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Review Article

Role of Epigenetics in Crop Improvement: Water and Heat Stress

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Abstract Overall transcriptomic activity of an organism is determined not only by its genetic makeup but also by epigenetic regulations. Reprogramming by epigenetic modification triggered by various environmental challenges contributes to the phenotypic diversity and defense against these challenges. Different stresses acts as stimulus and changes genic expression level by various mechanisms namely DNA methylation, histone modifications and miRNA. Epigenetics is a continuously progressing branch since the past two decades. Improvement of crop varieties with traditional breeding methods is tedious, time consuming, expensive and is unable to meet progressive living standards and demand in today's world whereas epigenetics holds great potential for the improvement of plant varieties with respect to yield and nutritional quality by creation of novel epialleles, transgenic RNAi. RNA interference has been used for the improvement of several crops like barley, wheat, soybean etc by reduction of various anti-nutritive factors or enhancement of flavor. Silencing of Omega – 3 fatty acid desaturase gene is achieved using RNAi technology for the reduction of linoleic acid and improvement of flavor. HvTX1 from barley shows increased expression during drought stress indicating its active role in drought tolerance which further can be exploited for the development of improved crop variety with increased drought tolerance. Also multiple HAT genes in rice are reported to be actively expressed under heat and water stress. Studies of epigenetic responses to different stresses can increase our understanding of plant stress adaptation and mechanism underlying them which can be further exploited for the development of improved crop varieties.

Keywords: Crop Improvement, Drought, Epigenetics, Heat, Stress

Introduction

Plants are sessile organism and hence are constantly challenged by various biotic and abiotic stresses like heat, water deficit and pathogens. Adaptation to these surrounding needs constant and frequent changes at molecular as well as morphological level. These efficient and effective controls are provided by epigenetic regulations which ultimately improve the survivability of plants by increasing the stress tolerance. Epigenetics as defined by Conrad professor of genetics at Edenberg University in 1940 is "the study of mitotically and/or meiotically heritable changes in gene function that cannot be explained by changes in DNA sequence" (Vincenzo et al. 1998). Now it is commonly referred to any chemical and heritable change in DNA and histone protein that leads to changes in chromatin structure and expression. It basically has evolved from word epigenesis which means the study of embryological growth and differentiation. Epigenetic profile related to specific phenotype and environmental cue becomes important to understand their contribution to crop improvement.

There are three epigenetic regulatory mechanism first DNA methylation second histone modification and third RNA interference (RNAi). DNA methylation is a chemical modification mainly catalyzed by cytosine methyltransferases which involves addition of methyl group in DNA sequence onto the position 5 of pyrimidine ring in the cytosine residue. It mostly occurs in a sequence specific manner, primarily within CpG dinucleotide. The addition of methyl group in DNA sequence acts as a binding site for various protein complexes that modifies the histone scaffolds containing the DNA and as a result changes the expression level of corresponding gene. Histones undergo both post translational modifications as well as have several variants which are expressed differentially under specific conditions. Linker histone variant H1-S is reported to be induced by drought in tomato (Scippa et al. 2004). Other than multiple variants histone undergo numerous modifications like acetylation, methylation, sumoylation, ubiqutination and phosphorylation. Acetylation and phosphorylation are mostly found out to

