ejpmr, 2017,4(10), 152-160

SJIF Impact Factor 4.161

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Review Article

ISSN 2394-3211

EJPMR

A BRIEF PREFACE ON STUDIES ON GENE TRAPPING

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Article Received on 03/08/2017 **Article Revised on 24/08/2017** **Article Accepted on 14/09/2017**

ABSTRACT

Every species has its own expression of genome; even though it has common alphabets the expression is different from species to species and genus to genus. During the evolution of species the genes get modified to reach more sophisticated sustainable stages to adopt the environmental conditions, in trying to adoptive conditions in evolutionary path genes give up its expression, redundant, changed their expression and over expressed. Apart from this, gene expression is regulated at different stages of life span of an organism. Some genes are expressed and some are silenced at every stages of life of an organism. Genes are expressed in the form of protein and non-proteins. These regulations give the cells to control their structure, cellular functions, morphogenesis, differentiation, dedifferentiation, receptor modulations and adaptive toward environment.

KEYWORDS: method to develop to monitor gene expression by random.

INTRODUCTION

Every species has its own expression of genome; even though it has common alphabets the expression is different from species to species and genus to genus. During the evolution of species the genes get modified to reach more sophisticated sustainable stages to adopt the environmental conditions, in trying to adoptive conditions in evolutionary path genes give up its expression, redundant, changed their expression and over expressed. Apart from this, gene expression is regulated at different stages of life span of an organism. Some genes are expressed and some are silenced at every stages of life of an organism. Genes are expressed in the form of protein and non-proteins. These regulations give the cells to control their structure, cellular functions, morphogenesis, differentiation, dedifferentiation, receptor modulations and adaptive toward environment. Genes are regulated and expressed only when the cell required product, depending up on the external environmental such as food availability, physical and chemical responses and stress and internal conditions like metabolism, protection, cell division. To fulfil the genetic application one must know the functions of each gene for that researches has been developing genetic engineering techniques based on mutagenesis to evaluate the function of gene by disruption by altering the phenotype expression but this mutagenesis not able to cover all the gene because of species having overlapping genes, sharing function with other genes and many genes functions at different stages of development. Mutation in these genes may s early lethality or pleiotropic may not give good results or mask the role of a particular gene. Apart from mutagenesis several gene expression profiles techniques have been developed such microarrays and chip technologies. These techniques analyzed and identified the expression profiles of many genes,[1] but one of limitation of this method is source of tissues used to isolate the RNA probe and low level or lack of expression of gene in many cells. To overcome these limitations and to analyze the expressions profile of genes researches have been developed a technique by inserting the reporter gene at randomly into the genome of an organism is called gene trapping.

