoxin found in cereal grains and is also a critical virulence factor for the *F. graminearum* infection. Chinese researchers (Wang et al., 2020) applied HIGS to produce transgenic wheat plants resistant to both *F. graminearum* and DON contamination by simultaneous silencing of three *F. graminearum* genes. As target genes for HIGS, the SGE1 (which encodes a critical regulator controlling DON biosynthesis), STE12 (which encodes a key transcription factor for the formation of the penetration structure), and PP1 (which encodes an essential phosphatase) genes were selected; based on them, a chimeric RNAi hairpin construction was designed, which could simultaneously silence three target genes. Four of 16 obtained transformants demonstrated slower growth rate (PP1 silencing), reduced DON production (SGE1 silencing), and a reduction of infection structures on the wheat lemma (STE12 silencing).

Chinese researchers (Jiao & Peng, 2018) found that wheat miRNA (miR1023) can inhibit *F. graminearum* invasion by targeting and silencing the gene FGSG_03101, which encodes the alpha/beta hydrolase of the fungus. When studying the molecular genetic mechanisms of increasing the resistance of wheat plants to *F. graminearum*, using natural multicomponent biostimulants, it was shown by the dot-blot hybridization method that biostimulants cause a significant increase in the production of protective si/miRNA in plant cells, and the mechanism of epigenetic inheritance of the resistance of treated plants was confirmed to this micromycete.

Stable transgenic wheat plants carrying the RNAi hairpin construction to the beta-1,3-glucan synthase gene Gls1 of *Fusarium culmorum* or a triple combination of Gls1 with two other target genes (Fgl1-secreted lipase, ChsV-chitin synthase V, MAP kinase Fmk1) also demonstrated increased resistance to fusarium head blight when inoculating leaves and ears (Chen et al., 2016).

Brown leaf rust of wheat caused by the fungus *Puccinia triticina* (Pt) is one of the most serious threats to sustainable wheat production in the world. Panwar et al. (2013b) developed a transient transformation system for the induction of HIGS against this pathogen. After Pt infection, the leaves of wheat plants transiently expressing hairpin RNA-generating constructions targeting the Pt pathogenicity genes (mitogenactivated protein kinase 1 (PtMAPK1), cyclophilin (PtCYC1) or calcineurin b (PtCNB)) demonstrated a 51–68% reduction in the symptoms of the disease and a decrease in fungal biomass by 59–69% 10 days after infection. The wheat plants also demonstrated a decrease in the symptoms after superinfection with stem rust (*Puccinia graminis*) or yellow stripe rust (*Puccinia striiformis*). In subsequent studies by this group, stable expression of RNAi hairpin constructions with a homology to the sequence of MAP kinase (PtMAPK1) or the gene encoding cyclophilin (PtCYC1) in susceptible wheat plants led to the efficient silencing of the corresponding genes in the interacting fungus, which determined a stability of the transgenic plants of T2 generation (Panwar et al., 2018). Indian scientists (Dutta et al., 2017) described a novel locus in wheat that shows differential expression during leaf rust infection and produces four trans-acting small interfering RNAs (tasiRNAs) that target α-gliadin, a leucine-rich repeat, transmembrane protein, glutathione-S-transferase and fatty acid desaturase.

Qi et al. (2018) used barley stripe mosaic virus (BSMV)-mediated HIGS to silence the protein kinase A (PsCPK1) gene of the *Puccinia striiformis* f. sp. *tritici* (Pst). They demonstrated that the PsCPK1 is an important pathogenicity factor for Pst and its knockdown led to a reduced virulence of the fungus. Two transgenic lines expressing the RNAi construction demonstrated high levels of stable resistance to Pst in the T3 and T4 generations. This group of researchers subsequently identified a glycine–serine-rich effector gene (PstGSRE1), which is induced during early infection. A transgenic expression of the PstGSRE1-RNAi constructions in wheat significantly decreased the virulence of Pst. It was demonstrated that PstGSRE1 acts on the transcription factor TaLOL2, a positive regulator of wheat immunity (Qi et al., 2019a). The transgenic wheat lines expressing dsRNA targeting the transcripts of the FUZ7 Pst gene, which is an important pathogenicity factor regulating infection and development of Pst, were created. A PsFUZ7-RNAi construction (stably expressed in two independent transgenic wheat lines) confers a strong resistance to Pst (Zhu et al., 2017).

To study the function of the wheat CSN5 gene (constitutive photomorphogenesis 9 (COP9) signalosome) in response to Pst infection, ten T1 generation transgenic lines silencing TaCSN5 were obtained using RNAi technology (Bai et al., 2021). The TaCSN5-RNAi lines demonstrated an increased resistance to Pst. In addition, the biomass of fungi was reduced by 8–20%. Stable transgenic plants of the T4 generation with TaCSN5 silencing demonstrated a broad-spectrum resistance to several races of the fungus.

To understand the mechanism of miRNA-regulated cellular functions during stripe rust infection of wheat, scientists (Gupta et al., 2012) investigated eight different miRNAs, namely miR159, miR164, miR167, miR171, miR444, miR408, miR1129 and miR1138, which are involved in three different independent cellular defense responses to infection and reported that 5 of them, miR167, miR171, miR444, miR1129, and miR1138, play key roles in wheat rust resistance. A target gene of wheat miR408, designated TaCLP1, was identified by degradomic sequencing (Feng et al., 2013). Accumulation of TaCLP1 and tae-miR408 transcripts showed contrasting divergent expression patterns in wheat response to *Puccinia striiformis* f. sp. *tritici*. Silencing of individual cDNA clones in Pst-infected wheat revealed that TaCLP1 positively regulates stripe rust resistance. Also, this group of researchers (Feng et al., 2014) isolated the full-length cDNA of the wheat miR164 target gene, which is a novel NAC transcription factor in the NAM subfamily, and designated it as TaNAC21/22. Accumulation of TaNAC21/22 and miR164 transcripts showed contrasting divergent expression patterns in wheat response to stripe rust infection. Silencing this gene revealed that TaNAC21/22 negatively regulates stripe rust resistance. Subsequently, these researchers (Feng et al., 2015) conducted an analysis of miRNAs that are synthesized in wheat when infected with *P. striiformis*, and identified other target genes, in particular those encoding the biosynthesis of a protein with a RabGAP/TBC domain, the "zinc fingers' and cysteine-rich receptor-like

protein kinase-41 and may play a significant role in the interaction of wheat resistance genes with avirulence genes in Pst.

Wang et al. (2017) found that microRNA-like RNA (milRNA) synthesized by the fungus *P. striiformis* was able to suppress the defense reactions of wheat plants. This RNA participates in "intergeneric" RNA interference, influencing the expression of the PR2 (β-1,3-glucanase) gene. Suppression of the synthesis of the milRNA precursor caused an increase in the resistance of wheat to the virulent strain of *P. striiformis*, and a decrease in its expression increased the sensitivity of plants to the avirulent strain of the pathogen.

The obvious effect of HIGS is shown in wheat infected with the fungus *Blumeria graminis* f. sp. *tritici* (Nowara et al., 2010). Transgenic plants expressing dsRNA to silence 1,3-b-glucanosyltransferase genes (GTF1 and GTF2) showed reduced symptoms of powdery mildew or haustoria formation/development and, therefore, greater resistance to the biotrophic pathogen. The possibility of inducing broad-spectrum resistance against this pathogen by RNAi of the barley Mlo ortholog in wheat using virus-induced gene silencing (VIGS) was explored. A clear correlation between resistance and the accumulation of Mlo-specific siRNAs was revealed, which raised the possibility of obtaining resistance to powdery mildew in wheat by RNAi (Riechen, 2007; Varallyay et al., 2012). Recently developed gene editing technology is used to achieve similar effects. For example, wheat plants highly resistant to powdery mildew infection were created with the simultaneous knockout of three TaMlo TALEN (effector nuclease-like transcription activator) homeologs. Transgenic wheat plants carrying mutations in the TaMLO-A1 allele were also obtained using CRISPR-Cas9 technology (Wang et al., 2014).

Stable transgenic lines of RNAi wheat were created for a simultaneous suppression of three genes of *B. graminis* f. sp. *tritici*, including SvrPm3a1/f1 (a virulence factor involved in the suppression of the powdery mildew resistance gene Pm3), Bgt_Bcg-6, and Bgt_Bcg-7. It was demonstrated that all target effectors are inhibited by HIGS, which leads to a reduced fungal virulence on mature wheat plants (Schaefer et al., 2020). Using Solexa high-throughput sequencing, 24 wheat miRNAs susceptible to powdery mildew were identified (Xin et al., 2010). Some of the conserved miRNAs showed differential expression in response to fungal infection: the expression of miR156, miR159, miR164, miR171 and miR396 decreased, while the expression of miR393, miR444 and miR827 increased.

A study by Yang et al. (2013) used the VIGS approach to establish the function of the TaCRK1 gene, which encodes a cysteine-rich receptor-like protein kinase, in the defense response of wheat to *Rhizoctonia cerealis* infection. The expression level of TaCRK1 was reduced in resistant plants infected with the fungus.

