

Epigenomics for Crop Improvement: Current Status and Future Perspectives

Suresh Kumar*

**Division of Biochemistry, ICAR-Indian Agricultural Research Institute, New Delhi, India.*

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ABSTRACT

Epigenome refers to the sum of all the epigenetic changes in DNA base (without altering the underlying nucleotide sequence), histone proteins and small-RNA biogenesis in a cell. Genome-wide epigenetic changes are being reported during cellular growth and development as well as during environmental stress, which are often associated with variation in gene expression. The level of gene expression and epigenetic changes may go back to the pre-stress state immediately after withdrawal of the stress. A well-known mechanism of epigenetic change has been the methylation of cytosine at 5th carbon. Additionally, certain amino acids of histone proteins are post-translationally modified that may affect transcription, chromosome segregation/condensation, and/or DNA repair processes. Small-RNAs play a crucial role in DNA methylation through the RNA-directed DNA methylation (RdDM) pathway. The epigenetic changes may be inherited over the generation that often results in phenotypic variations. It is becoming evident that epigenetic changes play important roles in acclimatization, stress tolerance, adaptation, and evolution processes. As the growing evidence on epigenetic variations suggest their effect on gene expression, it would be crucial to investigate the epigenetic machinery of gene regulation in plants, and its possible use in epigenome engineering/editing for crop improvement. This mini-review focuses on the basics of epigenomics, followed by the present status and prospects towards its usage for crop improvement to meet the challenges of sustainable food security for the global population.

Keywords: Crop improvement, DNA methylation, Epigenomics, Gene regulation, Histone modification, Stress memory

INTRODUCTION

Explaining genotypic variations with the rapid evolutionary changes under environmental pressure has become difficult using classical genetics alone. The rate of phenotypic variations and genetic mutations are considerably different, which cannot be explained merely based on genetics as the primary molecular mechanism. Additional mechanisms such as epigenetics can help to explain this enigma [1]. If epigenetics is considered as a complementary molecular mechanism, many of the phenotypic variations (e.g. the dissimilarity between the clones) can be explained easily [2].

Plants are sessile in nature and face multiple environmental stresses [3]. Until the last century, it was thought that isolation of the gene(s) associated with a trait of interest was sufficient to transfer the trait to a crop plant and to achieve the expected phenotype. Recently, definitive evidence has been gathered for the DNA to provide only part of the genetic information for a trait, and that chromatin changes also contribute to the expression of the trait. DNA (cytosine) methylation, post-translational modifications (acetylation, methylation, phosphorylation, etc.) of histones and regulatory RNAs (small non-coding RNAs or sncRNAs) define distinct chromatin/epigenetic states of the genome (epigenome), which vary with the changing environmental

conditions [4]. Thus, chromatin is a highly dynamic structure which carries various information: (i) the one encoded by the DNA sequence, and (ii) those provided by the epigenetic states. Since the epigenetic states of chromatin are variable, transfer of a trait from one species to another not only requires the transfer of the gene(s) associated with the trait but also the appropriate chromatin/epigenetic states to enable the trait to express. It is, therefore, essential to study the epigenetic states in the donor plant/species and to ensure proper re-establishment of the epigenetic state of the genes in the recipient plant/species for their expression under the appropriate (de)methylation level [5]. However, epigenetic mechanisms of gene regulation are yet to be fully understood and utilized as epialleles (the alleles that are genetically identical but epigenetically different due to the

Corresponding author: Dr. Suresh Kumar, USDA Norman E. Borlaug Fellow, IUSSTF Fellow, INSA V. Fellow, Principal Scientist, Division of Biochemistry, ICAR-Indian Agricultural Research Institute, New Delhi-110012, India, Tel: +91 11 25842038; Fax: +91 11 2584 6420; E-mail: sureshkumar3_in@yahoo.co.uk

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epigenetic modifications, showing variable expression) in crop improvement programs [6].

Considering the current molecular understanding, epigenetics can be defined as the studies of the molecular processes in and around DNA that control genome activity independent of the DNA nucleotide sequence which may be inherited through mitosis or meiosis [7]. These epigenetic mechanisms include DNA methylation, histone protein modifications, and biogenesis of sncRNAs [1]. Environmental factors have been reported to promote epigenetic variations. Several researchers have proposed the role of epigenetics in the evolution process, primarily as a sensible and responsive molecular mechanism in the natural selection [8].

