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Review Article

Role of DNA Methylation and Epialleles in Eukaryotes

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Abstract: Epialleles that emerge due to methylation variation in genetically identical individuals are gaining more interest due to their involvement in physiological and pathological processes. These are also important for transgenerational epigenetic inheritance and evolution. Both stable and metastable epialleles have their importance because of their contribution to the alteration of gene expression that may lead to useful traits or diseases. The main aim of this work lies in a comparative study between stable and metastable epialleles and the latest advancements that are helpful for the interpretation and analysis of DNA methylation and epialleles. However, there is so much to discover and understand because of the inadequate knowledge about methylomes of species as well as the naturally occurring epialleles in the wild. We will get more opportunities to apply this knowledge if we have a complete understanding of methylomes and epialleles and their contribution towards the normal functioning of an organism.

Keywords: Epialleles, DNA methylation, Stable Epialleles, Metastable Epialleles (MEs).

1. INTRODUCTION

Patterns of DNA methylation are the post replicative enzymatic modifications of DNA [1] that occur in all organisms including bacteria, plants, and animals and its correct order is essential for the health of the body, organs, and cells [2]. In mammals, cytosine methylation is only found at CpG sites [3], unlike mammals, plants contain an abundance of 5mC sites in symmetrical and non-symmetrical contexts at CpG, CpHpG, and CpHpH sites (H represent A, C or T) [4]. Methylation regulates the gene expression when there is methylation variation in the same gene of a species without any changes in the DNA sequences, it leads to the emergence of epialleles which can be stored and produce phenotypic variations [5] that can be stably transmitted across the generations [6]. Epialleles in the form of epigenetic variations are providing an insight into how an organism's genome functions during growth and development, health and disease, and in response to internal and external factors [7].

Variation in DNA methylation builds epialleles, the gene expression of which is controlled by alteration in methylation e.g., rRNA epialleles in *Arabidopsis thaliana* have altered epiallelic patterns in which active rRNA shows complete demethylation while silenced rRNA genes are fully methylated [8]. Epialleles can emerge in response to various environmental and genomic stresses and play important role in environmental adaptation and evolution [9]. Incompatibility between two strains of species can occur due to epigenetic variation or variation in epialleles without any change in the DNA sequence [10].

Two families of enzymes control the mechanism of methylation and demethylation, the DNA methyltransferases (DNMTs) and the ten-eleven translocation (TET) family of 5-methylcytosine deoxygenases. DNMTs generate 5-methylcytosine (5mC) by transferring a methyl group of S-adenosylmethionine (SAM) to the 5 positions of cytosine. However, the TET protein family converts 5mC into 5-hydroxymethylcytosine (5hmC), which acts as an intermediate in the demethylation process of DNA. These two families of enzymes

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play an important role in embryonic and post-natal mammalian development [11]. The DNMTs maintain CG methylation in plants and create transcriptional diversity based on the generation of independent epialleles [12]. There are also mobile small RNAs (sRNAs) of 24nt that regulate DNA methylation of transposable elements (TEs). e.g., the expression of some genes in the roots of *Arabidopsis* by DNA methylation is associated with sRNAs [13]. In addition to enzymes and sRNAs, some proteins regulate the DNA methylation dynamics e.g., recently a novel protein, rearranged L-myc fusion (Rlf) has been identified in a mouse that is involved in DNA hypomethylation of enhancer region and CGI shores at specific regions thus control transcriptional activity. The absence of it increases the DNA methylation of those specific regions [14]. In addition to the DNA methylation within gene-coding regions, non-coding regions are also equipped with methylation patterns and can affect gene expression [15], e.g., one study on the Peanut (*Arachis hypogaea*) methylome had shown DNA methylation at intergenic regions [16].

The DNA methylation patterns of eukaryotes are affected by different factors either genetic or environmental that have an equal influence on the next generation as demonstrated in Figure 1. This review focuses on the parameters that influence DNA methylation patterns and its effect on organisms in the form of stable and metastable epialleles moreover the latest advancements that are taking place in the field of epigenetics.

2. METHODOLOGY

Different search engines like Google Scholar, Science Direct, PubMed, Scopus, Medline, and Research Gate were used to collect the data for this work. The species names were confirmed and checked from Encyclopaedia Britannica. The genes and proteins were validated from the NCBI while the enzyme and its E.C number were authenticated from the Swiss-Prot databases.