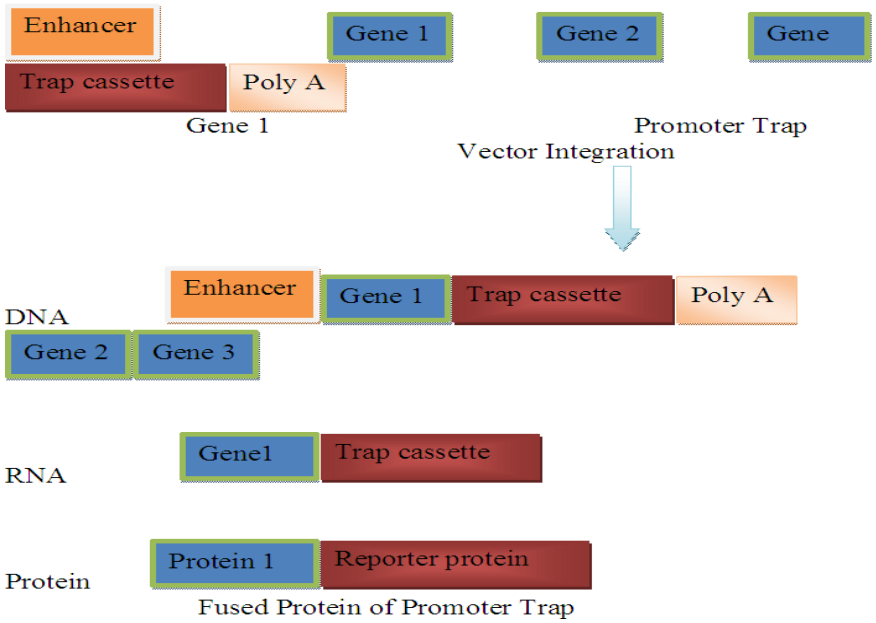
Gene trapping

Gene trapping is a method to develop to monitor gene expression by random insertion of reporter gene in to the genome. Gene trapping is a form of insertional mutagenesis specifically designed to disrupt gene function by producing intragenic integration events [Evans, M.J. 1998].The basis of geen trapping is strating by random insertion of a lacZ reporter gene into the E.coli genome, by fusing with a reporter gene helpful in monitorin the experssion of individual genes.[2] This approch modified and used in varoiuos level of species.There are different modes of gene trapping methods such as promoter trap,gene trap,enhancer trap and poly A trap ; all are responds to cis-acting regulatory sequence at the site of insertion.

Promoter Trap

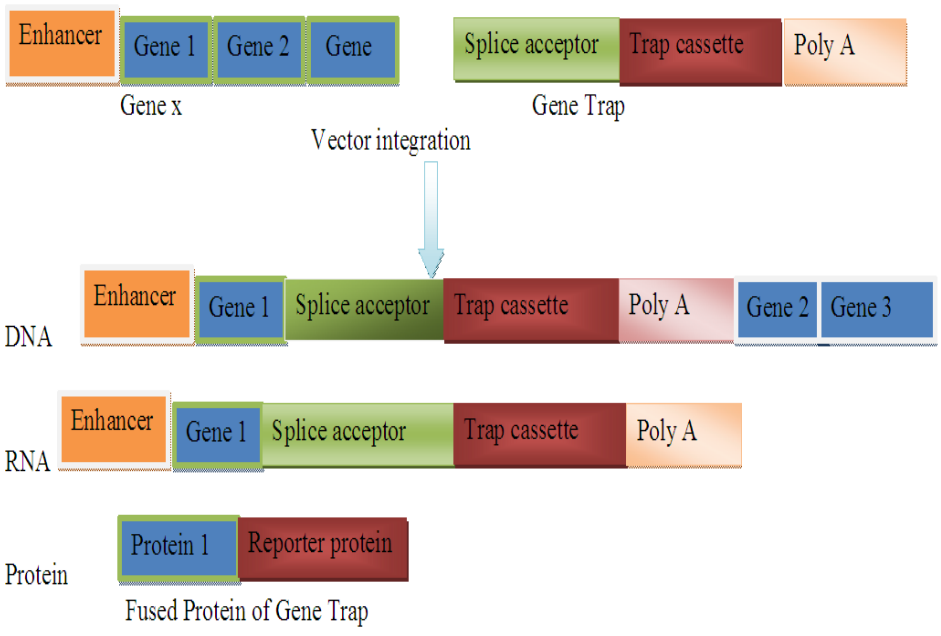
Promoter trap is a best tool for discovering the speciifc cell type markers which involves random insertion of promoterless reporter gene to produce transgenic cellines.owing to promoterlees reporter it will expressed only when it is in inserted into transcriptional unit in a

correct oreintation. The main drawback of promoter trapping is cannot be identified the transcriptionally silent loci in the target cells.



