Journal of Applied Horticulture, 20(1): 57-61, 2018

Chloroplast genes reveal hybridity in mango (*Mangifera indica* L.)

Muthukumar M.*, Anju Bajpai and S. Rajan

Division of Crop Improvement and Biotechnology, Central Institute for Subtropical Horticulture, Lucknow, India.
**E-mail: kumarbt@gmail.com*

Abstract

Mango (*Mangifera indica* L.) encompasses diverse varietiesof different traits owing to their evolution by open pollination and natural selection processes over several generations. Phylogenetic relatedness and confirmation of hybridity of out-crossed progenies in mango are the pre-requisites for improving the precision of selection in trait based breeding. In this context, two chloroplast genes namely *trn*L and *trn*F genes localized within the chloroplast LSC region were used for sequence characterization of 8 mango varieties/hybrids to affirm hybridity and trace their inheritance. Sequence annotation and analysis revealed that both these genes were able to discriminate the 8 varieties. The hybridity of Arunika, a progeny generated from a cross between Amrapali and Vanraj, was also confirmed. Sequence level variations in the hybrids in comparison with the parents indicated that the inheritance of chloroplast genes is not strictly maternal but could be even paternal or biparental in nature. Thus, chloroplast genes which were usually thought to be markers for plant species discrimination could also apparently be used as genetic markers for hybridity confirmation at the population level.

Key words: Mango, chloroplast genes, hybridity

Introduction

Mango (*Mangifera indica* L.), acclaimed to be "King of fruits" is closely related to cashewnut (*Anacardium occidentale* L.) by ancestry, being members of the same family Anacardiaceae. Mango, being native to South Asia especially the Indo-Burma region (major centre of origin), is esteemed as national fruit to India, Philippines and Pakistan because of the huge diversity in these countries. However, mango has a wider adaptability to both tropical and subtropical regions of the world (Vavilo, 1926 and Singh *et al.*, 2016). A huge variation exists at species level which is evident from the existence of wild species, such as *Mangifera indica*, *M. andamaninca*, *M. zylannica*, *M. grifithii*, *M. odarata*, *M. laurina*, *M. sylvatica*, *M. foetida.* Through a series of events of natural selections over generations, the present day varieties/ cultivars have evolved which present rich genetic diversity in India especially in the hot spot regions (Singh *et al.*, 2016). Therefore, characterization of these rich germplasm resources seems to be highly important in order to document and conserve the native cultivars vis-a-vis protecting the newly developed hybrids through breeding and improvement programmes.

Morphological descriptors and molecular markers have been extensively used in characterization of the mango genetic resources. Dominant marker systems like random amplified polymorphic DNA (RAPD), inter simple sequence repeats (ISSRs) and amplified fragment length polymorphism (AFLP) and co-dominant markers such as simple sequence repeats (SSRs) and single nucleotide polymorphism (SNPs) have been extensively used for developing genetic fingerprints as well as establishing the genetic relationship existing among a range of mango genotypes from different ecological niches (Bajpai *et al.*, 2008; 2016; Hirano *et al.*, 2010; Ravishankar *et al.*, 2015; Srivastava *et al.*, 2012; Sherman *et al.*, 2015). Although these marker systems were found to be useful in genetic discrimination between the cultivars, they were not efficient in confirming hybridity and establishing genetic inheritance. The failures in hybridity conformation and understanding the genetic relationship could be attributed to the following reasons; i) inherent limitation of heterozygous and heterogenous nature of the mango species, ii) limited genomic resources and unreleased whole genome sequence and iii) lack of genetic linkage map comprising of all chromsomes with marker positions.

