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Review

MicroRNAs as potential targets for improving rice yield via plant architecture modulation: Recent studies and future perspectives

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Ensuring agricultural food security is a major concern for the future world, and being the second most consumed crop, rice yield needs an urgent upliftment. Grain yield is a pleiotropic trait that employs a plethora of genes functioning in complex signalling cascades. The yield related genes are controlled by various regulatory factors including the microRNAs (miRNAs), the small 20–22 nucleotide (nt) non-coding RNAs, which have emerged as the master ribo-regulators of eukaryotic genes. Plant miRNAs can bind to highly complementary sequences in the target messenger RNAs (mRNAs) and negatively regulate gene expression to coordinate the various biological processes involved in plant development. In rice, an ideal plant architecture (IPA) has been regarded as the key to attain high yield and several miRNAs have been deciphered to play important roles in orchestrating vital regulatory procedures for achieving optimum plant morphological yield related traits like less unproductive tillers, more panicle branches and heavier grains. In this review, we present and discuss the various genetic engineering strategies undertaken to manipulate the miRNA-mRNA expression levels in order to achieve improved grain output by modulation of rice plant architecture and recent advances made in this regard.

Keywords. Gene regulation; genetic manipulation; grain yield; microRNAs; plant architecture; rice

1. Introduction

Global demand for food is continuously on the rise due to rapidly increasing world population. Due to its enhanced ability to easily adapt under different environmental conditions, rice (*Oryza sativa*) is grown all over the globe, thereby making it as the staple food for nearly half the humanity and feeding more than three billion people on earth (Birla *et al*. 2017). The total consumption of rice is expected to increase from the current 490 million tons in 2020 to around 650 million tons by 2050 (Milovanovic and Smutka 2017). Thus, in order to ensure food security of the future world, there is an urgent need to scale up the rice production rate by at least 40%. With the development of advanced genetic engineering techniques, this mammoth task of increasing rice production seems to be achievable.

The discovery of first small non-coding RNAs in the nematode *Caenorhabditis elegans* in 1993 opened up whole new avenues that led to formulation of newer technologies and ushered in an era of the RNA molecular world, which continues to expand even today. Considered to be evolved as a defence mechanism in organisms against the viral or transposon attack, small RNAs (sRNAs) have undergone modulation by adapting to regulate the action of endogenous genes (Borges and Martienssen 2015). sRNAs are

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known to be vital players in growth, development, reproduction and defence responses functioning in distinct, yet overlapping pathways leading to genetic and epigenetic control of key cellular processes (Borges and Martienssen 2015). These have helped to understand the intricacies of the control of gene function at molecular level and are the driving molecules involved in one of the most important and indispensable phenomenon of gene silencing occurring in all eukaryotes, popularly known as RNA interference (RNAi) (Mamta and Rajam 2018).

At present, several classes of sRNA molecules are known to us, viz., microRNAs (miRNAs), small interfering RNAs (siRNAs), PIWI-interacting RNA (piRNAs), and transfer RNA-derived small RNAs (tsRNAs) (Wang *et al*. 2019). Out of these, miRNAs and siRNAs are the two important small non-coding RNAs found in plants (Pareek *et al*. 2015). Both are short duplex RNA molecules that bring into effect their function by controlled repression of target gene's expression (Lam *et al*. 2015). However, despite of sharing similarities, both are different in their origin, mode of action and clinical implications (Lam *et al.* 2015). The origin of siRNAs are the double-stranded RNA (dsRNA) molecules which can be sourced as either endogenous or exogenous, whereas miRNAs are known to be derived from single-stranded RNAs (ssRNAs) transcribed from miRNA loci and are exclusively of endogenous origin (Lam *et al.* 2015; Pareek *et al*. 2015). The siRNAs are known to function transcriptionally as well as post-transcriptionally by activating auxiliary mechanisms such as histone modification, DNA methylation and degradation of messenger RNA (mRNA), whereas miRNAs primarily act via post-transcriptional gene silencing (PTGS) leading to mRNA degradation and translational repression (Guleria *et al.* 2011). While the former is known to target only one single gene at a time, the latter can silence multiple related genes to generate pronounced therapeutic effect (Lam *et al.* 2015). Also, compared to siRNAs, miRNAs are highly specific in their target down-regulation and are known to generate lesser undesirable off-target effects (Kamthan *et al.* 2015), making miRNA-mediated gene silencing much more efficient and effective strategy in agricultural biotechnology.

Plant miRNAs and their target mRNAs share a high degree of sequence similarity, which aids in specific binding between the two and leading to degradation of the latter (Pareek *et al*. 2015). This in turn results in lowering the abundance of these target mRNAs and down-regulation of the related gene product. Several discovered miRNAs have been found to control the expression of key regulatory proteins or transcription factors involved in important functions such as plant development or signal transduction, that includes auxin signalling, organ separation, organ polarity, floral organ identity, reproductive development, leaf growth and developmental transitions (Reviewed by Chen 2005). The high levels of sequence complementarity of miRNAs with their target mRNAs has enabled the researchers to predict these miRNA target genes with the help of bioinformatic analysis and design genetic engineering strategies targeting the suppression of desired genes by transgenic over-expression of such miRNAs and vice versa. Also, transgenic plants harbouring sRNAs have been considered much safer for human consumption by US Food and Drug Administration (FDA) (Kamthan *et al*. 2015; Rodrigues and Petrick 2020). Due to the vast influence of miRNAs in modulating the basic cellular, physiological and biochemical processes, plant miRNAs have been regarded as the master controllers of gene expression and are being extensively exploited for improving agronomic traits in crops, particularly yield.

Therefore, in this review, we will focus on the current understanding of biogenesis of plant miRNAs and their function, and the recent genetic engineering studies encompassing their role in enhancing rice yield via manipulation of plant architecture.

2. miRNAs: biogenesis and function

Plant-based miRNAs are short RNA molecules that are approximately 20–22 nts long (Pareek *et al*. 2015). These are generated as endogenous genes and are transcribed by the DNA-dependent RNA Polymerase II (Pol II) enzyme (Borges and Martienssen 2015). Although these sRNAs were discovered in 1993 but received wide attention of researchers only after a decade. With the gain in knowledge regarding their occurrence in the non-coding regions of the eukaryotic genome and their ongoing discoveries in diverse plant species, miRNAs have become rather ubiquitous in their prevalence and apparently presumed to be affecting most of the biological activities throughout the lifetime of the plant (Voinnet 2009; Shriram *et al*. 2016).

2.1 *Biogenesis of miRNAs*

The miRNA biogenesis pathway can be of two types, canonical and non-canonical, with the former being the

more prevalent one among plants (O'Brien *et al*. 2018) and is discussed in detail here (figure 1).