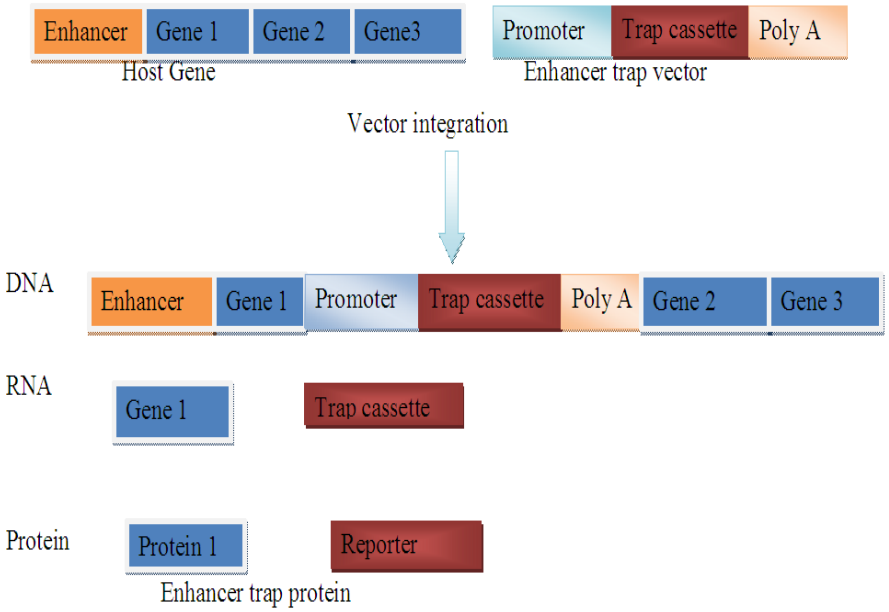
Gene Trap

Gene traps have promoterless reporter gene, splice acceptor sequence and ploy A at 3' tail and its activity can occur only when it is inserted in transcriptional unit .if the reporter gene is inserted in between the exon, the splice domain of the exon and splice acceptor of the reporter gene are spliced together and fused and the reporter gene transcribed. The splice acceptor utilizes the transcriptional unit by transcribing trapped gene followed by vector sequence and terminate at ploy A and produce a fused protein[gene and reporter protein] .if the reporter gene inserted into the exon but not in correct orientation the reporter will not transcribed.



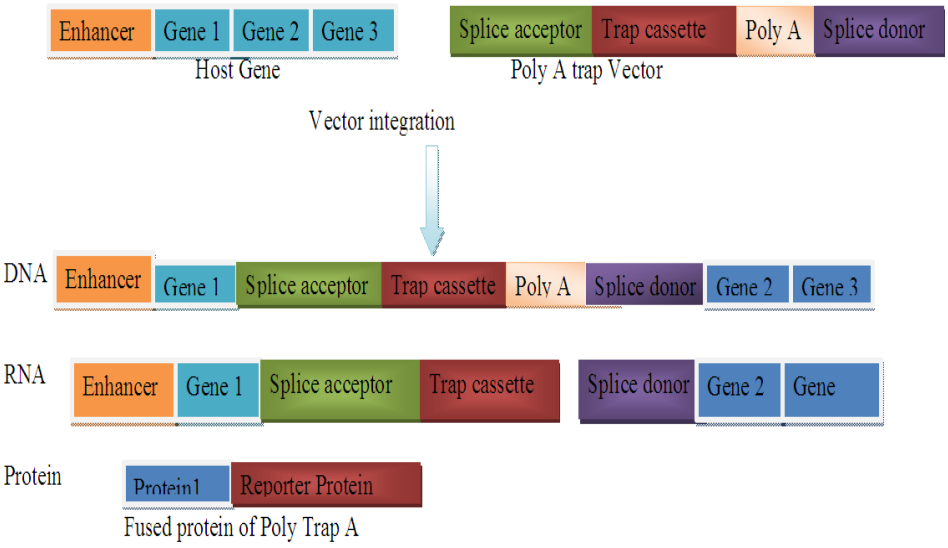
Enhancer trap

Enhancer trap method allows the hijacking of an enhancer from another gene, comprises the reporter gene with minimal promoter, containing a TATA box and transcription start site. It is unable to express the reporter gene alone but it expressed in the presence of endogenous enhancer element.



Poly A trap

Poly A trap gene cassette contains a promoter signal and a transcriptional start site and splice donor sequence lacks poly A signal, it trap the genes which are not expressed or at very low level of expression. Owning to having splice donor sequence the mRNA of a vector is fused with the exon of trapped gene and lacking of poly A signal in the cassette, the reporter gene RNA can be stabilised by trapping the endogenous gene poly A signal.



