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RESEARCH ARTICLE

RAPD ANALYSIS OF EMS MUTAGENISED MULBERRY GENOTYPE RFS_{135}

1* H.V. Anil Kumar, T. S. Muralidhar[2] and Munirajappa[3]

*[1] Department of Zoology, DVS College of Arts and Science, Shivvamogga-577201, Karnataka, India.
[2] P. G. Department of Biotechnology, R&D Center, Dayananda Sagar College of Biological Sciences, Kumaraswamy Layout, Bangalore-560078, India.
[3] Department of Sericulture & Life Sciences, Jnana Bharathi, Bangalore University, Bangalore – 560056, Karnataka, India

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ABSTRACT

EMS is a monofunctional-ethylating potent chemical mutagen, found to be mutagenic in wide variety of genetic test systems from viruses to mammals.EMS causes base-pair insertion or deletions and more extensive intragenic deletions in higher organisms. The alkyl group of an alkylating agent reacts with DNA that leads to change in the nucleotide sequence, thus resulting in point mutation. EMS is found to be more effective and safe for use in mulberry. In present investigation, the active bud sprouts of the mulberry genotype RFS_{135} was treated with EMS (0.1% and 0.3%) intermittently for the duration of twelve hours. The biological effects of EMS on the total genomic DNA of the treated plants were studied through RAPD technique. The M_1V_2 variant clones obtained from the plants treated with 0.1% and 0.3% concentrations of EMS revealed polymorphs of significantly variable size of different base pairs for two random primers used (OPW-04/05), thus indicating probable changes in the molecular characters of the total genomic DNA. Analysis of RAPD revealed genetic variation between control and EMS induced clones. Mutants screened at 0.1% and 0.3% showed higher levels of genetic variation and more unique alleles compared to the control due to EMS induced mutation.

INTRODUCTION

Mulberry is a perennial, deep-rooted, widely adaptable fast growing tree belonging to genus *Morus* L.order Urticales and family Moraceae. It is of great economic importance to sericulture industry and is extensively cultivated in more than 58 sericulturally important countries of both tropical and temperate regions around the world. There are 68 species in genus *Morus*, majority of them occurring in Asia. Mulberry is basically deciduous species of sub-tropical forests with arboreal habit and distributed in a wide area of tropical, sub-tropical, temperate and sub-arctic zones.[1,2] The wild mulberry species are distributed in the mountainous regions of Asia, Middle East, Central America and South America. Mulberry is the sole forage for monophagous lepidopteran insect silkworm *Bombyx mori* L. In crop improvement programme, several methods are used to increase the genetic variability within, by selection procedure, which attempts in identifying the beneficial genotypes. On the other hand, induction of mutations in crop improvement programme ensures the possibility of altering genes by exposure of seeds or other parts of the plant to chemical or physical mutagens. During 1950s and 1960s, when DNA had been well established as the genetic material and Watson and Crick's double helix model of DNA received recognition as basic organization of hereditary material, there was great interest in elucidating the mechanisms underlying induced genetic mutations, especially by chemical mutagens. Certain chemicals, such as Ethyl methane sulphonate (EMS), Methyl methane sulphonate (MMS), Diethyl sulphate (DES), can induce mutations. In particular, EMS, MMS, DES the chief alkylating agents are often applied for inducing point mutations. The alkyl group of an alkylating agent reacts with DNA, which may lead to changes in the nucleotide sequence and resulting in point mutation. Alkylating chemical is said to induce relatively few chromosome mutations however EMS reacts with DNA in variety of ways and produce a broad spectrum of mutagenic effects.[3]

EMS is a potent chemical mutagen that has been extensively used in genetic research and among chemical mutagens EMS has been found more potent safe and effective for mulberry.[4,5] EMS is a monofunctional-ethylating agent that is found to be mutagenic in wide variety of genetic test systems from viruses to mammals. In higher organisms, there is clear-cut evidence that, EMS is able to break chromosomes, although the mechanism involved is not well understood, but there is some evidence that EMS can cause base-pair insertion or deletions as well as more extensive intragenic deletions.[6]