2.1.1 *Transcription of miRNA genes to produce primary miRNAs:* Plant miRNAs are encoded by specific genes known as *MIRNA (MIR)* genes, which are present in the non-coding nuclear regions of the plant genome (Tyagi *et al.* 2019). There are more than several hundreds of *MIR* genes known to exist ubiquitously in a variety of plant species in diverse forms (Budak and Akpinar 2015). Sometimes, these have even been reported to occur within the exons of coding DNA strands and also in antisense strands (MacFarlane and Murphy 2010). According to their location in the plant genome, the *MIR* genes can be of two types, viz., intergenic i.e. located between two protein-coding genes, and intragenic or intronic, i.e., processed from introns of their host transcripts (Wang *et al.* 2019). The intergenic miRNAs are transcribed as independent units by Pol II and are mostly under the control of their own promoters, while intragenic or intronic miRNAs are processed from introns of their host transcripts (Budak and Akpinar 2015; Wang *et al.* 2019). Occasionally, some miRNAs may be synthesized as single long transcripts known as clusters and such clusters bearing regions of similarity are then grouped as families (O'Brien *et al.* 2018).

Biogenesis of miRNA in plants (figure 1) begins in the nucleus with the transcription of the *MIR* genes either from intra- or intergenic regions by the enzyme Pol II and results in the synthesis of long primary transcripts of miRNAs termed as the primary miRNAs (pri-miRNAs) (Djami-Tchatchou *et al*. 2017). Recent studies have revealed that this step is mediated by several proteins such as Negative On TATA Less2 (NOT2), Cell Division Cycle5 (CDC5) and Protein Phosphatase 4 Regulatory Subunit 3 (PP4R3A) that aid in recruiting Pol II and enable smooth transcription of the desired miRNAs (Fang *et al.* 2015).

2.1.2 *Primary miRNAs are processed to precursor miRNAs:* The pri-miRNAs are the usual Pol II products, which are capped at 5′ end, polyadenylated at 3′ end and sometimes even spliced (Rogers and Chen 2013). The pri-miRNA transcripts are generally of varying lengths (between 80–500 nts) and are then folded like an imperfect hairpin onto the complementarity region forming a stem-loop structure consisting of a terminal loop, an upper stem, the miRNA/miRNA* region, a lower stem, and two arms (Moro *et al.* 2018; Tyagi *et al.* 2019). The stem-loop structure of the pri-miRNA facilitates the cleavage action of RNase III endonuclease DICER-Like 1 (DCL-1) that acts specifically on dsRNAs in conjugation with another dsRNA-binding protein Hyponastic Leaves1 (HYL1) and the C2H2 zinc finger protein Serrate (SE) along with several other auxiliary factors located in nuclear processing centres called dicing bodies or D-bodies (Ren *et al.* 2012; Yang *et al.* 2006a). Together, these enzymes form the dicing complex or the microprocessor unit which is under tight functional control by HYL1 which undergoes phosphorylation by specific kinases such as Mitogen-Activated Protein Kinase3 (MAPK3) and SNF1-Related Protein Kinase2 (SnRK2), and dephosphorylation by the action of phosphatases like C-Terminal Domain Phosphataselike 1, 2 (CPL1/2), respectively (Yan *et al.* 2017; Yu *et al.* 2017). The outcome involves trimming of pri-miRNAs to precursor miRNAs (pre-miRNAs) which is further cleaved by DCL1 and results in the formation of a nascent miRNA–miRNA* duplex of 20-22 nt long (Bologna *et al*. 2013). It exhibits 2-nt 3′ overhangs at both strands and each strand possesses a 5′ end phosphate and two 3′ end hydroxyl groups (2′-OH and 3′-OH). In order to prevent its further degradation by small RNA degrading nuclease (SDN) class of exonucleases, this miRNA–miRNA* duplex is methylated at 2′-OH position of the 3′ end hydroxyl group by the HUA enhancer 1 (HEN1), an S-adenosyl L-methionine-dependent methyltransferase (Yang *et al.* 2006b).

2.1.3 *miRNAs maturation and assembly:* The methylated and stable miRNA–miRNA* duplex is then transported by the plant export protein HASTY (a homologue of animal exportin-5 protein) to cytoplasm where it recruits special effector proteins called ARGONAUTE (AGO) to enable cleavage of the target mRNAs (Park *et al.* 2005). The highly conserved AGO proteins contain two RNA binding domains, viz., PAZ and MID, while a third domain called PIWI possesses the endonucleolytic activity for target degradation (Swarts *et al.* 2014). At this stage, the miRNA–miRNA* duplex is also sorted into guide strand and passenger strand on the basis of their thermodynamic properties and the type of nt present on its 5′ end, e.g., guide miRNAs with a 5′ uracil are preferentially loaded onto *Arabidopsis* AGO1 proteins (Mi *et al.* 2008). In the final step of the pathway, the passenger miRNA is degraded by SDN while the mature guide miRNA is incorporated onto AGO proteins to form the miRNA induced silencing complex (miRISC), which is then activated to down-regulate the target mRNAs bearing

