

Review Article

Advances in DNA based Molecular Markers for the Improvement of Fruit Cultivars in Pakistan-A Review

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Abstract | Pakistan is an agricultural country and its population continues to grow rapidly at an alarming pace. The agricultural sector is playing a vital role in strengthening the economy of the country. Apart from traditional cereals cultivation, climatic conditions of Pakistan are feasible and appropriate for the cultivation of temperate, tropical and subtropical fruits. Thus, diverse environmental conditions allow the farmers to cultivate a variety of fruits which ultimately uplifting the burden from traditionally cultivated crops. In recent years, the cultivation and yield of different fruit trees are declining in the country. The key contributors to the decline in fruit crop yield include abiotic stresses, biotic stresses, certain pathogens and nematodes, market devaluation and access, urbanization, poor gardening, poverty climate change, small landholdings, exogenous factors and managerial decisions, etc. So, to counter these fruit yield declining factors the use of advanced molecular markers is a handy option for the improvement of the fruit germplasm in Pakistan. Additionally, they also have diverse applications in the area of genetic diversity, varietal identification studies, hybrid detection, disease diagnostics and sex differentiation by administering the powerful diagnostic polymorphism detection tools at specific loci and the genome level. This review focuses on exploring the application, recent advancements and future implications of DNA-based molecular markers with the context of their role in the genetic improvement of fruit germplasm that is widely grown in Pakistan like citrus, apples, mangoes, guavas, bananas, date palms and peaches, etc. Furthermore, by offering a piece of scientific information and addressing developments, notably in DNA-based molecular markers, this review paper may serve as a road map for obtaining high-yielding fruit varieties in Pakistan.

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Introduction

During the past few decades, various environmental factors are responsible for causing drastic changes in the climate of the world. These factors include irregular patterns of rainfall, floods, pests, insects and notably the virulence of novel diseases caused by multiple strains of microbes like bacteria, viruses and other pathogens. Furthermore, these factors are depicted as the direct indicators of climate changes experienced around the globe which ultimately results in the decline of yield and quality of crops. By considering climate changes and their effect on agricultural productivity at a global level it is likely to have a great impact on the overall production of crops, food availability, livestock production and many other nutritional and economic factors. It is estimated that in 2050, the population of the world will reach around 9.1 billion (Godfray *et al.*, 2010). Furthermore, the rapidly increasing population of the world along with the combination of unpredictable climatic changes are a global threat to food security precisely for the world's economic sector (Lesk *et al.*, 2016). Keeping in view the food requirement of the world in the scenario of the rapidly growing population and current decline rate of agricultural yield due to climatic change is the key concern for the researchers. Fruits are important as a source of nutrients and minerals and are also used as a staple food in some countries of the world. The productivity of fruit cultivars is at a high risk of decline due to unreliable climatic conditions worldwide. With the increasing population of the world, fruits requirement is also increased proportionally. To counter this alarming situation, improvement should be made in the traits directly related to the sustainable yield of crops. Pakistan is an agricultural country with a high population growth rate. Moreover, its economy is vastly dependent on the traditional agriculture and production of major cereal crops and cash crops like rice, wheat, maize, cotton, sugarcane which is not enough to meet the population demands (Rehman *et al.*, 2018a). Furthermore, Agriculture constitutes the largest sector of the economy and contributes 24% percent of Gross Domestic Product (GDP, 2020). As cultivation of cereal crops has a positive and important association with the overall GDP of Pakistan. The cultivation of several fruit varieties in Pakistan covers an area of 849,531 hectares providing about 722,328,3 tonnes of fruit production (FAOSTAT, 2018). The ecological conditions of Pakistan favor the cultivation and production of the popular commer-

cial fruits of the world such as Apple, Citrus, Mango, Guava, Banana, Apricot, Date palms and Melons (Figure 1). These fruit cultivars are very famous and demanding due to their unique taste and nutritional values. Fruits are cultivated in all the provinces of Pakistan so, during the year 2019 the fruits cultivated, areas of cultivation and production are given in (Table 1) whereas, percentage of fruit production and area harvested in Pakistan and four provinces are indicated in Figure 2, 3 and 4 respectively. Thus, the pomology sector could play an important role in galvanizing the economy of Pakistan.