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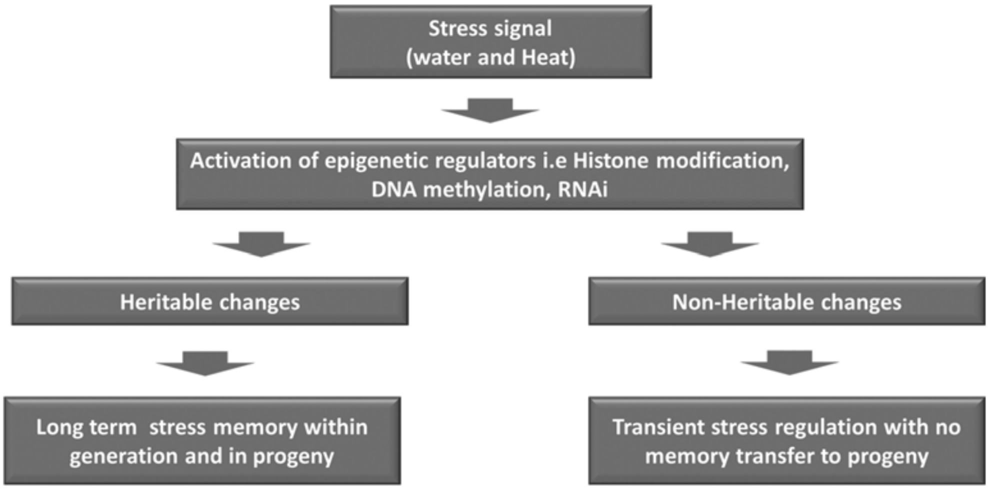
Stress signal
(water and Heat)
Activation of epigenetic regulators i.e Histone modification, DNA methylation, RNAi
Heritable changes
Non-Heritable changes
Long term stress memory within generation and in progeny
Transient stress regulation with no memory transfer to progeny

Fig. 1. Activation and effect of Epigenetics regulation in response to stresses. Epigenetic regulation can lead to either transient stress regulation or long term stress memory leading to non-heritable and heritable changes respectively.

induce gene expression while on the other hand modifications like sumoylation and biotinylation represses gene expression (Nathan et al. 2006; Camporeale et al. 2007). RNAi is an evolutionary conserved and sequence specific gene regulation. They were initially thought to play role in the defense mechanism against viruses and pathogens but later on found to play important role in the gene regulation as well. Plants exhibit mainly two types of RNAi, siRNA and miRNA which are synthesized as short single stranded RNA molecules of size ~20~30 nucleotide from a double stranded RNA precursor. Apart from showing individual effects epigenetic mechanism shows high degree of correlation and combinatorial interaction also. Observable points are the relationship between DNA methylation and siRNA, most siRNA clusters are heavily methylated also SUP32/UBP26 (a deubiquitination enzyme) mutant of Arabidopsis suppressed siRNA directed DNA methylation as well as dimethylation of H3K9 (Sridhar et al. 2007). Slat stress inducible protein GmPHD5 in soybean is found to interact with other DNA binding protein GmGNAT1 is responsible for a crosstalk between histone acetylation and methylation (Wu et al. 2011). Furthermore a high degree of methylation of H3K9m2 in a large number of genes has been observed in met1 mutant in *A. thaliana* indicating some kind of relationship between them (Deleris et al. 2012). Epigenetics holds great potential for the improvement of plant varieties with respect to yield, nutritional quality and stress tolerance by creation of novel epialleles, transgenic RNAi (Fig. 1).

Histone Modification and Crop Improvement

Histone proteins are found basically in eukaryotes and some archaea to which DNA wraps around and forms a highly compact structure known as nucleosome. Histone N terminal in the nucleosome known as tail is rich in basic amino acids like lysine and arginine. Histone undergo several covalent modifications like acetylation, methylation, phosphorylation, ubiquitination and biotinylation in response to various environmental stresses and regulates the transcription of wrapped DNA sequence. These modifications alter the packaging structure which either activates the DNA for the transcription or makes the structure even condensed so that transcription machinery is unable to bind to it.

Acetylation/Deacetylation

Acetylation of histone protein occurs at the N terminal lysine and leads to the activation of DNA sequence transcriptionally (Tang et al. 2016). What happens is the histone protein which is positively charged due to the addition of an acetyl group to the lysine leads to the reduction in the net positive charge of histone and as a result of which the electrostatic force of attraction between the negatively charged DNA and positively charged histone reduces which further leads to the loosening of the DNA supercoiled structure and activation of DNA transcriptionally (Shahbazian and Grunstein 2007).

