The Pharma Innovation Journal 2022; 11(7): 1884-1889

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; 11(7): 1884-1889

www.thepharmajournal.com
Received: 07-05-2022
Accepted: 11-06-2022

Shikha Jain
Ph.D. Research Scholar, Division of Fruits and Horticultural Technology, ICAR-Indian Agricultural Research Institute, New Delhi, India

Shikha Saini
Ph.D. Research Scholar, Division of Fruits and Horticultural Technology, ICAR-Indian Agricultural Research Institute, New Delhi, India

Koppula Satya Sai Kumar
M.Sc., Ag, Division of Genetics and Plant Breeding, IARI – Indian Institute of Agricultural Biotechnology, Ranchi, Jharkhand, India

Shubham Jagga
Ph.D. Research Scholar, Division of Fruits and Horticultural Technology, ICAR-Indian Agricultural Research Institute, New Delhi, India

Poonam Maurya
Ph.D. Research Scholar, Division of Fruits and Horticultural Technology, ICAR-Indian Agricultural Research Institute, New Delhi, India

Vinay Kumar
Ph.D. Research Scholar, Department of Horticulture, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, India

Bhargav Kiran
Ph.D. Research Scholar, Division of Vegetable Science, ICAR-Indian Agricultural Research Institute, New Delhi, India

Corresponding Author:
Shikha Jain
Ph.D. Research Scholar, Division of Fruits and Horticultural Technology, ICAR-Indian Agricultural Research Institute, New Delhi, India

Status of Genomic Resources in Apple Fruit Quality Traits Improvement: A Review

Shikha Jain, Shikha Saini, Koppula Satya Sai Kumar, Shubham Jagga, Poonam Maurya, Vinay Kumar and Bhargav Kiran

Abstract
The domesticated apple (*Malus* × *domestic a* Borkh.) is the main fruit crop of temperate regions of the world. The genetic improvement of apple can be done through various techniques including introduction, selection, hybridization, mutation and molecular techniques (genomics). Genetic improvement of fruit quality includes external fruit appearance, such as color, shape, and size, as well as internal traits such as aroma, texture, crispiness, and juiciness. Over the past ten years, apple genomic resources have been created, leading to the sequencing of the "Golden Delicious" genome with a genome size of 742.3Mbp. The creation of public databases such as the GDR (Genome Database for Rosaceae), NCBI, and the European HIDRAS Apple Breed offer excellent opportunities for improved collaboration between breeders and bioinformatics researchers. Genomics studies include structural, functional and comparative genomics. Although structural genomics was mainly based on microscopic research of chromosomes, it has now become a driving force in apple breeding research since the development of DNA marker technologies at the end of the 20th to the beginning of the 21st centuries. Apple now has access to a wide range of genomic materials, including whole genome sequences, various ESTs, numerous molecular markers, and high-density genetic maps. These resources have been used for sequencing, Quantitative Trait Loci (QTL) mapping, and marker assisted selection (MAS). The availability of whole genome sequence has provided new opportunities to explore the genetic basis of fruit quality traits.

Keywords: Bioinformatics, EST, genomics, markers, QTL

Introduction
Apple (*Malus domestica* Borkh) is the most important fruit crop in temperate regions of the world. Apple can be intentionally genetically improved through a variety of methods, such as introduction, selection, hybridization, mutation, and molecular approaches (genomics). Genetic resources are the most beneficial starting point for crop improvement and are essential for guaranteeing food and nutritional security. A higher yield, a pleasing appearance and eating quality, resistance to serious diseases and pests, good storage and shelf life, climatic adaptation and frost resistance, and an improvement in fruit quality are the broad goals for apple improvement. Fruit crop improvement is a difficult procedure that requires a number of systematic strategies to succeed.