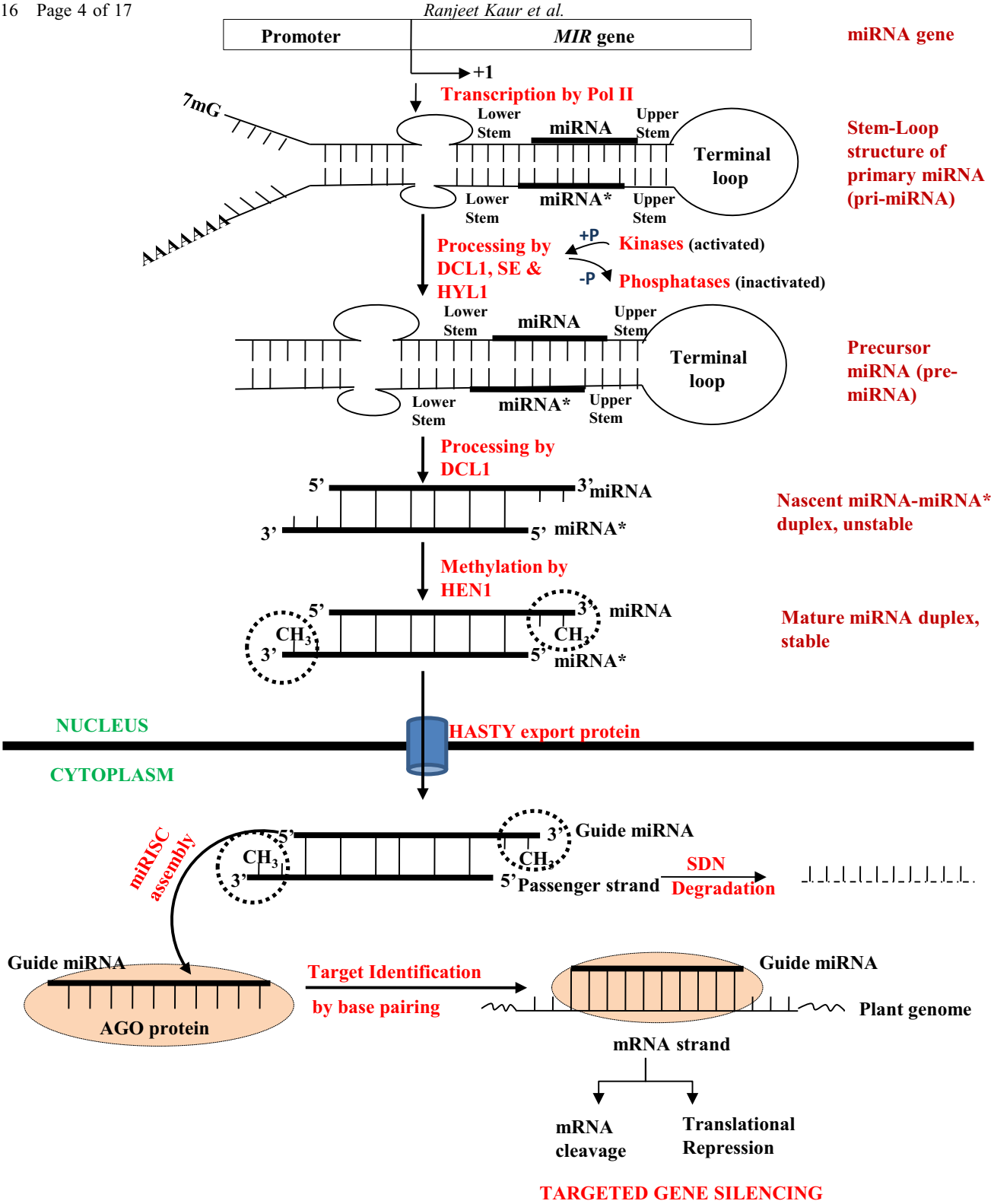

Figure 1. Schematic representation of miRNA biogenesis and gene silencing in plants: The miRNA biogenesis begins in the nucleus by the transcription of miRNA gene (*MIR*) by Pol II to produce pri-miRNA which is processed to pre-miRNA by the action of DICER-Like 1 (DCL-1), Hyponastic Leaves1 (HYL1) and Serrate (SE). Further processing by DCL1 leads to formation of miRNA/miRNA* duplex, which is stabilized by HUA Enhancer 1 (HEN1) methylation and exported to cytoplasm by HASTY. The passenger strand (miRNA*) is degraded by Small RNA Degrading Nuclease (SDN) while the guide miRNA strand is loaded onto Argonaute (AGO) to form the miRNA induced silencing complex (miRISC) which identifies the target miRNA by complementary base-pairing and silences it either by site-specific cleavage or translational repression.

sequence complementarity to the guide mRNA (Tyagi *et al.* 2019). However, recent studies in *Arabidopsis* have shown that it is the nucleus where the assembly of RISC takes place and it is then exported to the cytosol by another protein known as EXPO1 (Bologna *et al.* 2018).

2.2 *Function and operational mode of miRNAs*

The primary function of miRNA is the regulation of gene expression via PTGS by identifying its target mRNAs based on sequence complementarity. Most of the miRNA targets include important transcription factors (TFs) affecting several vital processes related to biotic and abiotic stress management as well as yield in plants. Their functions encompass an enormous variety and large spread effects that includes control of gene expression related to cellular metabolism, signal transduction, organ differentiation, reproduction, floral patterning, nutrient homeostasis and stress responses, auxin signalling, organ separation, organ polarity, floral organ identity, leaf growth and developmental transitions (reviewed in Tyagi *et al.* 2019). Studies have shown that one gene may be targeted by a single or multiple miRNAs and vice versa (Riffo-Campos *et al.* 2016). This makes it even more crucial and challenging for miRNAs to warrant careful binding with its targets so that no errors occur in target identification. The AGO protein binds to a small region of 6–8 nt at the 5′ end of the miRNA known as the seed which ensures high-affinity pairing between a miRNA and its target (Ameres and Zamore 2013). Once activated and loaded on to the RISC, miRNA can employ any of the following three major ways to silence the target genes: (i) site-specific cleavage of the target mRNA, (ii) translational repression of target genes, and (iii) miRNA-directed DNA methylation.

2.2.1 *Site-specific cleavage of the target mRNA:* The miRNA-directed cleavage of target mRNAs is the main mode of action of miRNA-based regulation in plants (Pareek *et al.* 2015). It is accomplished at a precise position of the target strand and is known as slicing (Yu *et al.* 2017). The action begins when the guide miRNA binds to the target mRNA with near-perfect sequence similarity, which is also an essential requirement for effective slicing of targets by the AGO endonucleases (Rogers and Chen 2013). The AGO proteins change their conformation to form a fold resembling RNase H with the aid of its PIWI domain and slices the phosphodiester bonds between the 10th and 11th nt from the 5′ end of the target mRNA (Ameres and Zamore 2013). The excised mRNA fragments are then degraded by the activity of non-specific nucleases such as exoribonuclease 4 (Souret *et al.* 2004). However, the miRNA remains intact even after the target cleavage and can guide the slicing of additional mRNAs (Pareek *et al.* 2015).

2.2.2 *Translational repression of target gene:* Plants exhibit translational repression of its target genes only in the absence of perfect base pairing between guide miRNA and target mRNA or due to lack of catalytic activity of AGO proteins (Tyagi *et al.* 2019). In this mode of action, the target is not sliced but rather silenced via translational repression and deadenylation, which leads to mRNA decay (Braun *et al.* 2012). However, it is less prevalent in plants as they lack the homologue for GW182 protein, an essential component of the deadenylation and translational repression in animals, and also do not allow seed mismatches unlike their animal counterparts (Iwakawa and Tomari 2013). Although, some studies have claimed that sequence similarity is not the major factor that dictates the mode of action employed by plant miRNAs (Yu *et al.* 2017). The mechanism of translational repression in plants remained largely unclear, until recent reports showed that miRNA-AGO1 RISC complex is the primary factor engaged in blocking translation by miRNAs in plants in the following ways: (a) binding to 5′ cap of target mRNA and blocking translation initiation, (b) inhibiting mRNA circularization, and (c) stalling ribosome assembly and movement (Iwakawa and Tomari 2013; Tyagi *et al.* 2019). Although mRNA cleavage is the major mode of action for gene silencing by miRNAs, studies have suggested that both the mechanisms, that is, miRNA-directed target slicing as well as translational repression, function in tandem at various stages in plant development and enhance the overall silencing efficiency by miRNAs (Iwakawa and Tomari 2015). However, studies such as ribosomal profiling along with transcript abundance should be performed in order to gain deeper insights of the miRNA action *in-vivo*.

2.2.3 *miRNA-directed DNA methylation:* Apart from regulation of gene expression at post-transcriptional level, a few miRNAs have been reported to act at transcriptional level also by mediating RNA-directed DNA methylation. This involves action of enzymes called methyltransferases which enable addition of a methyl group on the 5′ position of the nt which is

mostly a cytosine, and results in changing the expression pattern of desired genes. A novel population of long miRNAs (23–27 nt) produced from DCL3 enzyme and forming complex with AGO4 protein were reported in few plants such as *Arabidopsis*, rice and moss (reviewed in Jia *et al*. 2011). These are much different from the conventional canonical miRNAs (20–22 nt) and are also capable of epigenetic regulation at transcriptional level (Teotia *et al.* 2017), however their identity is still under debate. Recent findings have shown that miRNAs also direct the biogenesis of phased secondary siRNAs (phasiRNAs) which follow similar mechanism of recruiting AGO proteins to assemble RISC and regulate gene expression particularly in plant reproductive organs, affecting male fertility (Komiya 2017; Yu *et al.* 2017). Although studies are underway regarding the role of miRNAs in methylation of genes (Jha and Shankar 2014), the exact mechanism of miRNA-directed DNA methylation remains unclear and needs in-depth exploration.