Studies on *Drosophila melanogaster*

The drosophila melanogster is an insecta widely used in genetic engineering applications.the genome of Drosophila is sequenced in 2000 containing 15,682 genes. O'kane developed the sophisticated enhancer trap method in *Drosophila melanogsatre* fruit fly and provided information of cell specific type markers, novel gene expression pattern and some mutant phenotypes.[3] Mostly lac Z is used as a reporter gene in enhancer trap in Drosophila to identify the genes which are involved in a tissue specific and change in the gene expression during development.[4] Wang, S and Hazelring,T, used green fluorescent protein[GFP] isolated from Aequorea Victoria as a reporter gene to monitor the distribution of exuperantia protein [exu] which is a maternal originated protein play a crucial role in developing the polarity of oocyte by binding the mRNA . They found that the expression pattern of exu-GFP fused protein is similar to the expression pattern of exu and study the protein role at sub-cellular level.[5] Edward yeh and his co-team developed GAL4 enhancer trap with GFP reporter to monitor GFP expression in ovaries and larval nervous system and they described the dynamic changes in gene expression during the real time cell development in cultured egg chambers.[6] Insect's brain contains a pair of prominent neural centres called mushroom bodies[7] these are associated with function such as memory, learning, spatial recognition target location.[8,9] These are mainly three classes of neuron such as input, output and intrinsic, both the functional and structural studies showed that the functional properties provided by intrinsic neurons called Kenyon cells,[10,11] electron microscopic studies revealed that among there is a local difference in Kenyon cells in density and diameter,[12] these neural complexity may provide complex behaviour in mushroom bodies. To reveal the complexity of neurons Brand and Perrimon developed an enhancer trap P [GAL4] in that transposon is an enhancer trap with a reporter gene,[13] Insertion of these enhancer trap in the close proximity of transcriptional unit resultant the reporter to be expressed in a pattern by showed the enhancer regulatory properties. These expression patterns belong to neuronal trajectories and neuroanatomy. Ming yao yang. et.,al screened several collection of P[GAL4] enhancer trap lines to study the expression pattern of Kenyon cells in mushroom bodies at all levels of its developmental organisation like cell body, lobes, calyx and pedunculus, they strongly predicted that the function of Kenyon cell is different from each other.[14] Doreen Dawyuan Han et.,al., developed a hormone dependent enhancer trap line with tissue specific USA –linked gene expression for studying the role of polar follicle cells during oogenesis development by using a toxin diphtheria [GAL4-Human estrogen-UAS-linked transgene-Diphtheria enhancer trap] for cell ablation. They targeted to abolish GALER expressing border cells and posterior polar subpopulation of follicle cells reported that the failure of micropyle pore formation and defective deployment in the anteriorposterior polar oocyte and confirmed that posterior follicle cells are required for the formation of anterior and posterior axis in the oocyte.[15]

Studies on *Ciona intestinalis*

It is an ascidian and member of the phylum chordata. The genome of *C.intestinalis* is very small containing 155 Mbp genes with approximately 15,852 protein coding genes.[16] Because of availability of large EST database it is very useful in developmental biology studies and genetics approaches. Ci-Musashi is a RNA binding protein involved in cell differentiation of these organisms by binding the RAN and resulting asymmetric translation of RNA by repressing translation, its lead to different specficarion of tissues. Satoko Awazu.,et al., studied these gene by enhancer trapping method, they used GFP as a reporter gene. They concluded the GFP expression in the Ci[MiLRCiTPOgfp]2 cell lines show similar expression with Ci-Musashi expression and identified the enhancers of Ci-Musashi, it expressed in endostyle a row of tissue in the oral siphon and rows of languets of the pharyngeal gill and also revealed that many parts of the genomic region, the 5'upstream region, and introns regulates the Ci-Musashi expression. Enhancer activity of introns suggest that the regions are similar but with different enhancer activity. One of the enhancer characterizations of Fr3 contains e1 and e2 and these co-ordinately regulate the Ci-musashi expression.[17]