Need of Genomics
In order to produce gradual improvements in phenotypes such fruit size, yield, nutritional value, and aroma/taste, conventional breeding has historically been used to improve apples. This method entails choosing plants with desirable qualities over many successive generations. Traditional fruit tree breeding has improved consumer-driven qualities like fruit size, yield, nutritional value, aroma, and flavour while also introducing agronomic traits like disease resistance. However, it takes a lot of time and work to enhance fruit quality through conventional breeding. The advent of genetic engineering led to the rapid development of new varieties by allowing the direct introduction of genes into elite lines. With the assistance of modern molecular approaches such as marker-assisted selection, QTL mapping, genome editing *etc.* the process of developing new variety has accelerated.

Apple Genome Sequencing
Apple genome sequencing can be done by using a technique called Whole-Genome Shotgun (WGS). High molecular weight genomic DNA was extracted and cut up into smaller bits before being sequenced. The smaller bits of DNA were utilized to build 27 genomic libraries

for sequencing that ranged in size from 500 base pairs (BP) to 135 kilo base pairs (Kb). Both the Sanger Method based sequencers and the G2 sequencers were used. Ultimately, a total of 39.2 million reads accounting for approx. 13 billion sequenced nucleotides were generated, among which 26% were by the Sanger Method while 74% by the Roche/454 Genome Sequencers (Velasco *et al.*, 2010) [13]. Assembling of the 39.2 million reads has led to 122,146 contigs of an accumulative length of 604 Mbp, covering 81.3% of the genome with a new estimate of 742.3 Mbp. Using 1,643 genetic markers, 528.3 Mbp representing 103,076 contigs were anchored to 17 chromosomes. The anchored 528.3 Mbp sequences are gene rich regions and contain more than 90% of the apple genes assigned with chromosomes (Velasco *et al*., 2010) [13]. However, 138.4 Mbp (18.6% of the genome) have not been determined with their corresponding chromosomal origins although most (98%) are repetitive elements that make up nearly two thirds of the apple genome. In addition, an extensive final touch is needed to close the 100,000+ sequence gaps between and among the contigs anchored to chromosomes to enhance the utility of apple genome sequences.

The Apple Genome: A Delicious Promise

Since apples are a diploid species (2n = 2x = 34), their somatic cells, like those in leaves, have 34 chromosomes. However, all 17 monoploid (x) chromosomes in one full set of DNA define the genome of the apple. A multinational team of scientists led by Italy released a draft sequence of the apple genome in August 2010 as a result of the rapid developments of genome sequencing technologies (Velasco *et al*., 2010) [13]. The genome of 'Golden Delicious', one of the most widely grown apple varieties in the world, is the source of the published sequence. The release marks a major breakthrough in apple genetics and genomics. Several mysterious questions have been answered by the release, including the number of genes encoded by the apple genome, the origin of the 17 chromosomes of apple, and the origin of the domesticated apple.

Besides nuclear DNA, whole organelle genomes (chloroplast and mitochondrial DNA) of apple have also been sequenced. The mitochondrial (mt) DNA of apple has a size of 397 kbp and consists of 90% of non-coding DNA sequences. About 72% of the mtDNA is similar to DNA sequences of the nuclear genome and 20% consists of DNA sequences imported from other cell compartments. A complete mtDNA genome sequence is also obtainable for the apple rootstock *M. hupehensis* var. *pinyiensis*. This sequence is 25,608 bp longer than that of *M. domestica* and contains 38 protein-coding genes, 4 pseudogenes, 25 tRNA genes, and 3 rRNA genes. The chloroplast (cp) genomes of 47 genotypes belonging to different *Malus species* have been sequenced. *M. sylvestris* has contributed the most to the cp genome of cultivated apples. The cpDNA genome of *M. prunifolia* has been sequenced. The circular double-stranded genome has a size of 160 kbp and contains 129 genes, including 84 protein-coding genes, 37 tRNA genes and 8 rRNA genes.

Genomics Classification at a Glance

Genomics - It is the study of all the nucleotide sequences including structural genes, regulatory sequences and non – coding DNA segments in chromosomes of an organism. Term genomics was coined by Dr. Tom Roderick. It can be further divided into –

1. Structural genomics
2. Functional genomics – can be further sub – divided into Transcriptomics, proteomics and metabolomics.
3. Comparative genomics

Genomic Resources

Genomic resources include use of molecular markers (RFLP, RAPD, SSR, SCAR and SNP), QTL mapping, Marker assisted selection, genetic maps, transciptome, proteome, metabolome and epigenome.

