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Original Research Paper

Impact of carbohydrate metabolism pathways on bearing habit of mango (*Mangifera indica* L.) genotypes

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ABSTRACT

Heterozygosity is the major constraint in perennial fruit crop like mango for regular bearing breeding. Majority of the popular mango varieties have irregular bearing habit. Many external and internal factors affect the bearing habit of perennial fruit crops. Among internal factors, the level of carbohydrate reserves and phytohormones plays a major role on bearing habit of fruit crops like apple, citrus, mango, litchi *etc*., Therefore, present research work aimed to study the carbohydrate metabolism pathways in regular and irregular mango genotypes of varying origin. A total of 30 primers were designed using *in silico* mining of four key genes coding for citrate synthase, alcohol dehydrogenase, sucrose phosphate synthase and trehalose phosphate synthase. These genes play important role in sugar and starch metabolism in mango. Of these specific primers, 14 showed polymorphism among the genotypes studied. Gene diversity (GD), average number of alleles per locus (An), polymorphism information content (PIC) and major allele frequency (Maf) observed were 0.45, 2.14, 0.35, 0.59, respectively. Simple sequence repeats markers grouped 63.15% studied mango genotypes of regular bearers together. Further, these markers could be utilized in a greater number of genotypes for regularity.

Keywords : Carbohydrate metabolism, irregular bearing, mango, molecular markers

INTRODUCTION

Mango (*Mangifera indica* L.), belongs to the family Anacardiaceae, has an important place among the fruits of the world and is popularly called as king of fruits in India because of its wide uses and nutritional qualification. It is the most widely cultivated tropical fruit species in India and its cultivation also spread to other tropical and subtropical parts of the world. It occupies the highest area of 2,317 thousand ha among fruit crops and contributes 20, 386 thousand metric tons fruit production (NHB, 2020-21) in India. Globally, Asia accounts for 75% of world mango production. Whereas, India holds first rank among world's mango producing countries with a share of 38 percent in total world's mango production (FAOSTAT, 2019). In India, most of the commercial cultivars behave as irregular bearers in north Indian conditions whereas produce regular crops under south Indian conditions (tropical climate). The irregular bearing behaviour of mango is the major obstacle in getting good yields during "off-year" cropping. Irregularity in mango crop bearing is may be due to different factors like C:N ratio, hormonal imbalance, *etc*. Carbohydrate metabolism plays a very important role in bearing behaviour of fruit crops (Fischer *et al*. 2012). Carbohydrates reserves depicted as the key energy producing chemicals which play important role in floral induction process in many crop species (Wahl *et al.,* 2013). Draining out of carbohydrate and nitrogen reserves during "On" year is known to lead to a lean crop in the "Off" year as they are important for fruit bud initiation i.e., high C/N ratio helps for fruit bud initiation (Sharma *et al*., 2019, 2020). It is well studied about the catalytic activity of the enzymes Sucrose Phosphate Synthase, Trehalose Phosphate Synthase, Citrate Synthase, Alcohol Dehydrogenase in carbohydrate metabolism of plants (Brownleader, 1997). The genes related to the enzymes of carbohydrate metabolism (Trehalose phosphate synthase, Sucrose phosphate synthase, Citrate synthase, Alcohol dehydrogenase) have been studied

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by many researchers (Eldik *et al*., 1998, Coleman *et al*., 2010, Wahl *et al.,* 2013, Han *et al*., 2017, Benny *et al*., 2022) for their role in flowering related process of different plant species. Differential expression of the genes coding for sucrose synthase, sucrose phosphate synthase1, ATP synthase, polyphenol oxidase and auxin response factor are reported in the floral buds of mango cultivars Dashehari, Langra, Chausa and Amrapali (Bajpai *et al*., 2021). Carbohydrates levels in plants are generally analyzed by biochemical methods, recent advances in molecular biology and biotechnology fields helps us to find out the genes related to carbohydrate metabolism. In our present research we have designed carbohydrate metabolism specific primers for validation of regular and irregular bearing genotypes.