Plastid or chloroplasts with uniparental inheritance predominantly maternal in nature seems to be ideal choice for understanding the genetic relationship/kinship. Chloroplasts are unique organelles exclusive to the plant kingdom because they are the integral part of photosynthetic machinery (Cui *et al.*, 2006).Organellar genes representing chloroplast genes *viz*., *trn*L, *trn*L- *trn*F intergenic spacer region, *pet*B- *pet*D, *atp*B-*rbc*L, *rpL*20-*rpS*12 and mitochondrial gene namely *mat*K have been widely used for species discrimination and generation of DNA barcodes, the molecular signatures of a species in mango (Hidayat *et al.*, 2011; Fitmawati and Hartana, 2010). With the advancements in next generation sequencing platforms, chloroplast genes based sequence characterization of the mango varieties have not only become simple, efficient and cost effective but also facilitates establishing genetic relationship by reference mapping against the existing chloroplast genome map in mango reported by Azim *et al.*, 2014. Thus, in the present study, *trn*L and *trn*F genes with *trn*L-*trn*F intergenic spacer regions were used to characterize 8 mango varieties through PCR amplicon sequencing and computational analysis.

Materials and methods

Plant material and chemicals: Leaf samples from 8 mango varieties, *viz*., Amrapali, Dashehari, Vanraj, Langra, Janardhan Pasand, Arunika, Eldon, Tommy Atkins were collected from field gene bank at Central institute for Subtropical Horticulture, Lucknow. All the chemicals used in the study were of molecular

biology grade from Himedia Laboratories Pvt Ltd., Mumbai, India.

Chloroplast DNA isolation: Cholorplast DNA isolation was carried out using the protocol as described by Bookjans *et al.* (1984) with modifications. About 100 g of fresh leaves were homogenised in 50 mL of ice cold extraction buffer A (1M NaCl, 50 mM Tris-HCl (pH 8.0), 20 mM β-meraptoethanol) and filtered through a two layered cheese cloth. The filtrate was centrifuged at 1000 rpm for 1 min. and the supernatant re-centrifuged at 7000 rpm for 10 min. The pellet was again washed with extraction buffer A (5 mL) and was recovered by repeating centrifugation at 7000 rpm for 10 min. The pellet was suspended in 50 mL of extraction buffer B (0.15 M NaCl, 0.1 M EDTA, 2 % (v/v) DEPC) and incubated at RT for 15 min. SDS was added to the final concentration of 2 % and sodium acetate (3M, pH-4.8) to the final concentration of 20 % (v/v). The chloroplast was lysed at 37˚C for 45 min and was extracted with the equal volume of chloroform and centrifuged at 9000 rpm for 15 min. The extraction with chloroform was repeated until aqueous phase changed colourless. Potassium acetate (5 M, pH 5.2) was added to the aqueous phase to a final concentration of 2.5 M, incubated at -20 ˚C for 2 h and centrifuged at 10000 rpm for 10 min. 2 vol. of absolute ethanol was added to the aqueous and kept at -20 ˚C for 2 h. It was centrifuged at 12000 rpm for 10 min. Pellet was washed with 70 % ethanol, air dried, rehydrated in 10 mM $T_{10}E_1$ (Tris 10 mM, EDTA 1mM) and stored at -20 °C for further use.

Amplification of *trn*L and *trn*F genes and PCR product purification: Two chloroplast genes *trn*L and *trn*F were used for sequence characterization of the mango cultivars using universal primers (Table 1). Amplification of these two genes were done by standard polymerase chain reaction (PCR) carried at the following conditions comprising of 50 ng of DNA template, 1X buffer, 2.5 mM of 25 mM $Mgcl_2$, 0.2 mM of 10 mM dNTP, 0.5 µM forward and reverse primer, 1 % PVP, 1.5U of *Pfu* DNA Polymerase. The reaction was performed in a programme involving 1 cycle of 94 °C for 5 min, 35 cycles of 94 °C for 30 sec, 50.8 °C and 49.9 °C for 1 min (for *trn*L *and trn*F primers,respectively), 72 °C for 1 min followed by single cycle of 72 °C for 5 min. and held at 4 °C till end using thermal cycler (Helena Biosciences). Sixty µL of PCR products were checked on 1.5 % agarose gel for amplification, and the PCR products were purified using Biochem Gel extraction kit as per the instructions of the user manual.