Important fruit cultivars of Pakistan

Kinnow (*Citrus reticulata* Blanco): Kinnow is one of the economically significant fruit cultivars of Pakistan. In Pakistan citrus fruit or Kinnow (*Citrus reticulata*) is widely cultivated in tropical and subtropical areas. As compared to other citrus fruits farming of this cultivar is excessively adopted because of its high nutritional value, greater output and unique flavor. Currently, the yield of 'Kinnow' in Pakistan is far less than the other citrus-producing countries of the world. Shedding of unripened fruit from the trees is probably the major reason behind this decline drop in productivity (Ashraf *et al.*, 2013). In recent years the yield and quality of citrus fruit are highly affected by the due to changing climatic patterns of the region and tend to show high susceptibility response towards adverse climatic conditions as well. Likewise, the cultivation of 'kinnow' in areas affiliated with certain abiotic factors like temperature changes, precipitation and fog would be a great risk for production (Nawaz *et al.*, 2019). Furthermore, among the leading biotic factors, several microbes like bacteria, nematodes and viruses are drastically affecting the production of citrus by causing diseases like citrus canker, citrus withers tip and citrus greening (Anjum and Javaid, 2005). Citrus canker is caused by the bacterium (*Xanthomonas citri* subsp. *citri*), which affects the leaves, twigs and fruit of citrus plants causing the leaves to drop and unripe fruit to fall to the ground. The virulence of this disease occurs in large areas of the world's citrus-growing countries including Pakistan. Nearly 100% of the fruit that dropped were affected by canker (Graham *et al.*, 2006). Citrus wither tip is caused by the fungus (*Colletotrichum gloeosporioides*) is the most prevalent disease in Sargodha and is the most significant fungal disease contributing towards citrus decline in this area. According to the study conducted by (Raza *et al.*, 2019) the highest disease virulence

