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Stop the new gene, the alien: Predicted breakdown of MYB overexpression by ncRNA mediated gene regulations

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ABSTRACT

Acclimatization is a process that occurs in individual cells to a drastic change in micro and macro environments. When an organism is subjected to a new environment or a change in its normal growing conditions, the cellular mechanisms initiate a warning sign and over a period of time or over generations the acquired, modified traits are being communicated and fixed as a new trait. If there is lack of equilibrium within the cell due to over expression of a single gene or network of associated genes either manmade or due to mutations, the organism or plant tries to fix it by initiating gene regulatory mechanisms. According to our neutral theory of gene expression, always a cell tries to maintain its pH by modifying its cytosol through altered gene expression. In the present investigation, 198 AtMYB genes were analyzed and found to play an intrinsic photosystem linked network of 38 nodes where MYB being regulated by a set of 48 miRNAs. Members of the network have evidence-based link to energy related mechanisms. Altering gene expression to an extent where, the cell may not be able to fix it or a trait, which requires excessive energy loss escorts the organism's gene regulation by breakdown of the introduced sequence over few generations. Events with constitutive overexpression may suffer poor performance over the years based on gene network prevailing in the crop of interest. Hence, network rewiring with minimal energy expenses is concerned.

Keywords: Cytosolic pH balance, gene regulation, neutral theory of gene expression, over expression of genes

INTRODUCTION

Non-coding RNAs (ncRNAs) are potentially under explored part of biological gene regulation. Of the total RNA pool, many of them do not code a functional protein despite being transcribed but with important duties assigned other than coding. Splicing machinery decides in many instances on which to produce a functional protein. Though splice variants explained a few but consisted of translation machinery and its regulation though many left unexplained are to be explored. Many abundant structural and regulatory RNAs were distinguished and a few ncRNAs were confirmed of its involvement in imprinting and other cellular processes were identified by genetic studies. Further, small regulatory RNAs such as microRNAs were identified to regulate translation of mRNA to refine key genetic pathways (Kung *et* al., 2013). MYBs are a superfamily of transcription factors that mask regulatory roles in developmental processes and defense responses in plants. These are vital factors in regulatory networks controlling development, metabolism and responses to biotic and abiotic stresses (Dubos *et* al., 2010). MYB-type transcription factors (TFs) play fundamental roles in plant growth, development and respond to environmental stresses. There are 198 AtMYB genes identified in Arabidopsis genome sequence, among them, 126 are R2R3-MYB, 5 are R1R2R3-MYB, 64 are MYB-related, and 3 atypical MYB genes are reported. Most of MYB genes that are involved in response to diverse abiotic stress belonged to the R2R3-type group (Xiong *et* al., 2014).

MYB proteins are characterized by a highly-conserved DNA-binding domain: the MYB domain. Most MYB proteins function as transcription factors with varying numbers of MYB domain repeats conferring their ability to bind DNA. In plants MYB proteins and have been implicated in ABA response and operate with other transcription factors. Members of this family function in a diversity of plant-specific processes, as evidenced by their extensive functional characterization in Arabidopsis. In plants, for the first time MYB gene identified was C1 from Zea mays (Paz-Ares et al., 1987). The structure of MYB protein subfamily is different in plants containing mainly MYB protein subfamily characterized by the R2R3-type MYB domain. There are various functions of MYB family in plants that indicate their importance in the control of plant specific processes. Biological functions like phenylpropanoid metabolism biotic and abiotic stress (Segarra *et* al., 2009; Lippold *et* al., 2009), cell shape regulated by AmMIXTA (Noda *et* al., 1994), differentiation (Oppenheimer *et* al., 1991; Kang *et* al., 2009), hormone responses i.e. AtMYB2 (Urao *et* al., 1993), GaMYB and CpMYB (Gubler *et* al., 1995), formation of B-type cyclin (Ito et al., 2001) or during plant defense reactions by NtMYB1 are variedly controlled by the members of the MYB gene family. R2R3-MYB genes have been the extensively studied genes of MYB family. Myb1R is involved in regulation of circadian clock and telomeric DNA-binding protein (Schaffer *et* al., 1998).