Resistance to insects

RNAi was also used to control the pest insects that cause significant crop loss (Zhang et al., 2017; Yu et al., 2022). Some insects (particularly, Coleoptera (beetles)) were demonstrated to be highly sensitive to dsRNA (Baum & Roberts, 2014), so that only small amounts of ingested dsRNA can induce RNAi, knocking down both transcripts and important target genes of insect lethality. A special aspect of RNAi is that, in these highly sensitive insects, dsRNA is not only able to enter the intestinal cells but also spread to other tissues for inducing the systemic RNAi (Joga et al., 2016).

RNAi technology was applied to control Sitobion avenae by Xu et al. (2014), who used the carboxylesterase (CbE E4) gene of the pest as a target gene. The transgenic wheat lines expressing dsRNA CbE E4 of *S. avenae* were obtained and fed to aphid larvae. The expression of the CbE E4 gene in insects was reduced by 30–60%, and the number of aphids grown on the transgenic plants was lower than that on nontransgenic plants. In subsequent studies by these authors (Xu et al., 2017), lmf2-like fragment of the grain aphid lipase maturation factor gene was cloned and used to transform wheat. The expression of the lmf2-like gene was significantly reduced by 27.6% on the fifth day and by 57.6% on the tenth day after feeding the transgenic plants. The total number of aphids produced on modified plants was less than that produced on the control plants, and the difference became significant after two weeks. The results of studies by Zhao et al. (2018) indicate the fact that plant-mediated RNAi of the grain aphid chitin synthase 1 (CHS1) gene also confers insect resistance in common wheat. After feeding with transgenic T3 lines, the level of expression of CHS1 in the grain aphids decreased by 45–50%, and the number of aphids significantly decreased in the transgenic lines T4 and T5 in field conditions. The transgenic wheat plants expressing a 198-bp dsRNA fragment complementary to the grain aphid zinc finger protein (SaZFP) gene can efficiently increase its mortality and decrease daily fertility (Sun et al., 2019).

Silencing of key genes, including the ecdysone receptor (EcR) and the ultraspiracule protein (USP) in *Sitobion avenae*, reduced its survival and fecundity, providing a sustainable and transgenerational approach to enhance resistance in wheat (Yan et al., 2016). Oral administration of dsRNA SaEcR and dsSaUSP significantly reduced aphid survival through suppression of these two genes. The silencing effect was persistent and transmitted across generations, as evidenced by reduced survival and fecundity of both survivors and their offspring, even after crossing over to aphid-susceptible wheat plants. In addition, several potential RNAi targets have been validated by feeding or injecting the grain aphid with genes encoding catalase, acetylcholinesterase1, cytochrome *c* oxidase subunit precursor VIIc, zinc finger protein, secreted salivary peptide DSR32, salivary protein DSR33, serine protease 1 DSR48 and olfactory coreceptor (Yu et al., 2016).

Resistance to nematodes

One of the most promising approaches to the biocontrol of parasitic nematodes in cereal crops is RNAi (Lilley et al., 2007). A strategy is used in which nematodes, feeding on plants, consume dsRNA, which, entering their intestines, triggers the process of RNAi against their own genes, thus reducing fertility and causing parasite mortality. This can be achieved by developing transgenic plants capable of producing the required dsRNA that targets various nematode host genes as well as parasitization or effector genes (Dutta et al., 2015).

The wheat plants were transformed with seven target genes of the cereal cyst nematode *Heterodera avenae* for the analysis of HIGS. A transgenic expression of galectin, cathepsin L, vap1, serpin, flp12, RanBPM, and chitinase genes led to a decrease in *H. avenae* reproduction by 33–72% in the T1 generation. A similar level of resistance observed in T2 plants indicates a persistent effect of HIGS in subsequent generations. It is interesting that cysts isolated from RNAi plants were smaller in size with a semitransparent cuticle as compared with normal-sized, dark brown control cysts, suggesting a delay in the development of *H. avenae* due to HIGS (Dutta et al., 2020). The scientists also used RNAi to silence four *Heterodera avenae* genes, namely, nuclear hormone receptor, a protein binding the fruitin, intron-binding protein, and epsin (Gantasala et al., 2015). They reported that silencing these genes led to a decrease in the number of females and eggs 71%, 26%, and 60% due to silencing of the epsin, intron-binding protein, and pericarp-binding protein genes, respectively (Gantasala et al., 2015). A transgenic wheat line containing the HIGS construction for silencing the annexin gene of *Heterodera avenae* demonstrated a decrease in the nematode survival in plants (Chen et al., 2015). VIGS targeting of the Ha18764 effector of *H. avenae* protein family genes significantly attenuated the parasitism and reproductive status of this parasite in wheat (Yang et al., 2019).

RNAi suppression of pat-10, which controls troponin, and unc-87, which regulates calponin, of *Pratilenchus thornei*, which infects wheat roots, reduces its reproduction by 77–81% over a 5-week period (Tan et al., 2013). RNAi of the heat shock protein 90 (HSP90), isocitrate lyase (ICL) and cathepsin L cysteine proteinase (Mi-cpl-1) genes of the nematode *Meloidogyne incognita* significantly reduced their numbers or caused an increase in the male population, indicating unfavorable conditions for their reproduction in wheat (Lilley et al., 2007).

In addition, RNAi in wheat can be stimulated by multicomponent biostimulants, including Avercom, Avercom nova-2, Fitovit, and Violar, derived from metabolites of various soil streptomycetes, which enhance siRNA and miRNA regulation in wheat plants. These small RNAs are complementary to mRNAs of cereal cyst nematodes and thus inhibit their reproduction, ensuring plant resistance (Blyuss et al., 2019).

Resistance to abiotic stresses

Climate changes increase the regularity and severity of the effects of various abiotic stresses, which is an important reason for the significant reduction in wheat yields. Endogenous miRNAs and siRNAs are actively involved in abiotic stress responses, including osmoprotective function, abscisic acid response, antioxidant and auxin signaling by down-regulating target genes involved in abiotic stress response (Bharathi et al., 2023). miRNAs are critical factors that respond to stress and help the plant to withstand adverse environmental conditions such as drought, salinity, temperature extremes, metal toxicity, etc. Well-known miRNA target modules such as miR156-SPL, miR159-MYB, miR160-ARF, miR164-NAC (NAM, ATAF, CUC), miR167-ARF, miR169-NF-Y, miR319-TCP, miR394-LCR, miR396- GRF, and miR398-CSD play an important regulatory role in various stress environments to mitigate harmful effects (Bhogireddy et al., 2021).

Differential expression of miRNAs under different abiotic stresses was shown in wheat (Gupta et al., 2014). The expression profile of conserved miRNAs, namely miR159, miR164, miR168, miR172, miR393, miR397, miR529, and miR1029, in adaptation to osmotic, salt and cold stresses was investigated in C-306 wheat genotype. The expression of miR168 and miR397 was found to be down-regulated and miR172 was up-regulated under all stress conditions. However, miR164 and miR1029 were activated under cold and osmotic stress in contrast to salt stress, whereas miR529 only responds to cold and is not altered by osmotic and salt stress. miR393 showed up-regulation under osmotic and salt stress and down-regulation under cold stress (Gupta et al., 2014).

Drought

The main abiotic stress factor limiting the growth and yield of wheat is drought. All metabolic and physiological changes associated with drought are based on the regulation of gene expression at the level of transcription or translation (Ferdous et al., 2015). Hence, RNAi-based regulation plays an important role in drought response.

RNAi of the proline dehydrogenase (ProDH) gene, connected with proline catabolism, led to an increase in the level of its accumulation (2.6–4.1 times) in transgenic T1–T3 plants of winter and spring wheat under both optimal and stressful conditions and an increase their tolerance to soil drought (Dubrovna et al., 2020, 2022). The wheat NRX1 gene, which controls nucleoreduxin, has been shown to positively regulate its resistance to drought (Zhang et al., 2021), as transgenic lines silencing NRX1 created by RNAi technology had significantly reduced resistance to water deficit. Survival, chlorophyll, proline, soluble sugar, and antioxidant enzyme activity of RNAi lines were lower than wild-type plants. Transgenic wheat RNAi lines with reduced expression of the WZY2 dehydrin gene were also studied (Yu et al., 2019). Transgenic wheat showed lower relative water content, oxidation-related enzyme activity, and higher malondialdehyde content than non-transformed plants under osmotic stress. These results demonstrated the key function of the WZY2 gene in the response of plants to osmotic stress.