3. INFLUENCE OF GENETIC AND ENVIRONMENTAL FACTORS ON EPIALLELES

Epigenetics has now become an important aspect in the studies of various human diseases and disorders as well as of the issues related to environmental health which has now advanced into a separate field known as Environmental Epigenomics [17]. The study of the *A. thaliana* methylome and its association with climate changes has reported that many epialleles help to develop resistance against pests, diseases, help plants in the adaptation to the local environment as well as have a major role in the environmental adaptive evolution of plants [18]. Some epialleles emerge in response to environmental changes during the periconceptional period. These early embryonic environmental changes such as maternal nutrition and seasonal variation linked to epigenetic variation and human diseases, e.g., a genomically imprinted tumor suppressor gene *VTRNA2-1* is affected by the maternal environment during conception that remains highly stable for many years [19] (Table 1). The underlying cause of genetic variation in the formation of epialleles is rarely known, such genetic variation includes the insertion of structural variants (SVs) or transposable elements (TEs). Obligatory epialleles are completely dependent on genetic variations for their epigenetic state [20] as shown in Fig.1, its example is Avy epiallele in Agouti rodents [21].

Plants are always exposed to environmental stresses either internal or external, biotic or abiotic, they somehow adapt themselves to overcome the stresses. Epialleles play an important role in plants to tolerate such stresses by altering the methylation status under stress conditions as methylation regulates gene expressions [22] e.g., in rice (*Oryza sativa*) non-biotic stress i.e., N-deficiency results in an adaptive trait having hypercytosine methylation showing enhanced tolerance to N-deficiency [23]. Similarly, plants living in the heavy metal contaminated environment respond to heavy-metal stresses by altering methylation patterns, e.g., rice as a staple food crop known to be grown in heavy metal polluted paddy-fields were found to contain alteration in its DNA methylation showed tolerance against heavy metals such as Cu^{+2}, Cr^{+3}, Cd^{+2} or Hg^{+2}, which is inherited across the successive generations [24] (Table 2). An environmentally associated stable epiallele in *A. thaliana* is NMR19-4, its methylation affects the expression of Pheophytin Pheophorbide Hydrolase (PPH) and control chlorophyll degradation thus helping the plant adapt to its environment [25] (Table 2). The effect of environmental factors

on DNA methylation has also been studied in grapevine (*Vitis vinifera*) by exposing it to different environmental conditions such as elevated solar ultraviolet-B radiation (UV-B), water deficit (D), and treatment with abscisic acid (ABA), which can induce epigenetic variability and also translate into changes of biochemical composition [26]. DNA methylation increases phenotypic plasticity to help species adapt to their environment which has been studied in the two contrasting strains of hermaphrodite fish *Kryptolebias marmoratus* in two different environments, suggesting that DNA methylation mediates transgenerational stability of epialleles as well as phenotypic variation [27].

4. ROLE OF EPIALLELES IN PHENOTYPIC VARIATION

Plant traits can be controlled via epialleles through imprinted genes which show a strong association with epigenetic modification as imprinting variation occurs due to DNA methylation polymorphism that can bring phenotypic variation as well. By altering the methylation in imprinted genes, a repressed gene can be expressed, and novel traits can be brought [28].

Epigenetic variation accounts for more phenotypic diversity than was previously considered. The role of epialleles in the phenotypic variation has been studied in the mutation accumulation lines of *A. thaliana,* which contain methylation at most of its CGs sites [29]. As DNA methylation plays a part in the regulation of gene expression, the altered expression due to methylation brings forth disease or abnormal development or defensive response against environmental stresses. The coding region of the gene can be moderately or densely methylated which has been studied in *Arabidopsis* phytochrome A (*phy A*) gene, which is hypermethylated at the coding region resulting in silencing of this gene that leads to phenotypic variation and abnormal growth of seedlings [30] (Table 1). Another example in which a direct relationship between DNA methylation and gene expression can be deduced is from the wild-type and peloric flower of Toadflax plant (*Linaria vulgaris*), in which the epimutation has repressed the *Lcyc gene* causing a change in the symmetry of flowers from lateral to radial [31] (Table 2). Some current studies have reported that epigenetic regulation is also involved in fruit ripening, which causes changes in the DNA methylation of ripening-related genes thus controlling their expression. Such discoveries in epigenetics are important for the future perspective of agronomy [32].

5. ROLE OF EPIALLELES IN DEVELOPMENT

Just like phenotypic variations several findings have also demonstrated the role of epialleles in the

