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Role of miRNA for agricultural crop improvement

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Abstract

The conventional and modern crop improvement methods faces a prime problem is to balance the desirable character with one or more other beneficial characteristics in a new crop. This issue can be overwhelmed by utilizing regulatory genes, especially genes which provide more efficient and precise regulation in a target manner. MicroRNAs (miRNAs) is a type of short non-coding RNA which play a crucial role in gene expression at the post-transcriptional level and inhibition of translation by targeting mRNAs in eukaryotes. We herein are highlighting the biological role of miRNAs in agriculture important traits.

Keywords: miRNA, conserved and non conserved miRNA, applications of miRNA

Introduction

Addressing the challenges of food nutrition and security, and finding the ways out to tackle it is one of the important research areas which are being dealt by agriculture scientists around the globe. The world population is increasing geometrically but the land resources are constant. According to Varshney *et al.*, (2011) the world's population will reach up to 9 billion by next 30 years. Besides this abiotic stresses (climate change, drought, soil toxicity) and biotic stresses (microbial pathogens, herbivores, insects, etc.) also contribute to loss incrop productivity. This clearly indicates that the crop yield and food production should be geared up to meet the food demand of this population (Takeda *et al.*, 2008, Fedoroff *et al.*, 2010)^[40, 17]. With the recent advancement in the field of plant biotechnology and molecular biology various crop improvement techniques have been developed *viz.*, genetic engineering, molecular breeding, RNA interference (RNAi), Host Induced Gene Silencing (HIGS), Zinc Finger Nuclease (ZFN), and CRISPR-associated protein-9 nuclease (Cas9) (Tang and Chu, 2017)^[41]. Among these, the first two are not as recent as the rest of the aforementioned techniques which involve the development of gene-regulated plants (Nogoy *et al.*, 2018)^[34]. These techniques have been developed to overcome the problems associated with the conventional approaches but they simultaneously compromise the expression of one or more other desirable traits in the host plant (Tang and Chu, 2017)^[41].

There is always a quest among the researchers to develop natural, environmental friendly and sustainable methods for the enhancement of crop yield. In line with this, microRNAs (miRNAs) are one of the promising options in hands of the scientific community because these exist naturally, are non-coding, modulate the expression of genes, regulate plant metabolic processes at the post-transcriptional level, target messenger RNAs for cleavage and direct translational inhibition in eukaryotes (Sanan-Mishra *et al.*, 2009; Bej and Basak, 2014; Djami-Tchatchou and Dubery, 2015)^[37, 1, 14]. Recently, miRNAs have been explored by scientists to study their biogenesis, functional attributes, potential application in crop quality and yield improvement. Typically, miRNA are approximately 20-24 nucleotides in length and they bind to partially complementary sequences in target messenger RNAs (mRNAs) thereby regulating the gene expression at post-transcriptional level (Tang and Chu, 2017; Voinnet, 2009; Chen, 2009)^[41, 44, 8]. This review aims at discussing the previous researches and reviews related to miRNAs of plant origin, their role in expression of regulatory genes involved in plant development and stress responses, conservation and diversification of miRNAs among crops and their exploitation for the development of strategies for crop improvement.

Biogenesis of miRNA

Majority of the characterized miRNA genes is intergenic or oriented antisense to neighboring genes, therefore suspected to be transcribed as independent units. As much as 40% of miRNA genes may present in the introns of protein and non-protein coding genes or even in exons of long non-protein-coding transcripts. These are generally found in a sense orientation therefore, often are regulated together with their host genes.

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Exploitation of miRNA natural variation and their targets in agricultural crop

MicroRNAs (miRNAs) are 22 nucleotides, non-coding RNAs which enact vital role in regulation of gene expression for various important traits. During evolution, conserved miRNAs are under a strong filtering selection. Any change in the sequence of stem region or in mature miRNA sequence led to change their targeting specificity and may also build new miRNA-target interactions. Single nucleotide polymorphism (SNP) on mature miRNA may create interference between miRNA and target gene (Ferraro *et al.*, 2015) [19]. For example in Brassica juncea, miR SNP (C/T) was found at 5th position of mature miR2926 led deteriorated and unstable hairpin structure. There is complete loss of target function due to C/T transition of miR2926 (Singh *et al.*, 2017) [38]. In plants, primary miRNA (pri-miRNAs) are transcribed from miRNA gene and processing of pri-miRNA, form pre-miRNAs. Pre-miRNAs are further processed by different enzymes to form mature RNAs.