***Corresponding author:** profanilhv@rediffmail.com*

In the present investigation, attempts have been made to study the biological effects of EMS on the total genomic DNA of EMS induced mutants of mulberry population variety RFS_{135}. M_1V_2 variants were screened by phenotypic traits and Random Amplification Polymorphic DNA analysis was used for Molecular characterization to detect the mutated DNA segment. In mulberry RAPD, AFLP and RFLP has been extensively used by many researchers for the study of genetic diversity, morphological and molecular distinction among the species. However the present investigation attempts at exploring the detection of mutations through RAPD technique and asses the possible mutagenic effect of EMS on total genomic DNA.

MATERIALS AND METHODS

Mulberry genotype RFS_{135} was procured from Central sericulture germplasm research station, Hosur, TamilNadu (India).The genotypes was established at germplasm bank attached to the Department of Sericulture, Bangalore University, following the standard procedures of the National Bureau of Plant Genetic Resources (NBPGR).[7] and were maintained following the recommended package of practices.[8] Three replicates of disease free cuttings were prepared following the standard procedure[9] and were planted in earthen pots filled with propagation mixture and maintained under standard nursery conditions[10]. On sprouting the buds were treated intermittently every one-hour with 0.1% and 0.3% concentrations (v/v) of Ethyl methane sulphonate (EMS) solution, following cotton swab method for twelve hours[3]. The treatment was carried out from morning 8.00am to evening 6.00pm under bright sunshine. The treated populations were maintained in nursery for 90 days and the observations were made from the stage of initial responses of the treated buds[11]. The beneficial variants of M_1V_1 generation recovered at 0.1% and 0.3% EMS treated population of RFS_{135} were screened and used for further propagation of M_1V_2 populations under nursery conditions. After three months the saplings were transplanted to the field in separate blocks following RBD method with $3^{|}$ x $3^{|}$ spacing. Recommended package of practice was adopted to raise the saplings and the observations were carried out from 90^{th} day 180 days[12]. The comparative studies were carried to tally some common and consistent morpho-economic characters among population of M_1V_1 and M_1V_2 generations. The plants exhibiting maximum common morpho-economic traits at M_1V_2 generation were again carefully screened and isolated. Random amplified polymorphic DNA (RAPD) marker analysis was carried out, for molecular characterization and to assess the mutagenic effects of EMS on total genomic DNA of M_1V_2 beneficial variants.

Genomic DNA extraction

Leaf samples of M_1V_2 variants were collected from 90 days old (after pruning) primary branches of clonally propagated plants and stored immediately at -80°C for DNA extraction.Total genomic DNA was extracted by the DNA extraction protocol[13], modified[14]. 1g of leaf tissue was grinded in a precooled mortar and pestle using liquid nitrogen. The leaf powder was transferred into 50 ml polypropylene tube containing 20ml of the extraction buffer (3% w/v CTAB, 100mM Tris-Hcl, 20mM EDTA, 1.4M NaCl, and 0.1% w/v β-Mercaptoethanol) and incubated at 65°C for 1 hour with intermittent shaking. The mixture was cooled. 6ml of chloroform and iso-amyl alcohol (24:1) were added and centrifuged at 7000 rpm for 15 minutes. The supernatant was separated and the clean up step was repeated until clear supernatant was obtained. The clean supernatant was kept overnight at 4^0C by adding half volume of 5M Nacl and one volume of Isopropanol for precipitation of DNA. The DNA was pelleted by centrifugation at 10000 rpm for 20 minutes and pellet was washed with 70% ethanol.

Purification of DNA

The dried DNA pellet was resuspended in 300µl of TE (Tris-EDTA) buffer. Contaminating RNA was removed by digesting in 10µg of RNAase for 60 minutes at 37^0C. Proteins were removed by digestion in 25µg of Proteinase-k. The DNA was further purified by extracting twice with an equal volume of Phenol & Chloroform (1:1) and finally by equal volume of chloroform, following Phenol-Chloroform extraction method[15]. The DNA was precipitated by adding one volume of Isopropanol and centrifuging at 5000 rpm for 5 minutes. The extracted pure DNA pellet was dissolved in 300µl TE. The DNA concentration was determined by using UV-Visible spectrophotometer at 260nm and 280nm and further used for analysis of RAPD.