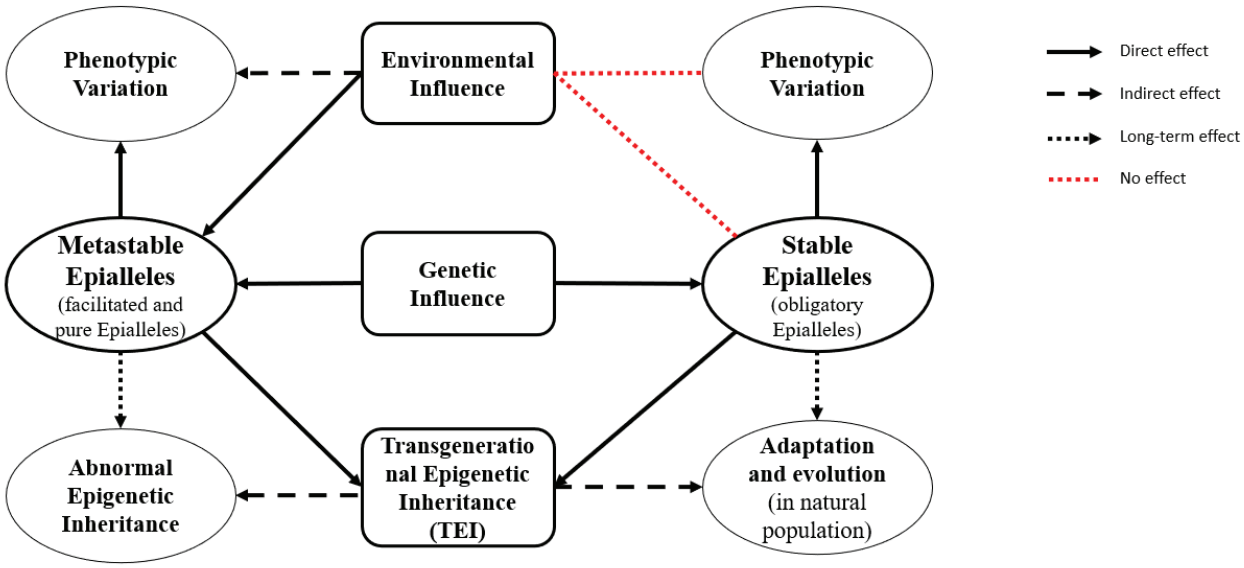

Fig. 1. Stable versus Metastable epialleles, their interaction with environmental and genetic factors leading to phenotypic variations, their inheritance to the next generation in the form of Transgenerational Epigenetic Inheritance (TEI) resulting in long term effect in the form of adaptation and evolution or abnormal epigenetic inheritance.

Table 1. Examples of Metastable Epialleles (MEs) in different species of Eukaryotes.

Species	Gene	Function	Methylation status	Phenotypic variation	Ref.
Human (*Homo sapiens*)	*VTRNA2-1*	Tumor suppressor gene	Hypermethylation	Leukaemia, lung and esophageal cancer	[19]
	POMC	Encodes melanocyte-stimulating hormone	Hypermethylation of VMR	Adiposity (BMI)	[42]
	UBXD2	Encode integral membrane protein of ER	Methylation patches at TSS (Transcription Start site)	Bladder cancer	[72]
	AQP11	Encodes channel proteins			
	FGF18	Encodes Fibroblast growth factor 18			
	MMP11	Extracellular remodeling and tumor invasion			
	TIMP1	Inactivates *MMP11* and other metalloproteinases			
	TGFBR	Provide instructions for making TGF- β receptor type-1			
	NUPR1	Encodes Nuclear protein 1			
	FAM124B	Encodes FAM124B protein	Hypermethylation (*FAM124B* and *ST6GALNAC1*) and hypomethylation (*NAV1* and *PER1*)	Breast cancer	[73]
	ST6GALNAC1	Encodes ST6GALNAC1 enzyme involves in glycosylation			
	NAV1	Encodes Neuron Navigator 1 protein			
	PER1	Encodes Period Circadian Regulator 1 protein			
	MAPK13, FASLG, PRF1, S100A13, RIPK3, IL-21R	Immune responses and activation	Altered methylation at coding site	Altering immune activation, Crohn's disease	[74]
	GSTM5	Glutathione metabolism	Alteration in promoter methylation	Glioblastoma	[75-77]
Agouti rodent *Dasyproctidae*)	$Cabp^{IAP}$	*Cabp* (encodes CDK5 activator binding protein)	hypomethylation and insertion of IAP (Intra-cisternal A particle) retrotransposon	Premature polyadenylation	[63]
	$Axin^{Fu}$	Encodes Axin protein, function in embryonic development	Methylation and insertion of IAP (Intra-cisternal A particle) retrotransposon	Formation of kinked tail	[62, 64, 100]
	A^{vy} (Agouti viable yellow)	Coat colour	Methylation and insertion of IAP (Intra-cisternal A particle) retrotransposon	Variation in coat colour, diabetes, obesity, susceptibility to tumors	[21, 62, 64, 68, 69]
Thale cress (*Arabidopsis thaliana*)	*phy A* (Phytochrome A)	Light sensitive pigments	Hypermethylation at coding region	Gene silencing, mutant phenotype, seedlings displaying short or intermediate length hypocotyls	[30]
Rice (*Oryza sativa*)	*Epi-ak1*	*OsAK1* encodes a rice adenylate kinase	Hypermethylation at promoter region	Gene silencing, albino leaves, panicle with abnormal chloroplast	[67]
African oil palm (*Elaeis guineensis*)	*EgDEF1*	Inflorescence development	Hypomethylation of Karma LINE transposon near *Karma* splice site	Sterile parthenocarpic flowers	[82]

Table 2. Examples of Stable Epialleles in different species of Eukaryotes.