Studies on Zebra fish

The Zebra fish is a freshwater fish; vertebrate, popular aquarium fish belongs to order Cypriniformes. Kerstin Howe et al. were reported that the zebra fish genome sequence has 1.4 GB and having 26,000 protein coding genes.[18] The genome of zebra fish is twofold less than the mouse genome. Nearly 1,400-2,400 genes are necessary for development of Zebra fish.[19,20] Most of gene trap in zebrafish is based on transposon based insertional mutagenesis to cause null or severe hypomorphic mutations know as[GBTs] "gene breaking transpsons"[21] and Tol2 transposon used for effective gene trap integration.[22] Darius Balciunas et.al succeeded in producing enhancer trap lines in zebra fish Danio rerio by using Sleeping Beauty transposon cassette with GFP reporter gene, they developed 9 enhancer trap lines of zebrafish with different tissue or organic specific GFP expressions pattern and predicted the novel gene in ET7 line which is in 30kb downstream of mkp3 locus pattern expression was resembled with GFP expression in the mid brain –hindbrain boundary, forebrain and ventricles it means the enhancer trap is under the control mkp3 enhancer element and in-situ hybridization studies showed that ET2 lines having the GPF expression specifically in caudal primary motor neurons.[23] Jorune Balciuniene et.al developed a GBT-R15 vector gene trap with Gal4-vp16 as a primary reporter and GFP as secondary reporter used to know the function of non-essential genes.[24]

Studies on Mouse

The rodent mouse belongs to chordate having approximately 23,000 genes. Several protocols have been developed to trap the genes in mouse and evaluate the functions,[25,26] Marjo Salminen et.al develop a poly A trap vector IRESbgalNeo [-pA] to trap the genes which are not active in undifferentiated ES cells, they developed different types of clones by electroporation method to predict the expression of embryos and in brains of post natal animals. They analysed chimeric mice embryos at 9.5.12.5 and 15.5 days post cotiumb[dpc]. The clone MS at 9.5 dpc showed strong expression in the ventral mesencephalon and weak expressionin body mesenchyme,at 12.5 dpc it showed expression in surface ectoderm, limb and wall muscles and at 15.5 dpc expressed in liver ,lung . The clone 6A-117 showed expression in gastrointestinal tract and nose area at 12.5 dpc and at 15.5 dpc showed expression in borders of developing bones and show weak expression in olfactory bulbs cortex and hypothalamus. The clone 8A-38 expressed in heart and vascular system at 9.5 dpc and 12.5 and 15.5 showed expression in CNS, Spinal ganglia, ribs and vertebrate.[27] Skarnes developed GT1 .8 geo vector to trap the genes responsible for transmembrane and membrane secreted proteins in mouse.[28] William L. Stanford et.al aimed to develop a gene trap vector both in-vivo and in-vitro to revealed the expression of different genes in the differentiate ES cells and characterise the large number of genes, they developed gene trap vector by using LacZ as a reporter gene. They developed different clones with different vectors and revealed the gene expression by using LacZ.

The clone k17G2 showed the lacz expression in hematopoietic lineages at in-vitro condition where as in vivo its show expression in hematopoietic, myocardium and nervous system and helped in identifying novel ORF and another clone GC11C7 showed the expression in hematopoietic in -vitro condition and heart, forebrain and optic vesicles at in-vivo condition and identified the placenta ESTs.[29]

Studies on Arabidopsis

Arabidopsis belongs to Brassicaceae family, it is the famous model organism for plant biology and the entire genome was sequenced. Because of its genome size many gene trapping techniques developed to know the gene expressions. Campisi et al develop enhancer trap by using GUS as a reporter gene to find the tissue and stage specific gene expression in leaves and inflorescences.[30] Dubreucg et al successfully trap the gene during the seed germination by using GUS as a reporter gene and by developing DFJ48 homozygous lines; they focused on AtEPR1 gene which has repeated motifs similar to extensins. They reported AtEPR1gene specifically expressed at radical protrusion of endosperm during seed germination and play a key role in cell wall structural modification.[31] Po-Pu Liu et al trap the genes by using GUS as a reporter gene in lag phase between the testa and endosperm rupture and succeed in detecting the GUS expression in embryo of Arabidopsis. They isolated many gene trap lines to characterise the seed germination genes such as TMH1 which shows the root specific expression in micropylar region of endosperm. The Gus expression in micropylar region reported that the transcriptional factor genes homeobox-leucine zipper protein and basic helix-loop –helix proteins potentially play a vital role in upstream events during the seed germination and involved in tissue specific expression of cell wall protein formation in germinating seeds apart from it some transcriptional factors play a crucial role in other stages of plant development like inflorescences and caulin leaves.[32] A circadian rhythm is a biological 24 hours cycle of living organisms, it synchronises external environment with internal physiological process and it is also influenced by a lot genes because of adaptive nature of genes towards nature. The circadian cycle regulates the transcription of genes. In Arabidopsis Todd P. Michael et.al studied the control of circardian cycle over the transcription of the genes by using in vivo enhancer trapping with a reporter gene luciferase through their experiment they found individual lines exhibit peak transcription rates during the circadian cycle. They identified 23circardian cycle controlled genes [CCG-ETs] and almost 36% of genome is under the influence of this cycle for its expression it suggests that key control of transcription genes is influenced by circardian cycle.[33] Swaminathan et al. find a novel gene of D class cyclin in Arabidopsis by developing enhancer trapped system.[34]