Amplification of DNA

The protocol of was followed with slight modification. A single decamer of arbitrary sequence was used in each PCR. Under optimized condition of PCR, informative and reproducible fingerprint profiles were carried out in 25µl reaction mixture. The reaction mixture contained template DNA (25ng), 10Pmol of primer (Operon Technologies USA, Inc), 2.5mM Mgcl2, 50Mm Kcl, 10mM Tris - Hcl and 0.1% Triton X - 100. One unit of Taq DNA polymerase (Bangalore Genie) and 250µM of each dNTPs (Bangalore Genie). Amplification was performed in the thermal cycler (Eppendorf, Master cycler) for 42 cycles. After initial denaturation at 94°C for 5 minutes, in each cycle denaturation for 1minute at 94°C, annealing for 1 minute at 33°C and extension for 2 minute at 72°C was programmed, with final step at 72°C for 8 minutes after 40^{th} cycle.[16]

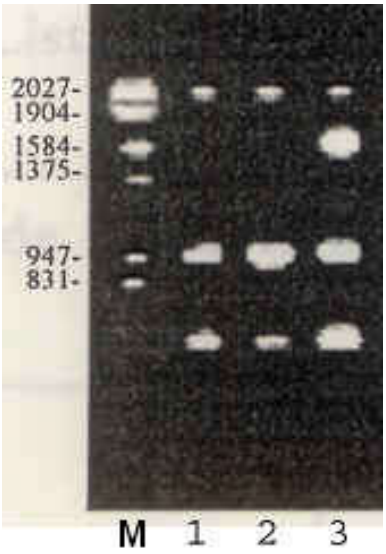
Electrophoresis of amplified DNA

Amplified DNA fragments were separated in 1.2% agarose gels and stained with ethidium bromide. Running buffer containing Tris-buffer, acetic acid and EDTA (ph 8.0) was used for electrophoresis. Wells were loaded with 25µl of a sample and 5µl of loading buffer (sucrose, bromophenol blue and Xylene cyanol). Electrophoresis was carried out for three hours at 45volts current. The gels were photographed under UV light using gel documentation system (Herolab).

RESULTS AND DISCUSSION

In the present study, the total genomic DNA of M_1V_2 variants of RFS_{135} recovered from 0.1% EMS treatment and control plants, amplified by random primer OPW-05-$5^{|}$GGCGGATAAG$3^{|}$, produced 3 fragments in control and clones of EMS treatment. The fragment was big in size at 947

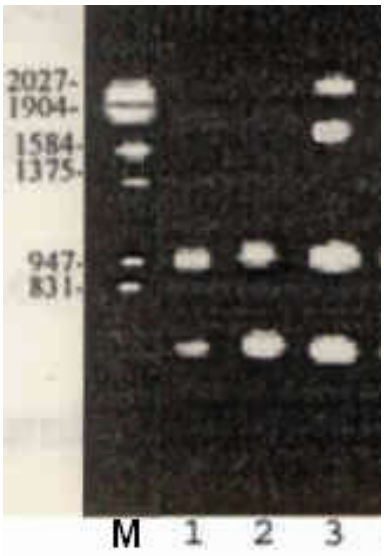
bp and slightly thicker fragment was generated at 2027 bp in the mutated clone (Lane-2). Further single diminishing fragment was observed below 831 bp in the mutant recovered from 0.1% EMS treatment, compared to control(Lane-1&2; Fig.1) (Plate-1).Total genomic DNA amplified by using random primer OPW-04-5[1]CAGAAGCGGA3[1] produced 2 fragments in control and the mutants obtained from 0.1% EMS treatment one each at 947 bp and below 831 bp. The fragments were big in size among the clones of 0.1% EMS treated when compared to control (Fig.2; Lane1&2) (Plate -1). The random primer OPW-05-5[1]GGCGGATAAG3[1] produced 4 fragments in the mutant clones recovered from 0.3% EMS treatment (Fig.1 Lane-3) and 2 fragments were produced in control population(Fig.1; Lane-1). The two fragments were noticed at 831 bp both in control and EMS treated clones. Two thick fragments were seen at 947 bp and 1584 bp and thin fragment was recorded at 2027 bp respectively in the mutant clones recovered from 0.3% EMS treatment (Fig.1; Lane-3) (Plate-1). In the total genomic DNA amplified by the random primer OPW-04-5[1]CAGAAGCGGA3[1], 4 DNA fragments were recorded in the clones recovered from 0.3% EMS treatment compared to two fragments in control. One each fragment was observed below 831 bp both in control and EMS treated plants. Another one fragment per control and EMS treated plants was augmented at 947 bp. Further, the two new fragments were observed at 1584 bp and 2027 bp in the mutants obtained from 0.3% EMS treatment (Fig. 2; Lane-3) All the fragments produced in the clones recovered from 0.3% EMS treatment were thicker in size when compared to control