Development of Research communities and databases

In order to combine molecular and phenotypic data from various, pedigree-verified populations, including crosses, breeding selections, and cultivars, the Apple Breed Database was developed. This database has uses outside of the European HIDRAS project, despite being made for it. The GDR (Genome Database for Rosaceae) is a combined Web database providing data on the genetics and genomics of Rosaceae in the USA. Even at National Centre for Biotechnology Information (NCBI) apple genomics database is present.

Fruit quality traits

Overall fruit quality is the most important target for every apple scion breeding program as the ultimate goal is to release a cultivar that appeals to consumers so that they will purchase, enjoy eating, and then repeat purchasing. However, breeding for fruit quality is highly complex. Selecting individuals based on one or two quality traits (*e.g*., crispness and sweetness) can be easily accomplished, but to develop a new cultivar with high overall fruit quality, breeders need to select individuals that are balanced for multiple traits, including appearance, texture, taste, aroma, and storability. Fruit quality traits include:

1. Fruit texture
2. Fruit shape
3. Fruit size
4. Fruit acidity
5. Fruit sugar content
6. Fruit firmness
7. Fruit colour
8. Fruit flavor

Inheritance pattern of fruit quality traits: Majority of the fruit quality traits are governed polygenically.

Table 1: Inheritance pattern of important fruit quality traits

Characters	Inheritance Pattern
Fruit size, Fruit shape, Russeting of skin, Fruit surface, Skin pigmentation, Acidity, Sweetness, Sourness, Texture	Polygenic
Red colour of shoot	Dominant
Cream yellow flesh colour	Single Dominant gene
Presence of Anthocyanin strips	Single Dominant gene
Mildew resistance	Polygenic
Flower colour (white to pink)	Three independent genes *A,B,C*

Table 2: Fruit quality attributed genes of apple

Preferred symbol	Original symbol	Gene Effect	Source
BP1,BP2	-	Bitter pit resistance	Coop 11
Caa, Cab	AK	Deciduous calyx	*M. zumi*
Ma	-	Mallic acid content	Lord Lambourne
Rf	-	Anthocyanin in fruit skin	Worcester Pearmain
Ru	-	Russet	D Arcy Spice

Table 3: Active breeding programmers and achievements of Asian countries

Country	Organization	Breeding objectives	Important varieties developed /released/Introduced
China	Hebei Changli Fruit Res. Institute	High quality	Yanshanhong (Ralls Janet x Richared Delicious)
	Zhengzhou Fruit Res. Institute	High quality	Huaguan (Golden Delicious x Fuji), Huashuai (Fuji x Starkrimson)
	Liaoning Fruit Research Institute	All of the advantages of Fuji	Golden Delicious 463 (russet resistant)
	Shenyang Agricultural University	-	Hanfu (Dongguang x Fuji)
	Res. Inst. of Pomology (CAAS)	-	Qiojin
India	SKUAST (K), J & K YSPUHF, H.P., HETC, Uttarakhand, CITH, Srinagar, IARI, RS, Shimla	Fruit quality, shelf life, high colour, early ripening and scab resistance	Lal Ambri, Sunehari, Akbar, Firdous, Shreen, Ambred, Ambrich, Ambstarking, Ambroyal, Chaubattia Anupam, Chaubattia Princess, Swarnima, CITH Lodh Apple 1, Pusa Gold, Pusa Amartara Pride
Japan	Aomori Apple Experiment Station	Self-compatibility	Self-compatible: Megumi, Mutsu, Sekaiichi, Hokuto, Natsumidori, Mellow; Self-thinning: Aori 9
	Akita Fruit Tree Exp. Sta.	-	Senshu, Akita Gold
	Gunma Agri. Res. Centre	-	Akagi, Yoko, Sinsekai, Gunma Meigetsu
	NIFTS, Marioke	Fruit quality, storage, disease resistance	Fuji, Akane Iwakami, Sansa, Kizashi
Korea (South)	Nat. Hort. Res. Inst. RDA	Storage life	Hongro, Kamhong, Seokwang - early, attractive colour
Russia	ARIH & ARRIGBFP, Michurinsk	-	Skala