The addition of acetyl group is done by HATs (Histone acetyl transferases). In eukaryotes HATs belongs to five different classes GNAT – GCN5 related N terminal acetyl transferse; MYST – MOZ, Ybf2/Sas3, Sas2, and Tip60; CBP - CREB – binding protein; TFII250 - TATA - binding protein-associated factors and the nuclear hormone-related HATs family (Sterner and Berger 2000) (Fig. 2). But in rice and *Arabidopsis thaliana* HATs belongs to four classes that is GNAT, CBP, MYST and TFII250. Different number of HATs have been reported in different plant species, in rice 8 different HATs have been reported so far belonging to above four different classes (Liu et al. 2012). Elongator, a histone

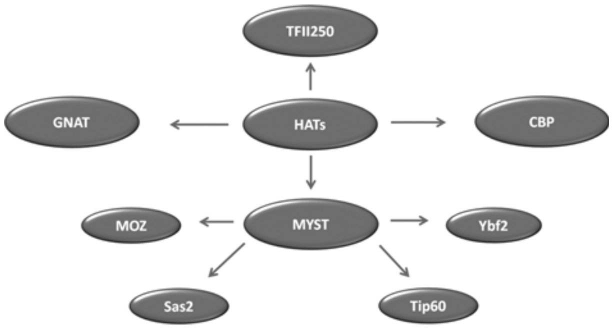

Fig. 2. Major classes of HATs in plants.

HAT complex consisting of six subunits, ELP1 to ELP6, and is highly conserved in eukaryotic organisms (Versées et al. 2010). In Arabidopsis, the elongator HAT complex is reported to be involved in drought responses (Chen et al. 2006; Hu et al. 2009). Not all the lysine at the N-terminal is susceptible to acetylation. Different residues in different histone proteins undergo modification for example in Arabidopsis, lysine residues of H4 (K5, K8, K12, K16, and K20) and histone H3 (K9, K14, K18, K23, and K27) are subjected to acetylation modifications (Earley et al. 2007).

When challenged with stress plants undergo diverse histone acetylation. Drought stress in *A.thaliana* induces an increase of H3K9ac in the promoter regions of stress-responsive genes, RD29A, RD29B, RD20, and RAP2.4 (Kim et al. 2008; Chen and Wu 2010). In Arabidopsis, ABO1 (ABA OVERLAY SENSITIVE 1) encodes a homolog of yeast elongator subunit ELP1. The abo1 mutant showed a drought resistant phenotype (Chen et al. 2006). Furthermore, the abo1 mutation enhanced ABA induced stomata closing. These findings suggest that the HAT elongator complex plays an important role in, drought and oxidative stress responses in *Arabidopsis* (Kim et al. 2008). In a study nitric oxide was found to increase the overall histone acetylation by inhibiting histone deacetylase in *Arabidopsis thaliana*. Functional analysis of the genes revealed that majority of them are involved in abiotic stress responses (Mengel et al. 2016). Study conducted on maize to find correlation of histone acetylation level in cell cycle genes under stress condition revealed an increase in total acetylation levels in the roots of maize seedlings in comparison with the control group indicating activation of several genes under the stress condition. Surprisingly, this increase was especially remarkable under heat stress. It was also observed that the signals like H3K9ac and H4k5ac, which are regarded as positive histone modification marks and are associated with gene activation, were evenly detected in the nucleus, on the other hand heterochromatin knobs and nucleoli barely showed any difference between control and treated samples (Zhao et al. 2014a). Maize seedlings showed upregulation of stress responsive gene ZmDREB2A (Zea mays dehydration-responsive element binding protein 2A) when grown under osmotic stress. Real-time PCR analysis of ZmDREB2A revealed that the H3K9 and H4K5 at the promotor region undergoes quick hypermethyation during stress indicating fast induction of the gene (Zhao et al. 2014b). In *Arabidopsis thaliana* histone deacetylase proteins HD2C and HD6 were found to regulate ABA responsive genes ABI1 and ABI2 when challenged with ABA stress by H3K9 and H3K14 acetylation in promoter region(Luo et al. 2012)**.** In a similar study conducted by Chen et al HDA6 mutant under ABA stress showed less expression of ABA responsive genes ABI1, ABI2 and RESPONSIVE TO DESSICATION 29B (RD29B) indicating HDA6 role in gene activation (Chen et al. 2010). HDA9, Arabidopsis histone deacetylase is found to modulate transcription of 60 genes under drought condition. HAD9 nonfunctional mutants showed higher degree of induction in these genes when compared to the wild type. Out of 60 genes, 40 showed increased expression while rest were downregulated indicating that HDA9 might be involved in the deacetylation of these genes. ChIP analysis of 14 upregulated genes revealed increase in H3K9ac in *had9* mutant indicating decreased acetylation of promotors in these genes (Zheng et al. 2016). Genomewide analysis of H3K4 trimethylation in rice revealed differential methylation of 4837 genes out of which 3927 showed increased expression while 910 showed decreased total transcript under drought conditions (Zong et al. 2013).