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MATERIALS AND METHODS

Sequence retrieval for bioinformatic analyses

About 198 AtMYB sequences of A. thaliana were retrieved from The Arabidopsis Information System (https://www.arabidopsis.org/). A set of mature miRNAs of A. thaliana downloaded from miRBase (Release 21, http://www.mirbase.org/) consists of a total number of 427 known mature miRNAs.

Target prediction

The MYB sequences of A. thaliana were used as query against the miRBase A. thaliana mature miRNAs sequences using psRNATarget web server with the following parameters: (1) maximum expectation value 3; (2) length for complementarities scoring value 20; (3) Range of central mismatch leading to translational inhibition 9-11 nucleotide. Server psRNATarget provides reverse complementary matching between miRNAs and its target transcript and finds the target site accessibility by calculating unpaired energy (UPE) necessary for opening the secondary structure around the miRNA target site.

Gene network prediction

Gene network was predicted based on co-expression datasets mined from available online resources.

RESULTS AND DISCUSSION

In the present investigation, 198 AtMYB genes were analysed and found to play an intrinsic photosystem linked network of 38 nodes where MYB being regulated by a set of 48 miRNAs (table 1). Members of the network have evidence-based link to energy related mechanisms. Forty-eight potential miRNAs targeting AtMYB were identified in A.thaliana using psRNATarget server with at least one target mRNA identified for most of the A. thaliana miRNA families. These identified miRNAs targets a variety of gene families with diverse biological and physiological functions.

MYB transcription factors genes network

The predicted evidence-based network of AtMYB genes is depicted in figure 1. The non-MYB members of the network are explained here.