Conservation and diversification of miRNAs among crops

Conserved miRNAs families

Functionally validated 36 miRNA families are evolutionarily conserved among different plant species. Eight miRNA families (MIR156, MIR159/319, MIR160, MIR166, MIR171, MIR408, MIR390/391 and MIR395) have been revealed in common ancestor of all embryophytes. The MIR396 family is present in common ancestor of tracheophytes. MIR397 and MIR398 families are present in the common ancestors of all spermatophytes. Ten miRNA families (MIR162, MIR164, MIR167, MIR168, MIR169, MIR172, MIR393, MIR394, MIR399 and MIR827) are present in all angiosperms. MIR529 and MIR536 were present in common ancestors of embryophytes but lost in common ancestor of eudicots and tracheophytes respectively (Cuperus *et al.*, 2011) [10]. Along with conserved miRNAs their targets are also conserved and play a crucial role in plant growth and development. This conserved property of these miRNA-target modules makes them more suitable to utilize in trait improvement. Recent studies revealed that in rice, miR164 –targeted NAC genes (OMTN1-OMTN6) are negative regulators of drought tolerance at the reproductive stage (Feng *et al.*, 2014) [18]. In maize, ZmNAC1 played vital role in lateral root development. Accumulation of ZmNAC1 gene transcript in roots of inbred line Zong3 was 1.8 fold higher expressions than 87-1 line.

And Zm miR164 showed higher expression in 87-1 inbred line than Zong3. miR164 down regulates the ZmNAC1 expression at post-transcriptional level (Li *et al.*, 2012) [26]. In wheat miR164 negatively regulates TaNAC21/22 (a novel candidate NAC gene of wheat) which provide resistance to stripe rust (Feng *et al.*, 2014) [18].

Throughout evolution, conserved miRNA can regulate a target gene in a conserved pathway. These findings suggest that the functional divergence of conserved miRNA should be taken into consideration when they are selected as candidates for crop improvement.

Non-conserved or species specific miRNAs

Large number of plant miRNA is present in few closely related species or specific species. These lineage-specific miRNAs are called non-conserved miRNAs. Non conserved miRNAs are considered as transient products and energy wasters because they are processed inexplicitly, weakly expressed and lack of functional targets. Despite this, in some cases they expressed abundantly in specific tissues to have physiological role for special environmental adaptations. MiR824 was first reported as a non conserved miRNA originated by duplication of founder gene Agamous-like 16 (AGL16). By lowering stringency in BLAST analysis, no miR824 orthologs could be identified in plant species (Rajagopalan *et al.*, 2006) [36]. MiR824 and its target AGL16 is highly conserved in brassicaceae, but identified in Arabidopsis thaliana. AGL16 expression is censorious for development of stomatal complex. Overexpressing miRNA resistant AGL16 significantly increases the density of higher order stomata but not wild type AGL16 in Arabidopsis (Kutter *et al.*, 2007) [24].

Other than table 1, for various traits large numbers of miRNAs are functionally validated in rice. For example, miR319 target osPCF5, osPCF6, osPCF8 and TCP21 genes responsible for leaf morphogenesis, cold tolerance and immune response (Yang *et al.*, 2013, Zhang *et al.*, 2016) [55, 58]. MiR398, miR528 and miR7695 are accountable for providing immune response by targeting SOD2, AO and osNramp6 genes respectively (Li *et al.*, 2014, Wu *et al.*, 2017, Campo *et al.*, 2013) [27, 49, 4]. In response to abiotic stresses (drought and salt alkali) miR393, miR396 and miR1848 responsible by targeting osAFB2, osGRF4/6/10 and osCYP51G3 genes respectively (Bian *et al.*, 2012, Che *et al.*, 2015, Xia *et al.*, 2015) [3, 5, 50].