MATERIALS AND METHODS

The present experiment was carried out on 19 genotypes of mango (Mango Field Gene Bank, IARI) of varying origin and bearing habit *viz*., 9 hybrids (regular bearer) released from ICAR-Indian Agricultural Research Institute, New Delhi (Pusa Arunima, Pusa Surya, Pusa Peetamber, Pusa Lalima, Pusa Shresth, Pusa Manohari, Pusa Deepshikha, Amrapali and Mallika), six irregular bearer genotypes namely Dashehari, Kesar, Alphonso, Bombay Green, Langra, Chausa, two south Indian genotypes of regular bearer namely Totapuri and Neelum and two exotic genotypes viz; Tommy Atkins and Sensation. During the course of investigation, blocks were maintained as per the recommended cultural practices. New flushing and healthy leaves from single tree of each genotype were plucked, put into labelled polyethylene bags and placed in an icebox. Samples were wrapped in aluminium foil, tagged properly, frozen in liquid nitrogen for a few seconds, and stored at –80°C until DNA extraction. Genomic DNA was extracted by the cetyltrimethylammonium bromide (CTAB) method with some modifications (Doyle and Doyle 1987).The genomic DNA was further purified by successive RNase treatment followed by phenol: chloroform extraction. The pellet dissolved in TE buffer and stored at -20 °C temperature. The quality of the extracted DNA was assessed by agarose gel electrophoresis and quantified using Nanodrop 8000 spectrophotometer (Thermo Scientific, USA).

A total of 4 key gene sequences coding for Trehalose phosphate synthase, Sucrose phosphate synthase, Citrate synthase and Alcohol dehydrogenase of *Mangifera indica* L. were retrieved from National Center for Biotechnology Information (NCBI, www.ncbi.nlm.nih.gov). These gene nucleotide sequences play important role in carbohydrate metabolism. A total of 30 primers were synthesized for wet lab validation. Carbohydrate metabolism genes coding for Trehalose phosphate synthase, Sucrose phosphate synthase, citrate synthase, Alcohol dehydrogenase generated 9, 10, 5 and 6 primers, respectively. Nucleotide accession number GU233771, GU233770 and GU233769 of *alcohol dehydrogenase* gene of M. *indica* L. var. Dashehari was used for simple sequence repeats (SSRs) mining. A total of 10 SSRs were identified and 6 primers were synthesized. For *citrate synthase* gene JN001196, XM_044609816, XM_044609329 nucleotide accession of mango varieties Jinhuang and Alphonso were used for SSRs mining. A total of 5 primers were generated from identified 5 SSRs sequences. *Trehalose phosphate synthase* gene (nucleotide accession number MH759789) sequence of mango variety Kensington Pride resulted into 13 SSRs and a total of 9 primers were generated. *Sucrose phosphate synthase* gene (nucleotide accession number AB724402, AB724401, AB724400 and AB724399) sequences of mango varieties namely N-13, Cat Trang, Glenn, Valencia Pride resulted into 25 SSRs sequences and a total of 10 primers were generated. Primer 3 software (www.frodo.wi/mit.edu/primer3) was used for primer designing. PCR was carried out in 10µl reaction mixture containing 0.5µl each primer (10 pico mole each of forward and reverse), 2 µl of 25ng/µl genomic DNA as template and 5µl of *Taq* polymerase buffer 2X master mix (G Bioscience, USA). The volume was made up to 10µl with sterile distilled water. Thermocycling was carried out in a PE-Thermo cycler (C1000 Touch Thermal cycler, Bio-Rad, USA). Initial denaturation carried out at 94 °C for 5 minutes followed by 35 cycles (denaturation at 94°C, annealing at 55 ° C and extension at 72°C for 1 minute). Final extension was carried out at 72°C for 10 minutes. PCR amplified products were resolved in 3% high resolution agarose gels (Sisco Research Laboratories Pvt. Ltd). Electrophoresis was carried out at 120 V for 3 to 4 hours. DNA profiles were visualized on UV trans-illuminator and photographed on gel documentation system (Alpha Innotech, USA). Power Marker 3.5 was used to calculate gene diversity, heterozygosity and polymorphic information content of the markers (Liu and Spencer, 2005).