3. miRNAs and their targeting for yield improvement of rice

Plant architecture is a multi-trait feature comprising of the height of the plant, shape and arrangement of leaves, branching pattern and floral morphology (Reinhardt and Kuhlemeier 2002). In order to characterize high yield traits in rice, an ideal plant architecture (IPA) has been proposed by breeder scientists and encompasses key rice features that influence grain yield in a defining manner (Wang and Li 2011). For a rice plant to be eligible for IPA, the tiller number should be low, unproductive tillers should be nil, number of grains per panicle should be high and stems should be thick as well as sturdy (Jiao *et al.* 2010; Peng *et al.* 2019). Modulation in any of these factors is destined to affect rice yield. Thus, in case of rice, the important yield parameters include tillering, panicle branching and grain size (Zheng and Qu 2015; Hu *et al.* 2018). These three factors are, however, dependent on each other and together they create IPA by a complex interplay of various modulators including miRNAs at different stages of rice plant development, *viz*., vegetative, reproductive and grain-filling.

In the past decade, miRNAs have emerged as the major regulators controlling the key genes involved in grain yield improvement. Numerous studies have been conducted involving miRNAs and scientists were also successful in deciphering the several key miRNAs that might be regulating gene expression in rice. Till date, as many as 684 miRNA sequences have been identified and deposited in miRbase version 22 (*http://www.mirbase.org*), a dedicated public database for published miRNA sequences (Kozomara *et al.* 2019). Knowledge of miRNAs involved in regulating plant architecture through functional genomics studies can lead to their targeted modulation and altering the rice plant architecture, thereby leading to enhanced yield. Keeping in mind the effect of miRNAs in three major traits involved in rice yield, we have categorized the yield-related miRNAs in the similar manner, *viz*. (i) miRNAs affecting tillering, (ii) miRNAs affecting panicle architecture, and (iii) miRNAs affecting grain morphology. Let us take a survey of the genetic engineering studies (table 1) undertaken to modulate rice plant architecture by targeting miRNAs involved in regulating these yield related traits.

3.1 *miRNAs affecting tillering*

Tillering is an important agronomic trait in rice and is known to affect grain yield (Li *et al.* 2003). A rice tiller is a specialized branch that arises from an axillary bud as lateral shoots at basal nodes with its own adventitious roots growing independently from the main culm (Li *et al.* 2003; Wang *et al.* 2018). As tillers give rise to panicles (on which arise the rice grains), branching or tillering in rice determines the number of panicle bearing tillers and also number of grains per panicle (Wang and Li 2011). Excessive tillering has been connected to the redirection of plant resources to production of more number of lateral branches while compromising on grain number and plant height accompanied with a rise in unproductive tillers, reduced panicle size and poor grain setting, thereby leading to a decrease in grain yield (Badshah *et al.* 2014; Wang *et al.* 2018). Thus, reduced tiller number is linked to improved panicle morphology, grain number, and plant architecture, making it an important feature required for achieving an IPA in rice to enhance the crop yield.

Being a complex process, tillering employs a multitude of genes being regulated either negatively or positively at different stages of rice growth by various miRNAs post-transcriptionally. In one of the significant researches, a conserved and highly expressed class of plant miRNAs, named *miR156*, were found to control the expression of *Squamosa Promoter Binding Protein-Like* (*SPL*) genes involved in regulation of plant architecture (Xie *et al.* 2006; Wang 2016). Out of the

Table 1. Genetic manipulation of miRNAs for yield improvement in rice via modulation of plant architecture traits

miRNA	Target gene(s)	Target gene function	Other genes/ hormones affected	Effect of miRNA overexpression on rice yield	Possible pathway/module	Reference(s)
miRNAs involved in tillering						
miR156 (miR156b, miR156h, miR156f)	*SPL14*	Reduces unproductive tillers	*TB1, LAX1, DEP1, DWARF53,* strigolactone	Negative	*miR156-SPL14-TB1-DWARF53*	Xie *et al.* (2006), Jiao *et al.* (2010), Liu *et al.* (2015, 2019), Luo *et al.* (2012) and Lu *et al.* (2013)
miR156f	*SPL7*	Reduces tiller number	Auxin	Negative	*miR156f–OsSPL7–OsGH3.8*	Dai *et al.* (2018) and Liu *et al.* (2019)
miR160	*ARF18*	Reduces number of tillers	Auxin	Negative	*miR160-ARF18-auxin*	Huang *et al.* (2016)
miR393	*TIR1, AFB2*	Increases number of productive tillers	Auxin, *IAA6*	Negative	*miR393-TIR1/AFB2-IAA6*	Xia *et al.* (2012) and Li *et al.* (2016a, b)
miR444a	*MADS57*	Regulation of tillering	*TB1, DWARF14*, strigolactone	Negative	*miR444a-MADS57-TB1-D14 (miMTD)*	Guo *et al.* (2013)
miRNAs involved in panicle architecture						
miR172	*SNB, IDS1*	Increases number of panicle branches and spikelets within the panicle	*APETALA 2*	Negative	-	Zhu *et al.* (2009), Lee and An (2012) and Lee *et al.* (2014)
miR396d	*GRF6*	Improves panicle and spikelet formation	*GIF1*	Negative	*miR396-GRF-GIF*	Liu *et al.* (2014)
miR396b	*GRF6*	Improves panicle branching	*TAWAWA1, MADS34*	Negative	*miR396-GRF-GIF*	Gao *et al.* (2015)
miR396a	*OsGRF1/2/6/8*	Improves panicle and spikelet formation	*FZP* and *LAX1*	Negative	*miR396-GRF-GIF*	Diao *et al.* (2018)
miR529a	*OsSPL2, OsSPL14, OsSPL17*	Panicle initiation and development	*TB1, DEP1, LAX1, SIZ11, CSLD4, Aux/IAA*	Negative	*miR529-SPL14-DEP1/LAX1/TB1*	Jeong *et al.* (2011) and Yue *et al.* (2017)
miR164b	*OsNAC2*	Enhances panicle branching and increases panicle length	*IPA1, DEP1,*	Negative	*miR164b-NAC2-IPA1/DEP1*	Jiang *et al.* (2018)
miR535	*OsSPL7/12/16*	Enhances panicle branching	*OsPIN1B, OsDEP1, OsLOG, OsSLR1*	Negative	-	Sun *et al.* (2019)