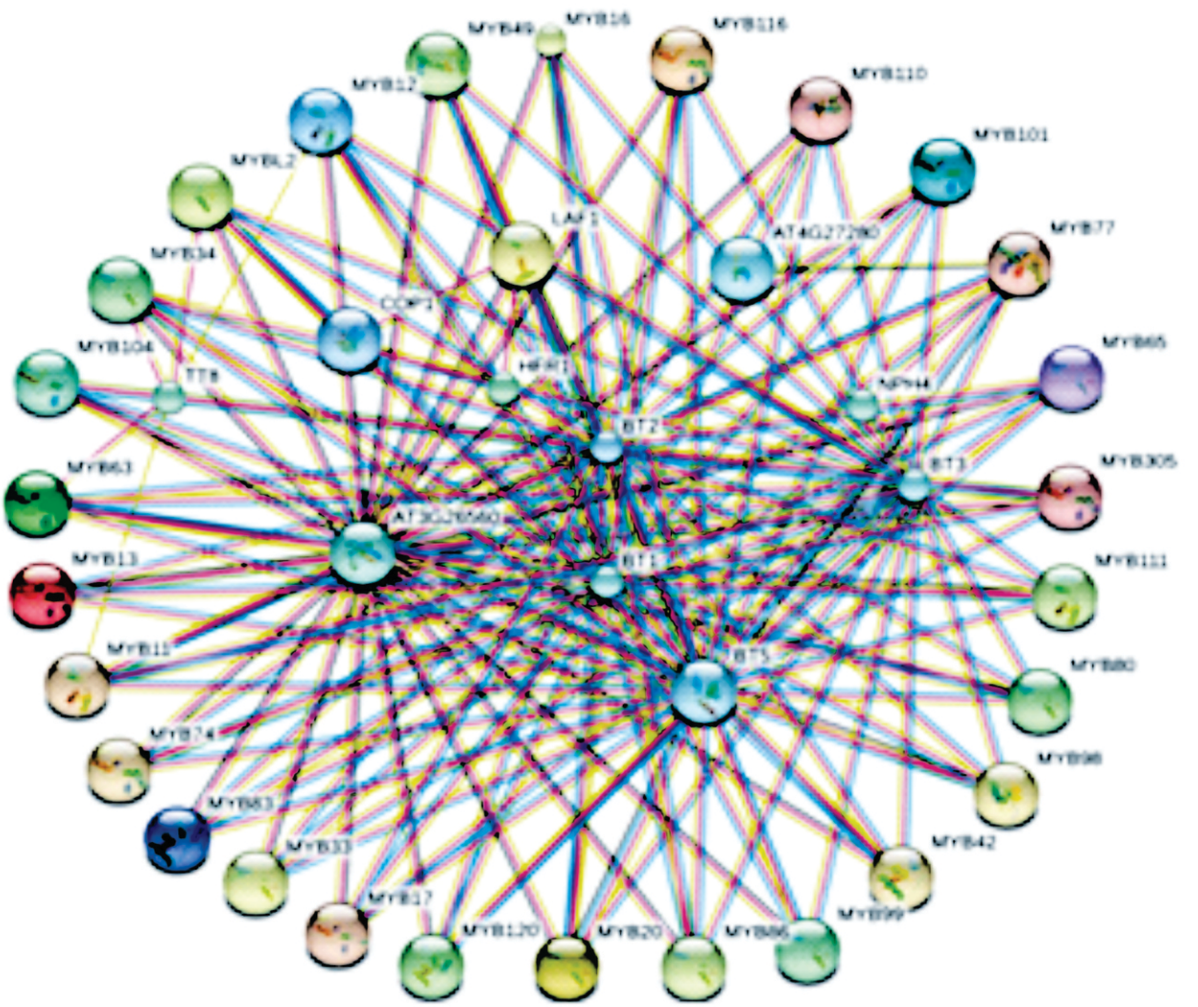

Fig.1: MYB gene network in Arabidopsis thaliana

BTB and TAZ domain
BT1 to BT5proteins have a typical domain structure that seen in plants. BT1 is a very short-lived protein constantly targeted for degradation by 26S proteasomes. Gene structure expression pattern and sequence analysis stipulate that BT1 and BT2 are closely concomitant. Molecular and phenotypic analyses of plants unveil notable redundancy amongst the BTB members. BTB plays a predominant role in development of male and female gametophyte (Robert *et al.,* 2009). BT3 and BT1 gene overtake BT2 gene functionally.

TT8
The role of TRANSPARENT TESTA 8 (TT8) is in the directive of flavonoid biosynthesis and formation of seed coat color. The effect of TT8 on seed Flavonoid Acid (FA) biosynthesis remains unheard. TT8 acted maternally to outturn seed FA biosynthesis and inhibited seed FA accumulation by downregulating a group of genes, which are either critical to embryonic development or FA biosynthesis. It has been observed that TT8 mutation reduced protein deposition in seeds during maturation. The main role of TT8 is to regulate FA biosynthesis in seeds (Baudry *et al.,* 2006).

COP1
CONSTITUTIVELY PHOTOMORPHOGENIC 1 (COP1), which is initially known as central repressor of seedling photo morphogenesis is known to be involved in the regulation of light input to the biological clock, modulating the circadian rhythm and flowering of plant. COP1 encompasses a RING-finger E3 ubiquitin ligase and works with SUPPRESSOR of phyA-105 (SPA) proteins to suppress photoperiodic flowering by synchronizing proteasome-mediated degradation of CONSTANS (CO), which is a central regulator of photoperiodic flowering. COP1 also indirectly modulate CO expression via the degradation of GIGANTEA (GI). It mainly helps in the regulation of photoperiodic flowering (Bauer *et al.,* 2004).

LAF1
LAF 1 transcription factor intricates photo morphogenesis in the presence of light by taking part in transmission of phytochrome-A signals to downstream responses. It probably plays role in activating expression of light-induced genes whereas in darkness, its degradation prevents the activation of light-induced genes (Jiao *et al.,* 2007).

HFR1
HFR 1 encodes a light directed, nuclear bHLH protein involved in phytochrome signaling. HFR 1 is said to interact with COP1, it co-localizes the nuclear specks and is ubiquitinylated by COP1. Mutants exhibit a long-hypocotyl phenotype only under far-red light but not under red light and are defective in other phytochrome A-related responses.

AT4G27280
These are the Calcium binding EF hand family proteins, which helps in calcium binding. They are also involved in response to karrikin. These proteins expressed in 23 diverse plant structures and co-localise in 13 growth stages (Walley *et al.,* 2007).