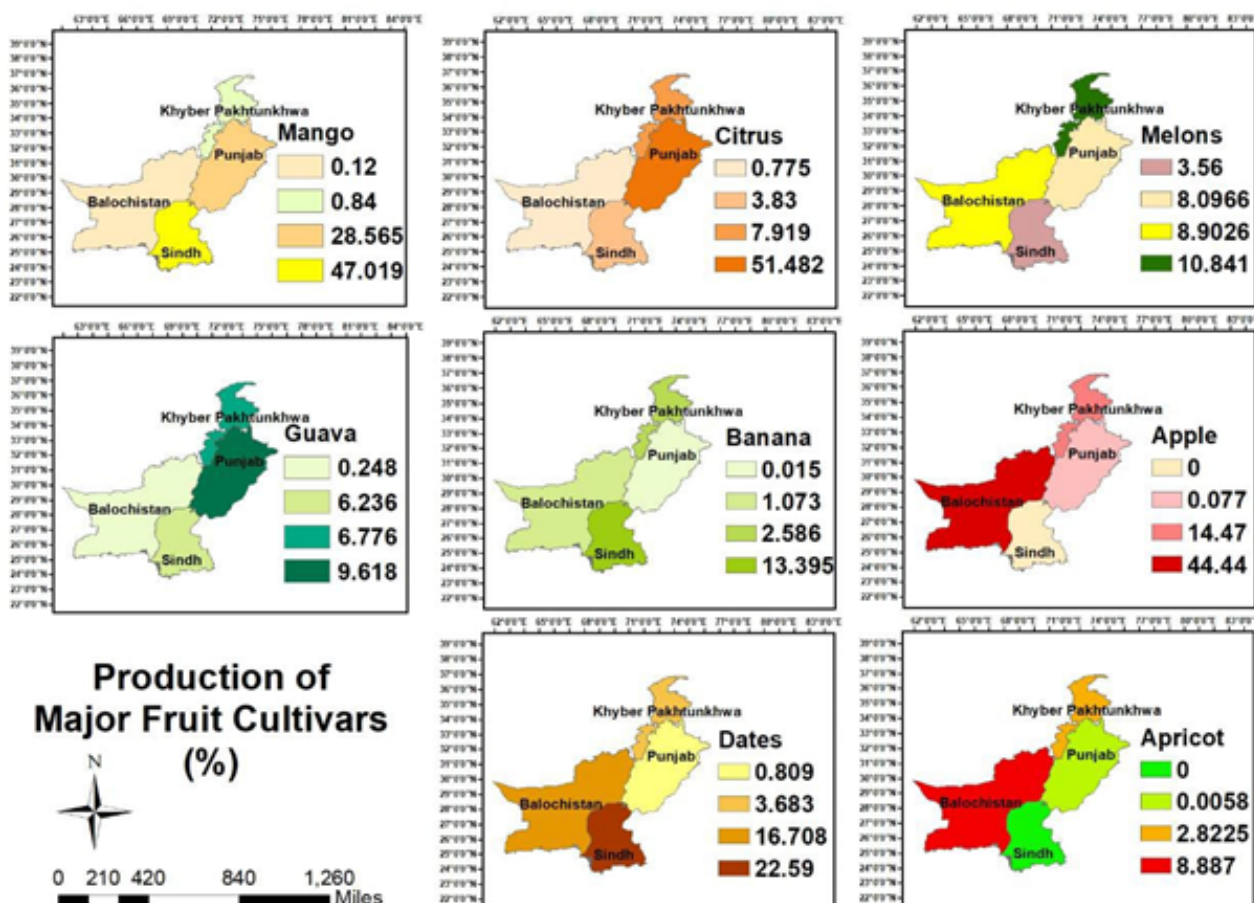


Figure 1: The production of major fruit cultivars in Pakistan.

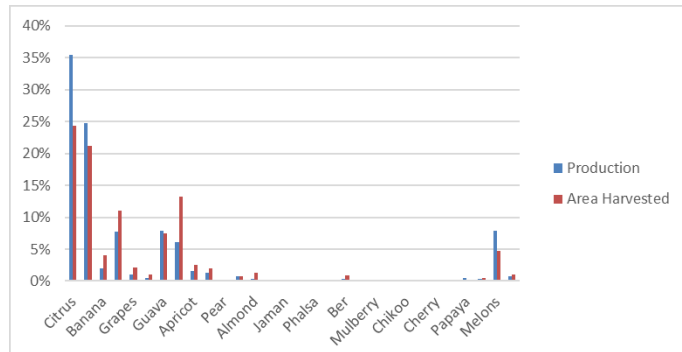


Figure 2: Production of fruit and area harvested (Percentage) in Pakistan 2019.

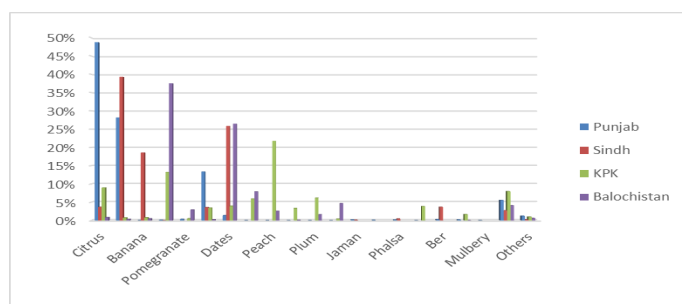


Figure 3: Fruit Area harvested (Percentage) in four provinces of Pakistan 2019.

32.22% and lowest in Shahpur 26.11% respectively. Citrus Greening (*Candidatus Liberibacter asiaticus*) is one of the most serious citrus plant diseases that prevail in the world. It is also known as Huanglongbing (HLB) or yellow dragon disease. Citrus greening disease destroyed about 60 million citrus trees in Africa and Asia (Shokrollah et al., 2011). The Asiatic form is prevalent in Pakistan, which is a threat to the citrus industry of Pakistan.

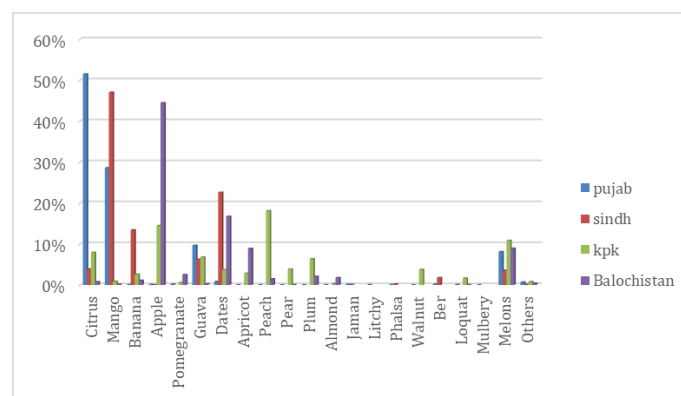


Figure 4: Fruit production (Percentage) in four provinces of Pakistan 2019.