Genomic Resources

1. Biochemical markers (Isozymes)

The first report on the use of isozymes in apple was in 1974, but this field of study did not become popular until the mid-1980s when several groups, used isozymes for cultivar identification and for genetic and linkage analysis. Isozyme studies were used to estimate genetic diversity in germplasm collections, resolve uncertainty in pedigrees, confirm hybrids, and to mark monogenic traits, such as Pgm-1 and *Vf* gene for resistance to apple scab.

2. Molecular markers

- Restriction fragment length polymorphisms (RFLPs) – 1st DNA-based marker system used to characterize and identify apple cultivars.
- Random amplified polymorphic DNAs (RAPDs) - Dominant markers, Non – reproducible.
- Amplified fragment length polymorphisms (AFLPs) - useful method to generate large no. of polymorphic bands without prior sequence information, Dominant marker.
- Sequence characterized amplified regions (SCARs) - were developed from AFLPs for the *Vf* gene for scab resistance.
- Simple sequence repeats (SSRs) - co-dominant markers, also useful in map alignment.
- Single nucleotide polymorphisms (SNPs) - can occur in both coding and non coding regions of genome. Those SNPs found within a coding sequence are of particular interest as they are more likely to alter the biological function of a protein. SNP maps helps in identifying multiple genes associated with such complex traits influencing apple fruit quality.

3. Genetic Linkage Maps

Hemmat *et al.* (1994) [6] integrated the isoenzyme, RFLP (Restriction fragment length polymorphisms), and RAPD (random amplified polymorphic DNA) markers scattered throughout 21 and 24 linkage groups (LG) to produce the first genetic map of apples from the cross of "Rome Beauty" and "White Angel." Conner *et al.* (1997) [4] enhanced the "Wijcik McIntosh" second batch of more saturated maps and scab-resistant selections from the Cornell breeding programme (NY 75441-67 and NY 75441-58). To match the number of haploid chromosomes in the apple (n = 17), the number of linkage groups has been reduced (19, 16, and 18, respectively). With the establishment of new, powerful and more reproducible DNA markers, the number of linkage mapping studies has increased rapidly. Currently, there are >60 different apple linkage maps available at the Genome Database for Rosaceae. These maps were established using different parental genotypes, with different DNA markers and for traits ranging from disease resistance to fruit quality, aroma, flower and fruit development, harvesting time and tree growth parameters. The biggest advance is the development of a multi-parental, high-density, integrated genetic linkage map (iGL Map) of apple. This map comprises 15,417 SNP markers. The iGL Map provides an excellent basis for QTL mapping studies& the evaluation of genome assemblies. For apple, 6K, 8K and 9K SNP chips are available, which were developed by the International Rosaceae SNP Consortium (IRSC). A 20K and a 480K SNP chip have also been established.

4. QTL Analysis

Many important traits are under polygenic control, *e.g.* fruit size, shape, fruit colour and fruit flavour. It is beneficial to

investigate the genetic basis of traits of interest to agronomists and map the appropriate loci so that marker-assisted breeding can use them. One of two broad approaches is used, either association mapping or linkage mapping based on biparental populations (also known as LD-based mapping). Establishing a connection between markers and the target locus within experimental populations is the foundation of linkage mapping. Using allegedly unrelated individuals, association mapping examines (either at the candidate gene or genome-wide level) the link between genotypes and phenotypes. Genetic mapping of QTLs involves identifying and determining the degree of association between these traits and a set of genetic markers. In 1st QTL study in apple, RAPDs were used to locate genes associated with juvenile tree growth and development in cross between 'Wijcik McIntosh' and 'NY 75441-58'. Significant QTLs were identified for fruit firmness, stiffness, crispness, granularity and juiciness.