Species	Gene	Function	Methylation status	Phenotypic variation	Ref.
Rice (*Oryza sativa*)	*Homeobox genes: DNA-binding protein, Elongation factor, Hsp70, YF25, SNF-FZ14, S3, CDPK-R, CAL-2, CAL-11, OsHMA4, OsHMA8*	Developmental processes	Hypomethylation	Tolerant response against heavy metal contamination	[24]
Thale cress (*Arabidopsis thaliana*)	*NMR19-4*	Regulates *PPH* expression	Methylation of downstream promoter	Adaptation to environment, regulates leaf senescence	[25]
	NLR genes, *At5g47260* and *At5g47260*	Innate immune responses	Hypermethylation and hypomethylation	Partial resistance against clubroot disease	[55]
	FWA	Flowering time	Hypomethylation	Delayed flowering	[94, 97]
Toadflax (*Linaria vulgaris*)	*Lcyc gene*	Establish flower symmetry	Hypermethylation	Radially symmetric peloric flowers	[31]
Tomato (*Solanum lycopersicum*)	*VTE3*	Encodes 2-methyl-6-phytyl-1,4-hydroquinone methyltransferase (VTE3)	Methylation of SINE retrotransposon at the promoter site of QTL loci	Regulates VTE content in tomato	[56]
Brown mustard (*Brassica juncea*)	*CaM9 genes*	Encodes calcium-binding proteins, calmodulin involve in signal transduction	Hypermethylation of promoter region	Reduced expression, aids in drought tolerance	[57]
Barley (*Hordeum vulgare*)	*Cly1 (cleistogamy 1)*	Encode AP2-protein causes swelling of lodicule results in non-cleistogamous flowers	Hypermethylation at upstream promoter region	Failure of lodicule to swell, cleistogamous flowers	[58]
Cotton (*Gossypium barbadense*)	*COL2D*	Photoperiod flowering	hypomethylation	Higher expression and loss of photoperiod sensitivity	[59]
Maize (*Zea mays*)	*P1-rr* *P1-pr*	*p1* (encodes a transcription factor that controls phlobaphene pigment accumulation in floral organs)	Methylation at enhancer	Increased transcription and pigmentation	[98, 99]
Honeybee (*Apis mallifera*)	*AmLAM*	Encodes conserved protein lysosomal α-mannosidase that is involved in the maturation and degradation of glycoprotein-linked oligosaccharides	Variation in methylation in response to environmental changes	Hypermethylation correlates to increase expression especially during early stages of development	[60, 61]

development of eukaryotes. In plants, epialleles have a role in organogenesis with DNA methylation changes in an organo-specific way, and epialleles showing phenotypic variation. In vitro study on the Sugar beet (*Beta vulgaris altissima*) cultivars showed that changes in DNA methylation during organogenesis lead to the alteration in root and shoot regeneration [33].

Like plants, DNA methylation is important for the development of mammals and represses certain genes during development [34]. During implantation-gastrulation transition, intermediate methylation states develop that are very susceptible to genetic and environmental variation and ultimately lead to phenotypic variation [35, 36]. Prenatal differentially methylated regions (P-DMRs) occur at regulatory regions that control the growth-related pathways, metabolism, and gestational timing. Prenatal malnutrition may

affect P-DRMs patterns [37] and can cause birth defects such as cleft lip, a very common birth defect in humans which is also associated with the methylation changes in nutrition-responsive metastable epiallele [38]. Another such study has supported the evidence that prenatal environment is very important for the development of healthy new-born and prenatal environment influences epigenetic modification. This study evaluated the effect of infertility and intracytoplasmic sperm injection on methylated patterns and compared in vivo and in vitro fertilized newborns and found a drastic difference in both. Many birth-related defects arose through metastable epialleles developed during conception from *in vitro* fertilization (IVF) [39]. The environment during the development of a newborn is very important and disruption may result in later-life diseases. The changes in the metastable epialleles during the gestational period may cause changes in childhood Body mass index (BMI) [40, 41]. Pro-opiomelanocortin (*POMC*) gene is expressed during early embryonic development and has a role in the regulation of body weight and contains variably methylated regions (VMR) and hypermethylation of VMR is related to obesity (BMI) [42] (Table 2).

The pollutants in our environment are hazardous to our bodies and genetic makeup and are also involved in epigenetic changes. One study has reported that exposure to bisphenol A (BPA) during fetal development has a significant effect on the DNA modifications that can give rise to birth defects [43]. Alteration in DNA methylation caused by nutrition and the environment during the prenatal period is now a target for the diagnosis and prevention of various Metastable-epialleles-associated-diseases. Following these studies, various epigenetic biomarkers and tools have been developed for the diagnosis of obesity and other metabolic syndromes related to MEs [44].