Many of the traits of economic importance are complex in nature controlled by the joint action/interactions of multiple genes. Recent findings indicate that heritable variations may also be caused by epigenetic changes in the genetic material. Propagation of epigenetic marks in plant takes much more direct route than that in the animal. It has also been reported that the rate of spontaneous epimutations is higher in the CG context because these sites are not retargeted by RdDM. DNA methylation generally refers to the addition of a methyl group at the 5th carbon of cytosine as a post-replicative event. In plants, cytosine methylation occurs in CG, CHG and CHH contexts (where H=A, C or T), while in somatic cells of animals/vertebrates, cytosine methylation is limited to CG context [9].

On the other hand, CHH methylation is maintained by Domains Rearranged Methyltransferase 2 (DRM2). Interestingly in *Arabidopsis*, DRM2 is responsible for *de novo* methylation in all the contexts of cytosine. DRM2 is recruited to the target loci by a specialized 24 nucleotide small interfering RNA (RNA-directed DNA methylation pathway) [10]. Cytosine methylation homeostasis is determined by the DNA methylation and demethylation processes. Promiscuous methylation is pruned by demethylases to create the desired methylation pattern. Demethylation of the promoter and/or coding region may also be required to activate the expression of specific genes under the changing environmental conditions or during the developmental stages of plant [11].

Histone proteins have numerous evolutionary conserved lysine (K) residues that are subjected to acetylation (ac), methylation (me), ubiquitylation (ub), etc. A variety of histone modifications and their possible combinations (e.g. H3K4me3 and H3K27Ac: activation marks and H3K9me3 and H3K27me3: repressive marks) regulate transcriptional potential of a gene. The level of histone acetylation is controlled by histone acetyltransferases (HAT) and histone deacetylases (HDAC). Histone lysine methylations have differential effects on transcriptional activity, depending on the site (K4, K9, K27) and mode (me1, me2, me3) of modifications. Histone lysine methylation can also be

reversed by the action of two different types of histone demethylases [12].

Studies indicate that the genome-wide hypomethylation induces biogenesis of 24 nt siRNAs, and activates *de novo* methylation pathways [13,14]. Studies suggest that epigenetics is more likely to be involved in the heritability of phenotypes in plants than in mammals. This might be attributed to two different activities. First, the RdDM pathway influences *de novo* establishment and maintenance of DNA methylation in the plant genome with the help of siRNAs. Second, unlike resetting of DNA methylation pattern during gametogenesis in mammals, DNA methyltransferases in plants are active during gametogenesis and embryogenesis; hence, the patterns of DNA methylation can be inherited from parent to progeny in plant. In-depth studies would be necessary to understand the role of RdDM pathway in the epigenetic regulation of genes and its deployment in epigenetic manipulation.

EPIGENETIC REGULATION OF DEVELOPMENTAL PROCESSES

Epigenetic changes in DNA methylation, histone modifications and ncRNA expression cause important biochemical, physiological and molecular consequences in plants. The epigenetic-phenotypes are now being explained based on the fundamental discoveries such as activation, excision and translocation of transposable elements, allelic interactions, transgene silencing and epialleles of the endogenous genes. In *Arabidopsis thaliana*, four bifunctional DNA glycosylases and AP lyases, namely DME (Demeter), DML2 (Demeter-Like 2), DML3 (Demeter-Like 3) and ROS1 (Repressor of Silencing 1) are known to recognize and remove methylated cytosines. ROS1, DML2, and DML3 generally function in vegetative tissues and demethylate specific loci in the genome [10]. These enzymes appear to counterbalance the RdDM pathway to fine-tune the methylation levels at particular genomic locations. Data indicate that apomictic seed development in plants is associated with dynamic transcriptional activity in ovule probably regulated through epigenetic mechanisms. Epigenetic model of regulation of apomixis shows that reversible changes in chromatin configuration might alter the expression of essential genes of the apomictic pathway at the different developmental stage or in different cell types [1]. Since the discovery of imprinted *R* gene in maize, dozens of imprinted genes have been identified in plants and epigenetics has been found to play a crucial role in this process [15,16]. Silencing of transposable elements in the male gametes is essential for genome stability and integrity. The decrease in methylation in pericarp on ripening of tomato suggests the involvement of DNA demethylation in fruit ripening [17]. Gliadins, the storage proteins in wheat and barley endosperm, require *TaDME* for their expression. RNAi-mediated suppression of *DME* resulted in a significant reduction in gliadins and LMWGs, but HMWGs remained