5. Marker Assisted Selection

For marker-assisted selection, markers that are tightly linked to the major genes responsible for the expression of critical traits (disease/pest resistance, fruit/nut quality, self-incompatibility, etc.) have been developed in apples. Marker-assisted breeding is widely used in apple to improve breeding efficiency as well as to reduce cost involved in breeding programmers. The concept of using MAS to enhance the efficiency of breeding in apple by increasing the precision of selection and enabling pyramiding of resistance genes, as well as reducing both the no. of generations and the resources required for new cv. development. *E.g.* of MAS in apples are related with resistances: (i) screening *Rvi6* (*Vf*) gene for scab apple resistance; and (ii) fire blight (FB-F7QTL) in combination with scab (alleles at the *Rvi6* and *Rvi4* loci), and powdery mildew (alleles at the *Pl*2 locus); and a marker-locus-trait association between a gene-based SCAR marker, the *Md-ACS1*-indel marker designed based on the *Md-ACS1* gene) and apple fruit postharvest storability.

6. Genome Wide Association Studies

Genome-wide association analysis can locate functional genes with polymorphic natural populations of unrelated individuals, rather than the populations derived from two-parental lines. This method assesses the correlation between phenotypic data and genome-wide genotypic data (commonly high-density genetic markers or SNPs), which are usually derived from high- throughput genome re-sequencing of the population. Genes or QTLs controlling complex fruit quality traits have been identified via GWAS in apple. A number of significant fruit quality parameters, including titratable acidity, soluble solids concentration, crispness, juiciness, flavour intensity, weight, skin colour, and fruit firmness, have been investigated using GWAS.

7. Malus EST Datasets

ESTs are established by partial sequences, for which transcripts are isolated randomly from cDNA libraries. ESTs have been used for finding new genes and their family members, elucidating phylogenetic relationships, and analyzing large-scale gene expression in various developmental stages and tissues. Expressed Sequence Tag (EST) sequencing represents an efficient alternative to whole genome sequencing, yielding information of the most expressed parts of the genes at a lower cost. It is also called gene signature which helps in cloning and characterization of full length genes. Malus cDNA libraries have been sequenced to varying depths, depending on library quality & novelty, to generate expressed sequence tags (ESTs). Malus cDNA libraries originate from a wide variety of different tissues and developmental time points. *E.g.*, libraries have been generated from a staged series of developing and ripening 'Royal Gala' fruit, including flower, whole fruit, fruit cortex, skin, and seed samples. Use of EST-based markers is very useful for genetic mapping and allows localization of known-function genes in the genome, enhancing probability of identifying specific genomic regions that control phenotypic traits. However, until recently, only a limited number of apple ESTs have been available because cDNA synthesis is very difficult due to extremely small amounts of mRNAs and the large quantity of phenolic compounds in ripened fruit tissues.