Table 1: List of functionally validated miRNAs in different plants

Sr. No	miRNA	Plant	Target gene(s)	classification	Agronomic traits	References
1.	miR156	Rice	OsSPL13, OsSPL14, OsSPL16,	SPLTFs	Grain size, yield, tillering, plant height	Tang and Chu (2017) [41]
		Maize	Tag1	SPLTFs	Juvenile-to-adult phase transition	Chuck <i>et al.</i> , 2007 [9]
		Wheat	TaTB1 and TaBa1	SPLTFs	Tillering and spikelet development	Liu <i>et al.</i> , 2017 [31]
		Soyabean	Gm SPL9d, WUSCHEL	SPLTFs	Axillary bud formation and branching	Su <i>et al.</i> , 2019
		Potato	StSPL3, StSPL6/9/13,	StLG1	Plant architecture, tuber yield	Bhogale <i>et al.</i> , 2014 [2]
		Tomato	SlySBP gene	SPLTF	Vegetative and reproductive development	Tang and Chu (2017) [41]
2.	miR157	Cotton	GhSPLs genes	SPLTFs	Floral organ growth, ovule production, auxin signal transduction	Liu <i>et al.</i> , 2017 [31]
		Tomato	LeSPL-CNR	SPLTF	Fruit ripening	Chen <i>et al.</i> , 2015 [6]
3.	miR159	Wheat	TaGAMYB1, TaGAMYB2	MYBTFs	Anther development, heat response	Wang <i>et al.</i> , 2012 [45]
		Rice	OsGAMYB, OsGAMYBL1	MYBTFs	Cell division, auxin, cytokinin and brassinosteroids biosynthesis	Zhao <i>et al.</i> , 2017 [59]
		Tomato	SGN-U567133	Uncharacterized protein	Leaf and flower development	Wu <i>et al.</i> , 2017 [49]

4.	miR160	Rice	ARF16, osARF18	ARFTF	Immune response, growth and development	Tang and Chu (2017) [41]
		Soyabean	GmARF10/16/17	ARFTFs	Nodule development	Tang and Chu (2017) [41]
		Tomato	SlARF10A	ARFTFs	Lamina outgrowth, ovary patterning, floral organ abscission	Damodharan <i>et al.</i> , 2016 [11]
		Wheat	HSP70, TPR domain protein ARF8, ARF18	ARFTF, ARFTFs	Heat stress, stress responsive regulator pattern in flag leaf and head	Goswami <i>et al.</i> , 2015 [20]
5.	miR162	Rice	OsTRE1	Trehalase	Drought tolerance	Tian <i>et al.</i> , 2015 [42]
		Rice	OMTN1-OMTN6	NACTFs	Drought tolerance	Fang <i>et al.</i> , 2014 [16]
		Maize	ZmNAC1	NACTF	Lateral root development	Li <i>et al.</i> , 2012 [26]
		Wheat	TaNAC21/22	NACTF	Immune response	Tang and Chu (2017) [41]
		Tomato	GOB	NACTF	Boundary specification	Berger <i>et al.</i> , 2009
6.	miR166	Rice	RDD1	DOFTF	Nutrient ion uptake and accumulation	Iwamoto <i>et al.</i> , 2016 [22]
		Maize	Rld1	classIII homeodomain/leucine Zipper TF	Leaf polarity	Tang and Chu (2017) [41]
		Cotton	Clp1	Ca ²⁺ dependent cysteine protease	Immune response	Zang <i>et al.</i> , 2016
7.	miR167	Rice	ARF8	ARFTF	Auxin response	Tang and Chu (2017) [41]
		Soyabean	GmARF8	ARFTF	Nodule and lateral root development	Wang <i>et al.</i> , 2015 [46]
		Tomato	SlARF6/8	ARFTFs	Female sterility, floral development	Liu <i>et al.</i> , 2014 [30]
8.	miR168	Rice	AGO1	AGO1	Immune response	Wu <i>et al.</i> , 2015 [48]
		Tomato	SIAGO1A,SIAGO1B	AGO1	Leaf epinasty, phase transition, fruit development	Xiang <i>et al.</i> , 2014 [51]
9.	miR172	Rice	SNB, OsIDS1, OsTOE1	AP2-like TFs	Spikelet determinacy, floral organ development, flowering time, panicle branching	Tang and Chu (2017) [41]
		Maize	gl15, ids1	AP2-like TFs	Juvenile-to-adult phase transition Sex determination, meristem cell fate	Tang and Chu (2017) [41]
		Barley	HvAP2	AP2-like TF	Grain density, cleistogamous flowering	Houston <i>et al.</i> , 2013 [21], Nair <i>et al.</i> , 2010 [33]
		Soyabean	GmAP2-2	AP2-like TF	Level of nodulation	Yan <i>et al.</i> , 2013 [54]
		Potato	RAP1	AP2-like TF	Flowering time, tuberization time	Tang and Chu (2017) [41]