6. ROLE OF EPIALLELES IN TRANSGENERATIONAL EPIGENETIC INHERITANCE (TEI) AND EVOLUTION

Epigenetic inheritance is the transfer of epigenetic variation from one generation to the next. The study of the methylome sequence of developing pollen in plants shows that methylation variation retains in the germline and is transmitted to the next generation, thus transgenerational epigenetic inheritance in plants is more common as compared to mammals, which erase epigenetic modification through germline programming [45]. As the dominant type of epigenetic modification in plants is cytosine methylation so epialleles that arise from cytosine methylation in plants have better heritability and stability and phenotypic variation is increased as compared to chromatin modification. Epialleles formed as an outcome of cytosine methylation contributes to the evolution and they evolve faster than unmethylated genes as identified by one study on genetically identical individuals of Clonal fish *Chrosomus eos-neogeus* found in different environments that contain the epiallelic variation in the absence of genetic variation and has evolved and adapted themselves according to their environmental conditions [46].

DNA methylation in plants is altered throughout its whole life starting from growth and development to its vegetative and reproductive phases and to counter various internal and external stresses [47]. Plants also inherit stress-tolerant epialleles from their parents [22]. The heritable epialleles as a result of environmental conditions contribute to phenotypic plasticity and fitness that can also influence plant evolution [48]. Certain loci enriched with CpG dinucleotides in a plant's genome can switch between alternative epiallelic states also called metastable epialleles that can sometimes transform into stable epialleles and can remain stable across generations. However, other loci with scarce CpG dinucleotides remain resistant to such switching [49]. Paramutation is an epigenetic allelic interaction in which one epiallele induces a heritable epigenetic change on another that can be stably inherited across generations and also linked to gene silencing. In the wild, the interaction between two naturally occurring epialleles can cause alteration in methylation and ultimately reactivation of silenced genes. Understanding paramutation through epigenetics may help to improve the cultivation of the crops [50].

Besides the beneficial effects, transgenerationally heritable epialleles also have some drawbacks such as inbreeding in an isolated population contributes to hybrid incompatibility, speciation, and reduces gene flow and epialleles play a major role in it. Hybrid incompatibility may

be due to hybrid progeny inheriting non-functional epialleles resulting in gene silencing which can stably inherit across generations and can affect a variety of beneficial plant traits [51].

7. STABLE AND NATURALLY OCCURRING EPIALLELES

In plants, epialleles can affect and alter the floral shape, vegetative and seed pigmentation, pathogen resistance, and development as well as respond to natural selection [25]. The study of transgenerational epigenetic variation in the wild strains of *A. thaliana* has shown that strains propagated by a single seed differ greatly in epialleles and contain differently methylated CG regions that are involved in plant evolution, alteration of transcription, phenotypic variation in the absence of genetic mutation and also affect the nearby genes [52, 53]. Stable DNA methylation brings about the emergence of stable epialleles that are transmissible through natural selection as well as inbreeding and follows Mendelian inheritance pattern and are independent of the environmental changes. Out of all three types of epialleles, obligatory epialleles are the most stable while the facilitated and pure epialleles remain mostly in a metastable state (Fig. 1). The heritable epigenetic changes although alter gene expression but they are unable to affect inherited differences among organisms [7, 54]. Few of the transgenerationally heritable stable epialleles have been reported that are mostly found in plants such as some naturally occurring epialleles show resistance against diseases, e.g., epigenetic variation in NLR genes show resistance against clubroot which is a type of root gall disease caused by the biotrophic pathogen Rhizaria (*Plasmodiophora brassicae*) in Brassicaceae crops [55]. Some naturally occurring stable epialleles in plants are responsible for the regulation of nutrients, e.g., naturally occurring epiallele VTE3 gene in tomato encodes an enzyme 2-methyl-6-phytyl-1, 4-hydroquinone methyltransferase (VTE3) (EC 2.1.1.295) that catalyzes the synthesis of γ- and α-tocopherols that form Vitamin E (VTE). This epiallele is regulated by the methylation of SINE retrotransposon located in the promoter region of VTE quantitative trait loci (QTL; $mQTL_{9\text{-}2\text{-}6}$) [56]. Some stable epialleles develop to counter stress, e.g., stress-responsive epialleles are formed in *Brassica juncea* under severe drought conditions which include hypomethylation of apoptosis gene and hypermethylation of genes involved in normal functioning, e.g., *CaM9* genes are hypermethylated at their promoter region that help combat stress conditions [57]. Others are known to control some useful traits such as epialleles that control plant's floral arrangement, e.g., some variants of barley show cleistogamy (closed flowering), which is due to the expression of *cly1 (cleistogamy 1)* epiallele, that is hypermethylated at upstream promoter region failing lodicule to swell forming cleistogamous flowers. Closed flowering is advantageous as it prevents pathogen entry into flowers [58]. Polyploidy can also induce genetic and epigenetic changes, e.g., induction of methylation changes on *COL2D* epiallele in allotetraploid cotton results in alternating gene expression in wild and domesticated cotton such, insight may help in using the epialleles in epigenetic engineering and breeding for better crop production [59]. Compared to plants and mammals, the genome of invertebrates is very sporadically methylated, and some studies are done to show the epialleles of invertebrates. *AmLAM* epiallele in honeybee *(Apis mallifera)* is the first studied epiallele in insects where differentially methylated regions result in alteration of phenotypic and behavioral plasticity to adapt to an ever-changing environment. An increase in methylation of certain regions is correlated with an increase in expression which in turn increases the energy and metabolism required during larval growth or adult building structure. The study was carried out to decipher the methylation patterns in sequence variants which have shown that a high level of flexibility exists in sequence variants [60, 61] (Table 2).