M: Marker, Lane-1: CC- Control, Lane-2: 1C- 0.1% EMS, Lane-3: 3C- 0.3% EMS

Fig. 1: RAPD of genomic DNA of mulberry genotype RFS_{135} generated by random primer OPW-05-5[1]GGCGGATAAG3[1] (Control and EMS treated)

The two primers produced total 12 polymorphic bands ranging from less than 831bp to 2027bp among the EMS treated clones compared to an average of 5 bands in control. The different polymorphic bands were detected at 0.1% and 0.3% EMS treatments. Averages of 5 polymorphic bands were detected from two primers at 0.1% treatment. The primer OPW-05 showed two high intensity polymorphic bands of



M: Marker, Lane-1: CC- Control, Lane-2: 1C- 0.1% EMS, Lane-3: 3C- 0.3% EMS

Fig. 2: RAPD of genomic DNA of mulberry genotype RFS_{135} generated by random primer OPW-04-5[1]CAGAAGCGGA3[1] (Control and EMS treated)

947bp and below 831bp among 0.1% EMS treated, further caused loss of bands at 2027bp inferring loss of certain genes. The 0.3% EMS treatment has altered the polymorphic band pattern annealed by two primers. The primer OPW-05 and OPW-04 yielded an average of four polymorphic bands among 0.3% EMS induced mutants. Primer OPW- 05 produced intensified band at 1904bp and moderate band at 947bp and below 831bp, whereas primer OPW-04 increased intensified polymorphic band at 2027bp, 947bp and below 831bp indicating the amplification of mutated genes. Contrastingly the decreased intensity of band was augmented at 1904bp, inferring the loss of certain genes. Primer OPW-05 augmented the magnification of polymorph at 1584bp at 0.3% treatment and 947bp at 0.1% treatment, further appearance of high intensified band below 831bp.The primer OPW-04 revealed the disappearance of polymorphic band at 2027bp in control and 0.1% EMS treatment, probably attributed to loss of certain genes. The magnification of polymorphs at 1584bp and below 831bp at 0.3% treatment annealed by OPW-05 primer signifies the new mutated genes. Similarly the primer OPW-04 annealed augmentation of two new bands at 2027bp and 1904bp among the mutants of 0.3% EMS treated clones, further appearance of two bands at 947bp and below 831bp among mutants of 0.1% and 0.3% EMS treated clones. Probably the high magnifications, appearance and loss of bands may be due to the point mutations. The variation in band intensities was stable at specific base pairs of DNA subjected to mutation in both primers, thus indicating the point mutation among EMS treated mulberry clones.These mutated segments of DNA and polymorphism is probably due to tetragonic and carcinogenic effects of EMS. It has been well established that EMS produces random mutations in genetic material by nucleotide substitution, specifically by guanine alkylation, typically producing only point mutations. It can induce mutations at a rate of $5x10^{-4}$ to $5x10^{-2}$ per gene without substantial killing. The ethyl group of EMS reacts with guanine in DNA, forming the abnormal base O-6-ethylguanine. During DNA replication, DNA polymerases the