Methylation

Two types of amino acid Lysine and arginine in histone can undergo methylation. Unlike acetylation methylation shows diverse pattern, a number of different arginine and lysine residues can undergo methylation (R3 of H2A, R3, K20 of H4 and K4, K9, K27, K36, R2, and R17 of H3 etc.) and that too with varying nature of methylation, these residues can be mono, di or tri methylated (arginine undergo mono and di methylation only while lysine can undergo mono, di and tri methylation). Depending on the nature of methylation it can either activate or deactivate a gene for example H3K4 trimethylation activates transcription on the other hand K9 and K27 dimethylation in H3 acts as a repressor. Methylation does not alter net charge on histone protein like acetylation but it does effect the hydrophobicity by the addition of methyl groups to the histone and hence may change histone DNA interactions or may create binding site for various proteins which restrict the binding of transcription machinery and inhibits the transcription.

Histone lysine methyl transferases (HKMT) and protein arginine methyl tranferases (PRMT) are responsible for the methylation of lysine and arginine respectively. AtATX1/ SDG27 Arabidopsis homolog of TRITHORAX1, is a methyltransferase of H3K4me3. Ding et al have reported that

Table 1. Different genes responsible for histone modification in various crops

Enzyme	Gene	Function	Plant	Reference
HATs	AtABO1	Drought and oxidative stress tolerance	Arabidopsis	(Chen et al. 2006)
	OsHAG702	Heat stress tolerance	Rice	(Liu et al. 2012)
	OsHAG703	Drought stress tolerance	Rice	(Liu et al. 2012; Fang et al. 2014)
	OsHAG704	Heat stress tolerance	Rice	(Liu et al. 2012)
	OsHAM701	Drought stress tolerance	Rice	(Liu et al. 2012; Fang et al. 2014)
	OsHAC701	Heat stress tolerance	Rice	(Liu et al. 2012)
	OsHAC703	Drought stress tolerance	Rice	(Liu et al. 2012; Fang et al. 2014)
	OsHAC704	Heat stress tolerance	Rice	(Liu et al. 2012)
	OsHAF701	Drought stress tolerance	Rice	(Liu et al. 2012; Fang et al. 2014)
HMTs	AtATX1	Drought stress tolerance	Arabidopsis	(Ding et al. 2011)
	HvTX1	Drought stress tolerance	Barley	(Papaefthimiou and Tsaftaris 2012)
HDMs	HvPKDM7	Drought stress tolerance	Barley	(Papaefthimiou and Tsaftaris 2012)
	AtMSI1	Drought stress tolerance	Arabidopsis	(Alexandre et al. 2009)
	AtCHR12	Drought and Heat stress tolerance	Arabidopsis	(Alexandre et al. 2009)
	AtBRM	Drought stress tolerance	Arabidopsis	(Berr et al. 2012)

AtATX1 is involved in drought and SA pathway responses (Liu et al. 2010; Ding et al. 2011) while on the other hand AtATX1 mutant displayed larger stomatal apertures, increased transpiration, and hence decreased tolerance to dehydration stress. HvTX1, barley TRX-like H3K4 methyltransferase, has been shown to be involved in drought stress. The transcription rate of HvTX1 were found increased under drought stress (Schwartz et al. 2010; Papaefthimiou and Tsaftaris 2012).