Table 1 (continued)

miRNA	Target gene(s)	Target gene function	Other genes/ hormones affected	Effect of miRNA overexpression on rice yield	Possible pathway/module	Reference(s)
OsmiR408	*OsUCL8*	Increasing panicle branches and number of effective grains per main panicle	-	Positive	-	Zhang *et al.* (2017a)
miRNAs involved in grain morphology						
miR396	*GRF4*	Larger grain size	*GIF1*, brassinosteroid-induced genes	Negative	*miR396-GIF1*-brassinosteroid	Li *et al.* (2016a, b), Duan *et al.* (2016) and Che *et al.* (2015)
miR396e and *miR396f*	*GRF8*	Improves grain size and panicle branching	*GIF1*	Negative	-	Zhang *et al.* (2020)
miR156	*SPL16*	Promotes grain filling and increased grain width	*GW7*	Negative	*miR156-OsSPL16-GW7*	Wang *et al.* (2012, 2015)
miR156	*SPL13*	Regulates cell size and enhances grain length	*GLW7*	Negative	*miR156-OsSPL13-GLW7*	Si *et al.* (2016)
miR397b	*OsLAC*	Increases panicle branching and enhances grain size	Brassinosteroid	Positive	*miR397-LAC*-brassinosteroid	Zhang *et al.* (2013)
miR167	*ARF8* and *ARF6*	Enhances grain size and quality	Several genes of the *GH3* family, auxin	Negative	*miR167-ARF8/6-GH3-IAA*	Yang *et al.* (2006c) and Xue *et al.* (2009)
miR1432	*OsACOT*	Promotes grain-filling and results in heavier grains	-	Negative	-	Zhao *et al.* (2019)
miR1848	*OsCYP51C*	Promotes grain filling and improves grain width	*OsGInF1*, *OsGW5* and *OsGW3*	Negative	miR1848-CYP51C-GW	Xia *et al.* (2015)
OsmiR159	*GAMYB* and *GAMYBL1*	Improves grain size	-	Negative	-	Zhao *et al.* (2017)
miR398	*Os07g46990*	Improves grain size and grain weight	-	Negative	-	Zhang *et al.* (2017b)

19 known SPL genes in rice, *OsSPL14* was deciphered to be directly involved in increased rice yield by reducing the tiller number and was found to be located on a major, co-dominant quantitative trait loci (QTL) of rice chromosome 8 known as *Wealthy Farmer's Panicle* (*WFP*) (Miura *et al.* 2010) or *IPA* (Jiao *et al.* 2010). At least 12 *OsmiR156* are known till date, out of which 10 members (*OsmiR156a* to *OsmiR156j*) encode for exactly same guide miRNA sequences and were found to show highly similar seed matches with 11 *OsSPL* genes, implying that a coordinated dialogue persists between *OsmiR156* and the *OsSPL* family (Liu *et al.*

2015). Together, *OsmiR156* and *SPL* genes form a regulatory hub that controls several developmental functions such as floral patterning, branching, juvenile/adult phase transition, leaf plastochron length, trichome morphology and organ size in *Arabidopsis* as well as in rice (Xie *et al.* 2006; Shikata *et al.* 2009; Wei *et al.* 2012; Liu *et al.* 2015; Wang and Wang 2015; Wang 2016).

Constitutive over-expression of different *OsmiR156* (*OsmiR156b*, *OsmiR156h* and *OsmiR156f*) under the control of maize ubiquitin promoter in rice resulted in reduced *OsSPL14* transcript levels and produced dwarf transgenic plants with multi-tillering phenotype and reduced yield (Xie *et al.* 2006; Liu *et al.* 2015). In an attempt to perturb the negative regulation of *OsSPL14* by *OsmiR156*, two independent studies elucidated that a single point mutation from C-to-T generated in the *OsmiR156*-binding site of *OsSPL14* without affecting the amino acid sequence, resulted in increased abundance of *OsSPL14* transcripts and achieved high yielding IPA by inhibition of the *OsmiR156*-directed transcriptional cleavage and translational repression of target *OsSPL14* mRNAs in rice (Jiao *et al.* 2010; Miura *et al.* 2010). Huang *et al.* (2016) performed a detailed analysis of *OsmiR160* using a similar strategy and found that over-expression of the mutant form of its target gene rice *Auxin Response Factor 18* (*OsARF1*) which was resistant to cleavage by *OsmiR160*, produced rice transgenic plants with reduced tiller number per plant, dwarf stature, rolled leaves and small seeds. In this study, the abundance of several auxin signalling genes was also reported to be modulated, indicating that negative regulation of *OsARF18* by *OsmiR160* is essential for rice growth and development and is mediated by affecting auxin signalling pathway (Huang *et al*. 2016).

In a recent study, ectopic expression of *OsmiR156f* was carried out under the control of stem-specific promoter for a gibberellin (GA) biosynthesis gene, *GA3ox2* in seasonal as well as ratooning rice (Liu *et al.* 2019). Contrary to the previous studies, it resulted in generation of rice transgenic lines with average height and more productive tillers that yielded high produce (Liu *et al.* 2019). This might be due to the fact that *miR156* controls apical dormancy and promotes plant branching, and regulation of its expression in a spatio-temporal manner proved to be beneficial for grain yield (Zhang and Wang 2016). Similar results were obtained in a previous study, where over-expression of *miR156* in switchgrass, a dedicated herbaceous bioenergy crop, caused doubling the plant biomass (Fu *et al.* 2012).

Transgenic overexpression of *OsmiR156f* showed an increased tiller number and reduced plant height, whereas over-expression of edited *OsmiR156f* having mutation for its target binding site led to production of rice plants with reduced tiller number and increased height (Dai *et al.* 2018). The researchers also found that the primary target gene for *OsmiR156f* was *OsSPL7* which binds directly to the *OsGH3.8* promoter, thus forming a *miR156f–OsSPL7–OsGH3.8* pathway that influenced tiller number and plant height in rice, and they speculated that this pathway may allow cross-talk between *miR156* and the phytohormone auxin. Silencing of *OsSPL14* by *miR156* was shown to down-regulate the expression of other down-stream genes such as *Teosinte Branched1 (TB1)*, *Lax Panicle 1 (LAX1)* and *Dense and Erect Panicle1* (*DEP1*), which control tillering and panicle size (Lu *et al.* 2013). In a recent study, *OsmiR156* was shown to control the spatio-temporal expression of tillering-related genes such as *TB1*, *LAX1* and *DWARF 53*, a negative regulator of *TB1* (Liu *et al.* 2019), thereby demonstrating the coordinated control of multiple yield-related genes by miRNAs through a complex network. Some studies have made speculations that strigolactones, known to inhibit axillary bud outgrowth in plants, function in tandem with *miR156* in regulating rice tiller outgrowth (Luo *et al.* 2012). However, an in- depth analysis needs to be conducted to understand the functioning of this pathway and its contribution in rice yield improvement.