process frequently by placing thymine, instead of cytosine, opposite O-6-ethylguanine, and original G:C base pair can be altered to A:T pair. This change in genetic information is often harmful to cell and can result in mutation[17]. Several works have been reported in many crops including mulberry substantiating that RAPD markers can quickly detect a large number of genetic polymorphisms, aiding to establish genetic diversities among species and variations in genetic population, which has been discussed below.

Studies on molecular genetic fingerprints of nine Curcuma species from Northeast India using PCR-based markers, revealed high degree of genetic polymorphism which assumed great significance for conservation and further improvement of Curcuma species[18]. Invitro mutants of loquat (*Eriobotrya japonica* Lindl. 'Dawuxing') treated with ethylmethane sulfonate (EMS) induced morphological, biochemical and molecular changes which were detected by RAPD amplification profiles. The variants showed high number of amplifications such as 8 (4.6%), 12 (6.8%) and 13 (7.0%)[19]. RAPD analysis for genetic variations among four mutants of black gram (high seed protein, tall, bushy, and dwarf mutants) induced by physical gamma rays and chemical Ethyl methane sulphonate yielded some advantageous mutants, further the genetic variants evaluated with 20 random primers, generated total 202 fragments scored with 58 polymorphic alleles, and the average was 10.1 alleles per locus and a range of 1–9 alleles. The average polymorphic rates were 38.37 among the mutants and parents through the 20 primers. Primers OPA-14 and OPI-04 revealed 35% of DNA polymorphism in their investigation[20]. RAPD technique applied for detection of induced variability in M_3 generation among the induced mutants of gamma kR ray (10 kR, 15 and 20 kR), ethylmethane sulphonate (0.05%, 0.1% and 0.2%) and combination of both (15 kR + 0.1% EMS, 20 kR + 0.2% EMS) of paparika (*Capsicum annnuum* L) produced polymorphic regions in mutant of consecutive generation, which was confirmed by the primers OPB-09, OPB -15, OPB-17, OPD-13 and OPC-03.Among them the primer OPC-15 produced polymorphic bands of 750bp size for the mutants of variety Ktpl-19 and Bydagi kaddi[21]. The RAPD analysis also has been reported to be efficient for studying the phylogenetic relationships among the groundnut genotypes.[22, 23] Twenty-one induced mutants belonging to different botanical types of groundnut were used to assess molecular diversity using RAPD analysis.The results had revealed that all 27 random primers augmented polymorphism, but OPV 16 (71.42%), OPB 13 (66.66%), OPJ 17 (53.84%), OPB 9 and OPB 19 (50%), OPK 18 (46.15%), OPA 07 (45.45%), and OPB 11 and OPA 12 (44.44%) were highly polymorphic. The number of amplicons varied from six to thirteen with an average of nine per primer, of which 3 were polymorphic. The polymorphism per primer ranged from 9.09 to 71.42 per cent with an average of 30.16 per cent.[24]

In studies conducted to estimate genetic variability obtained through the use of gamma rays induced somatic mutation for the improvement of sugarcane, screened through RAPD analysis showed that amplification products in 10 Gy of the five soma clones and its parent with nine primers yielded a total of 53 scorable bands. The number of fragments produced by various primers ranged from 3-11 with an average of 5.9 fragments per primer. The highest number of bands (11) was obtained with Primer B-10, while the lowest numbers (3) were obtained with primers B-08 and B-14.Similarly 20 Gy yielded a total of 48 scorable bands. The number of fragments produced by various primers ranged from 3-10 with an average of 5.3 fragments per primer. The highest number of bands (10) was obtained with Primer B-10, while the lowest number (3) was obtained with primer B-08 and no amplification observed in B-14. Further the number of fragments produced by various primers ranged from 3-11 with an average of 6.1 fragments per primer. The highest number of bands (11) was obtained with Primer B-10, while the lowest numbers (3) was obtained with primer B-14. In case of 30Gy amplified genomic product. [25]