NPH4
Auxin response factors (ARFs) are transcriptional factors that bind specifically to the DNA sequence 5'-TGTCTC-3' that exists in auxin-responsive promoter elements (AuxREs). It acts as a transcriptional activator of several tropic stimulus-induced (TSI) genes. These are involved in ethylene responses and regulate lateral root formation. It also mediates embryo axis formation and vascular tissues differentiation (Labusch *et al.,* 2013).

AT3G26560
An ATP dependent RNA helicase, which functions in changing expression of genes in a wide range of pathways associated with elongation growth and stress responses. Phytochrome family of photoreceptors (Devlin *et al.,* 2013) perceives photon quality.

DISCUSSION
Earlier reports explained upregulated MYB factors in various abiotic stress conditions (table 2). R2R3-type MYB transcription factor are primarily responsible for regulating drought stress response by integrating ABA and auxin signals (Seo *et al.,* 2009). OsMYB3R-2 overexpression showed increased tolerance to cold, drought, and salt stress. MYB transgenic plants are reported to be more tolerant to abscisic acid (AA) or NaCl stress. AtMYB41 transcription factor when overexpressed was controlling cell expansion, cuticle deposition and leaf surface permeability in response to abiotic stress (Cominelli *et al.,* 2008). Some proteins like dehydration responsive element binding protein 2A, COR15a and RCL2A have been upregulated in the presence of OsMYB3R. Transgenic overexpression patterns were observed when compared to wild type of Arabidopsis (Dai *et al.,* 2007). MYB transcription factors were involved in drought response, stomatal movement and regulation of flower development (Ambawat *et al.,* 2013). Overexpression of AtMYB44 conferred abiotic stress tolerance through enhanced stomatal closure in transgenic Arabidopsis (Jung *et al.,* 2008).
After thorough investigation of the network, it has been observed that MYB overexpression under various abiotic stress conditions is a common phenomenon at mRNA level and it may not be stable functional protein level due to the presence of regulatory elements. MYB overexpression under stress may not necessarily end up with stable transgenic line overtime as the network is indicating major complex of ncRNAs and their regulatory action.

ncRNAs Biosynthesis
These non-coding transcripts are always capped and polyadenylated and assumed not to have any biological function. LncRNAs are RNAs larger than 200 bp, which may or may not have enough coding potential. But lncRNAs are distinguishable from small regulatory RNAs such as miRNAs or piRNAs by its sequence length and structure. LncRNAs are transcribed by RNA polymerase II or III, and additionally, by polymerase IV/V in plants (Dinger *et al.,* 2009). LncRNAs are classified primarily based on four major features, namely,

genomic location, functions exerted on DNA or RNA, functioning mechanisms, and targeting mechanisms (Kim *et al.,* 2012). Typically, defined snRNAs and pri-miRs are in fact greater than 200 nucleotides (Li *et al.,* 2016). The mRNA primary coding sequence (CDS) plays a big role in the translation, while lncRNAs regulate a competing mRNA expression through the interactions between miRs, ceRNAs, piRNAs and by their higher-order structures and major interacting co-localized proteins (Liu *et al.,* 2015).

Mode of action

LncRNAs are extremely tissue and time-specific in terms of developmental expression patterns. In recent reports lncRNAs were found to interact with other macromolecules of the cell including DNA, RNA and transcription factors participating in various biological processes, such as DNA methylation, histone modification and chromatin remodeling resulting in the downregulation or overexpression of target genes or ceRNAs. All lncRNAs perform their functions mainly in four ways: as signals, decoys, guides or scaffolds. LncRNAs have the potential to regulate the expression of functional RNA and not necessarily a non-coding. Depending upon the regulation pattern, it can also produce functional RNA.

Breakdown of introduced gene expression

Silencing of introduced genes are also the function of miRs and lncRNAs; in many instances it needs case by case or gene by gene approach for detailed explorations. Functional analyses of lncRNAs have shown that they are potent cis- and trans-regulators of gene transcription, and act as scaffolds for chromatin-modifying complexes. As potent regulatory components involved in gene regulation from various aspects, lncRNAs can exert their effects during tissue development and in response to external stimuli (Ma *et al.,* 2013).