unchanged [18]. In a recent study, it was revealed that *MtDME* gets strongly induced in *Medicago truncatula* during nodule differentiation, and knockdown of *MtDME* resulted in morphological and functional alterations in the nodule [19]. Variation in DNA methylation and its effect on the expression of high-affinity potassium transporter under salt stress was reported to provide salt tolerance in wheat [20]. There is increasing evidence for the involvement of epigenetic regulations in various developmental processes in plants. Thus, understanding epigenetic regulation and functions of the machinery involved would be very much essential for epigenetic manipulation of plants for the trait of interest [21].

APPLICATIONS IN CROP IMPROVEMENT

Epigenetic changes can affect important traits in crop plants; therefore, creation/manipulation of stably inherited epigenetic variation could be a powerful tool in plant breeding. It can enable modification of traits in plant without altering the DNA sequence of the gene. Similarly, understanding the bases of phenotypic plasticity is crucial for crop breeding. Zheng et al. [22] reported that the genes of stress-responsive pathways showed accumulation of transgenerational epimutations and the DNA methylation patterns in the drought-responsive genes were affected by multi-generational drought. They reported that about 30% of the changes in methylation were stable and inherited, which corroborated with the earlier findings of Wang et al. [23] who reported 29% of the drought-induced DNA methylation to be maintained even after recovery to the normal condition. Kumar and Singh [7] also observed that 25% of the increase in methylation was retained in a rice genotype IR-64-DTY_{1.1} even after recovery from the drought stress. Thus, epigenetics can be considered as an important regulatory mechanism in plant's long-term adaptation and evolution under adverse environments. In *Arabidopsis*, DNA demethylases target promoter TEs to regulate stress-responsive genes. Therefore, manipulating DNA methylation of TEs in the promoter region (by recruiting DRM2 to the target loci) could be considered for epigenetic manipulation of stress tolerance in plants [24].

Certain epigenetic changes in plants persist even after withdrawal of the stress and may inherit over the generation in the form of epigenetic alleles. These heritable epigenetic alleles (epialleles) are now considered as another source of polymorphism which may be utilized in the breeding program. It is now apparent that somatically-acquired epigenetic changes in plants may be mitotically stable and meiotically heritable; hence the emphasis is given to the variations in DNA methylation as a source of variation [5]. A better understanding of the role and significance of this new source of genetic and phenotypic diversity in plants would be achieved as more data accumulates about the role of DNA methylation in plant evolution, domestication, and breeding. Identification and assessment of the importance of

epialleles in plant breeding require determination of (i) the extent of variation in epigenetic marks among the individuals, (ii) the degree to which the epimarks affect phenotype, and (iii) the extent to which the epimark-linked superior phenotypes are stably inherited. Although there are several challenging tasks, the technical potential to assess epigenetic variations between individuals and the estimation of the levels of epimark-associated phenotypic diversity does exist. With the increasing understating of epigenetic phenomena, it is expected that our potential to exploit epigenetics in crop improvement and nutritional management would get better, and will have significant implications in plant breeding [25].

Data indicates that F₁ hybrids are, in general, less methylated than their parental inbred. In general, hybrids are less methylated than their parental inbred, (ii) heterotic hybrids are less methylated than related non-heterotic hybrids, (iii) old and low-yielding inbred are highly methylated, (iv) new inbred, especially those selected for high and stable yield, have lower methylation level in comparison to their progenitors. DNA methylation can be considered as a regulatory mechanism that affects the expression of several genes important for the manifestation of heterosis. Repeated selfing carried out during the development of inbred, with more emphasis on combining ability of the inbred, leads to the gradual accumulation of methylated loci, which is released and/or re-patterned when the inbred are crossed to develop hybrids. The stressful growth conditions during the development of inbred result in more methylated DNA, and these stress-induced methylations and the linked suppression of genome activity could be at the core of higher yield of the hybrid [5].