RESULTS AND DISCUSSION

A total of 30 carbohydrate metabolism specific markers were designed (Online Resource 1). Carbohydrate metabolism genes coding for Trehalose phosphate synthase, Sucrose phosphate synthase, Citrate synthase, Alcohol dehydrogenase generated 9, 10, 5 and 6 primers, respectively (Online Resource 1).These markers were validated in 19 mango genotypes. Genomic DNA yield was found varied in all 19 studied mango genotypes and highest yield in Totapuri (1360.30 ng/µl) and lowest in Pusa Surya (405.80 ng/µl) with 752.91 ng/µl average yield. The average value of DNA quality on the basis of nanodrop reading (A260/280) was 1.68 and maximum value was found in Pusa Surya (1.79) and minimum value was found in Dashehari (1.65). However, A260/230 ratio was found maximum in Pusa Shresth (2.07) and minimum in Neelum (1.83) with 1.90 average values. A total of 14 markers were found polymorphic (Table 1). Agarose gel profile of mango genotypes using *alcohol dehydrogenase* gene-based primer NMAD1 shown in Fig. 1. The major allelic frequency (Maf) ranged from the 0.44 to 0.94 among the markers with a mean value of 0.59 per locus. The marker NMSPS4 had the highest allelic frequency (0.94), while NMTPS7 had the lowest value (0.44). Further, among all primers, maximum and minimum PIC value was found in NMTPS7 primers (0.49) and NMSPS4 (0.09), respectively. However, average PIC value was 0.35 per locus. The gene diversity of the primers was calculated which ranged from 0.09 to 0.58 with an average of 0.45 per locus. The NMTPS7 had the highest gene diversity (0.58), while the lowest value (0.09) was recorded in NMSPS4. The observed heterozygosity among primers was also estimated, which varied from 0.10 to 1.00 with an average value of 0.67 per locus.

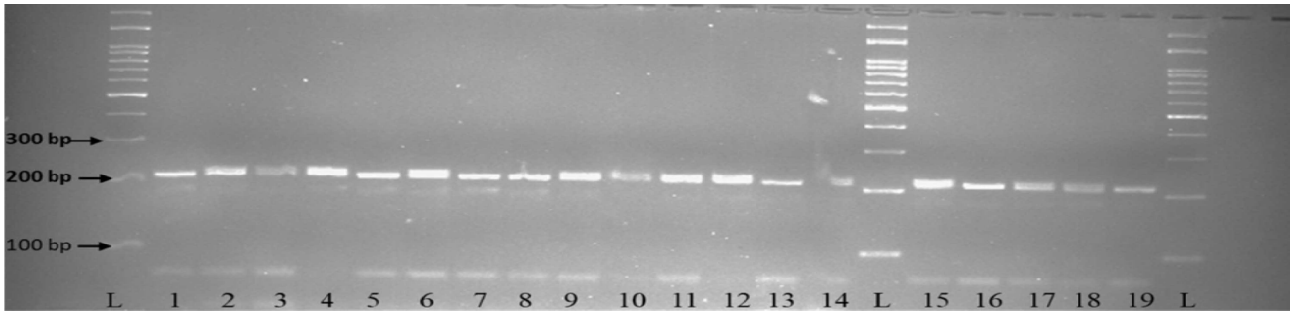

Fig. 1 : Agarose gel profile of mango genotypes using *alcohol dehydrogenase* gene-based primer NMAD1
L- 100 BP Ladder, 1. Pusa Arunima, 2. Pusa Surya, 3. Mallika, 4. Tommy Atkins, 5. Sensation, 6. Neelum, 7. Totapuri, 8. Pusa Shreshth, 9. Pusa Deepshikha, 10. Amrapali, 11. Pusa Manohari, 12. Pusa Peetamber, 13. Pusa Lalima, 14. Kesar, 15. Dashehari, 16. Bombay Green, 17. Langra, 18. Chausa, 19. Alphonso, L- 100 BP Ladder