Plant lncRNAs can act as precursors of miRNAs and other sRNAs, they either act directly or as processed to a shorter ncRNA for gene regulation. Under no situation, a cell favors the accumulation of a foreign protein or a gene product. The plant cell will be working towards a balance by feedback regulation which may either directly or indirectly affect their pH balance and osmotic potential (Yasin and Magadum, 2016a; Yasin and Magadum, 2016b). Some lncRNAs are primary transcripts of small regulatory RNAs such as miRNAs and siRNAs. Plant lncRNAs also act as miRNA target mimics (Mishra *et al.,* 2016). Target mimicry that emerged as a unique mechanism for regulating mRNA functions. Interactions between miRNAs and their authentic targets are blocked by the binding of decoy RNAs or ceRNAs to miRNAs via partial sequence complementarities during target mimicry (Liu *et al.,* 2015).

The various regulatory properties of lncRNAs in plants include the LDMAR lncRNA affects male fertility in rice, it functions to regulate PSMS (photoperiod-sensitive male sterility) and helps in providing a meaningful tool for generating hybrid rice. LncRNAs also direct protein re-localization in symbiosis as Enod40 which is one of the first lncRNAs that has been identified in plants, has role in regulation of symbiotic interactions between leguminous plants and soil bacteria. LncRNAs function as miRNA Target mimics which have been known by the resemblance of work in Arabidopsis thaliana with that of the miRNA sponges in animal system related to mechanism of lncRNA function that shows that some plant lncRNAs can interact with miRNAs as competitors and function as miRNA target mimics (Mishra *et al.,* 2016). Plant lncRNAs also help in the regulation of flowering as in A. thaliana, there are varied pathways which regulate expression of the floral inhibitor FLC (Flowering Locus C) to fine-tune flowering time. LncRNAs have role in RdDM also which is a plant specific pathway that include de novo DNA methylation that can be shown by many examples in

Table 1: List of identified miRNAs targeting MYB genes in Arabidopsis thaliana

miRNA_Accessions	Target Accessions	miRNA aligned fragment	Target *aligned* fragment	Target Genes	Target_Desc.
ath-miR5658	AT5G56110.1	AUGAUGAUGAUGAUGAUGAAA	AACCAUCAUCAUCAUCAACAU	AtMYB103, ATMYB80, MYB103, MS188	MYB domain protein
ath-miR319a	AT2G26950.1	UUGGACUGAAGGGAGCUCCCU	UUGGAGCUCCCUUCAUUCCAA	AtMYB104, MYB104	MYB domain protein
ath-miR319b	AT2G26950.1	UUGGACUGAAGGGAGCUCCCU	UUGGAGCUCCCUUCAUUCCAA	AtMYB104, MYB104	MYB domain protein
ath-miR159a	AT2G26950.1	UUUGGAUUGAAGGGAGCUCUA	UGGAGCUCCCUUCAUUCCAAG	AtMYB104, MYB104	MYB domain protein
ath-miR159b-3p	AT2G26950.1	UUUGGAUUGAAGGGAGCUCUU	UGGAGCUCCCUUCAUUCCAAG	AtMYB104, MYB104	MYB domain protein
ath-miR159c	AT2G26950.1	UUUGGAUUGAAGGGAGCUCCU	UGGAGCUCCCUUCAUUCCAAG	AtMYB104,MYB104	MYB domain protein
ath-miR858a	AT3G62610.1	UUUCGUUGUCUGUUCGACCUU	CGGGAAGAACAGACAACGAAA	ATMYB11, PFG2, MYB11	MYB domain protein
ath-miR858b	AT3G62610.1	UUCGUUGUCUGUUCGACCUUG	CCGGGAAGAACAGACAACGAA	ATMYB11, PFG2, MYB11	MYB domain protein
ath-miR5658	AT5G55020.1	AUGAUGAUGAUGAUGAUGAAA	AACCAUCAUCAUCAUCAUCAU	AtMYB120, MYB120	MYB domain protein
ath-miR159c	AT5G55020.1	UUUGGAUUGAAGGGAGCUCCU	AGCAGCUCCCUUCAAACCAAA	AtMYB120, MYB120	MYB domain protein
ath-miR858a	AT1G06180.1	UUUCGUUGUCUGUUCGACCUU	CUGGACGAACAGACAACGAAA	AtTMYB13, ATMYBLFGN, MYB13	MYB domain protein