MiRNA in hybrid crop breeding

Increasing population, change in climatic conditions, water scarcity, increasing temperature and decrease in cultivation area called for crop improvement. The emergence of one desirable trait in new crop variety along with deterioration of other valuable characteristics is the major limitation of both conventional and modern crop improvement methods. So miRNA provides genetic flexibility for fine regulation of genes at the post transcriptional and inhibiting translation by cleavage of RNA. The F1 hybrid progeny perform better and stronger than either parental lineage. This effect is called Heterosis and widely exploited in plant breeding. Heterosis is an effectual phenomenon to improve crops. Mi-RNA have role in differential expression of various traits in both hybrid and pure line varieties. Recent studies revealed that, under abiotic stress (salt and drought) in M017× B73 maize hybrid led to 2.5 and 1.8-fold upregulation of miR156 and miR166 respectively. And under abiotic stress miR171 and miR319 were down regulated in Mo17 and B73× M017 in contrast to B73 and M017× B73, which might indicate parental lines (Kong *et al* 2010). Recent studies revealed that miRNA play vital role in development of flower bud and pollen development. In flower development of soyabean gma-miR1566/GmSPL9 and gma-miR44136/GmPPR play important role in regulatory network (Ding *et al.*, 2018). MiR156 and SPL are very much conserved miRNA and gene family in plants respectively. In arabidopsis, differential expression pattern was observed at various stages of flower development by different members of miR156 and SPL (Xing *et al.*, 2010) [52].

Applications of miRNA

- 1. Enhancement of plant immunity:** Biotic stresses caused by viral, bacterial, fungal, insects and nematodes are the major restrictions to crop productivity. Viruses use multiple strategies to multiply and to spread through and between plants so they are especially difficult to control. Commercial varieties of potato highly resistant to three strains of PVY have been developed by expressing hpRNA derived from the 3' terminal part of the coat protein gene of PVY (Missiou *et al.*, 2004) [32]. In tomato, slmiR482f and slmiR5300 supresses NB-domain genes that impart resistance to *Fusarium oxysporum*. Glomerella leaf spot (GLS) of apple (*Malus domestica* Borkh.), caused by *Glomerella cingulata*, is an emerging fungal epidemic threatening the apple industry. A novel miRNA, Md-miRln20 negatively regulates resistance to *Glomerella* leaf spot by suppressing expression of NBS genes in apple (Zang *et al.*, 2019) [58].
- 2. Alteration of Plant Architecture and Flowering Time:** Carotenoid cleavage dioxygenase (CCD) genes play integral role in branch development. Transgenic kiwifruit plants containing RNAi construct for AcCCD8, resulted in reduce expression of AcCCD8 which correlated with delay leaf senescence and rise in branch development. Such type of betterment in plant architecture is anticipated to rise the number of flower and fruits (Ledger *et al.*, 2010) [25].
- 3. Modification of Flower Color:** The modification in flower colour to enhance the market value is done by genetic manipulation (RNAi) is more convenient than

conventional breeding. Anthocyanin and flavonoid are two pigments responsible for flower colour. The solubility of anthocyanins is enhanced by UDP glucose: flavonoid 3-o-glucosyltransferase (UFGT). In *Phalaenopsis*, suppressed *PeUFGT3* transcriptional activity caused the faded flower colour instead of red in normal expression (Chen *et al.*, 2011)^[7].

4. **Development of Seedless Fruits:** Absence of seeds in fruits increase their fruit market value as high yield can be obtained under unfavorable conditions. It is also a desirable agronomic trait which appreciated by consumers. In tomato plants, using RNA silencing the function of auxin response factor 7 (ARF 7) was suppressed to obtained seedless fruits. (De *et al.*, 2009)^[12].
5. **Secondary Metabolites for Nutraceutical and Pharmaceutical Applications-** Nutritional value can be enhanced by altering the accumulation of specific metabolites in fruits by RNA silencing. The down regulation of 9-cis-epoxy-carotenoid dioxygenase (NCED) enzyme of abscisic acid biosynthesis led to enhance the accumulation of upstream compounds like lycopene and β -carotene. In potato, by down regulating the expression of β -carotene hydroxylase (BCH) (converts β -carotene to Zeaxanthin) to enhance the β -carotene and lutene contents (Van *et al.*, 2007)^[43].
6. **Applications in Developing High Value Industrial Products:** Transgenic potatoes are used to produce human serum albumin but extraction and purification steps are the cost elements because of patatin contamination. Patatin is a family of glycoproteins depicting upto 40% of total soluble protein. Patatin amount was significantly decreased by nearly 99% at

both the protein and mRNA levels in transgenic potato particularly targeting patatin gene using the hpRNA approach, enabling fast purification of other potato glycoprotein or transgenically produced glycoproteins with minimum contamination (Kim *et al.*, 2008)^[23].