8. Reading the apple gene sequences – RNA - Seq

The transcriptome analysis provided by massive throughput RNA sequencing is now the most effective in the identification of annotated functional genes as well as small RNA molecules introduced from pathogens. It was successfully used for detection of pathogenic RNA transcripts, regulating apple rubbery wood disease resistance in the genome of Lord Lambourne cv. and gives valuable information about interaction between other viroid sequence coverage of plant genome, as well allowed to perform the expression analysis of genes involved in lignin synthesis. Such knowledge gives the huge impact for further breeding program directions (Jakovljevic *et al.*, 2017) [7]. RNA-seq technology contributes to the production of various sequence tag collections from different types of plant tissues, environmental conditions, Stress treatment and so on. The selected differentially expressed sequences are then compared with available plant genome databases, annotated and applied as functional molecular markers of traits of interests (Bai *et al.*, 2014;) [1]. RNA deep-sequencing technology is based on qualitative and quantitative measurements of sequence copy numbers and enables the identification of changes in transcript variants. This can be done by profiling of poly-A RNA template capturing, performed directly in cells (Ozsolak *et al.*, 2010) or fractionation of the RNA molecule converted to cDNA (cDNA libraries obtained after adapter attachment to one or both ends of the RNA molecule). Generally, transcriptome studies generate the big amount of read sequences (range of about 30–400 pair base length), which must be mapped and annotated by comparison with well described genome sequences. In case of non-model plants, for which no reference sequence of the genome is available, a novel transcript annotation can be assembled to the already published ones (in *e.g.* GeneBank, UniProt, Ensembl, TrEMBL, SwisProt) as de novo sequencing genome approach (Lopez-Maestre *et al.*, 2016) [8]. For *M. domestica*, functional annotation of genes was performed based on the available sequences of *A. thaliana*, papaya, rice, maize, grape, *Sorghum bicolor*, *Oryza sativa*, *Vitis vinifera* and *Cucumis sativus*. This created an opportunity to classify the gene sequences involved in the metabolic processes that produce volatiles, antioxidants, and pigments as well as predict their chromosome positions and ascertain their origin (Micheletti *et al.*, 2011) [9].

Table 4: Selected studies where RNA-seq-based approaches were used for transcriptome analysis in apple

Studies	References
Fruit acidity	Bai *et al.* (2015) [2]
Fruit skin colouration	Wang *et al*. (2015) [16]

9. Micro RNA (mi RNA)

MiRNAs are an extensive class of newly identified non-coding small RNAs approximately 20–24 nucleotides in length, which control gene expression at the post-transcriptional level by mRNA cleavage or translation inhibition. MiRNAs were discovered using cloning and sequencing methods, and computational prediction of miRNA precursors, pri-mi RNAs, from ESTs or whole genome sequences were performed in apple.

Table 5: Summary of mi RNA studies in apple

Source of miRNA discovery	Tissues, Treatments and Plant materials assessed	Remarks	References
miRBase Sequence Database	Vascular tissue and phloem sap	Potential targets for 21 miRNAs identified that encode putative proteins shown to be targets of corresponding miRNAs in several plant species (i.e. Arabidopsis, Oryza and Populus)	Varkonyi-Gasic *et al.* (2010) [12]
ESTs, miRBase and miR-RACE	Young and old leaves, young stem, flower bud, flower and developing fruits	56 potential targets were identified for the 16 apple miRNAs, most of which were transcription factors	Yu *et al.* (2011) [21]
Cloning and sequencing	-	42 apple-specific miRNAs or families, the identified miRNAs target 118 genes representing a wide range of enzymatic and regulatory activities	Xia *et al*. (2012) [17]
Computational identification from miRBase	-	26 miRNA families, the targets are involved in development, response to biotic and abiotic stresses, and other cellular processes	Ye *et al*. (2013) [20]

10. Exploring MYB Transcription Factors to Improve Fruit Quality

In eukaryotes, MYB family transcription factors represent huge family, which controls diverse function such as development, metabolism, and stress related response. The red color of apple skin requires accumulation of anthocyanin, which is controlled by the expression of anthocyanin biosynthetic gene expression. MYB transcription factor *MdMYBA* and *MdMYB10* positively regulates anthocyanin content in apple fruits by binding to the promoter region of anthocyanin biosynthesis genes.

11. Comparative genomics

The study of the relationships between the structural and functional attributes of genomes from various species is known as comparative genomics. Many horticulture crops' sequencing data have been deposited in public databases, and this data can be used for both fundamental and applied research in related genera. This will aid in clarifying the evolutionary connections between various species and improving phylogenetic classification. In Rosaceous sp., comparative genomic techniques have been applied. Available Rosaceae molecular data allow comparisons between apples and pears within the same family as well as a consensus between apples and pears and peaches. The genetic distance, based on DNA sequence divergence per base pair between members of the Rosaceae, is shown by data from a three-way sequence alignment between predicted gene space in apple (~84 Mbp) and experimentally derived EST data from pear (~14.9 Mbp) and peach (~18 Mbp). Nucleotide identity between the apple and pears' predicted gene spaces was calculated to be 96.35 percent. When the "Bartlett" and "La France" pear genetic linkage maps were compared to the "Discovery" and "Fiesta" apple reference maps, 66 apple SSR loci could be placed onto the homologous LGs of pear (Yamamoto *et al*., 2007) [19]. Gisbert *et al.* (2009) [5] constructed genetic linkage maps of the loquat cultivars "Algerie" and "Zaozhong-6" using SSR markers from apple and pears; when anchored SSR markers were employed, the loquat maps displayed a high degree of synteny with apple maps.