Up-regulation of *OsmiR393* resulted in targeted repression of two auxin receptor genes, *viz*., *Transport Inhibitor Response 1* (*OsTIR1*) and *Auxin-Signalling F-Box 2* (*OsAFB2*) resulting in increased productive tillers and early flowering which are yield enhancing traits (Xia *et al.* 2012). In another study, *OsmiR393* over-expressing transgenic lines were shown to accumulate OsIAA6, a key gene that represses auxin signalling, thereby curtailing the auxin sensitivity of the axillary buds and promoting rice tillering (Li *et al.* 2016a, b). Besides, it has also been reported to affect various processes, including leaf and root development in *Arabidopsis* (Si-Ammour *et al.* 2011), opening the possibility of exploiting its similar role in rice too for the purpose of yield improvement. Guo *et al.* (2013) reported that over-expression of *OsmiR444a* produced rice lines with fewer tillers. In this study, *OsMAD57* was found to interact with *OsTB1* and suppressed the expression of *DWARF14* (*D14*), a strigolactone-dependent inhibitor of rice tillering, and resulted in increased tiller number. However, its function was disrupted by its targeted silencing by *miR444a* which demonstrated that regulation of tillering was affected in

a complex mechanism involving *miR444a-MADS57-TB1-D14 (miMTD)* pathway (Guo *et al.* 2013).

3.2 *miRNAs affecting panicle architecture*

The rice inflorescence is known as the panicle and its arrangement or patterning is referred to as panicle architecture and consists of a rachis (main axis), the primary branches, the secondary branches and the spikelets (rice flowers), which ultimately develop into grains (Wang *et al.* 2018). Given the importance of the structures borne by the panicle, its architecture holds of great significance in agriculture as it directly influences the rice yield potential. Rice tillers produce the panicles when the shoot apical meristem gives rise to inflorescence meristems during the transition of the plant from vegetative to reproductive phase (Wang and Li 2011; Wang *et al.* 2018), thus making the genes and their corresponding miRNAs, which are involved in the spatio-temporal control of the different meristems in this phase transition, as the major determinants of the panicle development and morphology (Yang *et al.* 2019). Apart from this, the length of the rachis along with the number or length of primary and secondary panicle branches have also been found to influence the number of spikelets or grains per panicle which in turn affects the rice yield (Wang and Li 2011; Peng *et al.* 2019). Studies conducted on *Arabidopsis* have revealed quite a few such important factors influencing panicle architecture and similar homologous genes have been found in rice too.

Transgenic overexpression of *miR172b* in rice produced generic defects in floral organs, loss of spikelet determinacy and reduced grain weight (Zhu *et al.* 2009). In another study, over-expression of *miR172* resulted in silencing of two *APETALA2* (*AP2*) family genes viz., *Supernumerary Bract* (*OsSNB*) and *Indeterminate Spikelet 1* (*OsIDS1*) involved in establishment of floral meristems and inflorescence architecture, and the resulting transgenic rice plants displayed a significant decrease in the number of panicle branches and spikelets within the panicle, leading to loss of grain yield (Lee and An 2012). The same group further reported that the levels of *miR172* were increased while the transcript abundance of *OsIDS1* and *SNB* was reduced, leading to significant reduction in flowering time in aging transgenic rice plants over-expressing *miR172* (Lee *et al.* 2014). This speculated towards the role played by *OsmiR172* in flowering, which in turn is directly related to panicle development and improved rice yield. However, the exact mechanism by which *miR172* modulates flowering is still less understood and more research has to be conducted in this area before committing the function of *miR172* as an inducer of flowering.

miR396 influences panicle architecture and grain yield by targeting the growth regulating factors (GRFs), which execute their function via GRF-interacting factors (GIFs) forming a regulatory module of *miR396-GRF-GIF* (Liebsch and Palatnik 2020). Over-expression of *OsmiR396d* and silencing of its target gene OsGRF6 produced low-yielding transgenic rice lines with similar defects in panicle architecture such as open husks, long sterile lemmas, altered floral organ morphology and defective spikelets, whereas ectopic expression of *OsGRF6* totally alleviated these defects along with *OsGIF1* mediation (Liu *et al.* 2014). Repression of *miR396b* led to enhanced expression of panicle-branching factors *OsTAWAWA1* and *OsMADS34*, plausibly through the physical binding of *OsGRF6* to their promoters and the resulting transgenic rice plants showed improved panicle branching that accounted for increased grain yield (Gao *et al.* 2015). On the other hand, defective spikelets and abnormal inflorescence without the secondary branches was seen in short-heighted transgenic rice plants over-expressing *miR396* (Gao *et al.* 2015). Over-expression of *OsmiR396a* produced dwarf plants with smaller leaves and defective inflorescence architecture that included abnormal panicles and spikelets with a large proportion of rare conjoined-twin florets, resulting in decreased rice yield (Diao *et al.* 2018). Molecular analysis of the transgenic plants displayed a significant down-regulation in the expression of four GRF genes, *viz.*, *OsGRF1/2/6/8* and also indirectly modulated the transcript levels of related downstream genes such as *FZP* and *LAX1* (controlling panicle development) and various floral organ identity genes (involved in floral development) (Diao *et al.* 2018).

The spatio-temporal regulation of *SPL14* expression in different organs during different growth phases of rice has profound effects on plant architecture affecting the outcome of rice yield. The role of *SPL14/miR156* circuit does not limit to the fine control of tillering but rather extends to panicle initiation too. As discussed in the previous section on tillering, *miR156*-regulated expression of *SPL14* is predominant in the shoot tissues, where it is known to influence other tillering related genes such as *OsTB1* and *D53* (Liu *et al.* 2019). However, in panicles, the negative control of *SPL* genes is governed by *miR529*, which was shown to express preferentially at the reproductive stage, especially during panicle formation and post-embryonic

stages (Jeong *et al.* 2011). Sequence analysis of *OsmiR529a* revealed that it shared at least 14 nt with the *OsmiR156* family and also targeted similar genes belonging to the *SPL* gene family, but in different tissues and in different time periods (Jeong *et al.* 2011). Yue *et al.* (2017) reported that *miR529a* highly expressed in rice seedlings and panicles all throughout different developmental stages of panicle while the expression of *miR156* was extremely low in the panicle, suggesting that *miR529a* and not *miR156* is involved in panicle initiation. In the same study, over-expression of *miR529a* showed a severe decline in the expression levels of *OsSPL2, OsSPL14* and *OsSPL17* genes with a concomitant down-regulation of other down-stream panicle as well as branching related genes such as *TB1*, *DEP1*, *LAX1*, *SIZ11* (*SAP and Miz1*), *CSLD4* (*cellulose synthase-like D4*) and the *Aux/IAA* genes (Yue *et al.* 2017). The panicle architecture of these transgenic plants was also severely affected as it produced defective panicles with shorter rachis, fewer branches and lesser number of spikelets than the wild-type rice cultivar Nipponbare (Yue *et al.* 2017). From this study, it was inferred that *miR529* plays a vital role in panicle initiation and development at the reproductive stage, while *miR156* is critical for plant development at the vegetative stages in rice. The gradual shift in the relative abundance of the two miRNAs during the phase transition of rice plant displays the complex coordinated interplay between different miRNA species in regulating similar genes in a spatio-temporal manner, affecting the outcome of rice grain yield. However, a recent study showed that the over-expression of *miR156f* negatively regulated transcript levels of several panicle development genes such as *LAX1, LAX2, RCN2* and *RA2* by targeted silencing of *SPL7*, claiming that *miR156f* might be involved in panicle architecture modulation (Yang *et al.* 2019). Although they did not consider the effect of *miR529* in their study, the interaction between the two miRNAs need to be examined before jumping to any conclusions.