Table 1 : Genetic variability indices of the 14 polymorphic carbohydrate metabolism specific primers among the set of 19 mango genotypes

Marker ID	Maf*	An	GD	Ho	PIC
NMAD1	0.7105	2.0000	0.4114	0.5789	0.3267
NMAD2	0.5000	2.0000	0.5000	1.0000	0.3750
NMAD3	0.6579	2.0000	0.4501	0.6842	0.3488
NMAD4	0.5526	2.0000	0.4945	0.7895	0.3722
NMAD5	0.5000	2.0000	0.5000	0.8947	0.3750
NMAD6	0.6579	2.0000	0.4501	0.5789	0.3488
NMCS1	0.5526	2.0000	0.4945	0.5789	0.3722
NMCS2	0.4737	2.0000	0.5485	0.7368	0.4453
NMCS3	0.5000	3.0000	0.5000	0.5789	0.3750
NMSPS4	0.9474	2.0000	0.0997	0.1053	0.0948
NMSPS5	0.5526	2.0000	0.4945	0.8947	0.3722
NMSPS7	0.5789	2.0000	0.4875	0.7368	0.3687
NMTPS1	0.7105	2.0000	0.4114	0.5789	0.3267
NMTPS7	0.4474	3.0000	0.5886	0.6842	0.4997
Mean	0.5959	2.1429	0.4593	0.6729	0.3572

*Maf = Major allele frequency, An = Allele number, GD = Gene diversity, Ho = Observed heterozygosity, PIC = Polymorphism information content

A dendrogram generated based on molecular data grouped all the 19 genotypes of mango into one major cluster B and one out group A. Major cluster B, comprised most of the studied genotypes and further sub-divided into two clusters as B1 and B2. Cluster B2 further sub-divided into cluster B2.1 and B2.2. Only the Amrapali and Pusa Arunima genotypes were found in sub-cluster B2.1. Most of the mango genotypes (89.46%) come under sub-group B2.2 (Table 2). Genetic tree showed the relatedness among the studied mango genotypes (Fig.2). Operational taxonomic units (OTU) for all combinations given in Online Resource 2.

Table 2 : Distribution of mango genotypes into groups based on carbohydrate metabolism specific markers

Cluster	Alternate bearing genotypes (Bombay Green, Kesar, Dashehari, Alphonso, Langra, Chausa)	Regular bearing genotypes(Tommy Atkins, Pusa Arunima, Amrapali, Totapuri, Pusa Shreshth, Pusa Peetamber, Pusa Lalima, Pusa Deepshikha, Pusa Manohari, Neelum, Mallika, Pusa Surya, Sensation)	Total
A	1(5.2%)	0	5.2%
B.1	0	1 (5.2%)	5.2%
B.2	5(26.31%)	12 (63.15%)	89.46%