Manipulation of parental imprinting by epigenetic alteration may lead to the development of a superior endosperm, which has become a necessity for the improvement of seed crops. Understanding the epigenetic regulation of seed development would eventually uncover the mysteries behind apomixis, the asexual mode of reproduction through seeds wherein embryo develops without meiosis and double-fertilization leading to the production of progenies genetically identical to the mother plant [26]. If this mechanism could be deployed successfully in the commercial seed crops, hybrid vigor can be maintained indefinitely which may help to overcome the current limitations of plant breeders in maintaining hybrid vigor for more than one generation.

Zhang et al. [15] reported tissue-specific differentially methylated regions in sorghum and suggested that DNA methylation play an important role in regulating tissue-specific expression of the genes. Polycomb group (PcG) proteins are involved in controlling the expression of homeotic genes that are essential for the proper developmental processes in plants. The main component of the PcG complex in plants is methyltransferase (e.g. MEA in

Arabidopsis) that methylate histone to regulate expression of the homeotic genes for development of plant. In most of the plants, embryogenesis starts with asymmetric cell division, which gives rise to a polar embryo having a larger basal cell and a smaller apical cell. Cell division and differentiation during these processes are highly regulated that are influenced by epigenetic mechanism [1]. Demethylation of the promoter of the gliadins and LMWgs encoding genes in barley was reported to be important for the accumulation of gliadins and LMWgs. However, regulation of HMWgs expression was found to be independent of DNA (de)methylation. Due to the differential regulation of gliadin/LMWg and HMWg expression in wheat and barley, suppression of *TaDME* and *HvDME* has been proposed to be a potential strategy to eliminate gliadins and LMWgs that cannot be digested/tolerated by many people suffering from celiac disease [11].

Silencing of the transgene has frequently been observed as a major commercial risk of the transgenic technology, creating hindrance in the economic exploitation of transgenic plants [27]. Several strategies have been suggested to minimize silencing of the transgene at different stages of transgenic development. Silencing of transgenes also correlated with methylation of the transgenes. Methylation of the promoter correlates with transcriptional gene silencing, and methylation of the coding region is generally associated with post-transcriptional gene silencing. A better understanding of the mechanisms of epigenetically-enforced transgene silencing might help avoiding silencing of the gene of interest. One of the strategies suggested to avoid transgene silencing has been the careful designing of the transgene and thorough analyses of transformants at the molecular level [7].

Under osmotic stress, *P5CS* and δ -OAT genes were found to show DNA demethylation in mother plants, but it disappeared in the next generation, suggesting that DNA demethylation regulated expression of the genes [28]. One of the ways for plants to adapt to environmental stress is to remember a stress episode and to react more efficiently (faster and more strongly) upon subsequent exposures to the stress. At the molecular level, short-term memory results from a combination of mechanisms, including modification of the levels of stress-associated receptors, signaling components, and transcription factors. Multiple lines of evidence indicate that both short-term and transgenerational memories mainly rely on epigenetic modifications, and it can be exploited in developing tolerant crop plants [12]. However, fundamental investigations are required to understand whether stress-induced epialleles can be stabilized over several generations and consequently be utilized in crop breeding programs. The research challenges ahead include improving our understanding of the stability, reversibility, and heritability of epialleles. Epigenetic manipulation may become a valuable strategy in the future for crop improvement, as the approaches are available for

stochastic modulation of DNA methylation using chemical or by genetic means, followed by the forward or reverse selection of epialleles. However, we need to devise strategies to ensure stable retention of desirable epialleles within breeding materials and to develop techniques for targeted epigenetic manipulation. Eukaryotic genomes are complex in nature and genome complexity of many crop plants increases further because of their polyploid origins, which makes gene interaction networks complicated, and difficult to modulate for improved plasticity with inbuilt gene redundancy. Understanding how epigenetic changes are superimposed on the multiple gene copies to confer plasticity may provide a framework for the development of desirable crop variety enabled to cope up with the harsh multiple-stresses the crops are facing now due to the global climate changes. Currently, it is difficult to control epigenetic variations; mobilization of stress-responsive epigenetically-silenced TEs may contribute to the stable inheritance of stress-induced epigenetic changes.