ath-miR5658	AT5G15310.1	AUGAUGAUGAUGAUGAUGAAA	UCUCAACCUCAUCAUCAUCAU	AtTMYB16,ATMIXTA, MYB16	MYB domain protein
ath-miR858b	AT3G61250.1	UUCGUUGUCUGUUCGACCUUG	CCAGGAAGAACAGACAACGAG	AtMYB17, MYB17	MYB domain protein
ath-miR5658	AT4G25560.1	AUGAUGAUGAUGAUGAUGAAA	CUUCGUCUUCAUCAUCAUCAC	AtMYB18, MYB18	MYB domain protein
ath-miR858a	AT5G60890.1	UUUCGUUGUCUGUUCGACCUU	CGGGACGAACUGACAACGAAA	AtTMYB34,ATR1,MYB34	MYB domain protein
ath-miR5658	AT1G79180.1	AUGAUGAUGAUGAUGAUGAAA	AAGAAUCAUCAUCAUCAUCAC	AtTMYB63,MYB63	MYB domain protein
ath-miR159a	AT3G11440.1	UUUGGAUUGAAGGGAGCUCUA	UGGAGCUCCCUUCAUUCCAAU	AtTMYB65,MYB65	MYB domain protein
ath-miR159b-3p	AT3G11440.1	UUUGGAUUGAAGGGAGCUCUU	UGGAGCUCCCUUCAUUCCAAU	AtTMYB65,MYB65	MYB domain protein
ath-miR159c	AT3G11440.1	UUUGGAUUGAAGGGAGCUCCU	UGGAGCUCCCUUCAUUCCAAU	AtTMYB65,MYB65	MYB domain protein
ath-miR5658	AT4G05100.1	AUGAUGAUGAUGAUGAUGAAA	CAUCAUCAUCAUCAUCAUCAU	AtMYB74,MYB74	MYB domain protein
ath-miR5658	AT5G26660.1	AUGAUGAUGAUGAUGAUGAAA	GAUCAACAUCAUCAUCAUCAA	AtTMYB86, MYB86	MYB domain protein
ath-miR858b	AT1G71030.1	UUCGUUGUCUGUUCGACCUUG	CCAGGACGAACCGACAACGAA	AtTMYBL2,MYBL2	MYB-like
ath-miR159a	AT2G32460.1	UUUGGAUUGAAGGGAGCUCUA	UAGAGCUUCCUUCAAACCAAA	MYB101,AtMYB101,ATM1	MYB domain protein
ath-miR159b-3p	AT2G32460.1	UUUGGAUUGAAGGGAGCUCUU	UAGAGCUUCCUUCAAACCAAA	MYB101,AtMYB101,ATM1	MYB domain protein
ath-miR159c	AT2G32460.1	UUUGGAUUGAAGGGAGCUCCU	UAGAGCUUCCUUCAAACCAAA	MYB101,AtMYB101,ATM1	MYB domain protein
ath-miR5021	AT2G32460.1	UGAGAAGAAGAAGAAGAAAA	UUCUCUUCUUCAUCUUCUCA	MYB101,AtMYB101,ATM1	MYB domain protein
ath-miR414	AT3G29020.2	UCAUCUUCAUCAUCAUCGUCA	UGUAGAUGAUGAUGAAGAUGU	MYB110	MYB domain protein
ath-miR858a	AT5G49330.1	UUUCGUUGUCUGUUCGACCUU	CAGGAAGAACAGACAACGAAA	MYB111,AtMYB111,PFG3	MYB domain protein
ath-miR858b	AT5G49330.1	UUCGUUGUCUGUUCGACCUUG	CCAGGAAGAACAGACAACGAA	MYB111,AtMYB111,PFG3	MYB domain protein