Among miRNAs identified in wheat, relatively few respond to drought (Ferdous et al., 2015). Targets of miRNAs involved in drought response are genes whose products are involved in various cellular processes associated with water deficit, including auxin and abscisic acid signaling pathways, cell growth, photosynthesis, and respiration (Budak et al., 2015b). In response to stress, the expression of various miRNAs either increases or decreases. The same miRNAs can be both expressed and repressed in the same plant under drought conditions. In response to drought stress, tissue type is a determinant of miRNA expression pattern. The roots and leaves of plants are most susceptible to drought, as they directly contribute to the maintenance of high water potential and regulate osmotic pressure. Different tissue types can exhibit tissue-specific variations in miRNA expression. In addition, the same miRNA families can show different expression patterns in different tissues (Alptekin et al., 2017). For example, miR159 was shown to be up-regulated in wheat leaves, whereas it was down-regulated in roots (Gupta et al., 2014; Ma et al., 2015). Akdogan et al. (2016) compared drought-sensitive miRNAs in the root and leaf of the common wheat variety Sivas 111/33 using miRNA microarray screening. The analysis showed that 285 miRNAs (207 up-regulated and 78 down-regulated) and 244 miRNAs (115 up-regulated and 129 down-regulated) were differentially expressed in leaf and root tissues, respectively. Among the differentially expressed miRNAs, 23 were active only in leaves, and 26 miRNAs were only expressed in plant roots.

To understand the complex regulatory mechanisms governing wheat drought resistance, microRNA analysis of the highly drought-tolerant variety XF 20 was performed (Hua et al., 2019). Sequencing results confirmed the expression of 199 previously known miRNAs, and 32 new miRNAs were also identified. Analysis of target genes under drought conditions showed that differentially expressed miRNAs reduce antioxidant inhibition by increasing expression of antioxidant genes, while opposite processes occur for signal transduction genes. Novel miR08 and miR15 were down-regulated in wheat cultivar XF 20 in response to drought stress. Their targets may be mRNA genes for disease resistance proteins such as PRM1 and RPP13. Notably, miR15 also targets the mRNA of the gene encoding sucrose synthase. In wheat, sucrose synthase gene expression was recently shown to be significantly higher in drought-tolerant cultivars than in drought-sensitive cultivars (Nemati et al., 2018). Therefore, sucrose level is critical for wheat response to drought, and these results indicate that the response mechanism may involve the regulation of sucrose synthase through miR15. This miRNA also targets a phytochrome and a protein that regulates flowering time (PFT1, also called Mediator 25), which is involved in the drought response (Kazan, 2017). The target gene of miR2111c was found to be 6-phosphogluconate dehydrogenase (6-PGDH), one of the key enzymes of the oxidative pentose phosphate pathway. miR5054, whose expression was also down-regulated in response to drought, targets mRNA encoding epoxide hydrolase (EG), which is involved in lipid metabolism (Hua et al., 2019).

In a study by Ma et al. (2015), 14 conserved miRNAs were identified with down-regulation and 6 with up-regulation in two wheat genotypes under the effects of dehydration. It was established that the target gene of miR156 with up-regulation is the mRNA of the transcription factor SBP (squamosa promoter-binding-like protein), which is important for the growth and development of leaves. The up-regulated target of miR444c1 is the MIKC-type MADS-box transcription factor gene, which is involved in the regulation of plant development and stress response. miR398, whose target gene is superoxide dismutase, was found to be overexpressed in the drought-sensitive variety after dehydration. The expression of wheat miR628 was reduced only in the drought-sensitive variety, and its target gene is alpha/beta fold hydrolase (AFH), which is involved in the degradation of cell damage products. miR160a, miR164b, miR166h, miR169d, and miR444d.3 were down-regulated in the drought-tolerant variety but were up-regulated in the drought-sensitive variety. The miR160a target gene is the ARF gene family, which are key factors in auxin regulation (Guilfoyle & Hagen, 2007). The target of miR164b is the NAC family of transcription factors, which have functions associated with various abiotic stresses (Nakashima et al., 2012). The target of miR166h is the HD-ZIP4 gene of the HD-ZIP class III transcription factor. miR169d was repressed in the drought-tolerant cultivar after dehydration and targeted the CCAAT-box transcription factor, which is one of the most abundant elements in eukaryotic promoters. miR444d.3 was found to be down-regulated in the drought-tolerant variety, and its target is the initiation factor 3 (IF3) gene, which plays a central role in eukaryotic polypeptide chain elongation and may play a significant role in wheat drought resistance. The authors found that 46 conserved miRNAs and 321 novel miRNAs were differentially expressed in two wheat genotypes under dehydration stress conditions. Interestingly, 13 miRNAs showed opposite expression patterns in the two wheat genotypes - they were reduced in drought-resistant varieties, but increased in drought-sensitive varieties.

Indian scientists (Pandey et al., 2014) applied a combinatorial approach of high-throughput sequencing followed by computational miRNA prediction and identified 47 known, 49 novel, and 1030 potential novel miRNAs in wheat. They found that the expression level of miR156, miR160, miR166a, miR396d, miR1135, miR5139, tae_10, tae_15 and tae_44 doubled under water deficit. This analysis revealed that at least three wheat SPL genes, homologous to rice SPL 2, 11, and 16, are potential targets of miR156.

Salinization

Salinity is one of the major abiotic stresses that limit the growth and productivity of wheat plants (Zeeshan et al., 2021). Plants have developed various mechanisms to cope with salt stress, one of which is RNAi, which can affect gene regulation under salinity conditions (Islam et al., 2022). Most often, the formation of an adaptive response to salinity and drought is carried out by the same molecular mechanism or with the participation of the same transcription factors (Budak et al., 2015a).

Many miRNA families have shown induced expression in wheat in response to salt stress (Gupta et al., 2014; Wang et al., 2014; Islam et al., 2022). The expression of miR156, miR186, and miR393 is usually up-regulated during salt stress (Abdellatef et al., 2021). In wheat, miR171, which targets the MYB (myeloblastoma-like DNA binding domains) family of transcription factors, was found to be activated by salinity (Wang et al., 2014). Since both drought and salinity affect the osmotic balance of plant cells, miR171 may play a role in the regulation of osmotic balance under such stress conditions. Gupta et al. (2014) first reported that miR855 is activated in wheat during salinity. Wheat miR169 is down-regulated during salt stress by increasing the expression of the transcription factor NF-YA (nuclear factor Y subunit A) (Zhao et al., 2009).

Wheat miR408 is critical for plant adaptation to salt stress (Bai et al., 2018). It targets six genes that encode proteins related to biochemical metabolism, microtubule organization, and signal transduction. Transgenic lines overexpressing miR408 showed increased osmolyte content under salt stress conditions relative to wild-type plants, indicating that miR408-mediated salinity tolerance is associated with ABA signaling pathways.

It was shown that the expression of wheat miRNA depends on the dose of the stress factor (Pandey et al., 2014). Under salinity, a significant decrease (>3-fold change) in expression was observed for tae_15, tae_19 and tae_45. Interestingly, miR164 levels, which remained unchanged in response to 150 mM NaCl, showed a more than fourfold decrease with 250 mM NaCl. Under similar conditions, a contrasting response of miR5139 was observed, the expression level of which increased 1.8-fold at 150 mM NaCl and decreased 1.45-fold at a higher concentration (250 mM NaCl). miRNAs (tae_6, tae_15, tae_19, tae_27, and tae_45) showed decreased expression, with tae_45 showing the maximum (more than twofold) decrease, and tae_10 and tae_22 expression levels significantly increased in response to 150 mM salt stress. The expression of tae_7 and tae_44 (which remained unchanged at 150 mM NaCl) decreased at 250 mM NaCl.

To investigate root miRNA profiles of two wheat varieties Bezosta (sensitive) and Seri-82 (tolerant) under salinity, miRNA microarray analysis was used (Eren et al., 2015). A total of 44 differentially regulated miRNAs were identified, and 16 novel salinity-responsive miRNAs were identified for the first time in wheat. The expression of 3 miRNAs (hvu-miR5049a, ppt-miR1074 and osa-miR444b.2) was increased more than 260-fold in Bezosta variety under salt stress. Analysis of target genes revealed that miRNAs responsive to salt stress mainly regulate transcription factors such as bHLH135-like, AP2/ERBP, MADS-box and transporters.

Zeeshan et al. (2021) performed a genome-wide study using Illumina high-throughput sequencing and comprehensive in silico analysis to gain insight into the underlying mechanisms by which miRNAs confer salinity tolerance in the roots of two contrasting wheat cultivars, namely Suntop (salt resistant) and Sunmate (salt sensitive). A total of 191 miRNAs were identified in both cultivars, consisting of 110 known miRNAs and 81 novel miRNAs; 181 miRNAs were common to the two cultivars. The known miRNAs belonged to 35 families, which consisted of 23 conserved and 12 unique families. Salinity induced the induction of 43 and 75 miRNAs in Suntop and Sunmate cultivars, respectively. Among them, 14 known and 29 novel miRNAs were expressed in Suntop and 37 known and 38 novel miRNAs in Sunmate. In addition, seven miRNAs, including tae-miR156, tae-miR160, tae-miR171a-b, tae-miR319, tae-miR159a-b, tae-miR9657, and miR59, whose target genes are SPL, SCL6, PCF5, R2R3, and MYB CBL-CIPK, respectively, contributed to increasing the salinity resistance of the Suntop variety.