Studies conducted by Qian et al on maize have shown that 18 genes containing SET domain from maize showed differential expression under salt and drought stress suggesting a role of these 18 genes in drought and salt stress (Qian et al. 2014). Different genes responsible for histone modification in various crop under the influence of stress is given in Table 1.

DNA Methylation and Crop Improvement

DNA methylation is a chemical modification which arises from the addition of a methyl group to the nitrogenous base in the DNA strand in the sequence specific manner. These nitrogenous bases can be either cytosine or it can be adenine but mostly cytosine. Methylation of cytosine leads to the generation of 5-methyl cytosine. Being sequence specific in nature it occur at three kinds of positions CG, CHG, CHH (H being any base other than G) (Law and Jacobsen 2010). In case of mammals methylation is mostly seen only at CG sequences but in case of plants it occurs at all the three sequences (Feng et al. 2010). Studies have shown that the genome wide DNA methylation level at different positions in Arabidopsis is reported to be 24%, 6.7%, and 1.7% for CG, CHG, and CHH contexts, respectively. On the basis of the target sequence DNA methylation is classified either as symmetrical or asymmetrical methylation. CG and CHG methylation are termed as symmetrical and CHH methylation as asymmetrical.

DNA methylation shows different behavior according to the location of methylation but mostly is correlated with the repression of gene. Methylation in transposable elements and promoter region of a gene leads to the silencing (Chen et al. 2006; Li et al. 2012) on the other hand methylation inside gene body has shown to positive regulate gene expression (Lu et al. 2015). Increase in genomic DNA methylation may down regulate expression of the transcriptome slowing down the metabolism of the plant, which enables it to conserve energy for the sake of biotic or abiotic stress, and helps the plant to overcome the temporary challenge, similar to dormancy. In contrast, the declination in methylation of resistance-related genes favors chromatin activation and the expression of novel genes, which provides long-term or permanent resistance for stress.

When CAM plants encounter a water deficit, they switch over from C3-photosynthetic cycle to the CAM pathway, to reduce water loss and increase resistance to stress. This switchover to the CAM pathway is coupled with the promotion of genomic methylation and hypermethylation of satellite DNA (Dyachenko et al. 2006). Thus, hypermethylation of satellite DNA must be coupled with the synthesis of a specialized chromatin structure which can control the expression of a number of genes simultaneously, to enable the switchover to CAM as an adaptation to water stress. Hypermethylation is also detected in the root tip DNA of pea (*Pisum sativum L.)* under conditions of water deficit. MSAP analysis has revealed an increase in the methylation level of both the cytosine residues in CCGG motifs, particularly for the inner cytosine (Labra et al. 2002).

Table 2. DNA methylation modifications involved in biotic and abiotic stresses in different plant varieties

Gene	Plant	Stress	Methylation status	Reference
Asr1	Tomato	Drought stress	CG hypermethylation and CHH hypomethylation	(González et al. 2011)
Asr2	Tomato	Drought stress	CHH hypomethylation in regulatory region	(González et al. 2013)
NtGPDL	Tobacco	Cold	Hypomethylation	(Choi and Sano 2007)
ZmMI1	Maize	Cold	Root-specific hypomethylation	(Steward et al. 2002)
Glyma11g02400	Soybean	Salinity	Demethylation	(Song et al. 2012)
Glyma16g27950	soybean	Salinity	Hypomethylation	(Song et al. 2012)
Glyma20g30840	soybean	Salinity	Hypomethylation	(Song et al. 2012)
Genome wide	Maize	Cold stress	Global methylation shift	(Tan 2010)
Genome wide	Rice	Drought stress	Genotype-dependent differential methylation	(Wang et al. 2011)