ath-miR858b	AT1G25340.1	UUCGUUGUCUGUUCGACCUUG	CCAGGAAGAACAGACAACGAU	MYB116,AtMYB116	MYB domain protein
ath-miR858a	AT2G47460.1	UUUCGUUGUCUGUUCGACCUU	CAGGGAGAACAGACAACGAAA	MYB12,AtMYB12,PFG1	MYB domain protein
ath-miR858a	AT2G47460.1	UUUCGUUGUCUGUUCGACCUU	CAGGGAGAACAGACAACGAAA	MYB12,AtMYB12,PFG1	MYB domain protein
ath-miR858a	AT1G66230.1	UUUCGUUGUCUGUUCGACCUU	CAGGAAGAACAGACAACGAAA	MYB20,AtMYB20	MYB domain protein
ath-miR858b	AT1G66230.1	UUCGUUGUCUGUUCGACCUUG	CCAGGAAGAACAGACAACGAA	MYB20,AtMYB20	MYB domain protein
ath-miR858a	AT3G24310.1	UUUCGUUGUCUGUUCGACCUU	CGGGAAGAACAGACAACGAAA	MYB305,AtMYB71	MYB domain protein
ath-miR858b	AT3G24310.1	UUCGUUGUCUGUUCGACCUUG	CCGGGAAGAACAGACAACGAA	MYB305,AtMYB71	MYB domain protein
ath-miR159a	AT5G06100.2	UUUGGAUUGAAGGGAGCUCUA	UGGAGCUCCCUUCAUUCCAAU	MYB33,AtMYB33	MYB domain protein
ath-miR159b-3p	AT5G06100.2	UUUGGAUUGAAGGGAGCUCUU	UGGAGCUCCCUUCAUUCCAAU	MYB33,AtMYB33	MYB domain protein
ath-miR159c	AT5G06100.2	UUUGGAUUGAAGGGAGCUCCU	UGGAGCUCCCUUCAUUCCAAU	MYB33,AtMYB33	MYB domain protein
ath-miR858a	AT4G12350.1	UUUCGUUGUCUGUUCGACCUU	CAGGAAGAACAGACAACGAAA	MYB42,AtMYB42	MYB domain protein
ath-miR5658	AT5G54230.1	AUGAUGAUGAUGAUGAUGAAA	UUUCAUCAUCAUCAUGAUCAU	MYB49,AtMYB49	MYB domain protein
ath-miR5021	AT3G50060.1	UGAGAAGAAGAAGAAGAA-AA	UUCUUCUUCUUCUUCUUCUCU	MYB77	MYB domain protein
ath-miR858b	AT3G08500.1	UUCGUUGUCUGUUCGACCUUG	CCAGGUAGAACAGACAACGAG	MYB83,AtMYB83	MYB domain protein
ath-miR858a	AT3G08500.1	UUUCGUUGUCUGUUCGACCUU	CAGGUAGAACAGACAACGAGA	MYB83,AtMYB83	MYB domain protein
ath-miR5658	AT4G18770.1	AUGAUGAUGAUGAUGAUGAAA	UUUGAUCAUCAUCAUCAUCAU	MYB98,AtMYB98	MYB domain protein
ath-miR5658	AT4G18770.1	AUGAUGAUGAUGAUGAUGAAA	CUUCUUCUUCAUCAUCAUCAU	MYB98,AtMYB98	MYB domain protein
ath-miR414	AT4G18770.1	UCAUCUUCAUCAUCAUCGUCA	ACAUGAUGAUGAUGAGGAUGA	MYB98,AtMYB98	MYB domain protein
ath-miR858b	AT5G62320.1	UUCGUUGUCUGUUCGACCUUG	CCAGGAAGAACAGACAACGAU	MYB99,AtMYBCU15,AtMYB99	MYB domain protein