7. **Prolongation of Shelf-life:** The post-harvest degradation and spoilage is one of the main causes of economic loss in horticultural plants. This loss can be overcome by increasing the shelf life of vegetables and fruits by delayed ripening process. In climacteric fruits like tomato, initiation of ripening is mainly controlled by ethylene that regulates a series of ripening-specific genes (Osorio *et al.*, 2011)^[35]. The down-regulation of 1-aminocyclopropane-1- carboxylate oxidase (ACCO), a gene of ethylene biosynthesis pathway effectively decreased the rate of ethylene production in the ripening tomato fruits of transgenic plants by increasing shelf life of more than 120 days with same levels of titratable acid, total soluble sugar, total soluble solids and amino acids as the control plants (Xiong *et al.*, 2005)^[53].
8. **Removal of Allergens and Toxic Compounds:** Along with useful compounds plants are known to have many nutritionally undesirable compounds and toxins of several types, elimination of which from plants is usually a complicated and costly process. Numerous studies have proven RNA silencing as a powerful technology to make the plants toxin-free. RNA silencing of Carrot allergen Dau c 1 that belongs to pathogenesis-related 10 (PR 10) family of plant protein were down-regulating by RNA silencing. It resulted in transgenic carrot lines revealing greatly reduced specific allergenic reactivity in patients in skin prick tests (Eady *et al.*, 2008)^[15].

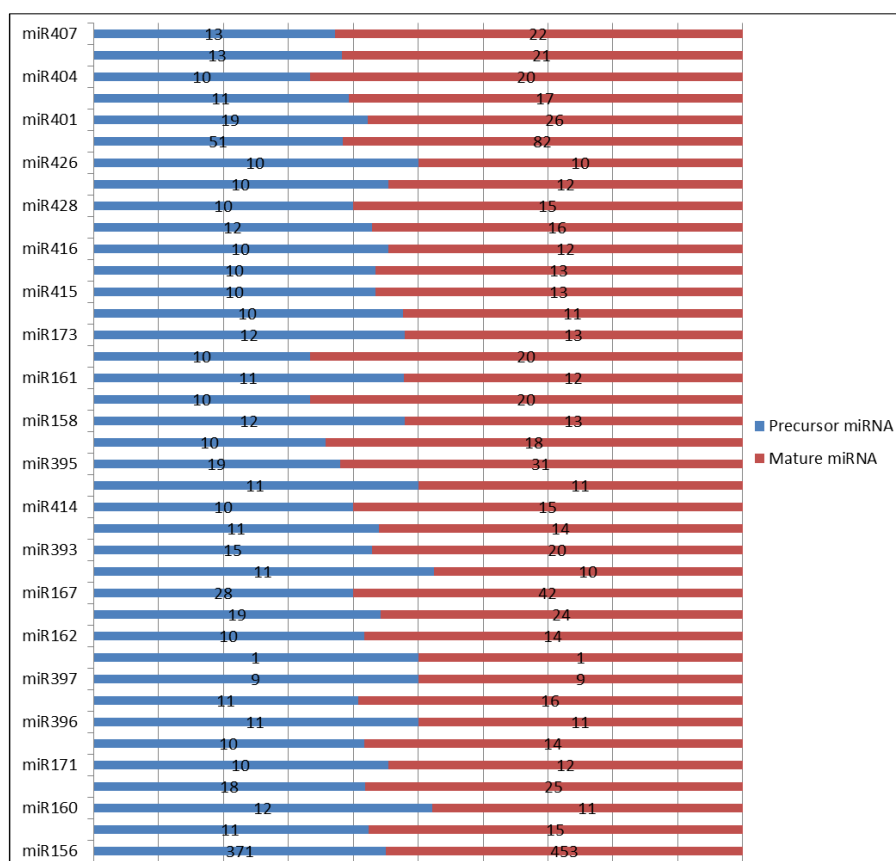


Fig 1: Number of mature miRNAs and their precursors in plants deposited in miRBase 22.1

Conclusion

The biological role of various miRNA-target modules from diverse crop species revealed the fundamental importance of miRNAs in the suppression of target gene expressions and also showed more complicated and diverse functions in the complex regulatory networks. The more knowledge of the function of miRNAs in these networks will not only increase our understanding of plant biological processes, but will also allow breeders to choose suitable targets for modulating agriculturally important traits.