Studies conducted on tomato showed change in the DNA methylation pattern of *Asr* gene. Under water deficit conditions the regulatory region of tomato Asr2,27 spanning 968 nt upstream of the transcription start site there were considerable levels of the three types of methylation (CG, CHG, and CHH) in that region under normal water conditions. After stress, the overall CHH methylation showed a slight but significant decrease (González et al. 2013). Cytosine methylation in the gene body of Asr2 was also studied. It was observed that methylation occurred at the few existing CG sites under both normal and water deficit environmental conditions. In contrast, very few of the numerous CHG and CHH sites were methylated under either normal or non-water stress conditions. It is worth mentioning that after the water stress, two distinct clones appeared to be strongly methylated in all contexts, leading to an overall increase in CHG and CHH methylation when analyzed comprehensively. In a study of the effect of cold on maize seedlings revealed that under the exposure of cold stress genome wide DNA methylation in root tissues but screening of genome revealed a segment, 1.8-kb designated as ZmMI1 that was demethylated during chilling. The same segment under normal conditions shows a methylation percentage of about 38% which is almost 2 folds higher than other regions. This gene segment represents a part of a putative protein coding gene indicating its active role under the chilling tolerance (Steward et al. 2002). In a similar study of DNA methylation pattern in cold grown maize 28 differentially amplified fragments were obtained successfully. In silico analysis of these fragments showed their role in several processes like photosynthesis, hormone regulation, transposon activation and in cold response (Shan et al. 2013). Study of the expression level of different DNA methytranferse in rice showed key role of OsCMT2 in rice. The expression level of OsCMT2 coding mRNA under drought condition revealed six to four folds reduction. This study also showed role of OsCMT3 role in drought. Under drought stress four to six folds reduction was observed in transcript accumulation in rice seedlings (Sharma et al. 2009) The comparative analysis of DNA methylation in different crop is given in Table 2.

In a study conducted on rice *Oryzae sativa* L it was found out that drought have a huge impact on the total DNA methylation pattern. Drought was able to induce an average total of 12.1% methylation differences when accounted across different tissue, genotype and developmental stage. Stress conditions such as drought, heat and heavy metal stress tend to increase demethylation. It was also observed that DNA methylation also shows tissue specificity. The overall DNA methylation level at the same developmental stage was lesser in roots than in leaves indicating a significant role of roots than leaves under drought. A total of 29% such DNA methylation/de-methylation sites were observed that did not revert back to the normal condition even after recovery (Wang et al. 2011). Another heat stress related study in rice showed reduction in seed size which is controlled by OsFIE1 (fertilization independent endosperm). Analysis showed that the expression of OsFIE1 is controlled by two factors DNA methylation and H3K9me2 methylation. Further analysis revealed that under heat stress both DNA methylation as well as histone methylation showed a decline (DNA methylation declined by 8.8% and 6.6% with respect to CH and CHG context) (Folsom et al. 2014). Global methylation analysis in *Popuplus tricocarpa* under drought conditions revealed increased methylation level under drought stress (10.04%) as compared to the well watered conditions (7.75%). Single base methylome analysis also showed that the CG and CHG methylations were higher as compared to the CHH methylation. Furthermore analysis of UTRs, coding regions and repetitive sequence revealed that methylation in coding sequence was higher as compared to UTRs on the other hand repetitive sequence showed higher methylation than coding region (Liang et al. 2014). DNA methylation has been found to control cell growth and expansion under heat stress in a study on growth pattern of tobacco BY-2 cells. Exposure to moderate heat (35°C) inhibited 70% growth accompanied with decrease in transcript level of NtCycA13, NtCyc29 and CDKB1-1 transcripts and increase in NtCycD3-1 level. McrBC assay based DNA methylation analysis revealed hypomethylation in

NtCycD3-1 genes whereas hypermethylation in NtEXPA-5. NtcycA13 and CDKB1-1 showed no change In methylation pattern under heat stress and normal condition (Centomani et al. 2015).

MicroRNAs and Crop Improvement

MicroRNAs (miRNAs) are a class of small RNA molecules of about 25 nucleotides (nts) in length which are synthesized endogenously and are generally genetically conserved. Multiple isoform present in some plants are considered to have risen by the process of gene duplication and divergence. miRNA are known to play diverse functions processes including cell proliferation, cell death, immunity, and control of leaf and flower development and in the abiotic and biotic stresses as well (Ruiz-ferrer and Voinnet 2009; Ragupathy et al. 2016). All these function are performed by the regulation of gene expression in plants by targeting complementary mRNA sequences for degradation or repression of expression. Mostly the miRNA genes are annotated to intergenic regions, and in animal are arranged in clusteres but unlike animals plants miRNAs are not arranged in clusters (Budak and Akpinar 2015).