It was found that under the influence of salinity, the tolerant variety Suntop decreased the expression of miR156, which negatively regulated the target gene SPL3. miR160, which targets auxin-responsive factor 8-like protein (ARF8), was down-regulated in Suntop and up-regulated in Sunmate, which is associated with the down-regulation of ARF8. miR171a and miR171b, which target the transcription factor SCL6, were repressed in Suntop but activated in Sunmate. This study shows that miR319 target gene – PCF5 can enhance salinity tolerance in Suntop. Overexpression of miR319 in transgenic lines upregulates potassium transporter genes, increasing K^+ concentration TaHKT and TaAKT gene expression. The regulation of miR159a-b and miR9657 was suppressed in Suntop, thereby increasing the expression of their target genes, which encode MYB-related transcription factors. This suggests that MYB-related transcription factors play a role in salt stress tolerance. Expression of a novel miR59 targeting two genes, TraesCS6B01G465600.1 and Traes CS6B01G465600.2, encoding CBL-interacting serine/threonine protein kinase 7, was unchanged in Suntop but increased in Sunmate. The TaCB L3–TaCIPK29 complex regulates the antioxidant system, and transporter genes protect wheat from salt stress (Deng et al., 2013). Therefore, miR59 and its potential targets (CBL-interacting serine/threonine protein kinase 7) also play a positive role in Suntop salt tolerance.

Temperature stress

The growth of plants and their productivity is affected by an important ecological parameter – the temperature. Temperature stress can be conditionally divided into hot and cold. miRNAs are directly involved in adaptation to heat and cold stress, acting as post-transcriptional regulators of gene expression (Alptekin et al., 2017; Zuo et al., 2021; Sun et al., 2022). Temperature stress-related miRNAs are believed to be involved in the regulation of general stress-responsive genes, such as miR398, which silences the superoxide dismutase genes CSD1, CSD2, and the copper chaperone CSD, and is also involved in reducing the accumulation of reactive oxygen species (ROS) (Song et al., 2019). Several miRNAs responsive to heat and cold stress have been identified and experimentally characterized under different stress conditions in different wheat tissues (Tang et al., 2012; Wang et al., 2012; Gupta et al., 2014). Altered expression of miR159, miR164, miR167, miR172, miR319 and miR398 was found in response to both heat and cold stress (Gupta et al., 2014; Wang et al., 2014). Interestingly, several miRNAs showed opposite expression patterns under heat and cold. For example, miR164, which targets heat shock protein 17 (HSP17), is activated during cold stress but down-regulated in response to heat stress in wheat (Gupta et al., 2014; Kumar et al., 2015). Another wheat miRNA, miR319, which targets the transcription factor MYB, also shows activated expression under cold stress but is down-regulated under heat stress. The suppressed expression of miR160 and miR164 likely induces the expression of heat shock proteins and maintains viability at high temperatures. Conversely, increased expression of the same miRNAs during cold stress indicates a change in the regulatory role of heat shock proteins under the influence of cold. Several miRNAs detected during both heat and cold exposure in different tissues show similar expression patterns. For example, miR167, which targets auxin response factor (ARF), and miR169, which targets nuclear transcription factor Y (NF-Y), are up-regulated in wheat under both heat and cold stress (Gupta et al., 2014; Kumar et al., 2015).

Many miRNAs are involved in heat stress response in wheat plants, including miR156, miR159, miR172, miR396 (Wang et al., 2012; Gupta et al., 2014). Indian researchers described a tissue-specific expression pattern of many miRNAs under heat stress in wheat, where miR3466, miR5652, and miR5064 showed differential expression in root, stem, and leaf tissues (Kumar et al., 2015). The activity of miR5652 was found to be highly cultivar-dependent, as its expression had significant differences between heat-sensitive and tolerant wheat cultivars. Wheat miRNAs showed differential expression in response to heat stress (Xin et al., 2010). Among 32 miRNA families, nine conserved miRNAs were sensitive to heat. miR172 was significantly down-regulated, and miR156, miR159, miR160, miR166, miR168, miR169, miR393, and miR827 were activated under heat stress. Indian scientists (Goswami et al., 2014) identified several heat-responsive miRNAs in wheat using the Illumina Hiseq 2000 method. Validation of the identified miRNAs in endosperm tissues of heat-tolerant (HD2985) and heat-sensitive (NIAW) wheat varieties using real-time PCR revealed activation 4 miRNAs (miR156, miR167,

miR395b and miR398) and down-regulation of 6 miRNAs (miR159a, miR159b, miR160, miR171a, miR319 and miR1117) in response to heat stress. Analysis of the identified miRNAs showed that their target genes are HSF3, HSFA4a, HSP17, HSP70 and superoxide dismutase (CSD). The expression of the identified target genes under heat stress (42 °C, 2 hours) increased by 2.34 and 1.33 times (HSF3); 2.45 and 1.44 times (HSFA4a); 3.9 and 1.9 times (HSP17); 5.6 and 2.4 times (HSP70); 1.9 and 1.2 times (CSD); 2.7 and 1.6 times (catalase) in wheat varieties HD2985 and NIAW compared to the control. Later, Kumar et al. (2015) found 53 and 46 mature miRNAs in control and heat-stressed (42 ºC, 2 h) HD2985 wheat samples, among which 37 new miRNAs were identified. Six novel miRNAs were confirmed to be heat sensitive.

In another study (Ravichandran et al., 2019), wheat miRNAs regulated by heat stress were identified and their target genes related to thermotolerance were confirmed. Leaf tissues collected from control and heat-stressed Chinese Spring variety wheat plants were analyzed through 1 and 4 days after the stress period. They identified 202 mature miRNAs, of which 36 were differentially changed over time in response to heat stress. PARE sequencing confirmed the targets of miR156, miR159, miR166 and miR393 families, miR398 as a squamosa-like promoter binding protein (SPL), MYB transcription factor, leucine zipper homeobox protein, and superoxide dismutase-responsive protein, respectively. Monocot-specific miR528 was significantly activated after heat stress to regulate antioxidant activity.

Down-regulation of miR159a, whose target gene encodes the transcription factor MYB3, plays an important role in the response to cold stress in wheat (Ma et al., 2015). miR394, miR397, miR319, miR396, miR408, miR402 were found to be associated with cold stress response via CBF-dependent pathway and ROS accumulation. The miR394-LCR module is involved in plant cold stress response (Song et al., 2016). Chinese researchers (Tang et al., 2012) performed deep sequencing of small RNAs obtained from ear tissues of male-sterile wheat line TGMS under cold stress and under control conditions and identified a total of 78 unique miRNA sequences from 30 families and transactive small interfering RNA (tasiRNA), derived from two TAS3 genes. They identified six miRNAs and one tasiRNA (tasiRNA-ARF) as cold stress responsive mRNAs in tissues ear of the TGMS line. These data indicated that miR167 and tasiRNA-ARF play a significant role in the regulation of the auxin signaling pathway and possibly in cold stress responses.

Conclusion

Today, RNAi has become an attractive tool, which is used not only to decipher the function of important wheat genes, but also to obtain plants with improved and new traits by manipulating both desirable and undesirable genes. This technology is used to increase the nutritional value of wheat grain, and is also used to obtain plants with increased resistance to various abiotic (especially drought) and biotic stresses, such as attacks by pathogens and pests (viruses, bacteria, fungi, insects and nematodes). Data on the functioning of RNAi mechanisms allow the development of very effective strategies for combating a complex of harmful organisms in agrocenoses, which contributes to increasing the yield of this crop, while avoiding the use of environmentally hazardous pesticides. The possibilities provided by RNAi technology enrich the genetic pool of plants and expand the range of traits that can be transferred by introgression of stable transgenic RNAi plants into commercial crops, obtaining improved varieties with many characteristics that cannot be obtained in one variety by traditional breeding. The advent of advanced tools, such as microarrays and deep sequencing, is expanding the application of RNAi for targeted gene silencing to improve various wheat traits. This can also be achieved by combining the beneficial properties of RNAi with other advanced and innovative technologies such as gene pyramiding and gene editing. Despite some limitations, strategies for wheat improvement based on small non-coding RNAs have enormous potential for increasing its productivity, improving nutritional properties and resistance to environmental stressors.

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References

Abdellatef, E., Kamal, N. M., & Tsujimoto, H. (2021). Tuning beforehand: A foresight on RNA interference (RNAi) and in vitro-derived dsRNAs to enhance crop resilience to biotic and abiotic stresses. International Journal of Molecular Sciences, 22(14), 7687.

Akbar, S., Wei, Y., & Zhang, M.-Q. (2022). RNA interference: Promising approach to combat plant viruses. International Journal of Molecular Sciences, 23(10), 5312.

Akdogan, G., Tufekci, E. D., Uranbey, S., & Unver, T. (2016). miRNA-based drought regulation in wheat. Functional and Integrative Genomics, 16(3), 221–233.

Ali, N., Datta, N., & Datta, K. (2010). RNA interference in designing transgenic crops. GM Crops, 1(4), 207–213.

Alptekin, B., Langridge, P., & Budak, H. (2017). Abiotic stress miRNomes in the Triticeae. Functional Integrative Genomics, 17, 145–170.

Altenbach, S. B., Tanaka, C. K., & Allen, P. V. (2014a). Quantitative proteomic analysis of wheat grain proteins reveals differential effects of silencing of omega-5 gliadin genes in transgenic lines. Journal of Cereal Sciense, 59(2), 118–125.