References

1. Bej S, Basak J. MicroRNAs: the potential biomarkers in plant stress response. *Am. J Plant Sci.* 2014;5:748-759. DOI: 10.4236/ajps.2014.55089
2. Bhogale S, *et al.* MicroRNA156: a potential graft-transmissible microRNA that modulates plant architecture and tuberization in *Solanum tuberosum* ssp. *andigena*. *Plant Physiol.* 2014;164:1011-1027.
3. Bian H, *et al.* Distinctive expression patterns and roles of the miRNA393/ TIR1 homolog module in regulating flag leaf inclination and primary and crown root growth in rice (*Oryza sativa*). *New Phytol.* 2012;196:149-161.
4. Campo S, *et al.* Identification of a novel microRNA (miRNA) from rice that targets an alternatively spliced transcript of the Nrmp6 (Natural resistance-associated macrophage protein 6) gene involved in pathogen resistance. *New Phytol.* 2013;199:212-227.
5. Che R, *et al.* Control of grain size and rice yield by GL2-mediated brassinosteroid responses. *Nat. Plants.* 2015;2:15195.
6. Chen W, *et al.* Tuning LeSPL-CNR expression by SlymiR157 affects tomato fruit ripening. *Sci. Rep.* 2015;5:7852.
7. Chen WH, Hsu CY, Cheng HY, Chang H, Chen HH, Ger MJ. Downregulation of putative UDP-glucose: flavonoid 3-O-glucosyltransferase gene alters flower coloring in *Phalaenopsis*. *Plant cell reports.* 2011;30(6):1007-1017. <https://doi.org/10.1007/s00299-011-1006-1>
8. Chen X. Small RNAs and their roles in plant development. *Annu. Rev. Cell Dev. Biol.* 2009;25:21-44.
9. Chuck G, Cigan AM, Saetern K, Hake S. The heterochronic maize mutant *Corngrass1* results from overexpression of a tandem microRNA. *Nat. Genet.* 2007;39:544-549.
10. Cuperus JT, Fahlgren N, Carrington JC. Evolution and functional diversification of MIRNA genes. *The Plant cell.* 2011;23(2):431-442. <https://doi.org/10.1105/tpc.110.082784>
11. Damodharan S, Zhao D, Arazi T. A common miRNA160-based mechanism regulates ovary patterning, floral organ abscission and lamina outgrowth in tomato. *Plant J.* 2016;86:458-471.
12. De Jong M, Mariani C, Vriezen WH. The role of auxin and gibberellin in tomato fruit set. *J Exp. Bot.* 2009;60:1523-1532.
13. Ding X, Zhang H, Ruan H, *et al.* Exploration of miRNA-mediated fertility regulation network of cytoplasmic male sterility during flower bud development in soybean. *3 Biotech.* 2019;9:22. <https://doi.org/10.1007/s13205-018-1543-1>
14. Djami-Tchatchou AT, Dubery IA. Lipopolysaccharide perception leads to dynamic alterations in the microtranscriptomes of *Arabidopsis thaliana* cells and leaf tissues. *BMC Plant Biol.* 2015;15:79. DOI: 10.1186/s12870-015-0465-x.
15. Eady CC, Kanoi T, Kato M, Porter NG, Davis S, Shaw M, *et al.* Silencing onion lachrymatory factor synthase causes a significant change in the sulfur secondary metabolite profile. *Plant Physiol.*, 2008, 147, 2096-2106.
16. Fang Y, Xie K, Xiong L. Conserved miR164-targeted NAC genes negatively regulate drought resistance in rice. *J Exp. Bot.* 2014;65:2119-2135.
17. Fedoroff NV. The past, present and future of crop genetic modification. *New Biotechnol.* 2010;27:461-465.
18. Feng H, *et al.* The target gene of tae-miR164, a novel NAC transcription factor from the NAM subfamily, negatively regulates resistance of wheat to stripe rust. *Mol. Plant Pathol.* 2014;15:284-296.
19. Ferrao LFV, Caixeta ET, Pena G, Zambolim EM, Cruz CD, Zambolim L. New EST-SSR markers of *Coffea Arabica*: transferability and application to studies of molecular characterization and genetic mapping. *Mol. Breed.* 2015;35:1-5. DOI: 10.1007/s11032-015-0247-z
20. Goswami, Suneha Kumar, Ranjeet Rai, Raj. 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. *Aust. J Crop Sci.* 2014;8:697-705.
21. Houston K, *et al.* Variation in the interaction between alleles of HvAPETALA2 and microRNA172 determines the density of grains on the barley inflorescence. *Proc. Natl Acad. Sci. USA.* 2013;110:16675-16680.
22. Iwamoto M, Tagiri A. MicroRNA-targeted transcription factor gene *RDD1* promotes nutrient ion uptake and accumulation in rice. *Plant J.* 2016;85:466-477.
23. Kim YS, Lee YH, Kim HS, Kim MS, Hahn KW, Ko JH, *et al.* Development of patatin knockdown potato tubers using RNA interference (RNAi) technology, for the production of human-therapeutic glycoproteins. *BMC Biotechnol.* 2008;8:36.
24. Kutter C, Scho BH, Stadler M, Meins F Jr, Si-Ammour A. MicroRNA-mediated regulation of stomatal development in *Arabidopsis*. *Plant Cell.* 2007;19:2417-2429.
25. Ledger SE, Janssen BJ, Karunairetnam S, Wang T, Snowden KC. Modified CAROTENOID CLEAVAGE DIOXYGENASE8 expression correlates with altered branching in kiwifruit (*Actinidia chinensis*). *The New phytologist.* 2010;188(3):803-813. <https://doi.org/10.1111/j.1469-8137.2010.03394.x>
26. Li J, Guo G, Guo W. miRNA164-directed cleavage of *ZmNAC1* confers lateral root development in maize (*Zea mays* L.). *BMC Plant Biol.* 2012;12:220. <https://doi.org/10.1186/1471-2229-12-220>
27. Li Y, *et al.* Multiple rice microRNAs are involved in immunity against the blast fungus *Magnaporthe oryzae*. *Plant Physiol.* 2014;164:1077-1092.
28. Liu Haipei, Able Amanda J, Able Jason A. Genotypic water-deficit stress responses in durum wheat: association between physiological traits, microRNA regulatory modules and yield components. *Functional Plant Biology.* 2017;44:538-551.
29. Liu J, Cheng X, Liu P, Sun J. miR156-Targeted SBP-Box Transcription Factors Interact with DWARF53 to