The first small RNA to be discovered was lin-4 in 1993 in nematodes by a genetic screening process (Lee et al. 1993). Later on, the discovery of lin-14 the substrate of lin-4 revealed that miRNA play an important role in regulation of genes (Wightman et al. 1993). In Plants, first miRNA was discovered in Arabidopsis thaliana ten years after the discovery of animal miRNA (Park et al. 2002). After that several thousand miRNAs have now been reported in various plants (Kozomara and Griffiths-jones 2010). The plants in which miRNAs have reported are Arabidopsis, rice, populus, maize, sorghum, soybean, sugarcane etc. With the help of cloning and size-fractionated RNA techniques along with the recent advancements and exploitation of technologies like high throughput sequencing techniques and computation tools for the prediction of the putative target as well as miRNA sequences hundreds of miRNA sequences have been discovered (An et al. 2013; Wei et al. 2015).

miRNA Synthesis in Plants

Most of the miRNA are synthesized from their own genes with independent transcription unit but can also be synthesized in combination with the host gene. RNA polymerase II solely yields primary miRNA (pri-miRNA). Many different enzymes are responsible for the processing of the pri-miRNA into mature miRNA. Processing is a two-step process, initial step is performed by DCL1, SE (zinc finger motif containing) and HYL1 which belongs to dsRNA binding protein family in Arabidopsis to produce a pre-miRNA. This pre-miRNA is again processed by HYL1 and DCL1 to yield miRNA/miRNA duplex (Kurihara and Watanabe 2004) (Kurihara and Watanabe 2004). HEN1 performs methylation of miRNA/miRNA duplex (Yu et al. 2012) then HASTY, a plant homolog of Exportin-5 carry out the transportation of duplex from nucleus to cytoplasm (Yi et al. 2003). After transportation an unidentified helicase uncoils the miRNA duplex and with the help of AGO protein one strand is degraded and other strand termed as guide strand is destined to mature miRNA remains (Fig. 3).

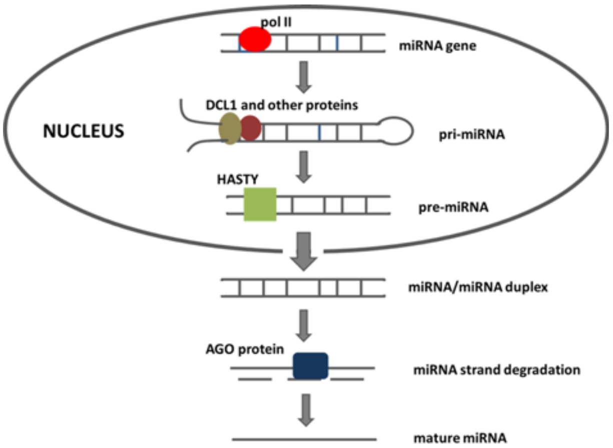

Fig. 3. miRNA synthesis : miRNA genes are transcribed by RNA pol II to produce hairpin loop structure called pri-miRNA. After transcription, pri-miRNA is processed by DCL1 and other proteins to form pre-miRNA. miRNA duplex is then methylated by HEN1 in nucleus and transported to cytoplasm with the help of HASTY, an Exportin5 homolog, for loading into Argonaute (AGO) complex that target complementary mRNA for cleavage.