The putative mutants (*v*M3) induced by EMS and EB were hardened and screened using RAPD–PCR analysis with 30 arbitrary decamer primers to isolate the solid mutants. Percentage of mutants identified after marker-assisted selection was higher in EMS (28.5) compared to EB (8.8) treatment. Further the polymorphic RAPD primers were effectively used for selection of solid mutants in grape cv. PS. Marker-assisted selection served as a potential tool for increasing selection efficiency by allowing earlier selection and also reducing the plant population size. The effect of EMS and EB mutagens for *in vitro* mutagenesis of grape plantlets was prominent when tested with RAPD analysis. Seven primers out of thirty were able to identify solid mutants. These selected RAPD primers generated fifteen RAPD markers. The size of the amplification product ranged from 400 to 2500 bp.[26] Investigation undertaken with inter-simple sequence repeat (ISSR) and random amplified polymorphic DNA (RAPD) markers to find out the possibility of using these DNA markers to confirm the identity of genotypes in a particular species. Fifteen ISSR and 15 RAPD primers generated 86% and 78% polymorphism, respectively, among 19 mulberry genotypes. The polymorphism among the species varied from 50% to 57% in ISSR markers and 31% to 53% in RAPD markers.[27] Studies in mulberry by using 17 random amplified polymorphic DNA (RAPD) and 11 inter-simple sequence repeat (ISSR) primers the genetic relationships among popular varieties were analyzed. The RAPD and ISSR primers revealed more than 75% polymorphism among the varieties. The genetic similarity estimated from RAPD markers varied from 0.645, between Kajli and Victory-1 to 0.887, between Kanva-2 and Bilidevalaya. Similarly, the genetic similarity estimated from the ISSR markers ranged from 0.600, between Kajli and Victory-1, to 0.873 between Kanva-2 and $BC_2$59.[28] Similar studies elucidated the genetic relationships among 18 mulberry genotypes collected from India and Japan using 15 Inter Simple Sequence Repeat (ISSR) and 15 Random Amplified Polymorphic DNA (RAPD) primers. The ISSR primers generated 81.13% polymorphism while the RAPDs generated 71.78% polymorphism. The polymorphic index of the primers identified UBC-812, UBC-826, UBC827, UBC-881, OPA-01, OPA-02, OPA-04 and OPH-17 as informative primers in mulberry.[29] Several workers have showed that RAPD markers, which can quickly detect a large number of genetic polymorphisms, that have led to the creation of genetic maps in a number of woody fruit crops[30] and detection of mutations in soybean[31], Brazilian rice[32], citrus[33], pear[34] and cherry[35], including changes due to DNA damage[36]. However our present studies focussed upon the RAPD analysis for identification of mutation in EMS induced

mutant clones of mulberry RFS_{135}, which were well established by the structural alterations of DNA manifested by polymorphisms of different base pairs, showing significant gain and loss of gene expression.This confirms the opinion that the estimate on the existence of mutation and structural alterations in plant DNA due to impact of EMS treatment on the bases of DNA patterns could be obtained by RAPD markers with the set of primers.[37] In our study, EMS mutagenised Mulberry genotype RFS_{135} can be a potent variant variety with wide economic importance leading to gain of valuable genetic resources, for sustainable utilization and conservation, emphasizing the assessment of genetic diversity. Further investigation is required for the isolation of specific gene sequences, established in M_1V_2 mutants of mulberry RFS_{135}, leading to PCR based assay in gene matching and construction by applying bioinformatic tools. However the specific bands obtained in the present investigation could be used as molecular markers to screen the mutants in mulberry breeding programmes further strengthening the commercial utility in sericulture.

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Competing Interests Statement

"The Authors declare no Competing Interests".