Amplicon sequencing and sequence analysis: The purified PCR products were sequenced by Sanger sequencing method ABI biosystems 454 sequencer (Chromous Biotech, Bengaluru). Double pass sequence data of 8 mango cultivars were edited using BioEdit and contigs were assembled using contig assembly program (CAP3). The conserved domains were used as the motif to align the genes and position on the mango chloroplast genome and determine the actual sequence variations. The contigs were used for multiple sequence alignment and done through Clustal Omega, MUSCLE and ProbCons accessible and open source utility software at http://www.phylogeny.fr/index.cgi and the phylogenetic analysis was also carried out using the software TreeDyn accessible at http://www.phylogeny.fr/one_task.cgi?task_type=treedyn.

Table 1. Details of universal primers used in the study for amplification of chloroplast genes

Primer code	Sequence (5'-3')	Product size (bp)	Reference
*trn*L	F: CGAAATCGGTAGACGCTACG R: GGGGATAGAGGGACTTGAAC	550	Taberlet *et al.*, 1991
*trn*F	F: GGTTCAAGTCCCTCTATCCC R: ATTTGAACTGGTGACACGAG	400	Taberlet *et al.*, 1991

Results and discussion

Whole genome and sequence information in perennial fruit species/plants speeds up the breeding program. The draft genome of mango with a genome size of 450 Mbp has been reported (Singh *et al.*, 2014); yet the complete genome sequence needs to be released. With the limitations in genome information understanding genetic inheritance is cumbersome. Previously, genetic differentiation of the mango genetic resources have been extensively carried out using co-dominant markers like minisatellite and microsatellite markers (Bajpai *et al.*, 2008; 2016; Ravishankar *et al.*, 2015; Srivastava *et al.*, 2012; Sherman *et al.*, 2015), which could hardly classify the cultivars based on the genetic lineage and neither could prove inheritance pattern. Most recently sequence based markers such as SNP markers have been reported in mango (Singh *et al.*, 2016) which could also be complemented with the sequence analysis of chloroplast genes for phylogenetic reconstruction and understand inheritance of genes. Inheritance pattern of the genes is one of the key factors that determine the efficiency of hybridisation and selection in tree breeding programs.

Chloroplasts are unique to plant kingdom and some photosynthetic microorganisms. A typical chloroplast genome in higher plants is organized into a double stranded circular DNA molecule with genome size ranging between 120 and 160 kb (Odintsova and Yurina, 2006; Azim *et al.*, 2014). Chloroplast genome of mango was recently reported with genome size of 151 kb comprising of large and small single copy regions (LSC, SSC; respectively) separated by 27 kb duplicated inverted repeat regions (IR_A and IR_B) (Azim *et al.*, 2014). Chloroplast genes have been widely used as markers for DNA barcoding and for species level discrimination in mango (Fitmawati and Hartana, 2010)

In this context, two chloroplast genes namely *trn*L and *trn*F genes falling in the LSC region of the chloroplast were used for the sequence characterization of 8 mango varieties. PCR amplification produced amplicons of invariably same size in all 8 mango varieties, 550 bp and 400 bp for *trn*L and *trn*F genes, respectively (Fig. 1). This could be attributed to the highly conserved nature of the gene size and content in the chloroplast genome of angiosperms (Olmstead and Palmer, 1994). Perhaps, *trn*L-F region of cpDNA is conservative with low rate evolution whereas the *trn*L-F intergenic spacer is non-coding and hypervariable in nature (Bayer *et al.*, 2000). Some studies on non-coding region of cpDNA showed higher variations and mutations than coding regions (Baldwin, 1995; Fitmawati and Hartana, 2010).