Studies on Rice

Rice is the one of the most important staple food for world, because of its small genome and having many cDNA libraries it is used as model research plant for biologist. Jeaon et al., in the genotype of Dongjin and Wu et al., in the genotype of Zhonghua develop rice mutant libraries by using enhancer and gene trap techniques [35 and 36]. Changyin Wu et al. developed an enhancer system GAL4/VP16 –USA with a minimal promoter -48 CaMv and a reporter gene GUS to produce trapped lines in rice. They compared GUS expression levels between the calli and leaves, Leaves and root and finally vegetative tissues and reproductive tissues at T_0 plant stage and they showed the GUS expression were stably inherited to T_1 generation and finally concluded that GAL4/VP16-USA is useful in producing enhancer trap lines in rice.[37] Alexander A.T. Johnson et al. developed an enchancer system of pC4956:ET15 with GAL4 gene and GFP gene with a minimal promoter, they produced 13000 enhancer trap lines in rice with expression of GFP in all major organs of the rice at several stages of development.[38] Dacheng Liang et al stabilished a binary GAL4-VP16-UAS transactivation system for finding gene functions, they developed two types of transgenic lines such as pattern lines and target lines. Pattern lines were created by using a minimal promoter with transactivator gene GAL4-VP16 and GUSplus reporter gene controlled by upstream activation sequence in Zhonghua 11a variety of japonica cultivar

where as target lines were constructed by EGPF reporter and target genes of interest in Zhonghua 11. Later hybrid lines were produced by crossing the pattern lines and target lines to generate mutations by gain or loss of function of genes for this they tested transactivation of 10 transcription factor genes in the hybrids which induced ectopic expression of the target gene and they provide an evidence that the binary system useful in knowing the gene function in the rice and provided more than 1000 organ-specific pattern lines have been identified especially the genes which are partially redundant functions of many transcription factors. For example the gene OsMADS15 whose function is unknown was isolated by Moon et.al in 1999[39 and 40] by this study this gene has been reported as a negative regulator α-amylase which plays a vital role in seed germination as a result it have a negative effect on reproductive process in rice.[41] Hang Gyeong Chin et al. develop a gene trap system by using heterologous transposons of maize [Activator] Ac/Ds [dissociation] to evaluate the efficiency of gene trapping by analyze single and simple insertions of T-DNA in rice and the behaviour of Ac/Ds transposable elements.the Ac/Ds trap system consist of Ac transposon with CaMV 35S promoter, trapDs with splice acceptor, GUS reporter gene and a modified bacterial cytochrome P450 used as a counter selection marker. Approximately 80% of Ds mobilized in primary transposition and 18% were mobilized into secondary transposition in shoots of newly developed through this they provide molecular information for establishment of effective gene tagging system in rice.[42]