Over the last century, genetic improvement of crops and modern agronomic practices has underpinned a massive increase in crop yield and productivity. However, most of these gains have been achieved by utilizing the 'Green Revolution' technologies in a period of relative climate stability [29], compared to the current period of increased climate change and variability. To facilitate climate resilient agriculture in the future, we need to understand the molecular and mechanistic basis of genotype $\times$ environment interactions ($G \times E$) and the emergent property of crop plant plasticity facilitated by epigenetic mechanisms. Epigenetic manipulation may provide a way to achieve the desired variations and adaptive advantages without manipulating DNA sequence. Importantly, epialleles may alter the expression of the gene(s) controlling cellular/physiological processes during plant development. Stable inheritance of such adaptive epialleles may provide increased fitness/adaptability to the plant in the changing environmental conditions.

FUTURE PERSPECTIVES

In recent years, tremendous progress has been witnessed towards understanding the epigenetic regulation of gene expression in plants, particularly in Arabidopsis. The proteins involved in DNA (de)methylation, histone modification, and the mechanisms of ncRNA mediated regulation of developmental processes in plants are becoming clear day-by-day. However, many areas of epigenetics remain to be explored. We still know only a little about the factors that regulate the targeting of active DNA demethylation during developmental stages. Does DNA (de)methylation interplay with other epigenetic features or chromatin features? Future research should aim at identifying more developmental processes in different species that involve epigenetic regulation. Assessing the contribution of transgenerational epimarks to heritable

phenotypic variation has been a major challenge as many of the chromatin (DNA methylation and histone modification) changes and gene expression variants co-segregate with DNA sequence polymorphisms.

Nonetheless, there is evidence that plants possess heritable epiallelic variations that can be associated with the trait of interest and utilized for crop improvement. Although it had been difficult to alter DNA methylation and chromatin states in a locus-specific manner, the situation is changing rapidly with the advances in genome editing tools like the CRISPR-Cas9 system. Catalytically inactive Cas9 (dCas9) can be fused with methylases and/or demethylases to manipulate DNA methylation in a site-specific manner [8]. Thus, we can anticipate that soon epigenome editing will provide a means to assess the role of a QTL in epiallelic variations which may provide an exciting new route for the improvement of crop plants. With the modern tools and techniques in molecular biology and biotechnology, it is expected that soon we may achieve a comprehensive understanding of this amazing biological phenomenon, and we might be able to use it for the development of climate-resilient crops for the benefits of humankind. However, this will need a deeper understanding of the interactions between crop genomes and how their genomic regulatory networks contribute to the plasticity of phenotype.

Genetic engineering technology offers novel approaches for biotic and abiotic stress management with several advantages over the conventional methods. However, a few drawbacks like gene silencing due to the epigenetic changes are also there which can be managed as mentioned above. Plant-incorporated protectants like *Bt* gene have been one of the modern biotechnology approaches to protect crop plants which have provided several products in the global market [30,31]. Considering the biosafety uses of genetically modified organisms developed through the genetic manipulation of crop plants [32-34], the epigenetic engineering (which is supposed to have limited biosafety issues, if any) would be a preferred approach [2]. However, necessary safety guidelines framed in the country by the regulatory authorities must be followed for personnel, laboratory and environmental safety [35,36]. Thus, epigenome engineering not only provides unprecedented opportunities for understanding the epigenetic mechanisms of growth and development, but also to manipulate the biological system to improve stress tolerance against the changing climatic conditions.