Varietal differentiation on the basis of sequence variations in chloroplast genes: Amplicon sequencing of *trn*L and *trn*F genes of the 8 mango varieties confirmed through BLAST analysis revealed highest sequence similarity scores, maximum identity and coverage and lowest e-value with the respective genes. Annotations also indicated that the sequence similarity of *trn*L and *trn*F genes were more close to *C. Sinensis* which was in

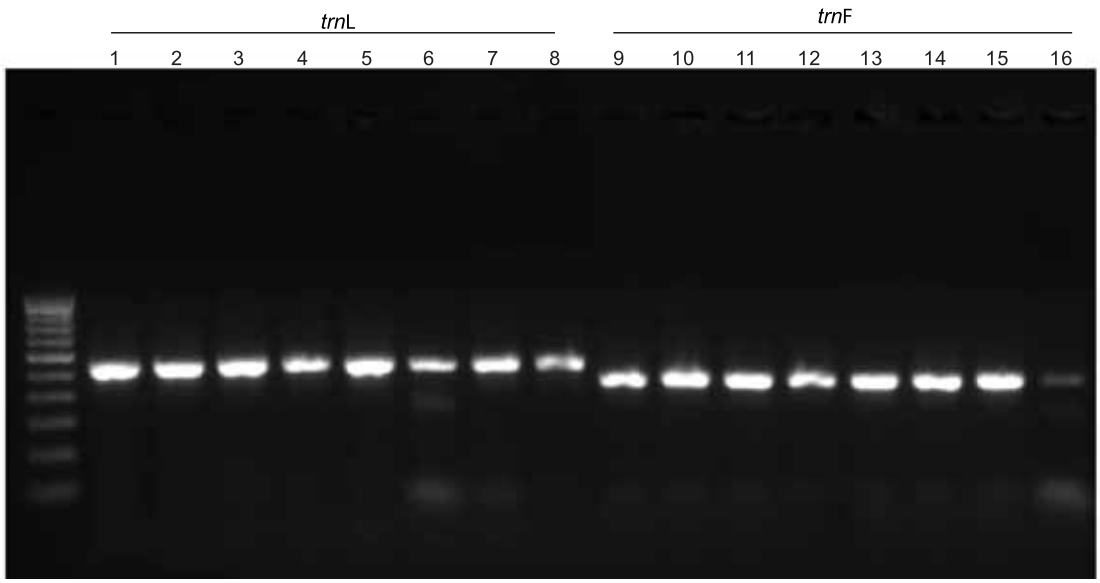

Fig. 1. Agarose gel (1.5 %) profile of PCR amplicons of *trn*L (550 bp) and *trn*F (400 bp) genes in 8 mango varieties. 1. Amrapali, 2. Dashehari, 3. Langra, 4. Tommy Atkins, 5. Eldon, 6. Vanraj, 7. Janardhan Pasand, 8. Arunika

corroboration of the earlier report that mango chloroplast genome has highest sequence similarity with Citrus cp genome (Azim *et al.*, 2014). These sequences have been submitted in the NCBI database assigned with specific gene bank accession numbers as shown in Table 2.

Multiple sequence alignment (MSA) through three algorithms such as Clustal Omega, MUSCLE and ProbCons detected the same type of alignment pattern indicating accuracy of the sequence data eliminating sequencing errors or bias. Distinct variations in both

Table 2. Description of the mango cultivars/hybrids and their gene bank accession numbers

Name of cultivar	Gene bank accession number	
	tRNA-Leu (*trn*L) gene	*trn*F gene with *trn*L-*trn*F intergenic spacer region
M. indica cv Amrapali	JQ726832	JX185672
M. indica cv. Dashehari	JQ726833	JX185673
M. indica cv Janardhan Pasand	JQ726834	JX185674
M. indica cv. Langra	JQ726835	JX185675
M. indica cv. Tommy atkins	JQ726836	JX185676
M. indica cv. Vanraj	JQ726837	JX185677
M. indica cv. Eldon	JQ726838	JX185678
M. indica cv. Arunika	JQ726839	JX185679