model plants, such as maize and Arabidopsis. Genome-wide identification of lncRNAs in plants has helped in providing a newwayt to identify the broadly diverse mechanisms of lncRNAs.

Emerging techniques that identify large amounts of lncRNAs with different characteristics have produced substantial advances in our knowledge. In plants, we can identify lncRNAs at the genome-wide level, test their different expression patterns, analyze their distribution and expression levels in different developmental stages and tissues, investigate the action of lncRNAs on their targets, and so on. Thus, we can improve our understanding of the genetic mechanism of lncRNAs in plants and extend our knowledge of the roles of lncRNAs in all species (Quan *et al.*, 2015).

In crop plants, some of the epigenetic mechanisms of ncRNAs have also been known and the complex network of ncRNAs shows the potential regulatory roles of ncRNAs in plants. Thus, ncRNAs in plants can be considered as essential elements of gene regulation. Despite the fact that, few plant lncRNAs have been identified and functionally investigated till date, there are findings which explains that plant lncRNAs have important role in regulating complex gene regulatory networks involved in plant development and stress tolerance (Crespi *et al.*, 1994; Ding *et al.*, 2012; Heo and Sung 2011; Mishra *et al.*, 2016).

They carry out their task by regulating the expression levels of target genes from transcription to translation. Although there are preceding biological importance of ncRNAs, together with low sequence conservation, diversity of their molecular mechanisms, and interaction with other proteins

Table 2: List of MYB overexpression events reported

Gene	Plant	Impact	Citation
OsMYB48-1	Rice	Overexpression significantly improved tolerance to simulat ed drought and salinity stress. Positive role in drought and salinity tolerance by regulating stress induced ABA synthesis.	Xiong *et al*., 2014
MsMYB31	Banana	Overexpression in transgenic banana plants evaluated its potential role in regulating biosynthesis of lignin and polyphenols.	Tak *et al*., 2017
OsMYB3R-2	*Arabidopsis thaliana*	Overexpression showed increased tolerance to cold, drought, salt stress and the seed germination of transgenic plants was more tolerant to abscisic acid or NaCl than that of wild type.	Dai *et al*., 2007
BplMYB46	*Betula platyphylla*	Overexpressing or silencing, it improves salt and osmotic stress tolerance, higher lignin and cellulose content and lower hemicelluloses content.	Guo *et al*., 2016
AtMYB37	*Arabidopsis thaliana*	Overexpression confers hypersensitive phenotypes to exogenous ABA in all the major ABA responses. Improves plant tolerance to drought, enhances growth of mature plants and seed productivity.	
TaPlMP1	Transgenic Wheat	Overexpression significally enhanced resistance to the fungal pathogen *Bipolaris sorokiniana* and drought stresses.	Zhang *et al*., 2012
GmMYB12B2	Transgenic *Arabidopsis thaliana*	Overexpression induced by UV irradiation and salt treatment, but no response was detected under low temperature, drought and ABA stresses. It might be involved in response of plants to UV radiation and salt stresses.	Li *et al*., 2016
AtMYB28 or AtMYB99	*Arabidopsis Thaliana*	Vital role in development and response to abiotic stress. Over expression confers hypersensitivity to exogenous ABA during seed germination, cotyledon greening and early seedling growth.	
OsMYB2	Rice	Enhanced upregulation of genes encoding proline synthase and transporters. Plays important role in tolerance of rice to salt, cold and dehydration stress.	
AtMYB44	Transgenic *Arabidopsis thaliana*	Enhances stomatal closure to confer abiotic stress. Reduced expression of genes encoding PP2Cs which have been described as negative regulats of ABA signaling.	Jung *et al*., 2008

are highly sequence-specific. However, ncRNA application on crop plant engineering is a critical task at present with available resources. As we are in genomic era and whole genome of many crop species are available still its exploration for crop improvement is in infancy and we are still exploring genome wide markers for mapping and marker- aided selection of agronomically important crop traits (Chaudhary *et al.,* 2016). The present investigation indicated that MYB overexpression under stress might not necessarily end up with stable transgenic line overtime as the network is indicating major complex of ncRNAs and their regulatory action. For efficient application, such tools like ncRNAs in molecular breeding, those difficulties ought to be solved through distinctive additional cases of plant ncRNAs and developing new set of markers and tools for ncRNAs targeted SMART crop breeding.

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