8. METASTABLE EPIALLELES (MES)

Metastable Epialleles (MEs) emerge because of their inter-individual epigenetic variation at the genomic loci of the same species. MEs are responsible for the phenotypic variation among genetically identical individuals. They switch between stable and unstable states and are particularly susceptible to environmental influence [62]. Obligatory epialleles remain stable and have no environmental influences, facilitated and pure epialleles are metastable and are influenced greatly by the genetic and environmental factors [7] (Figure 1).

MEs are formed due to alteration in normal methylation by either exposure to different environmental factors or the introduction of the genetic variants such as methylation and insertion of intracisternal A particle (IAP) in genes, e.g., $Cabp^{IAP}$ [63], $Axin^{Fu}$ and A^{vy} genes in Agouti rodent are particularly vulnerable to environmental changes [62, 64] (Table 2). MEs have also been reported in various developmental disorders associated with mammals and they have a potential of transgenerational epigenetic inheritance [65] that is beneficial for some plants as sometimes they provide useful traits for breeding and adaptation thus increasing the plants' productivity [66].

Very few naturally occurring metastable epialleles have been reported. A naturally occurring epiallele *Epi-ak1* is found in rice in which due to changes in DNA methylation can cause albino leaves with abnormal chloroplast and malformed thylakoid membrane [67]. Some naturally occurring MEs are also found in mammals, e.g., A^{vy} allele responsible for the coat colour in agouti rodent, the methylation at the Intra-cisternal A particle (IAP) where the transcription of A^{vy} allele starts, causes ectopic expression of agouti gene resulting in variation in coat colour, obesity, diabetes and susceptibility to tumors [21, 68, 69] (Table 1).

Mutations can disrupt DNA methylation targeting genes which ultimately change the gene transcription and expression [70]. Inter-individual epiallelic variations have been observed in many eukaryotic species that remain temporarily stable. Non-genetically determined epialleles at the transcription site are related to a variety of diseases in humans, e.g., hypomethylation of promoters has been observed in various cancers [71]. The methylation status of many human genes has provided evidence that MEs are involved in several common human diseases and disorders such as bladder cancer [72], Breast cancer [73], Crohn's disease [74], glioblastoma [75, 76], and many more, suggesting their potential role in the development of tumorigenesis and malignancies [77] (Table 1). Aberrant DNA methylation can also cause some of the serious imprinting disorders in humans, such as Uniparental disomy (UPD), Angelman, Prader-Willi, Beckwith-Wiedemann syndrome, multi-locus imprinting disturbances (MLID), Transient neonatal diabetes mellitus, Silver-Russell syndrome, and Pseudohypoparathyroidism type 1b [78].

Epialleles or epigenetic variations are part of many diseases, and the disease itself can induce epigenetic changes in an organism. Many studies have conducted a comparison to determine the epigenetic variation of affected and unaffected individuals, e.g., DNA methylation profiling of Guthrie cards of many neonates lead to the identification of temporarily stable epialleles present at birth in humans, such a method is very powerful and cost-effective for the diagnosis of epigenetic variation-relevant diseases [79]. Methylomes of malnourished children have shown the potential effect of malnutrition during development, which elicit epiallelic alterations resulting in cognitive and behavioral disorders in humans, which is a major problem worldwide [80]. Patients with Schizophrenia and non-psychiatric control have been measured for the methylation status, they were found to contain significant variation in DNA methylation of Spatio-temporal regulatory genes [81]. As in plants, MEs are also the cause of a somaclonal variation which is a problem for a variety of economically important crops, e.g., a study was done on the somaclonal mantled plants of African oil palm (*Elaeis guineensis*), an important oil-bearing crop, has found hypomethylation at Karma LINE retrotransposon of *EgDEF1* gene resulting in mantled progeny producing abnormal fruits with very low oil yield [82] (Table 1).