Table 6: Details of whole-genome sequencing in Rosaceae crops

	Pyrus Bretschneideri	Pyrus communis	Malus × domestica	Prunus persica	Prunus mume	Fragaria Vesca
Common name	Chinese pear	European pear	Apple	Peach	Japanese apricot	Woodland strawberry
Cultivar name	Dangshansuli	Bartlett	Golden Delicious	Lovell	BJFU1210120008	Hawaii 4 (PI551572)
No. of contigs	25,312	182,196	122,146	–	45,592	–
No. of scaffolds	2103	142,083	–	391	29,989	3263
Genome assembly size (Mbp)	512.0	577.3	603.9	215.9	237	209.8
Coverage (%)	97.1	96.2	81.3	81.5	84.6	95
Estimated genome size (Mbp)	527	600	742.3	265	280	240
No. of putative genes	42,812	43,419	57,386	27,852	31,390	34,809
No. of pseudo-chromosomes	–	–	17	8	8	7
References	Wu *et al*., 2013 [16]	Chagne *et al*., 2014 [3]	Velasco *et al*., 2010 [13]	Verde *et al*., 2013 [14]	Zhang *et al*., 2012 [18]	Shulaev *et al*., 2011 [11]

Conclusion

- Identification of genes that mediate horticulturally important characters such as improved fruit quality (anthocyanin, mallic acid content *etc*.) has accelerated in the past decade after apple genome sequencing.
- Latest genomic technologies and developing genome resources like genome sequencing, marker-assisted selection, high-density genetic map, transcriptome analysis, ESTs, GWAS and miRNA database are used to genetic control of important horticultural traits with the aim of fruit quality improvement.
- Metabolomics analysis combined with genomic/ Transcriptomics analysis can reveal the genetic basis of compounds that affect fruit skin colour and guide breeding.

Future Thrust

- Deep mining of genomic data to provide new molecular markers to uncover the basis of fruit quality traits.
- There is also a need for more international collaboration especially from developed countries already extensively researching apple fruit crop improvement. Ultimately, fruit crop improvement programs for fruit quality will lead to not only profitable return on production but also improve food security, nutrition and sustainable production in many developing countries.
- In future, it may be possible to create new varieties via genome- editing or other genome technology, because some of the genes controlling fruit traits are conserved among different species.
- It is to be determined that how environmental factors affect gene expression, and consequently, fruit quality, to inform the development of new cultivation strategies.