Studies on other plants

Barley is a cereal grain of grass family, diploid species with 12 chromosomes. Katina Lazarow et al. used the maize transposable elements Ac/Ds for establishing a two component gene trap system for barley. They construct vector with the wild type Ac transposase under the control of native Ac promoter, Ds element with uid A reporter gene encoding β-glucourodinase and a splice acceptor and transformed into barley using particle bombardment. They found 11% frequency of transposition in F1 generation and Independent events of transposition takes place in gene trap lines of barley was observed at multiple sites of GTDsB. They developed several gene trap lines and observed GUS expression indifferent organs such as the GT32 gene trap line shows GUS activity in grain and seedling and GT82 showed GUS activity in immature floret, node and leaf.[43] The legume plant Lotus japonicas is belong to Fabaceae family, used as a model plant for nodulation studies. Webba et al. introduced a promoter less GUS reporter into Lotus japonicas to study the events associated with calcium binding protein with root epidermis and root hairs which are necessary for nodulation pathway by developing promoter trap lines.[44] Diana Mihaela Buzas et al. developed a promoter trapped system with GUS as a reporter gene to monitor gene expression used for the development of nodule and lateral root formation, they developed different lines such as CHEETAH, FATAMORGANA,TIMPA and VASCO lines. They found the GUS expression in CHEETAH associated with lateral root and nodule primordium and involes in both pericycle and cortical cell intiation, in FATAMORGANA showed in nodule primordium which identifies a new type of cellular function with the primordium. In TIMPA lines a Sub class of cortical events occurs in curled root hair in the formation of nodule primordium involved in cortex differentiation, the GUS expression in VASCO lines revealed a saddle like structure which are specific for nodule vasculature showed the pericycle differentiation, this GUS expression give a clear statement that the ontogeny of different cellular types play a role in organising the meristematic centres of nodules and lateralroots.[45] The activation tagging and enhancer trapping techniques succeed in identifying the genes responsible for tree development of Populus trichocarpa, the black cotton wood belongs to salicaceae family.[46] Sukmin Ko et .al succeded in producing enhancer trap lines in somatic embryogenesis in the carrot with at 64% high frequency by using enhancer trap vectors [pETVs] with GUS reporter gene and minimal promoter.[47] Rafael Meissener et al., construct vectors such as D3378-GUS,Bam35s-AS,DSE and DSG for both gene tagging and enhancer trapping with NPTll gene as a transformation marker confers resistance towards Kanamycin in tomato and they proved trapping system was active in Micro-tom.[48] The As/Ds tagging system play a vital role in trap the genes and to isolate the genes in for example Cfa gene for cladosporium resistance and Dcl gene for Chloroplast development in tobacco and in tomato respectively.[49,50]

DISCUSSION

Research has been going to reveal the genome sequence of many species, owning to development of new techniques in DNA sequencing technology so many species genome sequence already revealed. Even though many of the genes identified in the genome sequence but the function of many genes are still unknown. It is necessary to know functions of each and every gene to face the different challenges in world of genomics. A lot of techniques have been exploits to know the function of genes and creating so many genomic libraries, in that gene trap technique is a powerful method to characterized and discovered the a lot of gene with unknown functions. The advantage of Gene traps techniques is based on expression pattern of genes and reporter genes which is a part of gene trap and it's not depending upon the mutational changes in the genes. It mainly identified of two classes of genes those are functionally redundant genes and genes which are expressed at different stages of life of an organisms. The reporter gene play a major role in gene traps by tagging the functional regulatory elements associated with genes in the host genome and provides valuable information about markers, regulatory genes, foetal sedentary genes. Some of the gene trap techniques utilize transposons

along with reporter which generates large collection of gene trap lines by having diverse expression in the gene reporters and these are used for the isolation and characterisation of diverse genes which are expressed in different stages of life development. The transposons trap shows high level of diversity in the expression of genes because of having transpoase enzyme, which helps in jumping of trasposons elemrnt foem on eplace to another within the genome of organism. The trap lines provide information for specific cell markers and identification of new cells and cell lineages in higher plants. In case of plants the gene trap has advantages in knowing the genes responsible for avoiding physical stress like salt, light, water and biological stress like disease causing agents, especially in rice which is a major food crop for growing populations in the world wide to increasing the yield by reducing the both biotic and abiotic stress. The present review is focused on various studies conducting on different species and gives the information about gene trapping techniques and its success in knowing the function of genes in view of future aspects; here we discussed contribution of gene trap works towards the cell developmental, gene identification, transcriptional regulatory elements, circardian cycle and seed germination studies. The gene trap creates a disruption in gene which resultant subtle the phenotypic expression, this disruption of gene fucttion by gene trap but it's not problematic for gene dentification such as fuctionally redundant gens although it have a lot of advantages some draw back are there such as the gene trap cannot be used for genes those are switched off permanenetly of Itgaration of multiple copies of the gene. sometimes fail in genetrating paticualr gene of interested.

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