Altenbach, S. B., Tanaka, C. K., & Seabourn, B. W. (2014b). Silencing of omega-5 gliadins in transgenic wheat eliminates a major source of environmental variability and improves dough mixing properties of flour. BMC Plant Biology, 14, 393.

Bai, Q., Wang, X., Chen, X., Shi, G., Liu, Z., Guo, C., & Xiao, K. (2018). Wheat miRNA Taemir408 acts as an essential mediator in plant tolerance to Pi deprivation and salt stress via modulating stress-associated physiological processes. Frontiers in Plant Science, 18(9), 499.

Bai, X., Huang, X., Tian, S., Peng, H., Zhan, G., Goher, F., Guo, J., Kang, Z., & Guo, J. (2021). RNAi-mediated stable silencing of $TaCSN_5$ confers broad-spectrum resistance to *Puccinia striiformis* f. sp. *tritici*. Molecular Plant Pathology, 22(4), 410–421.

Banerjee, P. (2020). Plant abiotic stress responses and microRNAs. In: Maitra, S., & Pramanick, B. (Eds.). Advanced agriculture. New Delhi Publishers, New Delhi. Pp. 109–118.

Barro, F., Iehisa, J. C., Giménez, M. J., García-Molina, M. D., Ozuna, C. V., Comino, I., Sousa, C., & Gil-Humanes, J. (2016). Targeting of prolamins by RNAi in bread wheat: Effectiveness of seven silencing-fragment combinations for obtaining lines devoid of coeliac disease epitopes from highly immunogenic gliadins. Plant Biotechnology Journal, 14(3), 986–996.

Baulcombe, D. (2019). How virus resistance provided a mechanistic foundation for RNA silencing. The Plant Cell, 31, 1395–1396.

Baum, J. A., & Roberts, J. K. (2014). Progress towards RNAi-mediated insect pest management. Advances in Insect Physiology, 47, 250–295.

Bharathi, J., Anandan, R., Benjamin, L., Muneer, S., & Prakash, M. (2023). Recent trends and advances of RNA interference (RNAi) to improve agricultural crops and enhance their resilience to biotic and abiotic stresses. Plant Physiology and Biochemistry Journal, 194, 600–618.

Bhogireddy, S., Mangrauthia, S., Kumar, R., Pandey, A., Singh, S., Jain, A., Budak, H., Varshney, R., & Kudapa, H. (2021). Regulatory non coding RNAs: A new frontier in regulation of plant biology. Functional and Integrative Genomics, 21, 313–330.

Bilir, Ö., Göl, D., Hong, Y., McDowell, J. M., & Tör, M. (2022). Small RNA-based plant protection against diseases. Frontiers in Plant Science, 13, 951097.

Blyuss, K. B., Fatehi, F., Tsygankova, V. A., Biliavska, L. O., Iutynska, G. O., Yemets, A. I., & Blume, Y. B. (2019). RNAi-based biocontrol of wheat nematodes using natural poly-component biostimulants. Frontiers in Plant Science, 10, 483.

Brosnan, C. A., & Voinnet, O. (2009). The long and the short of noncoding RNAs. Current Opinion in Cell Biology, 21(3), 416–425.

Budak, H., & Akpinar, B. A. (2015). Plant miRNAs: Biogenesis, organization and origins. Functional Integrative Genomics, 15(5), 523–531.

Budak, H., Hussain, B., Khan, Z., Ozturk, N. Z., & Ullah, N. (2015a). From genetics to functional genomics: Improvement in drought signaling and tolerance in wheat. Frontiers in Plant Science, 6, 1012.

Budak, H., Kantar, M., Bulut, R., & Akpinar, B. A. (2015b). Stress responsive miRNAs and isomiRs in cereals. Plant Science, 235, 1–13.

Canto-Pastor, A., Santos, B. A., Valli, A. A., Summers, W., Schornack, S., & Baulcombe, D. C. (2019). Enhanced resistance to bacterial and oomycete pathogens by short tandem target mimic RNAs in tomato. Proceedings of the National Academy of Sciences of the United States of America, 116(7), 2755–2760.