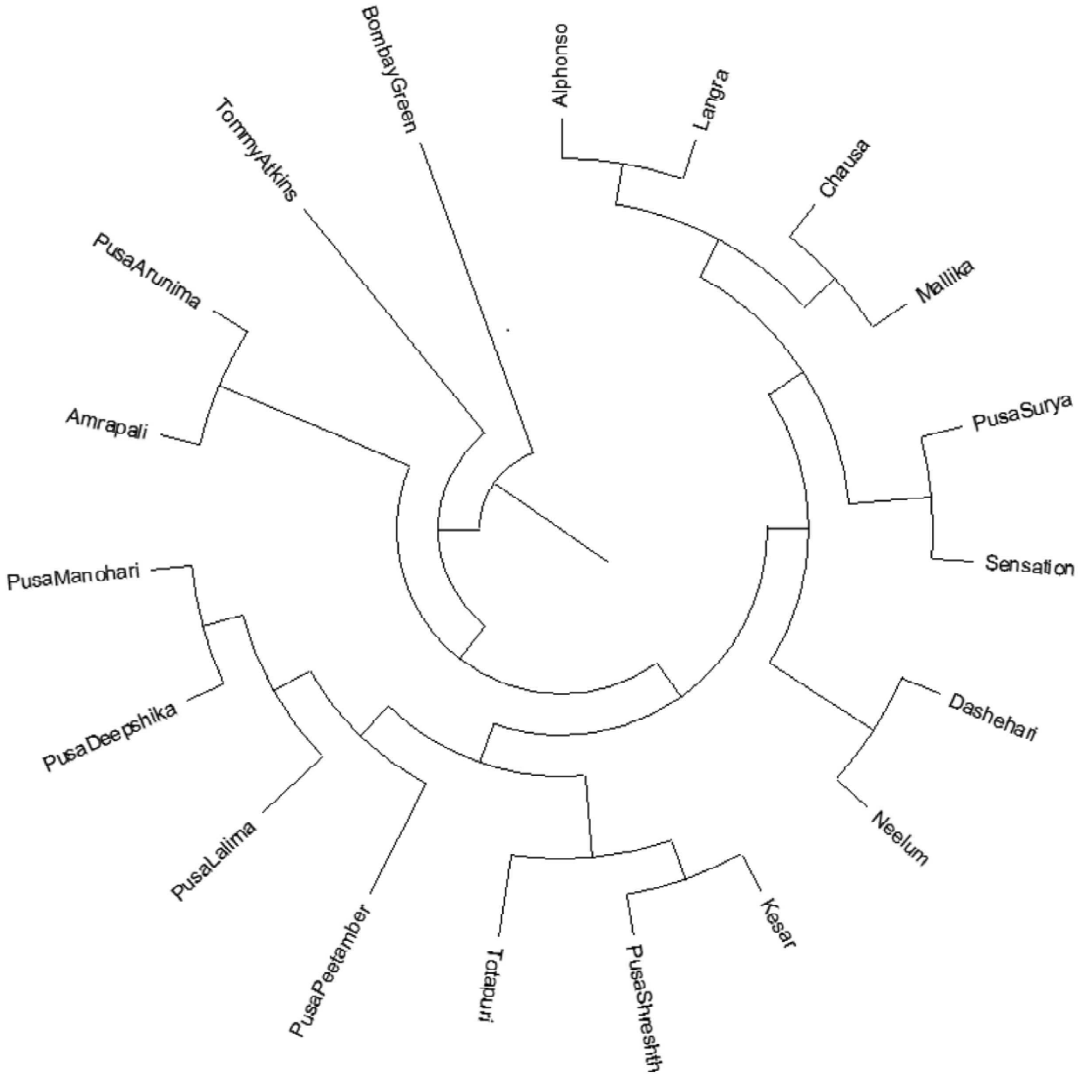

Fig. 2 : Genetic tree of 19 mango genotypes using carbohydrate metabolism specifc primers

The SSR markers were used with a view to characterize and analyze the 19 mango genotypes with respect to bearing habit (regular or alternate bearing) of mango tree. Out of 30 SSR markers used, 14 were found polymorphic. PIC values aid in forecasting the potential use of DNA markers for genotypes assessment in molecular breeding. Markers with high PIC values (NMTPS7) have greater potential in showing allelic variation according to Spandana (2012) findings in Sesamum crop. And our SSR markers exhibited lower level of gene diversity (0.45). Low level of genetic diversity indicates the frequent use of only few parents in breeding among selected cultivars (Kumar *et al*., 2013). Though the dendrogram in the present study did not indicate very clear pattern of clustering according to the bearing habit. The cluster B2 consists of 63.15 % regular bearing genotypes as one group which may indicate that these markers have some potential to use and to improve in future studies for differentiating mango cultivars based on their bearing nature.

CONCLUSION

For characterization and evolution of mango genotypes with respect to bearing habit, SSR markers can be used as they are globally accepted for their efficient and effective management and analysis of the genetic diversity of the germplasm. Though clustering is not clear in dendrogram, our markers grouped more than 60 % regular bearing cultivars in one cluster which need further improvement in future studies. Therefore, the research work further will be helpful in selection of suitable recombinants and hybrids having regular bearing habit in early nursery stage itself overcoming the problem of long gestation periods and other economic constraints.

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