CONCLUSION

Our understanding of the foods, their production, and uses in maintaining and optimizing health is continuously being improved. The global population is speculated to reach 9 billion by 2050. This 2-3 billion upsurge in the global population would require increasing food production by 70% [37]. Providing adequate food to the global population is only the preliminary challenge; the major challenges would

be to produce the food in a safe and sustainable manner [38,39] under the increasingly unfavorable environmental conditions [36]. Although plants have the innate capability to survive under adverse climatic conditions, yet crop plants need improvement in their efficiency to produce more and more nutritious food even under unfavorable climatic conditions. Properly harnessing the epigenetic variation is must to provide new opportunities for crop improvement and boost the production. The coming years are likely to realize increased opportunities for monitoring and manipulating crop epigenomes. Because gene expression profile provides the primary account of the epigenotype to phenotype effect, it becomes essential to dissect the relative contributions of genetic and epigenetic variations on gene expression. The knowledge of epigenetic variation might allow exploitation of different epigenetic marks towards the development/selection of superior genotype at the early stage of plant growth.

The views expressed are those of the author only. These may not be the views of the institution or organization the author is associated with.

REFERENCES

1. Kumar S (2017) Epigenetic control of apomixis: A new perspective of an old enigma. *Adv Plants Agric Res* 7: 227-233.
2. Kumar S (2018) Epigenomics of plant responses to environmental stress. *Epigenomes* 2: 6.
3. Kumar S, Beena AS, Awana M, Singh A (2017) Physiological, biochemical, epigenetic and molecular analyses of wheat (*Triticum aestivum*) genotypes with contrasting salt tolerance. *Front Plant Sci* 8: 1-20.
4. Kumar S, Chinnusamy V, Mohapatra T (2018) Epigenetics of modified DNA bases: 5-methylcytosine and beyond. *Front Genet* 9: 1-14.
5. Kumar S, Singh AK, Mohapatra T (2017) Epigenetics: History, present status and future perspective. *Indian J Genet Plant Breeding* 77: 445-463.
6. Kumar S (2018) Environmental stress, food safety, and global health: Biochemical, genetic and epigenetic perspectives. *Med Safety Global Health* 7: 2.
7. Kumar S, Singh A (2016) Epigenetic regulation of abiotic stress tolerance in plants. *Adv Plants Agric Res* 5: 517-521.
8. Kumar S (2019). Epigenetics and epigenomics for crop improvement: Current opinion. *Adv Biotechnol Microbiol* 14: 555879.
9. Wang X, Li Q, Yuan Q, Kumar S, Li Y, et al. (2016) The cytosolic Fe-S cluster assembly component MET18 is required for the full enzymatic activity of ROS1 in active DNA demethylation. *Sci Rep* 6: 26443.