- Regulate *Teosinte branched1* and *Barren stalk1* Expression in Bread Wheat. *Plant physiology*. 2017;174(3):1931-1948. <https://doi.org/10.1104/pp.17.00445>
30. Liu N, *et al.* Down-regulation of *AUXIN RESPONSE FACTORS 6* and *8* by microRNA 167 leads to floral development defects and female sterility in tomato. *J Exp. Bot.* 2014;65:2507-2520.
 31. Liu N, Tu L, Wang L, Hu H, Xu J, Zhang X. MicroRNA 157-targeted SPL genes regulate floral organ size and ovule production in cotton. *BMC plant biology*. 2017;17(1):7. <https://doi.org/10.1186/s12870-016-0969-z>
 32. Missiou A, Kalantidis K, Boutla A, Tzortzakaki S, Tabler M, Tsagris M, Generation of transgenic potato plants highly resistant to potato virus Y (PVY) through RNA silencing. *Mol. Breed.* 2004;14:185-197.
 33. Nair SK, *et al.* Cleistogamous flowering in barley arises from the suppression of microRNA-guided *HvAP2* mRNA cleavage. *Proc. Natl Acad. Sci. USA*. 2010;107:490-495.
 34. Nogoy FM, Niño MC, Song JY, *et al.* Plant microRNAs in molecular breeding. *Plant Biotechnol Rep.* 2018;12:15-25. <https://doi.org/10.1007/s11816-018-0468-9>.
 35. Osorio S, Alba R, Damasceno CM, Lopez-Casado G, Lohse M, Zanor MI, *et al.* Systems biology of tomato fruit development: combined transcript, protein, and metabolite analysis of tomato transcription factor (nor, rin) and ethylene receptor (Nr) mutants reveals novel regulatory interactions. *Plant Physiol.* 2011;157:405-425.
 36. Rajagopalan R, Vaucheret H, Trejo J, Bartel DP. A diverse and evolutionarily fluid set of microRNAs in *Arabidopsis thaliana*. *Genes Dev.* 2006;20:3407-3425.
 37. Sanan-Mishra N, Kumar V, Sopory SK, Mukherjee SK. Cloning and validation of novel miRNA from basmati rice indicates cross talk between abiotic and biotic stresses. *Mol. Genet. Genom.* 2009;282:463-474. DOI: 10.1007/s00438-009-0478-y
 38. Singh I, Smita S, Mishra DC, Kumar S, Singh BK, Rai A. Abiotic Stress Responsive miRNA-Target Network and Related Markers (SNP, SSR) in Brassica juncea. *Front. Plant Sci.* 2017;8:1943. DOI: 10.3389/fpls.2017.01943
 39. Sun Z, Su C, Yun J, Jiang Q, Wang L, Wang Y, *et al.* Genetic improvement of the shoot architecture and yield in soya bean plants via the manipulation of GmmiR156b. *Plant biotechnology journal.* 2019;17(1):50-62. <https://doi.org/10.1111/pbi.12946>
 40. Takeda S, Matsuoka M. Genetic approaches to crop improvement: responding to environmental and population changes. *Nat. Rev. Genet.* 2008;9:444-457.
 41. Tang J, Chu C. MicroRNAs in crop improvement: fine-tuners for complex traits. *Nat. Plants.* 2017;3:17077. DOI: 10.1038/nplants.2017.77
 42. Tian C, Zuo Z, Qiu JL. Identification and characterization of ABA-responsive microRNAs in rice. *J Genet. Genomics.* 2015;42:393-402.
 43. Van Eck J, Conlin B, Garvin DF, Mason H, Navarre DA, Brown CR. Enhancing beta-carotene content in potato by RNA-mediated silencing of the beta-carotene hydroxylase gene. *Amer. J Potato Res.* 2007;84:331-342.