*trn*L (Fig.2A) and *trn*F (Fig.2B) genes discriminating the 8 mango varieties were observed in the trailing ends of the sequences. In *trn*L MSA result, the presence of T in Amrapali and absence in Vanraj and Arunika at 480bpposition was observed which indicated the hybridity of Arunika, a progeny of cross between Amrapali and Vanraj. Similarly, at 396th position G-A base substitution was observed in Eldon over other varieties. A four base indel (AAAT) for *trn*F with *trn*L-F intergenic spacer region in Eldon discriminated it from Tommy Atkins while a single base T deletion for *trn*L gene in Tommy Atkins differentiated it from Eldon. Such kind of single nucleotide variations for *trn*L-*trn*F intergenic spacer region have been reported in citrus (Urasaki *et al.*, 2005). The *trn*L-F intergenic spacer region was reported to be a powerful marker for discrimination of gametophytes in Pteridaceae family because of its highest primer universality and discriminatory ability scores (Chen *et al.*, 2013). Similarly, Janardhan Pasand was differentiated from other 7 varieties by a three base change in *trn*L gene from CCC to AGT. Variations in the introns as well as intergenic spacer regions and presence of 51 short repeats were earlier predicted to be associated with extensive DNA rearrangements in mango chloroplast genome (Azim *et al.*, 2014). This could be explained based on the two theories of evolution of chloroplast genes, exon theory of genes and insertional theory of introns, which pertain to presence or absence of introns in primordial genes. Group II introns in tRNA-Leu gene showed no conserved region for introns and structural mutations in the form of indels and transversions in tRNA intron (Drabkova *et al.*, 2004).

Phylogenetic relationship among mango varieties: The cpDNA markers are commonly used in the phylogenetic studies because they are easily isolated, purified, characterized and cloned. In the present study, phylogenetic analysis of sequences of two chloroplast genes in a set of 8 mango cultivars formed two distinct groups (Fig. 3) in which; group 1 included Dashehari, Amrapali, Vanraj and Arunika with Eldon as outgroup and the group 2 comprised of Tommy Atkins, Janardhan Pasand and Langra. The maximum similarity was obtained in hybrid Arunika with the parent Vanraj for *trn*F and *trn*L genes. A stricking observation in the phylogenetic classification based on the *trn*F gene revealed grouping of Dashehari with hybrids Amrapali and Arunika which could be attributed to the reason that both these hybrids inherited the lineage of Dashehari as one of the parents. Vanraj also fall in the same group along with Arunika and Amrapali which coincided with the parental lineage. Janardhan Pasand and Langra could not be differentiated by both the choloroplast genes and were falling in the same group along with Tommy Atkins. Exotic variety Eldon formed an outgroup which was also evident in the sequence variations (Fig 2). Sequence variations in *trn*L-F region of cpDNA has been more widely used in phylogenetic studies to discriminate the crop at generic and specific levels (Hansen *et al.*, 2006; Hidayat *et al.*, 2011; Fitmawati and Hartana, 2010;

Fig. 2. Sequence based variations among 8 mango varieties for chloroplast genes A) *trn*L gene and B) *trn*L-*trn*F intergenic spacer regions. The vertical rectangular boxes highlight genes with single base change (base substitution or indel), small rectangular box (in *trn*L region) indicates 3 base variations and square box (in *trn*F region) indicates indels.

Urasaki *et al.*, 2005; Daniell *et al.*, 2016). However, in practice, detecting useful polymorphism at the population level has been considered to be difficult due to the low level of substitutions (slow substitution rates) in plant chloroplast genomes. In contrast to these earlier studies, in the present study, it is evident that the chloroplast genes can also be useful for discriminating the varieties at the population level.