9. LATEST ADVANCEMENTS IN EPIGENETICS CONCERNING EPIALLELES

In the current era, many epigenetic markers have been discovered and are used for the diagnosis of various diseases like cancer and other genetic disorders. Although many studies have proved that numerous common human diseases and disorders contained abnormal DNA methylation [83], but their comprehension and analyses are quite difficult. Different methods have been developed to analyze them including the use of inversion probes that quantify epialleles in the bisulphite treated genomic DNA [84]. Another such technique is methylation-sensitive amplified polymorphism (MSAP) that is used for the quantification and detection of tissue origin, it has been used to detect and quantify the

origin of tissues of salmon and veal in processed food using epialleles as biomarkers [85]. Another useful approach to quantify epialleles at a single-molecular level using DNA ligases is epialleles quantification (EpiQ), which is feasible for routine practice in quantifying heterogeneous methylation patterns [86]. Many other methodologies have been developed for the detection and interpretation of DNA methylated patterns, another significant method is the electrochemical method in which the recognition element (enzyme, probe, or antibody) is used that binds and interact with the selective region and recognizes the methylated region [87]. A very substantial method is the Epiallele-sensitive droplet digital PCR (EAST-*dd*PCR) that is used for the quantification and analysis of epialleles which may be helpful in the diagnostics of many diseases [88]. Multilayer microfluid PCR-HRM-based device is developed for the detection of DNA methylation heterogeneity in samples with very low DNA such as liquid biopsies [89]. Though many studies have confirmed the epigenetic biomarker as a more convenient tool, there is still so much to overcome before their practical application in the clinical setting.

One of the most advanced technologies that have been employed in epigenetics is CRISPR/dCas9 targeting methylation system that is helpful in many aspects and will be a breakthrough to treat diseases that are caused by epigenetic changes. One of the comparative studies between the dCas9-KRAB system and short-hairpin RNA [shRNA] has demonstrated that the repression of *phosphatase and tensin homolog (PTEN)* transcription in the neural cells of the adult mammalian central nervous system (CNS) using CRISPR/dCas9 is more effective compared to the shRNAs targeting as the most successful strategy to improve axon regeneration in the damaged CNS is through the *PTEN* repression [90]. Another purpose of this method is to study the methylation status of genes and their role in controlling their expression as CRISPR/dCas9 has been used along with short catalytically inactive guide RNAs (gRNAs) to express endogenous genes [91]. This epigenome editing technique can be promising to cure Imprinting diseases (IDs) in which activation and suppression of disturbed imprinted genes is a way to control the disease [92]. CRISPR-based epigenome editing technologies can also provide the best way to treat various types of human cancers. These technologies are very effective in regulating gene expression without changing the DNA sequence so they can be used to treat cancers in which tumour suppresser genes and oncogenes have been silenced due to the methylation of their promoters [93]. These techniques can also be used effectively in agronomy to regulate the expression of different genes e.g., expression of A. thaliana stable epiallele *FWA* and *CACTA1* transposon has been controlled by demethylating their methylated region using a fusion of human demethylase Ten-Eleven Translocation1 (TET1cd) and artificial zinc finger (ZF). This system has provided the opportunity to control the expression of epialleles, reactivate the silent epialleles, and introduce new traits in cultivated plants [94] (Table 2). One study has combined two genetically identical plant genomes with different DNA methylation of *A.thaliana* producing an epihybrid with an epigenomic shock that contains novel epialleles and regulated transposons with more mobility in the genome due to a decrease in their DNA methylation [95].

Some studies have also provided evidence that DNA methylation can survive in the fossils which gave us a great way to study palaeogenomics of DNA methylation. Using independent Methylated Binding Domains (MBD)-based enrichment method, DNA samples of various ancient extinct organisms have been studied for their methylation profiles concluding that DNA methylation can survive in a variety of tissues, environmental conditions, and over a long fossil range [96].

10. FUTURE PERSPECTIVES

The Era of epigenetics has just started and there is much more to discover about DNA methylation and epialleles. There is a need for a deep understanding of the formation of epialleles and their contribution to nature. Complete methylome sequences of organisms will help the researchers to discover more useful epigenetic markers for the diagnosis of diseases and a more deep understanding of methylation patterns will be useful in the introduction of novel traits in cultivated species, similarly, distribution of naturally occurring epialleles in the wild, as well as stable epialleles in humans and animals, account for more discoveries. Despite so much data about epialleles, naturally

occurring epialleles remained less and stable epialleles in animals are rarely documented. In the future, more studies may help us in the discoveries of epigenetic shocks and epihybrids in the wild with wide epigenetic diversity and novel epialleles that may lead us to more advances in epigenetics. One of the most important steps forward in epigenetics is CRISPR/dCas9 de/methylation system that has opened a door for many opportunities to cure some hard-to-treat human diseases like cancers and genetic disorders [90, 91]. What we need in the future is an effective epigenetic editing system that has practical applications in therapeutics.