Chen, C. L., Liu, S. S., Liu, Q., Niu, J. H., Liu, P., Zhao, J. L., & Jian, H. (2015). An annexin-like protein from the cereal cyst nematode *Heterodera avenae* suppresses plant defense. PLoS One, 10(4), e0122256.
Chen, W., Kastner, C., Nowara, D., Oliveira-Garcia, E., Rutten, T., Zhao, Y., Deising, H., Kumlehn, J., & Schweizer, P. (2016). Host-induced silencing of *Fusarium culmorum* genes protects wheat from infection. Journal of Experimental Botany, 67(17), 4979–4991.
Cheng, W., Song, X. S., Li, H. P., Cao, L. H., Sun, K., Qiu, X. L., Xu, Y. B., Yang, P., Huang, T., Zhang, J. B., Qu, B., & Liao, Y. C. (2015). Host-induced gene silencing of an essential chitin synthase gene confers durable resistance to *Fusarium* head blight and seedling blight in wheat. Plant Biotechnology Journal, 13(9), 1335–1345.
Cruz, L. F., Rupp, J. L. S., Trick, H. N., & Fellers, J. P. (2014). Stable resistance to Wheat streak mosaic virus in wheat mediated by RNAi. In Vitro Cellular and Developmental Biology-Plant, 50(6), 665–672.
D'Ario, M., Griffiths-Jones, S., & Kim, M. (2017). Small RNAs: Big impact on plant development. Trends in Plant Science, 22(12), 1056–1068.
Dalakouras, A., Wassenegger, M., Dadami, E., Ganopoulos, I., Pappas, M., & Papadopoulou, K. (2020). Genetically modified organism-free RNA interference: Exogenous application of RNA molecules in plants. Plant Physiology, 182(1), 38–50.
Deng, P., Muhammad, S., Cao, M., & Wu, L. (2018). Biogenesis and regulatory hierarchy of phased small interfering RNAs in plants. Plant Biotechnology Journal, 16(5), 965–975.
Deng, X., Hu, W., Wei, S., Zhou, S., Zhang, F., Han, J., Chen, L., Li, Y., Feng, J., Fang, B., Luo, Q., Li, S., Liu, Y., Yang, G., & He, G. (2013). TaCIPK29, a CBL-interacting protein kinase gene from wheat, confers salt stress tolerance in transgenic tobacco. PLoS One, 8(7), e69881.
Dubrovna, O. V., Priadkina, G. O., Mykhalska, S. I., & Komisarenko, A. G. (2022). Drought-tolerance of transgenic winter wheat with partial suppression of the proline dehydrogenase gene. Regulatory Mechanism sin Biosystems, 13(4), 385–392.
Dubrovna, O. V., Stasik, O. O., Priadkina, G. O., Zborivska, O. V., & Sokolovska-Sergienko, O. G. (2020). Resistance of genetically modified wheat plants, containing a double-stranded RNA suppressor of the proline dehydrogenase gene, to soil moisture deficiency. Agricultural Science and Practice, 7(2), 24–34.
Dutta, S., Kumar, D., Jha, S., Prabhu, K. V., Kumar, M., & Mukhopadhyay, K. (2017). Identification and molecular characterization of a trans-acting small interfering RNA producing locus regulating leaf rust responsive gene expression in wheat (*Triticum aestivum* L.). Planta, 246(5), 939–957.
Dutta, T. K., Banakar, P., & Rao, U. (2015). The status of RNAi-based transgenic research in plant nematology. Frontiers in Microbiology, 5, 760.
Dutta, T. K., Papolu, P. R., Singh, D., Sreevathsa, R., & Rao, U. (2020). Expression interference of a number of *Heterodera avenae* conserved genes perturbs nematode parasitic success in *Triticum aestivum*. Plant Science, 301, e110670.
Eren, H., Pekmezci, M. Y., Okay, S., Turktas, M., Inal, B., Ilhan, E., Atak, M., Erayman, M., & Unver, T. (2015). Hexaploid wheat (*Triticum aestivum*) root miRNome analysis in response to salt stress. Annals of Applied Biology, 167(2), 208–216.
Fahim, M., Ayala-Navarrete, L., Milla, A. A., & Larkin, P. J. (2010). Hairpin RNA derived from viral NIa gene confers immunity to wheat streak mosaic virus infection in transgenic wheat plants. Plant Biotechnology Journal, 8, 821–834.
Fahim, M., Millar, A. A., Wood, C. C., & Larkin, P. J. (2012). Resistance to wheat streak mosaic virus generated by expression of an artificial polycistronic microRNA in wheat. Plant Biotechnology Journal, 10(2), 150–163.
Feng, H., Duan, X., Zhang, Q., Li, X., Wang, B., Huang, L., Wang, X., & Kang, Z. (2014). The target gene of tae-miR164, a novel NAC transcription factor from the NAM subfamily, negatively regulates resistance of wheat to stripe rust. Molecular Plant Pathology, 15(3), 284–296.
Feng, H., Wang, B., Zhang, Q., Fu, Y., Huang, L., Wang, X., & Kang, Z. (2015). Exploration of microRNAs and their targets engaging in the resistance interaction between wheat and stripe rust. Frontiers in Plant Science, 6, 469.
Feng, H., Zhang, Q., Wang, Q., Wang, X., Liu, J., & Li, M. (2013). Target of tae-miR408, a chemocyanin-like protein gene (TaCLP1), plays positive roles in wheat response to high-salinity, heavy cupric stress and stripe rust. Plant Molecular Biology, 83(4–5), 433–443.
Ferdous, J., Hussain, S. S., & Shi, B. J. (2015). Role of microRNAs in plant drought tolerance. Plant Biotechnology Journal, 13, 293–305.
Figueroa, M., Hammond-Kosack, K., & Solomon, P. (2018). A review of wheat diseases – a field perspective. Molecular Plant Pathology, 19(6), 1523–1536.
Fire, A., Xu, S., Montgomery, M. K., Kostas, S. A., Driver, S. E., & Mello, C. C. (1998). Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. Nature, 391, 806–811.
Fletcher, S. J., Reeves, P. T., Hoang, B. T., & Mitter, N. A. (2020). Perspective on RNAi-based biopesticides. Frontiers in Plant Science, 11, e00051.
Gantasala, N. P., Kumar, M., Banakar, P., Thakur, P. K., & Rao, U. (2015). Functional validation of genes in cereal cyst nematode, *Heterodera avenae*, using siRNA gene silencing. In: Dababat, A. A., Muminjanov, H., & Smiley, R. (Eds.). Nematodes of small grain cereals: Current status and research. FAO, Ankara. Pp. 353–356.
Gasparis, S., Kała, M., Przyborowski, M., Orczyk, W., & Nadolska-Orczyk, A. (2017). Artificial microRNA-based specific gene silencing of grain hardness genes in polyploid cereals appeared to be not stable over transgenic plant generations. Frontiers in Plant Science, 9(7), 02017.
Gasparis, S., Orczyk, W., Zalewski, W., & Nadolska-Orczyk, A. (2011). The RNA-mediated silencing of one of the Pin genes in allohexaploid wheat simultaneously decreases the expression of the other, and increases grain hardness. Jornal of Experimental Botany, 62(11), 4025–4036.
Ghag, S. B. (2017). Host induced gene silencing, an emerging science to engineer crop resistance against harmful plant pathogens. Physiological and Molecular Plant Pathology, 100, 242–254.
Gil-Humanes, J., Piston, F., Gimenez, M. J., Martin, A., & Barro, F. (2012). The introgression of RNAi silencing of γ-gliadins into commercial lines of bread wheat changes the mixing and technological properties of the dough. PLoS One, 7(9), e45937.
Gil-Humanes, J., Piston, F., Hernando, A., Alvarez, J. B., Shewry, P. R., & Barro, F. (2008). Silencing of γ-gliadins by RNA interference (RNAi) in bread wheat. Jornal of Cereal Science, 48, 565–568.
Gil-Humanes, J., Pistón, F., Tollefsen, S., Sollid, L. M., & Barro, F. (2010). Effective shutdown in the expression of celiac disease-related wheat gliadin T-cell epitopes by RNA interference. Proceedings of the National Academy of Sciences of the United States of America, 107(39), 17023–17028.
Godwin, I. D., Williams, S. B., Pandit, P. S., & Laidlaw, H. K. (2009). Multifunctional grains for the future: Genetic engineering for enhanced and novel cereal quality. In Vitro Cellular and Developmental Biology – Plant, 45(4), 383–399.
Goswami, S., Kumar, R. R., & Rai, R. D. (2014). Heat-responsive microRNAs regulate the transcription factors and heat shock proteins in modulating thermo stability of starch biosynthesis enzymes in wheat (*Triticum aestivum* L.) under the heat stress. Australian Journal of Crop Science, 8(5), 697–705.
Guilfoyle, T. J., & Hagen, G. (2007). Auxin response factors. Current Opinion in Plant Biology, 10(5), 453–460.
Gupta, O. P., Meena, N., Sharma, I., & Sharma, P. (2014). Differential regulation of microRNAs in response to osmotic, salt and cold stresses in wheat. Molecular Biology Reports, 7, 4623–4629.
Gupta, O. P., Permar, V., Koundal, V., Singh, U. D., & Praveen, S. (2012). MicroRNA regulated defense responses in *Triticum aestivum* L. during *Puccinia graminis* f.sp. *tritici* infection. Molecular Biology Reports, 39, 817–822.
Halder, K., Chaudhuri, A., Abdin, M. Z., & Datta, A. (2023). Tweaking the small non-coding RNAs to improve desirable traits in plant. International Journal of Molecular Sciences, 24(4), 3143.
Hamilton, A. J., & Baulcombe, D. C. (1999). A species of small antisense RNA in posttranscriptional gene silencing in plants. Science, 286(5441), 950–952.
Hernández-Soto, A., & Chacón-Cerdas, R. (2021). RNAi crop protection advances. International Journal of Molecular Sciences, 22(22), 12148.
Hua, Y., Zhang, C., Shi, W., & Chen, H. (2019). High-throughput sequencing reveals microRNAs and their targets in response to drought stress in wheat (*Triticum aestivum* L.). Biotechnology and Biotechnological Equipment, 33, 465–471.
Islam, W., Waheed, A., Naveed, H., & Zeng, F. (2022). MicroRNAs mediated plant responses to salt stress. Cells, 11(18), 2806.
Jiao, J., & Peng, D. (2018). Wheat MicroRNA1023 suppresses invasion of *Fusarium graminearum* via targeting and silencing FGSG_03101. Journal of Plant Interactions, 13, 514–521.
Joga, M. R., Zotti, M. J., Smagghe, G., & Christiaens, O. (2016). RNAi efficiency, systemic properties, and novel delivery methods for pest insect control: What we know so far. Frontiers in Physiology, 7, 553.
Kamthan, A., Chaudhuri, A., Kamthan, M., & Datta, A. (2015). Small RNAs in plants: Recent development and application for crop improvement. Frontiers in Plant Sciense, 6, 208.
Kapoor, D., Bhardwaj, S., Landi, M., Sharma, A., Ramakrishnan, M., & Sharma, A. (2020). The impact of drought in plant metabolism: How to exploit tolerance mechanisms to increase crop production. Applied Sciences, 10(16), 5692.
Kaur, R., Choudhury, A., Chauhan, S., Ghosh, A., Tiwari, R., & Rajam, M. (2021). RNA interference and crop protection against biotic stresses. Physiology and Molecular Biology of Plants, 27(10), 2357–2377.
Kazan, K. (2017). The multitalented mediator25. Frontiers in Plant Science, 8, 999.
Koch, A., & Kogel, K. H. (2014). New wind in the sails: improving the agronomic value of crop plant sthrough RNAi-mediated gene silencing. Plant Biotechnology Journal, 12(7), 821–831.
Kong, X., Yang, M., Le, B. H., He, W., & Hou, Y. (2022). The master role of SiRNAs in plant immunity. Molecular Plant Pathology, 23(10), 1565–1574.
Kumar, K., Gambhir, G., Dass, A., Tripathi, A. K., Singh, A., Jha, A. K., Yadava, P., Choudhary, M., & Rakshit, S. (2020). Genetically modified crops: current status and future prospects. Planta, 251(4), 91.
Kumar, R. R., Pathak, H., Sharma, S. K., Kala, Y. K., Nirjal, M. K., Singh, G. P., Goswami, S., & Rai, R. D. (2015). Novel and conserved heat-responsive