REFERENCES

1. Jolly, M.S. and Dandin, S.B. 1986. Collection, Conservation and evaluation of mulberry (*Morus* spp.) Germplasm, C.S.R. & T.I., Mysore.
2. Sanjappa, M. 1989. Geographical distribution and exploration of the genus *Morus* L. (Moraceae). Genetic resources of mulberry and utilization. Ed. by Sengupta, K. and Dandin, S.B., C.S.R. & T.I., Mysore, 4-7.
3. Broertjes, C. and van Harten, A.M. 1988. Developments in Crop Science 12. Applied Mutation Breeding for Vegetatively Propagated Crops. Elsevier science publishing company Inc., New York, U.S.A.
4. Sastry, 1983. Evaluation and utilization of existing genetic variability in Mulberry. National Seminar on Silk Research and Development, Bangalore, March 10-13, p. 2.
5. Yang, J.H. and Yang, X.H. 1991. Polyploid breeding of mulberry. In : Practical mulberry breeding. Si Chuan Tech. Publishing House : 206-218.
6. Gary, A. Sega. 1984. A review of the genetic effects of ethyl methanesulfonate. Mutation Reviews in Genetic Toxicology, Vol. 134, Issues 2-3, Sept-Nov, pp. 113-142.
7. Chahal, G.S. and Gosal, S.S. 2002. Principles and procedure of Plant Breeding – Biotechnological and conventional Approaches, Narosa Publishing House, New Delhi.
8. Krishnaswami, S. 1971. On some aspects of the improved techniques of rearing mulberry silkworm. Indian Silk. Vol. 10(2) : 7-9.
9. Sujathamma, P. and S.B. Dandin. 1998. Evaluation of mulberry (*Morus* spp.) genotypes for yield under rayalaseema conditions of Andhra Pradesh. Indian J. Seric., Vol. 37, No. 1, 13-16.
10. Hartmann and Kester. 1986. Plant propagation, principles and practices. 4th edition, Prentice Hall of India Pvt. Ltd., New Delhi.
11. Dandin, S.B. and Kumar, R. 1989. Evaluation of mulberry genotypes for different growth and yield parameters. In : Genetic resources of mulberry and utilization. Edt. by Sengupta and Dandin, S.B. C.S.R. & T.I. Mysore, 142-152.
12. Thangavelu, K., Mukherjee, P., Tikader, A., Ravindran, S., Goel, A.K., Ananda Rao, A., Girish Naik, V., Sekar, S. 1997. Catalogue on Mulberry (*Morus* spp.) Germplasm, Vol. 1.
13. Dellaporta, S. L. Wood.J and Hicks. J.B. 1983. A plant miniprepartion: Version II. Plant Molecular biology Reporter, Vol 1,19-21.
14. Porebski. S. Bailey, G. and Baum. B.R. 1997. Modification of CTAB DNA extraction protocol for plants containing high polysaccharides and polyphenol components. Plant molecular Biology Reporter Vol 15, 8-15.
15. Sambrook. J. Fritsch. E.F. and Maniatis.T. 1989. Molecular cloning: a laboratory manual. 2nd edition. Cold Spring Harbor Laboratory Press. Cold Spring Harbor. NY
16. Williams.J.G.K, Kulelik. A.R, Livak.K.J. Rafalski. F.A and Tingey. S.V. 1990 DNA polymorphisms amplified by arbitrary primers are usefull as genetic markers. Nucleic acids Research. Vol. 18, 6531 – 6535.
17. Sameer Hasan M. Qari. 2010 "DNA-RAPD Fingerprinting and Cytogenetic Screening of Genotoxic and Antigenotoxic Effects of Aqueous Extracts of *Costus Speciosus* (Koen.) JKAU: Sci., Vol. 22 No. 1, pp: 133-152.
18. Das. A. Kesari. V. Satyanarayana VM. Parida.A. Rangan. L**.** 2011.Genetic relationship of *Curcuma Species* from Northeast India using PCR- based Markers. Mol Biotechnol. Jan 21. [in press]
19. Y.Q. Wang, N. Luo, J.Q. Li, Q.X. Deng, H.Y. Liang. *Eriobotrya japonica*, mutation breeding. Pod isozyme and RAPD analysis of plants regenerated from EMS-treated shoot tips in 'Dawuxing' loquat.SHS Acta Horticulturae 750: II International Symposium on Loquat Morphology
20. D. Arulbalachandran, L. Mullainathan, S. Karthigayan, S. T. Somasundaram and S. Velu. Genetic variation in mutants of black gram (*Vigna mungo* (L.) Hepper) evaluated by RAPD markers. Journal of crop science and Biotechnology.Volume 13, Number 1, 1-6
21. G Ashok Kumar and V Ponnuswami. 2010. Effect of Physical and Chemical Mutagenesis on DNA Polymorphism in *Paprika*. Research Journal of Agricultural Sciences, 1(3): 193-195.
22. Halward, T., Stalker, T., Larue, E., Kochert, G., 1992. Use of singleprimer DNA amplifications in genetic studies of peanut (*Arachis hypogaea L.*). Plant Mol. Biol.,18 : 315-325.
23. Raina, S. N., Rani, V., Kojima, T., Ogihara, Y., Singh, K. P.,Devarumath, R. M., 2001, RAPD and ISSR fingerprints as useful genetic markers for analysis of genetic diversity, varietal identification, and phylogenetic