 44. Voinnet O. Origin, biogenesis, and activity of plant microRNAs. *Cell.* 2009;136:669-687.
 45. Wang Y, Sun F, Cao H, Peng H, Ni Z, *et al.* TamiR159 Directed Wheat TaGAMYB Cleavage and Its Involvement in anther Development and Heat Response. *PLoS ONE.* 2012;7(11):e48445. DOI: 10.1371/journal.pone.0048445.
 46. Wang Y, *et al.* MicroRNA167-directed regulation of the auxin response factors *GmARF8a* and *GmARF8b* is required for soybean nodulation and lateral root development. *Plant Physiol.* 2015;168:984-999.
 47. Waterhouse PM, Graham MW, Wang M-B. Virus resistance and gene silencing in plants can be induced by simultaneous expression of sense and antisense RNA. *Proc. Natl. Acad. Sci. U.S.A.* 1998;95(23):13959-13964.
 48. Wu J, *et al.* Viral-inducible Argonaute18 confers broad-spectrum virus resistance in rice by sequestering a host microRNA. *E-Life.* 2015;4:e05733.
 49. Wu JG, *et al.* ROS accumulation and antiviral defence control by microRNA528 in rice. *Nat. Plants.* 2017;3:16203.
 50. Xia K, *et al.* Rice microRNA osa-miR1848 targets the obtusifoliol 14a-demethylase gene *OsCYP51G3* and mediates the biosynthesis of phytosterols and brassinosteroids during development and in response to stress. *New Phytol.* 2015;208:790-802.
 51. Xian Z, *et al.* miR168 influences phase transition, leaf epinasty, and fruit development via *SIAGO1s* in tomato. *J Exp. Bot.* 2014;65:6655-6666.
 52. Xing SP, Salinas M, Höhmann S, Berndtgen R, Huijser P. miR156-targeted and nontargeted SBP-Box transcription factors act in concert to secure male fertility in *Arabidopsis*. *Plant Cell.* 2010;22:3935-3950. <https://doi.org/10.1105/tpc.110.07934>
 53. Xiong AS, Yao QH, Peng RH, Li X, Han PL, Fan HQ. Different effects on ACC oxidase gene silencing triggered by RNA interference in transgenic tomato. *Plant Cell Rep.* 2005;23:639-646.
 54. Yan Z, *et al.* miR172 regulates soybean nodulation. *Mol. Plant Microbe Interact.* 2013;26:1371-1377.
 55. Yang C, *et al.* Overexpression of microRNA319 impacts leaf morphogenesis and leads to enhanced cold tolerance in rice (*Oryza sativa* L.). *Plant Cell Environ.* 2013;36:2207-2218.
 56. Zhang C, *et al.* Suppression of jasmonic acid-mediated defense by viral-inducible microRNA319 facilitates virus infection in rice. *Mol. Plant.* 2016;9:1302-1314.
 57. Zhang T, *et al.* Cotton plants export microRNAs to inhibit virulence gene expression in a fungal pathogen. *Nat. Plants.* 2016;2:16153.
 58. Zhang Yi, Zhang Qiulei, Hao Li, Wang Shengnan, Wang Shengyuan, Zhang Wenna, *et al.* A novel miRNA negatively regulates resistance to Glomerella leaf spot by suppressing expression of an NBS gene in apple. *Horticulture Research.* 2019;6:93. 10.1038/s41438-019-0175.
 59. Zhao Y, Wen H, Teotia S, *et al.* Suppression of microRNA159 impacts multiple agronomic traits in rice (*Oryza sativa* L.). *BMC Plant Biol.* 2017;17:215. <https://doi.org/10.1186/s12870-017-1171-7>