10. Law JA, Jacobsen SE (2010) Establishing, maintaining and modifying DNA methylation patterns in plants and animals. *Nat Rev Genet* 11: 204-220.
11. Li Y, Kumar S, Qian W (2018) Active DNA demethylation: Mechanism and role in plant development. *Plant Cell Rep* 37: 77-85.
12. Kumar S (2018) Epigenetic memory of stress responses in plants. *J Phytochem Biochem* 2: e102.
13. Blevins T, Pontes O, Pikaard CS, Meins F Jr (2009) Heterochromatic siRNAs and DDM1 independently silence aberrant 5S rDNA transcripts in Arabidopsis. *PLoS One* 4: e5932.
14. Teixeira FK, Heredia F, Sarazin A, Roudier F, Boccara M, et al. (2009) A role for RNAi in the selective correction of DNA methylation defects. *Science* 323: 1600-1604.
15. Zhang M, Xu C, von Wettstein D, Liu B (2011) Tissue-specific differences in cytosine methylation and their association with differential gene expression in *Sorghum bicolor*. *Plant Physiol* 156: 1955-1966.
16. He S, Sun Y, Yang Q, Zhang X, Huang Q, et al. (2017) A novel imprinted gene NUWA controls mitochondrial function in early seed development in Arabidopsis. *PLoS Genet* 13: e1006553.
17. Lang Z, Wang Y, Tang K, Tang D, Datsenka T, et al. (2017) Critical roles of DNA demethylation in the activation of ripening-induced genes and inhibition of ripening-repressed genes in tomato fruit. *Proc Natl Acad Sci U S A* 114: 4511-4519.
18. Wen S, Wen N, Pang J, Langen G, Brew-Appiah RA, et al. (2012) Structural genes of wheat and barley 5-methylcytosine DNA glycosylases and their potential applications for human health. *Proc Natl Acad Sci U S A* 109: 20543-20548.
19. Satge C, Moreau S, Sallet E, Lefort G, Auriac MC, et al. (2016) Reprogramming of DNA methylation is critical for nodule development in *Medicago truncatula*. *Nat Plants* 2: 16166.
20. Kumar S, Beena AS, Awana M, Singh A (2017) Salt-induced tissue-specific cytosine methylation downregulates expression of HKT genes in contrasting wheat (*Triticum aestivum* L.) genotypes. *DNA Cell Biol* 36: 283-394.
21. Singh A, Krishnan V, Vinutha T, Kumar S (2018) Biochemical, physiological and molecular approaches for improving salt tolerance in crop plants - A review. In: *Engineering Practices for Management of Soil Salinity* (Eds. Goyal MR, Gupta SK, Singh A) Apple Academic Press, pp: 159-208.
22. Zheng X, Chen L, Xia H, Wei H, Lou O, et al. (2017) Transgenerational epimutations induced by multi-generation drought imposition mediate rice plant's adaptation to drought condition. *Sci Rep* 7: 39843.
23. Wang W-S, Pan YJ, Zhao XQ, Dwivedi D, Zhu LH, et al. (2011) Drought-induced site-specific DNA methylation and its association with drought tolerance in rice (*Oryza sativa* L.). *J Exp Bot* 62: 1951-1960.
24. Xu R, Qin R, Li H, Li D, Li L, et al. (2017) Generation of targeted mutant rice using a CRISPR-Cpf1 system. *Plant Biotechnol J* 15: 713-717.
25. Kumar S, Krishnan V (2017) Phytochemistry and functional food: The needs of healthy life. *J Phytochem Biochem* 1: 103.
26. Yadav CB, Anuj, Kumar S, Gupta MG, Bhat V (2012) Genetic linkage maps of the chromosomal regions associated with apomictic and sexual modes of reproduction in *Cenchrus ciliaris*. *Mol Breed* 30: 239-250.
27. Kumar S, Arul L, Talwar D (2010) Generation of marker-free Bt transgenic indica rice and evaluation of its yellow stem borer resistance. *J Appl Genet* 51: 243-257.
28. Zhang Y-Y, Fischer M, Colot VV, Bossdorf O (2013) Epigenetic variation creates potential for evolution of plant phenotypic plasticity. *New Phytol* 197: 314-322.
29. Kumar S, Singh A (2015) Biopesticides: present status and the future prospects. *J Fertil Pesticides* 6: e129.
30. Sharma A, Kumar S, Bhatnagar RK (2011) Bacillus thuringiensis protein Cry6B (BGSC ID 4D8) is toxic to larvae of *Hypera postica*. *Curr Microbiol* 62: 597-605.
31. Kumar S (2011) Biotechnological advancements in alfalfa improvement. *J Appl Genet* 52: 111-124.
32. Kumar S, Arul L, Talwar D, Raina SK (2006) PCR amplification of minimal gene expression cassette: An alternative, low cost and easy approach to 'clean DNA' transformation. *Curr Sci* 91: 930-934.
33. Kumar S, Chandra A, Pandey KC (2008) Bacillus thuringiensis (Bt) transgenic crop: An environment friendly insect-pest management strategy. *J Environ Biol* 29: 641-653.
34. Kumar S (2014) Biosafety issues of genetically modified organisms. *Biosafety* 3: e150.
35. Kumar S (2012) Biopesticides: A need for food and environmental safety. *J Biofertil Biopesticide* 3: e107.
36. Kumar S (2015) Biopesticide: An environment friendly pest management strategy. *J Biofertil Biopesticide* 6: 1.

37. Kumar S (2013) The role of biopesticides in sustainably feeding the nine billion global populations. *J Biofertil Biopesticide* 4: e114.
38. Kumar S (2012) Biosafety issues in laboratory research. *Biosafety* 1: e116.
39. Kumar S (2015) Biosafety and biosecurity issues in biotechnology research. *Biosafety* 4: e153.