relationships in peanut (*Arachis hypogaea*) cultivars and wild species. Genome, 44:763-772.

24. Varasha Kumari, M. V. C. Gowda and Ramesh Bha**t.** 2009. Molecular characterization of induced mutants in groundnut using random amplified polymorphic DNA markers. Karnataka J. Agric. Sci, 22(2): 276-27.
25. Imtiaz Ahmed Khan, M. Umar Dahot and Abdullah Khatri. 2007. Study of genetic variability in sugar cane induced through mutation breeding. Pak. J. Bot, 39(5):1489-1501.
26. Ramakrishna Namdeo Khawale,Vimala Yerramilli, Sanjay Kumar Singh. 2007 Molecular marker-assisted selection of *in vitro* chemical mutagen-induced grapevine mutants Current Science, Vol. 92, No(8): 1056-1060.
27. K. Vijayan, P.P. Srivastava, and A.K. Awasthi. 2004. Analysis of phylogenetic relationship among five mulberry (*Morus*) species using molecular markers. Genome 47:439–448.
28. K. Vijayan, A. K. Awasthi, P. P. Srivastava, B. Saratchandra (2004) " Genetic analysis of Indian mulberry varieties through molecular markers". Hereditas. 141 (1), 8–14.
29. K. Vijayan. 2004. "Genetic relationships of Japanese and Indian mulberry (*Morus* spp) genotypes revealed by DNA fingerprinting". Journal Plant Systematics and Evolution Publisher Springer Wien. Volume 243, Numbers 3-4. P 221-232.
30. Luo, S., He, P., Zheng, X., Zhou, P., Sci. Hortic., 2002, 93, 19–28.
31. Atak, C., Alikarnanoglub, S., Acik, L., Canbolatb, Y., Mutat. Res., 2004, 556, 35–44.
32. Sandhu, S. S., Bastos, C. R., Azini, L. E.,Neto, A. T., Colombo, C., Genet. Mol. Res., 2002, 1, 359–370.
33. Deng, Z. N., Gentile, E., Nicolosi, E., Domina, F., Vardi, A., Tribulato, E., J. Hortic. Sci., 1995, 70, 117–125.
34. Schiliro, E., Predieri, S. and Bertaccini, A., Plant Mol. Biol. Rep., 2001, 19, 217a–h
35. Yang, H. and Schmidth, H., Euphytica, 1994, 77, 89–92.
36. Atienzar, F. A., Venier, P., Jha, A. N., Depledge, M. H., Mutat. Res. /Genet. Toxicol. Environ Mutagen. 2002. 521,151–163.
37. Wachira F N, Waugh R, Hackett C A and Powell A. 1995. Detection of genetic diversity in tea using RAPD markers. Genome 38: 201-210.

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