Gain of function for *miR164b* or loss of function for its target gene *OsNAC2* negatively impacted panicle architecture as it produced transgenic rice plants with shorter panicles and reduced grain yield, while over-expression of *OsmiR164b*-resistant *OsNAC2* promoted panicle branching with enhanced panicle length producing at least 58% more grains in field conditions (Jiang *et al.* 2018). Molecular analysis of the high yielding transgenic rice plants revealed significant up-regulation of plant architecture-related genes *IPA1* and *DEP1*, indicating the existence of a complex pathway in regulating panicle architecture and rice yield. Recently, Sun *et al.* (2019) reported that *OsmiR535* over-expression greatly influenced plant architecture resulting in plants with dwarf stature, more but shorter panicles with fewer primary/secondary panicle branches and longer grains without affecting the grain width. Further analysis in the same study revealed that *OsmiR535* had high sequence similarity to *miR156* and *miR529*, and its abundance level varied from very low during the vegetative growth to very high in young panicles, just like spatio-temporal expression pattern of *OsmiR529*. *OsmiR535* targeted the expression of *OsSPL7/12/16* and other down-stream panicle related genes, such as *OsPIN1B*, *OsDEP1*, *OsLOG* and *OsSLR1* (Sun *et al.* 2019). In contrast to all other reports on miRNAs which involved increasing rice yield by repressing the target miRNA, Zhang *et al.* (2017a) showed that over-expression of *OsmiR408* positively regulated grain yield in rice by increasing panicle branches (primary branches ~14% and secondary branches ~22%) and number of effective grains per main panicle (~18%) by down-regulating its downstream target, *OsUCL8*, which is an *uclacyanin* (*UCL*) gene of the phytocyanin family.

3.3 *miRNAs affecting grain morphology*

Rice grain weight is another important trait that directly affects yield as well as quality of rice and is determined by three main characteristics controlling seed size, *viz*., grain width, length and thickness (Duan *et al.* 2016). Grain filling rate and filling period has also shown to influence the grain weight and ultimately affects crop yield (Peng *et al.* 2019). During the seed development, slower grain-filling rate causes differential distribution of photosynthetic products among spikelets which produces grains that are under-developed and lighter in weight, thereby affecting rice grain quality and yield (Peng *et al.* 2014).

OsmiR396c was shown to block the expression of *OsGRF4*, whereas this action was perturbed by a mutation in the *OsGRF4* which was shown to interact with *OsGIF1* and resulted in producing larger grain size and enhancing grain yield (Li *et al.* 2016a, b). In a study by Duan *et al.* (2016), *GRF4* was targeted by *OsmiR396* but a 2 bp substitution mutation in the related QTL, grain size 2 (GS2) was shown to alleviate the silencing effect of *miR396*. This promoted grain size and grain weight, resulting in increased grain yield. Similar yet independent study conducted by Che *et al.* (2015) showed that mutations in *OsGRF4* blocked the effect of *miR396* and produced heavier

grains (~27%) that enhanced grain yield (~16%) by regulating many brassinosteroid-induced genes (Che *et al.* 2015). Recently, grain size and panicle branching was improved in transgenic rice plants carrying a disruption of the *miR396*-targeting site in *OsGRF8* as well as by knockout of *miR396e* and *miR396f* (Zhang *et al.* 2020).

Transgenic overexpression of *miR156* target gene *OsSPL16* (encoded by grain weight associated QTL *GW8*) was shown to promote cell division and grain filling, with positive consequences for grain width and yield in rice whereas its repression resulted in formation of slender grains with better quality of appearance in rice cultivar Basmati (Wang *et al.* 2012). It was found that *SPL16* expression was inversely related to another QTL *GW7* associated with grain quality and length (Wang *et al.* 2015). In another study, *miR156*-targeted *OsSPL13* (encoded by grain length and weight associated QTL *GLW7*) was shown to positively regulate cell size in the grain hull, resulting in enhanced rice grain length and yield (Si *et al.* 2016). Thus, *miR156* can be associated with the fine control of rice grain shape either by *OsSPL16-GW7* or *OsSPL13-GLW7* module, and its regulated expression can be exploited to produce rice grains with enhanced weight and quality, thereby influencing yield and market value.

Zhang *et al.* (2013) found that *OsmiR397* was highly expressed in young panicles and grains of wild-type rice varieties and targeted the *OsLAC* gene that encoded a laccase like protein promoting plant sensitivity to brassinosteroids. Over-expression of *miR397b* increased branching of panicles and enhanced grain size that included an increase of 12.4, 11.7, and 2% in the parameters for grain length, grain width, and grain thickness, respectively. This amounted to an increased grain yield by at least 25% in field conditions via the brassinosteroid pathway (Zhang *et al.* 2013). This was contrary to other miRNAs negatively regulating grain yield and displayed how miRNAs can contribute a positive regulatory role in controlling plant architecture and enhancing yield.

Elevated auxin content and expression of auxin response factors (ARFs) during early stages of grain-filling speculated the positive correlation of auxin with superior grain quality (Yang *et al.* 2006c; Wang *et al.* 2018). However, *miR167* was shown to mediate targeted cleavage of *ARF8* and *ARF6* which was accompanied by the decrease in levels of several genes of the *GH3* family involved in controlling the cellular concentration of free auxins (Yang *et al.* 2006c; Xue *et al.* 2009), suggesting that *OsmiR167* plays a key role in rice grain development via auxin signalling pathway. In order to get a holistic view of the role of various miRNAs in rice grain development, Peng *et al.* (2013) performed a detailed expression analysis of various miRNAs and their target genes at different developmental stages of rice grain filling and their data revealed that the elevated auxin levels triggered the abundance of *miR167* which gradually increased while the auxin content declined during the course of various rice grain filling periods, indicating a possible role of *miR167* in rice grain development through regulating auxin content in the immature grains. In a similar study by the same group, similar trends for other microRNA-mediated auxin signals, such as *miR164*, *miR160* and *miR390* in regulating auxin signals during grain development were also detected, but their roles are yet to be understood (Peng *et al.* 2014). Thus, *auxin-miR167-ARF8-OsGH3.2-IAA* pathway is an important the regulatory hub operating in the developing rice grains towards regulation of grain filling process and final grain weight in rice, thereby modulating rice yield.