miRNA technology can be used to improve crop by targeting several traits like enhancing shelf life, crop yield improvement by modifying crop height, branching and size, seedless fruit development, enhanced nutritional value . RNAi has been exploited for the development of a tomato variety with increased level of flavonoids and carotenoids which are very beneficial for human health. By this technology a high market value seedless tomato variety has also been achieved by down-regulating a chalcone synthase, gene involved in flavonoid biosynthesis in tomato (Schijlen et al. 2007). Development of stress tolerant crop has successfully been achieved by the use of RNAi technology. Canola with down regulated expression of farnesyl transferase was developed which shows more resistance towards seed abortion during flowering induced by water deficiency (Wang et al. 2009). Transgenic rice plants with tolerance to drought were developed by silencing of activated C-kinase1 receptor(Da-hong et al. 2009). In rice RINGfinger E3 ligase gene-OsDSG1 knockdown leads to enhanced drought tolerance (Park et al. 2010). Recent study showed that overexpression of a miR319 gene from rice in the bentgrass leads to

Table 3. Exploitation of miRNA technology in crop improvement

Crops	Improtant traits	Gene/Enzyme related	Reference
Cotton	Used to produce cottonseed with reduced amount of glossypol a toxic compound	d-cadinene synthase	(Sunilkumar et al. 2006)
Maize	Drought tolerance	PDH, POK, MAPK, PLD	(Wei L et al. 2009)
Wheat	Drought resistance	11 different miRNA are upregulated under drought exposure	(Kantar et al. 2011)
	Production of wheat with low starch branching enzyme to reduce the total starch content which will ultimately reduce the risk of various diseases	silence the gene for starch branching isoenzymes[SBEIIa and SBEIIb]	(Regina et al. 2010)
Rice	Drought tolerance	miR170 miR171 miR172	(Zhou et al. 2010)
	Cold tolerance	PCF5/PCF8	(Yang et al. 2013)
	Production of rice variety with low glutamine content beneficial to the health.	low glutamine content	(Butardo et al. 2011)
Soybean	Silencing of Omega-3 fatty acid desaturase gene in soybean using RNAi	reduce α-linolenic acid	(Flores et al. 2008)
	Drought tolerance	GmNFYA3	(Ni et al. 2013)
Barley	Drought tolerance	NFY-A	(Ferdous et al. 2016)
Common bean	Drought tolerance	NAF transcription factor	(Sosa-valencia et al. 2016)
Cow pea	Drought tolerance	ARF10 Tranferase family protein Leucine repeat rich transmembrane protein	(Barrera-Figueroa et al. 2011)
Chick pea	Drought tolerance	BHLH23 ERF/AP2	(Hajyzadeh et al. 2015)
Tomato	Drought tolerance	SlNF-YA 1/2/3	(Zhang et al. 2011)
Arabidopsis	Heat stress tolerance	CSD1CSD2CCS	(Guan et al. 2013)
	Drought tolerance	NFY-A5	(Li et al. 2008)
Sugarcane	Drought tolerance	NAC translational factor Auxin responsive factor Glyceraldehyde 3 phosphatedehydrogenase	(Ferreira et al. 2012)

increased draught tolerance due to increased leaf wax content. Studies showed considerable change in miR319 expression in rice and sugarcane during cold condition (Thiebaut et al. 2012). Today miRNA technology has emerged as a powerful tool of molecular engineering for the crop improvement. Exploitation of miRNA technology for various traits improvement is given in Table 3.

Conclusions

Several genes are expressed differentially under the influence of stress which is controlled by the processes histone variants, histone N-tail modifications, and DNA methylation have been shown to regulate plant development under stress. Studies of mechanism and nature of these different modifications can give a proper insight on the different genes and the specific regions in them which are responsible for adaptation to various abiotic stresses like water and heat leading to the development of better understanding of the pathway to be targeted for the crop improvement. Genetic manipulation using RNAi technology can be used to enhance crop quality as various agronomic traits can be manipulated and improved by changing the gene expression and therefore changing the metabolic pathways using RNAi technology and nutritional value of the products can be improved. Food safety which is a major concern can be improved by inhibiting the synthesis of naturally occurring metabolites that are toxic in nature from the edible portion of the plant. It also provides an additional means to meet the nutritional requirements of the world population

Competing Interests

The authors declare that they have no competing interest.

Author's Contributions

NKS designed the work, SS studied the literature, collected the data and wrote manuscript. AKY and PS helped in writing the manuscript.

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