Inheritance of chloroplast genes in hybrid mango progenies: In a hybrid progeny, chloroplast genome is expected to have uniparental inheritance (mostly maternal) while the nuclear genome is inherited equally from both the parents (Daniell *et al.*, 2016). In the present study based on sequence variations, it was possible to infer the hybridity and chloroplast inheritance. Amrapali is a hybrid developed from a cross between Dashehari and Neelum. By principle, if the cpDNA inheritance is maternal, then the allelic pattern or sequence in Amrapali was expected to be inherited from Dashehari. Similarly, in a hybrid named Arunika descended from a cross between Amrapali and Vanraj, cpDNA was supposed to be inherited from maternal chloroplast of Amrapali. A single base variation in *trn*L gene *i.e.*, the presence of A in Dashehari in place of C in Amrapali, Vanraj and Arunika discriminated Dashehari from the other three. For *trn*F gene with *trn*L- *trn*F intergenic spacer region a single base deletion was noticed in Dashehari and Vanraj in contrast to the presence of a base T in hybrids, *viz*., Amrapali and Arunika. This not only confirms the hybridity but also evinces that inheritance of a chloroplast genome is paternal. Earlier, incomplete paternal inheritance of chloroplast genes especially *trn*L-*trn*F intergenic spacer region has been reported in citrus (Urasaki *et al.*, 2005), whose chloroplast genome is very close to mango chloroplast genome (Bausher *et al.*, 2006; Azim *et al.*, 2014). It is reported earlier that many land plants deviate from the maternal pattern of organelle inheritance and show paternal or biparental inheritance (Hansen *et al.*, 2006). From our findings, it is evident that there may be possibility of paternal or biparental inheritance of chloroplast genome in mango because of the heterogenous and heterozygous nature of the species, which needs further validation through screening a larger population of hybrids along with their parents.

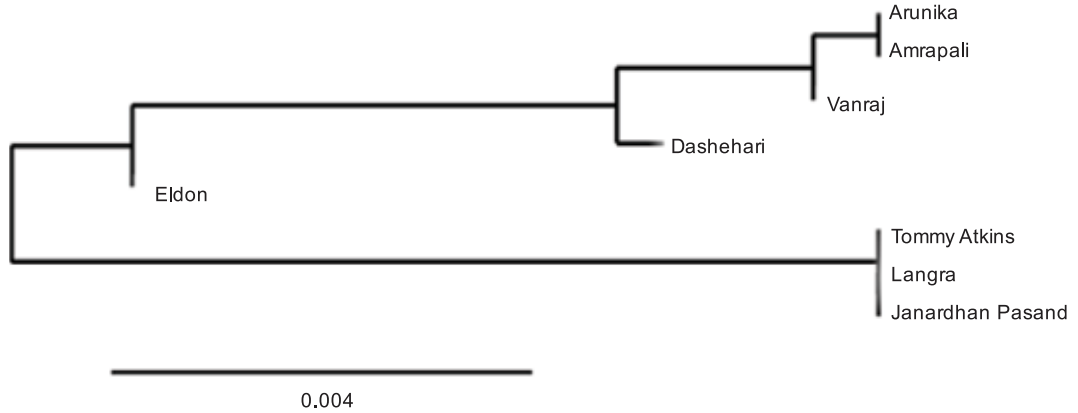

Fig. 3. Phylogenetic tree based on sequence variation detected in *trn*L gene among 8 mango varieties. The tree was generated using TreeDyn software accessible through http://www.phylogeny.fr/one_task.cgi?task_type=treedyn using the Newick format of alignment file generated from MSA in ProbCons.

Chloroplast genes have been used extensively in taxonomy and evolutionary research for the following advantages: (1) smaller size, high copy number, and simple structure; (2) conserved in gene content and arrangement over mitochondrial and nuclear genomes; (3) mostly maternally inherited and lacks genetic re-assortment that interferes with determining the molecular phylogenetic relationships (Hansen *et al.*, 2006; Daniell *et al.*, 2016). In addition, information about the chloroplast genome could be used for chloroplast transformation (Maliga, 2004), and development of crops with improved photosynthetic efficiency (Bock and Khan, 2004; Clarke *et al.*, 2011). Sequence level variations in two chloroplast genes detected in 8 mango varieties not only indicates their potentials as genetic markers for varietal differentiation but also facilitated evidences of deviation of chloroplast inheritance from maternal to paternal or biparental nature in mango. This study opens new vistas for exploring NGS platforms for amplicon sequencing of cp genes in mango hybrids and parents to confirm the hybridity and also to study the influence of chloroplast inheritance in the hybrid progenies for enhancing photosynthetic efficiency and developing climate resilient mango hybrids.