microRNAs in wheat (*Triticum aestivum* L.). Functional and Integrative Genomics, 15(3), 323–348.
Lacombe, S., Bangratz, M., Ta, H., Nguyen, T., Gantet, P., & Brugidou, C. (2021). Optimized RNA-silencing strategies for Rice Ragged Stunt Virus resistance in rice. Plants, 10(10), 2008.
Lee, R. C., Feinbaum, R. L., & Ambros, V. (1993). The *C. elegans* heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. Cell, 75(5), 843–854.
Li, C., & Zhang, B. (2016). MicroRNAs in control of plant development. Journal Cellula Physiology, 231(2), 303–313.
Li, Z., Liu, Y., & Berger, P. H. (2005). Transgenic silencing in wheat transformed with the WSMV-CP gene. Biotechnology, 4(1), 62–68.
Lilley, C. J., Bakhetia, M., Charlton, W. L., & Urwin, P. E. (2007). Recent progress in the development of RNA interference for plant parasitic nematodes. Molecular Plant Pathology, 8, 701–711.
Liu, P., Zhang, X., Zhang, F., Xu, M., Ye, Z., Wang, K., Liu, S., Han, X., Cheng, Y., Zhong, K., Zhang, T., Li, L., Ma, Y., Chen, M., Chen, J., & Yang, J. (2021). A virus-derived siRNA activates plant immunity by interfering with ROS scavenging. Molecular Plant, 14(7), 1088–1103.
Liu, Q., Yang, T., Yu, T., Zhang, S., Mao, X., Zhao, J., Wang, X., Dong, J., & Liu, B. (2017). Integrating small RNA sequencing with QTL mapping for identification of miRNAs and their target genes associated with heat tolerance at the flowering stage in rice. Frontiers in Plant Science, 8, 43.
Liu, S., Geng, S., Li, A., Mao, Y., & Mao, L. (2021). RNAi technology for plant protection and its application in wheat. aBIOTECH, 2, 365–374.
Lu, W., Li, J., Liu, F., Gu, J., Guo, C., Xu, L., Zhang, H., & Xiao, K. (2011). Expression pattern of wheat miRNAs under salinity stress and prediction of salt-inducible miRNAs targets. Frontiers of Agriculture in China, 5, 413–422.
Ma, X., Xin, Z., Wang, Z., Yang, Q., Guo, S., Guo, X., Cao, L., & Lin, T. (2015). Identification and comparative analysis of differentially expressed miRNAs in leaves of two wheat (*Triticum aestivum* L.) genotypes during dehydration stress. BMC Plant Biology, 15, 21.
Machado, A. K., Brown, N. A., Urban, M., Kanyuka, K., & Hammond-Kosack, K. E. (2018). RNAi as an emerging approach to control Fusarium head blight disease and mycotoxin contamination in cereals. Pest Management Science, 74(4), 790–799.
Mezzetti, B., Smagghe, G., Arpaia, S., & Christiaens, O. (2020). RNAi: What is its position in agriculture? Journal of Pest Science, 93(4), 1125–1130.
Nakashima, K., Takasaki, H., Mizoi, J., Shinozaki, K., & Shinozaki, K. Y. (2012). NAC transcription factors in plant abiotic stress responses. Biochimica et Biophysica Acta, 1819(2), 97–103.
Nemati, F., Ghanati, F., Ahmadi Gavlighi, H., & Sharifi, M. (2018). Comparison of sucrose metabolism in wheat seedlings during drought stress and subsequent recovery. Biologia Plantarum, 62, 595–599.
Nowara, D., Gay, A., Lacomme, C., Shaw, J., Ridout, C., Douchkov, D., Hensel, G., Kumlehn, J., & Schweizer, P. (2010). HIGS: Host-induced gene silencing in the obligate biotrophic fungal pathogen *Blumeria graminis*. Plant Cell, 22, 3130–3141.
Nowsherwan, I., Shabbir, G., Malik, S. I., & Ilyas, M. (2018). Effect of drought stress on different physiological traits in bread wheat. Journal of Agriculture, 16(1), 1.
Pandey, R., Joshi, G., Bhardwaj, A. R., Agarwal, M., & Katiyar-Agarwal, S. A. (2014). Comprehensive genome-wide study on tissue specific and abiotic stress-specific miRNAs in *Triticum aestivum*. PLoS One, 9(4), e95800.
Panwar, V., Jordan, M., McCallum, B., & Bakkeren, G. (2018). Host-induced silencing of essential genes in *Puccinia triticina* through transgenic expression of RNAi sequences reduces severity of leaf rust infection in wheat. Plant Biotechnology Journal, 16, 1013–1023.
Panwar, V., McCallum, B., & Bakkeren, G. (2013a). Host-induced gene silencing of wheat leaf rust fungus *Puccinia triticina* pathogenicity genes mediated by the Barley stripe mosaic virus. Plant Molecular Biology, 81, 595–608.
Panwar, V., McCallum, B., & Bakkeren, G. (2013b). Endogenous silencing of *Puccinia triticina* pathogenicity genes through in planta-expressed sequences leads to the suppression of rust diseases on wheat. Plant Journal, 73(3), 521–532.
Pistón, F., Gil-Humanes, J., Rodríguez-Quijano, M., & Barro, F. (2011). Down-regulating γ-gliadins in bread wheat leads to non-specific increases in other gluten proteins and has no major effect on dough gluten strength. PLoS One, 6(9), e24754.
Qi, T., Guo, J., Liu, P., He, F., Wan, C., Islam, M., Tyler, B. M., Kang, Z., & Guo, J. (2019a). Stripe rust effector PstGSRE1 disrupts nuclear localization of ROS-promoting transcription factor TaLOL2 to defeat ROS-induced defense in wheat. Molecular Plant, 12(12), 1624–1638.
Qi, T., Guo, J., Peng, H., Liu, P., Kang, Z., & Guo, J. (2019b). Host-induced gene silencing: A powerful strategy to control diseases of wheat and barley. International Journal of Molecular Sciences, 20(1), 206.
Qi, T., Zhu, X., Tan, C., Liu, P., Guo, J., Kang, Z., & Guo, J. (2018). Host-induced gene silencing of an important pathogenicity factor PsCPK1 in *Puccinia striiformis* f. sp. *tritici* enhances resistance of wheat to stripe rust. Plant Biotechnology Journal, 16, 797–807.
Ragupathy, R., Ravichandran, S., Mahdi, M. S. R., Huang, D., Reimer, E., Domaratzki, M., & Cloutier, S. (2016). Deep sequencing of wheat sRNA transcriptome reveals distinct temporal expression pattern of miRNAs in response to heat, light and UV. Scientific Reports, 6, 39373.
Rajam, M. V. (2020). RNA silencing technology: A boon for crop improvement. Journal of Biosciences, 45(1), 1–5.
Ravichandran, S., Ragupathy, R., Edwards, T., Domaratzki, M., & Cloutier, S. (2019). MicroRNA-guided regulation of heat stress response in wheat. BMC Genomics, 20(1), 488.
Regina, A., Bird, A., Topping, D., Bowden, S., Freeman, J., Barsby, T., Kosar-Hashemi, B., Li, Z., Rahman, S., & Morell, M. (2006). High-amylose wheat generated by RNA interference improves indices of large-bowel health in rats. Proceedings of the National Academy of Sciences USA, 103(10), 3546–3551.
Riechen, J. (2007). Establishment of broad-spectrum resistance against *Blumeria graminis* f.sp. *tritici* in *Triticum aestivum* by RNAi-mediated knock-down of MLO. Journal für Verbraucherschutz und Lebensmittelsicherheit, 2(suppl.), 120.
Rodrigues, T. B., & Petrick, J. S. (2020). Safety considerations for humans and other vertebrates regarding agricultural uses of externally applied RNA molecules. Frontiers in Plant Science, 11, 407.
Sang, H., & Kim, J. (2020). Advanced strategies to control plant pathogenic fungi by host-induced gene silencing (HIGS) and spray-induced gene silencing (SIGS). Plant Biotechnology Reports, 14, 1.
Schaefer, K. L., Parlange, F., Buchmann, G., Jung, E., Wehrli, A., Herren, G., Müller, M. C., Stehlin, J., Schmid, R., Wicker, T., Keller, B., & Bourras, S. (2020). Cross-kingdom RNAi of pathogen effectors leads to quantitative adult plant resistace in wheat. Frontiers in Plant Sciences, 11, 253.
Shewry, P. R. (2009). Wheat. Journal of Experimental Botany, 60(6), 1537–1553.
Shiferaw, B., Smale, M., Braun, H., Duveiller, E., Reynolds, M., & Muricho, G. (2013). Crops that feed the world 10. Past successes and future challenges to the role played by wheat in global food security. Food Security, 5(3), 291–317.
Shoup Rupp, J. L., Cruz, L. F., Trick, H. N., & Fellers, J. P. (2016). RNAi-mediated, stable resistance to *Triticum* mosaic virus in wheat. Crop Science, 56(4), 1602–1610.
Singh, K., Dardick, C., & Kindu, J. K. (2019). RNAi-mediated resistance against viruses in perinnial fruit plants. Plants, 8(10), 359.
Sivamani, E., Brey, C. W., Talbert, L. E., Young, M. A., Dyer, W. E., Kaniewski, W. K., & Qu, R. (2002). Resistance to wheat streak mosaic virus in transgenic wheat engineered with the viral coat protein gene. Transgenic Research, 11(1), 31–41.
Sivamani, E., Brey, C., Dyer, W. E., Talbert, L. E., & Qu, R. (2000). Resistance to wheat streak mosaic virus in transgenic wheat expressing the viral replicase (Nlb) gene. Molecular Breeding, 6(5), 469–477.
Skopelitis, D. S., Hill, K., Klesen, S., Marco, C. F., von Born, P., Chitwood, D. H., & Timmermans, C. P. M. (2018). Gating of miRNA movement at defined cell-cell interfaces governs their impact as positional signals. Nature Communications, 9(1), 3107.
Song, J. B., Gao, S., Wang, Y., Li, B. W., Zhang, Y. L., & Yang, Z. M. (2016). miR394 and its target gene LCR are involved in cold stress response in *Arabidopsis*. Plant Gene, 5, 56–64.
Song, X., Li, Y., Cao, X., & Qi, Y. (2019). MicroRNAs and their regulatory roles in plant-environment interactions. Annual Review of Plant Biology, 70, 489–525.
Sun, L., Wen, J., Peng, H., Yao, Y., Hu, Z., Ni, Z., Sun, Q., & Xin, M. (2022). The genetic and molecular basis for improving heat stress tolerance in wheat. aBIOTECH, 3, 25–39.
Sun, Q., Liu, X., Yang, J., Liu, W., Du, Q., Wang, H., Fu, C., & Li, W-X. (2018). MicroRNA528 affects lodging resistance of maize by regulating lignin biosynthesis under nitrogen-luxury conditions. Molecular Plant, 11(6), 806–814.
Sun, Y., Sparks, C., Jones, H., Riley, M., Francis, F., Du, W., & Xia, L. (2019). Silencing an essential gene involved in infestation and digestion in grain aphid through plant-mediated RNA interference generates aphid-resistant wheat plants. Plant Biotechnology Journal, 17(5), 852–854.
Sunkar, R., Li, Y. F., & Jagadeeswaran, G. (2012). Functions of microRNAs in plant stress responses. Trends in Plant Science, 17(4), 196–203.
Tan, J-A. C. H., Jones, M. G. K., & Fosu-Nyarko, J. (2013). Gene silencing in root lesion nematodes (*Pratylenchus* spp.) significantly reduces reproduction in a plant host. Experimental Parasitology, 133(2), 166–178.
Tang, Z., Zhang, L., Xu, C., Yuan, S., Zhang, F., Zheng, Y., & Zhao, C. (2012). Uncovering small RNA-mediated responses to cold stress in a wheat thermosensitive genic malesterile line by deep sequencing. Plant Physiology, 159(2), 721–738.
Tiwari, M., Sharma, D., & Trivedi, P. K. (2014). Artificial microRNA mediated gene silencing in plants: Progress and perspectives. Plant Molecular Biology, 86(1–2), 1–18.
Varallyay, E., Giczey, G., & Burgyan, J. (2012). Virus-induced gene silencing of Mlo genes induces powdery mildew resistance in *Triticum aestivum*. Archives of Virology, 157, 1345–1350.