Many naturally occurring epialleles account for agronomic traits and are beneficial for humans such as traits related to the nutritional availability, cleistogamy, regulation of flowering time and development, as well as stress-tolerant and disease-resistant traits in plants. A better understanding of such beneficial traits may be helpful for agricultural purposes, e.g., a good insight into the role of epialleles in polyploidy such as allotetraploid may be useful to cultivate crops with more valuable traits and improve the knowledge about epigenetic engineering and breeding [59]. Similarly, a major problem we face during the cultivation of crops through cloning is a somaclonal variation which is proved to be related to the epigenetic changes, more work is needed to be done to understand the epialleles that are involved in somaclonal variation to overcome problems [82]. Epigenetics is advancing more considering its interaction with other fields such as agronomy, biotechnology, bioengineering, diagnostics, paleontology, evolution, environmental biology, etc., more such discoveries will be useful to better understand the role of epialleles and DNA methylation in different areas of biology (Fig. 2).

11. CONCLUSION

Considering all the data that has been reviewed, it is concluded that DNA methylation and epialleles have an important role in transforming an organism and through transgenerational epigenetic inheritance, the changes are passed to the next generation. All in all, their involvement in many humans concerning diseases and agronomic traits are making them equally practical but it is also important to interpret the epigenetic data in a systematic and well-organized way due to susceptibility of DNA methylation to biotic and abiotic factors. However, there are still some unanswered questions that need our attention to work on like despite so much data about the stable and metastable epialleles there is

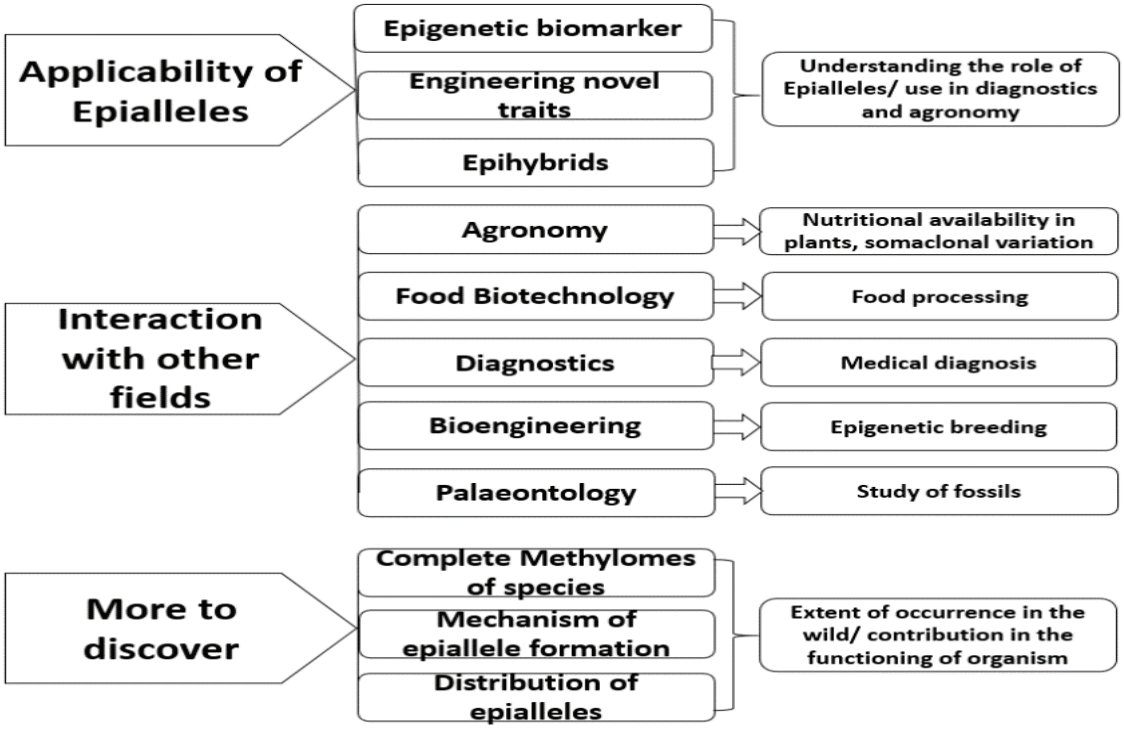

Fig. 2. Flow diagram of applicability of epialleles, its interaction with other fields, and the way forward.

still no borderline that separates them, so the factors that transform these epialleles need to be studied well to have a clear-cut understanding. Similarly, the DNA methylation status of the prominent species of different phyla are unreported showing a gap in the phylogenesis of the methylomes. Exploring these aspects will enhance our study in the better application of this knowledge.

12. CONFLICT OF INTEREST

The authors declare no conflict of interest.

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