References

Azim, M.K., I.A. Khan and Y. Zhang, 2014. Characterization of mango (*Mangifera indica* L.) transcriptome and chloroplast genome. *Plant Mol Biol*., 85: 193-208.

Bajpai, A., M. Kumar, Israr Ahmad, K.V. Ravishankar, V.A. Parthasarthy, Bhuwon Sthapit, Ramanatha Rao, J.P. Verma and S. Rajan, 2016. Molecular and morphological diversity in locally grown non-commercial (heirloom) mango varieties of North India. *J. Environ. Biol.*, 37(2): 221-228.

Bajpai, A., N. Srivastava, R. Chandra and S. Rajan, 2008. Genetic diversity and discrimination of *Mangifera indica* (L.) accessions using ISSR and RAPD markers. *Ind. J. Hort*., 65: 377-382.

Baldwin, B.G., M.J. Sanderson, J.M. Porter, M.F. Wojeichowski, C.S. Cambell and M.J. Donoghue, 1995. The ITS region of nuclear ribosomal DNA: a valuable source of evidence an angiosperm phylogeny.*Annu. Miss. Bot. Gard*., 82: 247–277.

Bausher, M.G., N.D. Singh, S.B. Lee, R.K. Jansen and H. Daniell. 2006. The complete chloroplast genome sequence of *Citrus sinensis*(L.) Osbeck var ‚Ridge Pineapple': organization and phylogenetic relationships to other angiosperms.*BMC Plant Biol.*,6: 21.

Bayer, R.J.B., C.F. Puttock and S.A. Kelchner, 2000. Phylogeny of south African Gnaphilieae (Asteraceae) based on two-coding sequences. *Am. J. Bot*., 87: 259–272.

Bock, R. and M.S. Khan, 2004. Taming plastids for a green future. *Trends Biotechnol.,* 22: 311-318.

Bookjans, G., B.M. Stummann and K.W. Henningsen, 1984. Preparation of chloroplast DNA from pea plastids isolated in a medium of high ionic-strength. *Anal. Biochem*., 141: 2440-247.

Chen, C.W., Y.M. Huang, L.Y. Kuo, Q.D. Nguyen, H.T. Luu, J.R. Callado, D.R. Farrar and W.L. Chiou, 2013. *trnL-F* is a powerful marker for DNA identification of field vittarioid gametophytes (Pteridaceae). *Annals of Botany*, 111(4): 663-673.

Clarke, J.L. and Daniell, H, 2011. Plastid biotechnology for crop production: present status and future perspectives. *Plant Mol. Biol.*, 76(3-5): 211-220.

Cui, L., N. Veeraraghavan, A. Richter, K. Wall, R.K. Jansen, J. Leebens-Mack, I. Makalowska and C.W. dePamphilis, 2006. Chloroplast DB: the chloroplast genome database. *Nucl. Acids Res.,* 34: D692-696.

Daniell, H., C.S. Lin, M. Yu and W.J. Chang, 2016. Chloroplast genomes: diversity, evolution, and applications in genetic engineering. *Genome Biol.*, 17(1): 134.

Drabkova, L., J. Kirschner, C. Vcek and V. Paces, 2004. *Trn*L- *trn*F intergenic spacer and *trn*L intron define major clades within *Luzula*and *Juncus* (Juncaceae): importance of structural mutations. *J. Mol. Evol*., 59(1): 1-10.

Fitmawati and A. Hartana, 2010. Phylogenetic study of *Mangiferalaurina* and its related species using cpDNA*trn*L-F spacer markers. *HAYATI Journal of Biosciences*, 17(1): 9-14.

Hansen, A.K., L.K. Escobar, L.E. Gilbert and R.K. Jansen, 2006. Paternal, maternal, and biparental inheritance of the chloroplast genome in *Passiflora* (Passifloraceae): implications for phylogenetic studies. *Am. J. Bot*., 94(1): 42-46.