In another study by Zhao *et al.* (2019), down-regulation of *OsmiR1432* released its target gene rice *Acyl-CoA thioesterase* (*OsACOT*) from suppression and promoted grain-filling in rice, resulting in heavier grains and improved grain yield by at least 17%. In the same study, similar results of enhanced grain yield were obtained in transgenic rice plants over-expressing *miR1432*-resistant form of *OsACOT* and grain weight was increased up to 46.7 % via improved grain filling rate. *OsmiR1848* target gene *OsCYP51C* gene was found to belong to the larger cytochrome P450 family and involved in phytosterol and brassinosteroid biosynthesis (Xia *et al.* 2015). However, in *OsmiR1848* over-expression rice plants, the expression of three rice genes involved in grain filling and grain width namely *Grain Incomplete Filling 1* (*OsGInF1)*, *OsGW5* and *OsGW3* were down-regulated, which implied that *OsmiR1848* might be indirectly involved in the control of grain size and quality in rice through regulation of phytosterol and brassinosteroid pathways (Xia *et al.* 2015). Repression of *OsmiR159* caused elevated abundance of its two targets genes, *OsGAMYB* and *OsGAMYBL1* (*GAMYB-LIKE 1*) and negatively impacted multiple agronomic traits including grain size by suppressing cell division, suggesting that *miR159* levels can be modulated to achieve increased grain size and high yield in rice (Zhao *et al.* 2017). Grain size and grain weight was shown to be positively affected by inhibition of *miR398* levels in transgenic rice plants (Zhang *et al.* 2017b). In this study, the authors down-regulated the

target miRNA in transgenic plants by applying a new technique known as the short tandem target mimic (STTM) that was found to be highly specific and stable for multiple generations.

4. Conclusions and future perspectives

The major challenge for today's agriculture is the amalgamation of modern crop improvement technologies with the existing molecular breeding approaches to generate plants that can produce well and ensure food security for the teeming millions. An ideal rice plant loaded with perfect features for achieving the capacity for high yield in the field conditions is every farmer's dream. The employment of miRNAs in fine tuning of the rice gene expression has enlightened a new direction for modulating complex yield related quantitative traits encompassing plant architecture.

The biogenesis of plant miRNAs is very interesting phenomenon and is tightly regulated at each step. The miRNA synthesis involves decoding of miRNA genes into stem-looped pri-miRNAs transcripts that are processed to pre-miRNAs by the microprocessor unit followed by their conversion to mature miRNAs, which are then loaded onto special effector AGO proteins to form RISC. The guide miRNA carries the crucial complementary sequence, which can identify the target mRNA and down-regulate it mostly through site-specific cleavage and sometimes by repressing the translational process or DNA methylation. Despite of numerous studies involving miRNA biogenesis and functional mechanism, there still exists large research gap involving several unanswered questions such as how is this biogenesis triggered and adjusted throughout the course of plant development; how do miRNAs decide their mode of action; why do plant miRNAs incorporate stringent miRNA-mRNA pairing whereas their animal counterparts allow few mismatches. These areas need to be explored and key factors involved should be identified to bring more transparency to the handling of miRNAs for genetic manipulation *in vivo*.

Being a complicated trait, rice grain yield is primarily controlled by plant architecture which mostly depends on tillering ability of plant, panicle morphologies and grain size. Accordingly, a myriad of genes/loci are associated with these traits which are regulated mainly by miRNAs. Other traits such as dwarf stature as well as thick and sturdy stems which improve lodging resistance in rice also contribute to IPA and aid in yield increase. Dwarf stature, which was an important trait for high yielding rice plants during green revolution, was found to be inversely correlated to tiller outgrowth of the plant. Known miRNAs contributing to lodging resistance in plants are scarce. Recent studies have shown the contribution of miRNAs to produce stronger stems in maize and creeping bentgrass. Similar studies can be conducted in rice too to unravel novel miRNAs contributing to lodging resistance and hence improved yield. Besides, optimal light reception by proper leaf angle can positively affect photosynthetic capability of the plant and enhance grain yield, hence leaf inclination is also considered to be an indirect factor for IPA and has been found to be under the control of several miRNAs through brassinosteroid pathway.

During the course of this study on miRNAs, a number of trends were noted. Firstly, up- or down-regulation of a single miRNA or its mRNA results in the differential expression of several of its downstream genes which are involved in modulation of plant morphology or other yield related traits. Secondly, the miRNA-mRNAs worked in the form of different modules/nodes which are involved in regulating vital processes in plant development. Sometimes, same miRNA can target multiple genes and work in different modules as seen in case of *miR156* which can block the expression of different genes from *SPL* family and operate in various modules/nodes to alter the plant architecture. Thirdly, auxin has emerged as the key phytohormone that is involved in these mechanisms along with brassinosteroids. For instance, *miR160* targeted *ARF18* while *miR393* targeted auxin receptor genes *TIR1* and *AFB2*; all of these repressions altered tillering outgrowth of plants and negatively affected rice grain yield. Similarly, *miR396* and *miR397* were shown to mediate their effects on grain size with a concomitant alteration in the levels of brassinosteroids. Fourthly, some miRNAs were found to be expressed in spatio-temporal manner and regulated various plant pathways; as seen in case of *miR156* and *miR529*. Both of these miRNAs targeted *SPL14*, but in different tissues and during different growth stages, thereby affecting development of different plant architectural features. Fifth, it was found that some rice cultivars had a natural mutation in their loci which perturbed the repressing action of their homologous miRNA molecules and resulted in ideal plant architecture that corresponded to high yield. For instance, mutation in *WFP*, which encoded *SPL14*, was not blocked by *miR156* and resulted in reduced tillers, and *miR396* could not down-regulate the mutated version of *GS2* encoding *GRF4*, which

resulted in increased grain size. Lastly, not all miRNA over-expression leads to yield loss, some were found to be positively influencing the rice yield; as was seen in the case of *miR408* and *miR397*. However, the knowledge obtained so far is just tip of the iceberg and more research should be focussed on the discovery as well as characterization of novel miRNAs and their target genes involved in rice yield improvement. Deep sequencing and RNA sequencing techniques are being used to uncover the unknown miRNAs and their sequences.

Genetic engineering strategies can be employed to comprehend the role of miRNAs in context of plant development and then manipulate these known miRNAs or their target genes to achieve enhanced yield. The most common technique that we came across was over-expression or silencing of the candidate miRNA or its target mRNA and studying its effect on the plant morphology and grain yield. Researchers have also over-expressed mutant forms of target genes which carried point mutation in the miRNA target sites but without affecting the protein sequence. Such mutant genes could evade miRNA mediated down-regulation and thus promoted high yielding traits in rice. Also, studies nowadays comprise of studying the expression levels of down-stream genes/hormones that might be affected by the gain or loss of function of the target miRNA or mRNA. This has enabled to get a holistic view of the cellular metabolome and the multi-gene interaction modules/pathways that might be involved in the miRNA regulation of yield related pathways, thereby unfolding the large scale impact of miRNAs on plant architecture modulation and grain yield. Apart from the regular silencing of genes, other newer techniques such as target mimicry, STTM and CRISPR-CAS9 gene editing tool are also rapidly gaining popularity for blocking the miRNA expression. Numerous genes/loci are known to influence grain yield characteristics of rice plant and yet their regulatory mechanisms are unknown which might involve a small non-coding homologous dsRNA in the form of miRNA. In-depth analysis of such postulated mRNAs and their miRNAs needs to be done to understand the *in vivo* functioning of the various signalling cascades in order to achieve optimum plant development and morphological features to enhance yield. Given the environmental conditions being friendly and constant, the plant morphology can be orchestrated by genetic manipulation of miRNA-mRNA modules to obtain ideal plant architecture, which can be extrapolated to produce high yielding rice plants.

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