Wang, B., Fei Sun, Y., Son, N., Wei, J., Wang, X., Feng, H., Yin, Z., & Kang, Z. (2014). MicroRNAs involving in cold, wounding and salt stresses in *Triticum aestivum* L. Plant Physiology and Biochemistry, 80, 90–96.

Wang, B., Sun, Y., Song, N., Zhao, M., Liu, R., Feng, H., Wang, X., & Kang, Z. (2017). *Puccinia striiformis* f. sp. *tritici* microRNA-like RNA 1 (Pst-milR1), an important pathogenicity factor of Pst, impairs wheat resistance to Pst by suppressing the wheat pathogenesis-related 2 gene. New Phytologist, 215(1), 338–350.

Wang, M., Wu, L., Mei, Y., Zhao, Y., Ma, Z., Zhang, X., & Chen, Y. (2020). Host-induced gene silencing of multiple genes of *Fusarium graminearum* enhances resistance to *Fusarium* head blight in wheat. Plant Biotechnology Journal, 18(12), 2373–2375.

Wang, Y., Sun, F., Cao, H., Peng, H., Ni, Z., Sun, Q., & Yao, Y. (2012). TamiR159 directed wheat TaGAMYB cleavage and its involvement in anther development and heat response. PLoS One, 7(11), e48445.

Wen, S., Wen, N., Pang, J., Langen, G., Brew-Appiah, R. A., Mejias, J. H., Osorio, C., Yang, M., Gemini, R., Moehs, C. P., Zemetra, R. S., Kogel, K. H., Liu, B., Wang, X., von Wettstein, D., & Rustgi, S. (2012). Structural genes of wheat and barley 5-methylcytosine DNA glycosylases and their potential applications for human health. Proceedings of the National Academy of Sciences of the United States of America, 109(50), 20543–20548.

Wieser, H., Koehler, P., Folck, A., & Becker, D. (2006). Characterization of wheat with strongly reduced α-gliadin content. In: Lookhart, G. L., Ng, P. K. W. (eds.). Proceedings of the 9th Annual Gluten Workshop. San Fransisco, California, United States of America. American Association of Cereal Chemists, St. Paul, Minnesota. Pp. 13–16.

Xin, M., Wang, Y., Yao, Y., Xie, C., Peng, H., Ni, Z., & Sun, Q. (2010). Diverse set of microRNAs are responsive to powdery mildew infection and heat stress in wheat (*Triticum aestivum* L.). BMC Plant Biology, 10, 123.

Xu, L., Duan, X., Lv, Y., Zhang, X., Nie, Z., Xie, C., Ni, Z., & Liang, R. (2014). Silencing of an aphid carboxylesterase gene by use of plant-mediated RNAi impairs *Sitobion avenae* tolerance of Phoxim insecticides. Transgenic Research, 23(2), 389–396.

Xu, L., Hou, Q., Zhao, Y., Lu, L., Lim, B., Ni, Z., & Liang, R. (2017). Silencing of a lipase maturation factor 2-like gene by wheat-mediated RNAi reduces the survivability and reproductive capacity of the grain aphid *Sitobion avenae*. Archives of Insect Biochemistry and Physiology, 95, e21392.

Yan, T., Chen, H., Sun, Y., Yu, X., & Xia, L. (2016). RNA interference of the ecdysone receptor genes EcR and USP in grain aphid (*Sitobion avenae* F.) affects its survival and fecundity upon feeding on wheat plants. International Journal of Molecular Sciences, 17(12), 2098.

Yang, K., Rong, W., Qi, L., Li, J., Wei, X., & Zhang, Z. (2013). Isolation and characterization of a novel wheat cysteine-rich receptor-like kinase gene induced by *Rhizoctonia cerealis*. Scientific Reports, 3, 3021.

Yang, S., Dai, Y., Chen, Y., Yang, J., Yang, D., Liu, Q., & Jian, H. (2019). A novel G16B09-like effector from *Heterodera avenae* suppresses plant defenses and promotes parasitism. Frontiers in Plant Science, 10, 66.

Younis, A., Siddiqu, M. I., Kim, C.-K., & Lim, K.-B. (2014). RNA interference (RNAi) induced gene silencing: A promising approach of hi-tech plant breeding. International Journal of Biological Sciences, 10(10), 1150–1158.

Yu, H., Wang, Y., Fu, F., & Li, W. (2022). Transgenic improvement for biotic resistance of crops. International Journal of Biological Sciences, 23(22), 14370.

Yu, R., Xu, X., Liang, Y., Tian, H., Pan, Z., Jinm, S., Wang, N., & Zhang, W. (2014). The insect ecdysone receptor is a good potential target for RNAi based pest control. International Journal of Biological Sciences, 10(10), 1171–1180.

Yu, X. D., Liu, Z. C., Huang, S. L., Chen, Z. Q., Sun, Y. W., Duan, P. F., Ma, Y. Z., & Xia, L. Q. (2016). RNAi-mediated plant protection against aphids. Pest Management Science, 72(6), 1090–1098.

Yu, Y., Zhang, Y., Chen, X., & Chen, Y. (2019). Plant noncoding RNAs: Hidden players in development and stress responses. Annual Review of Cell and Developmental Biology, 35, 407–431.

Yu, Z., Wang, X., Mu, X., & Zhang, L. (2019). RNAi mediated silencing of dehydrin gene WZY2 confers osmotic stress intolerance in transgenic wheat. Functional Plant Biology, 46(10), 877–884.

Zeeshan, M., Qiu, C. W., Naz, S., Cao, F., & Wu, F. (2021). Genome-wide discovery of mimas with differential expression patterns in responses to salinity in the two contrasting wheat cultivars. International Journal of Molecular Sciences, 22(22), 12556.

Zhang, B., & Wang, Q. (2016). MicroRNA, a new target for engineering new crop cultivars. Bioengineered, 7(1), 7–10.

Zhang, J., Khan, S. A., Heckel, D. G., & Bock, R. (2017). Next-generation insect-resistant plants: RNAi-mediated crop protection. Trends Biotechnology, 35, 871–882.

Zhang, Y., Zhou, J., Wei, F., Song, T., Yu, Y., Yu, M., Fan, Q., Yang, Y., Xue, G., & Zhang, X. (2021). Nucleoredoxin gene TaNRX1 positively regulates drought tolerance in transgenic wheat (*Triticum aestivum* L.). Frontiers in Plant Science, 12, 756338.

Zhao, B., Ge, L., Liang, R., Li, W., Ruan, K., Lin, H., & Jin, Y. (2009). Members of miR-169 family are induced by high salinity and transiently inhibit the NF-YA transcription factor. BMC Molecular Biology, 10, 29.

Zhao, Y., Sui, X., Xu, L., Liu, G., Lu, L., You, M., Xie, C., Li, B., Ni, Z., & Liang, R. (2018). Plant-mediated RNAi of grain aphid CHS1 gene confers common wheat resistance against aphids. Pest Management Science, 74(12), 2754–2760.

Zhu, X., Qi, T., Yang, Q., He, F., Tan, C., Ma, W., Voegele, R. T., Kang, Z., & Guo, J. (2017). Host-induced gene silencing of the MAPKK gene PsFUZ7 confers stable resistance to wheat stripe rust. Plant Physiology, 175(4), 1853–1863.

Zuo, Z.-F., He, W., Li, J., Mo, B., & Liu, L. (2021). Small RNAs: The essential regulators in plant thermotolerance. Frontiers in Plant Science, 12, 726762.