Hidayat, T., A. Pancoro, D. Kusumawaty and W. Eiadthong, 2011. Development matK gene as DNA barcode to assess evolutionary relationship of important tropical forest tree genus *Mangifera* (Anacardiaceae) in Indonesia and Thailand. *JurnalTeknologi*, 59: 17-20.

Hirano, R., O.T. Htun and K.N. Watanabe, 2010. Myanmar mango landraces reveal genetic uniqueness over common cultivars from Florida, India, and Southeast Asia. *Genome,* 53: 321-30.

Maliga, P, 2004. Plastid transformation of higher plants. *Annu. Rev. Plant Biol*., 55: 289-313.

Odintsova, M.S. and N.P. Yurina, 2006. Chloroplast genomics of land plants and algae, in biotechnological applications of photosynthetic proteins: Biochips, Biosensors and Biodevices. Springer US.

Olmstead, R.G. and J.D. Palmer, 1994. Chloroplast DNA systematics: a review of methods and data analysis. *Am. J. Bot*., 81: 1205-1224.

Ravishankar, K.V., P. Bommisetty, A. Bajpai, N. Srivastava, B.H. Mani, C. Vasugi, S. Rajan and M.R. Dinesh, 2015. Genetic diversity and population structure analysis of mango (*Mangifera indica*) cultivars assessed by microsatellite markers. *Trees*, 29(3): 775-783.

Sherman, A., M. Rubinstein, R. Eshed, M. Benita, M. Ish-Shalom, M. Sharabi-Schwager, A. Rozen, D. Saada, Y.M. Cohen and R. Ophir, 2015. Mango (*Mangifera indica* L.) germplasm diversity based on single nucleotide polymorphisms derived from the transcriptome. *BMC Plant Biology***,** 15(1): 277.

Singh, N.K., A.K. Mahato, P.K. Jayaswal, A. Singh, S. Singh, N. Singh, V. Rai, S.V. Amitha Mithra, K. Gaikwad, N. Sharma, S. Lal, N. Srivastava, J. Prakash, U. Kalidindi, S.K. Singh, A.K. Singh, K. Khan, R.K. Mishra, S. Rajan, A. Bajpai, B.S. Sandhya, P. Nischita, K.V. Ravishankar, M.R. Dinesh, S. Jaiswal, M.A. Iquebal, A. Rai, D. Kumar and T.R. Sharma, 2016. Origin, diversity and genome sequence of mango (*Mangifera indica* L.). *Indian Journal of History of Science*, 51.2.2: 355-368.

Singh, N.K., A.K. Mahato, N. Sharma, K. Gaikwad, M. Srivastava, K. Tiwari, V. Dogra, H.C. Rawal, P.K. Jayaswal, A. Singh, V. Rai, S.V. Amitha Mithra A. Bajpai, M.R. Dinesh, K.V. Ravishankar, S. Rajan, A. Rai, A.K. Singh and T.R. Sharma, 2014. A draft genome of king of fruit, mango (*Mangifera indica* L.). *Plant and Animal Genome Conference XVII*. Jan 10-14, San Diego, CA. P045.

Srivastava, N., A. Bajpai, R. Chandra, S. Rajan, M. Muthukumar and M.K. Srivastava, 2012. Comparison of PCR based marker systems for genetic analysis in different cultivars of mango. *J. Environ. Biol.,* 33: 159-166.

Taberlet, P., L. Gielly, G. Pautou and J. Bouvet, 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Mol. Biol*., 17(5): 1105-1109.

Urasaki, N., N. Yoshida, T. Uehara, H. Inoue, S. Onda, H. Kinjyo, S. Kawan and O. Hideyasu, 2005. Single nucleotide polymorphism in Shiikuwasha (*Citrus depressa*Hayata) chloroplast DNA, *trn*L-*trn*F. *Jpn. J. Trop. Agr.,* 49(3): 246-251.

Vavilov, N.I, 1926. Centres of Origin of Cultivated Plants. *Bulletin of Applied Botany of Genetics and Plant-Breeding*, 16: 1-248.

Received: November, 2017; Revised: November, 2017; Accepted: December, 2017