References

1. Bai Y, Dougherty L, Xu K. Towards an improved apple reference transcriptome using RNA-seq. *Molecular* Genetics and Genomics. 2014;289:472–438.
2. Bai Y, Dougherty L, Cheng LL, Zhong GY, Xu KN. Uncovering co-expression gene network modules regulating fruit acidity in diverse apples. BMC Genom, 2015, 16.
3. Chagne D, Crowhurst RN, Pindo M, Thrimawithana A, Deng C, Ireland H, *et al*. The draft genome sequence of European pear (*Pyrus communis* L. 'Bartlett'). PLoS ONE. 2014;9:92644.
4. Conner P, Brown S, Weeden N. Randomly amplified polymorphic DNA-based genetic linkage maps of three apple cultivars. J. Amer. Soc. Hort. Sci. 1997;122(3):350-359.
5. Gisbert AD, Martinez-Calvo J, Llacer G, Badenes ML, Romero C. Development of two loquat (*Eriobotrya japonica* (Thunb.) Lindl.) Linkage maps based on AFLPs and SSR markers from different Rosaceae species. Mol. Breed. 2009;23:523–538.
6. Hemmat M, Weeden NF, Manganaris AG, Lawson DM. Molecular marker linkage map for apple. Journal of Heredity. 1994;85:4-11.
7. Jakovljevic V, Otten P, Berwarth C, Jelkman W. Analysis of the apple rubbery woody disease by next generation sequencing of total RNA. European Journal of Plant Pathology. 2017;148:637–646.
8. Lopez-Maestre H, Brinza L, Marchet C, Kielbassa J, Bastien S, Boutigny M, *et al*. SNP calling from RNA-SEQ data without a reference genome: identification, quantification, differential analysis and impact on the protein sequence. Nucleic Acid Research. 2016;1:1-13.
9. Micheletti D, Troggio M, Zharkikh A, Costa F, Malnoy M, Velasco R, *et al*. Genetic divesresity of the genus Malus and implication of linkage mapping with SNPs. Tree Genetics and Genomes. 2011;7:857–868.
10. Ozsolak F, Ting DT, Wittner SB, Branningan BW, Suchismita P, Bardeesy N, *et al*. Amplification-free digital gene expression profiling from minute cell quantities. Nature Methods. 2010;7:619–621.
11. Shulaev V, Sargent DJ, Crowhurst RN, Mockler TC, Folkerts O, Mane SP *et al*. The genome of woodland strawberry (*Fragaria Vesca*). Nat. Genet. 2011;43:109–116.
12. Varkonyi-Gasic E, Gould N, Sandanayaka M. Characterization of microRNAs from apple (*Malus Domestica* 'Royal Gala') vascular tissue and phloem sap. BMC Plant Biol. 2010;10:159.
13. Velasco R, Zharkikh A, Affourtit J. The genome of the domesticated apple (*Malus* × *domestica* Borkh.). Nature Genetics. 2010;42:833–839.
14. Verde I, Abbott AG, Scalabrin S, Jung S, Shu S, Marroni F, *et al*. The high-quality draft genome of peach (*Prunus Persica*) identifies unique patterns of genetic diversity, domestication and genome evolution. Nat. Genet. 2013;45:487–494.
15. Wang H, Niu QW, Wu HW, Liu J, Ye J, Yu N, *et al*. Analysis of non-coding transcriptome in rice and maize uncovers roles of conserved lncRNAs associated with agriculture traits. Plant J. 2015;84:404–416.
16. Wu J, Wang Z, Shi Z, Zhang S, Ming R, Zhu S, *et al*. The genome of the pear (*Pyrus Bretschneideri* Rehd.). Genome Res. 2013;23:396–408.
17. Xia R, Zhu H, An YQ. Apple miRNAs and tarsi RNAs with novel regulatory networks. Genome Biol. 2012;13:R47.
18. Xu Y, Feng S, Jiao Q, Liu C, Zhang W, Chen W, *et al*. Comparison of MdMYB1 sequences and expression of anthocyanin biosynthetic and regulatory genes between *Malus domestica* Borkh. cultivar 'Ralls' and its blushed sport. Euphytica. 2012;185:157-170.
19. Yamamoto T, Kimura T, Terakami S, Nishitani C, Sawamura Y, Saito T, *et al*. Integrated reference genetic linkage maps of pear based on SSR and AFLP markers. Breed. Sci. 2007;57:321-329.
20. Ye K, Chen Y, Hu X, Guo J. Computational identification of microRNAs and their targets in apple. Genes Genom. 2013;35:377–85.
21. Yu H, Song C, Jia Q. Computational identification of microRNAs in apple expressed sequence tags and validation of their precise sequences by miR-RACE. Physiol Plant. 2011;141:56–70.
22. Zhang Q, Chen W, Sun L, Zhao F, Huang B, Yang W, *et al*. The genome of Prunusmume. Nat. Commun. 2012;3:1318.