Note: Figures source: Fruit, Vegetables and Condiments Statistics of Pakistan 2019. (FAOSTAT, 2019).

was recorded higher in Kotmomin 42.22%, Sargodha 36.11%, Sahiwal 34.44%, Silanwali 36.11%, Bhalwal

Table 1: Production of various fruit cultivars of Pakistan (FAOSTAT, 2019).

Common names	Scientific names	Varieties	Major producing areas	Total area harvested (ha)	Total yield (hg/ha)
Oranges	<i>Citrus</i> spp	Sweet orange, Bitter orange, Lime, Mandarin	Mainly Punjab, Faisalabad, Jhung, Sar-godha, (KPK) Hazara, Mardan, Sindh Nawabshah, Sakhar	136435	118386
Mango	<i>Mangifera indica</i>	Langra, Zafran, Dusehri, Desi, Kala, Chunsai and Sindhri	Sindh and Punjab (Muzaffargarh, Multan, Rahinyar khan, Bahawalpur)	214800 Combined data	105690 Combined data
Banana	<i>Musa paradisiaca</i>	Cavendish dwarf (Barsai)	Sindh province	29735	45623
Apple	<i>Malus</i> spp	Red delicious, Gaja, Mashadi, Amri, Kala kullu	Kashmir, Rawalpindi District, Punjab, Balochistan	73995	67503
Watermelons	<i>Citrullus lanatus</i>	Sygar baby, Sweet beauty, Golden midget	Punjab province, Bahawalpur, Khan-ewal, D.G Khan, Shaikupura, Multan, Vehari and Bahawalnagar.	37714	149887
Guava	<i>Eugenia</i> spp	Thadharami, Ryali, Sindhi, Seedless, Ramzani, Allahabadi, Indian, Benazir.	Sindh (Larkana, Mirpurkhas, Hyderabad, Khairpur, NausheroFeroz, Matyari, TandoAllahyar, Punjab (Lahore, Shangla Hills, Qasoor, Gujranwala, Sharaqpur), KPK (Kohat, Haripur, Bannu)	214800 Combined data	105690 Combined data
Apricot	<i>Prunus armeniaca</i>	MarGhulam, Shakanda, Shakarfo, Halman, Habi, khakas, narie, Sakardu local, NiliPawand, Shaipawand, Charmagzi, Astor-1	KPK, Balochistan, upper Punjab and mainly in Northern Areas (Gilgit-Baltistan, Skardu)	19372	54069
Peach	<i>Prunus</i> spp	Golden, Coronet, Texas- 455	KPK (Peshawar and swat)	16185	59148
Pear	<i>Pyrus communis</i>	Shugri, Psycho, Batung, Puno-Batung	Gilgit-Baltistan (Bagrote, Haramosh valleys), Hunza-Nagar	1698	88799
Grapes	<i>Vitis</i> spp	King Rubbi, Attock Collection, Babar house, Abbasin-2000, Fazil Shah, Cardinal Italia	Balochistan, KPK (Peshawar)	16311	41661
Dates	<i>Phoenix</i> spp	Begum Junngi, Aseel, Dhakki	Balochistan (Panjgur) and Sindh (mainly Khairpur, Sukkur), Punjab (Muzaffargarh, Multan, Bahawalpur, Jhung), KPK (Dera Ismail khan)	104836	46079
Almonds	<i>Prunus</i> spp	Jordonala, Non Parral, Ktha, Kaghzi, Mission, Utra	Balochistan, KPK	9602	20823

Note: Combined data: mangoes mangosteen and guavas, KPK: Khyber Pakhtunkhwa, D.G Khan: Dera Ghazi khan.

Apple (*Malus domestica* L.): Apple is another extensively grown fruit cultivar of Pakistan. It belongs to the family Rosaceae. A large number of apple varieties including Red delicious, Golden delicious, Gaja, Mashadi, Amri, Kala Kullu and Kashmiri are widely cultivated in different provinces of Pakistan (Hussain *et al.*, 2020). Balochistan has a large share of apple production varieties like Red delicious, Gaja, Amri and Golden delicious are widely grown in Kalat, Loralai, Mastung and Quetta. Likewise, in KPK the famous village named as Gwalera in swat producing

the varieties called Aamri, Shana and Sheesha and in other areas of KPK the varieties like Harri, Fransisi (French), King Star, Kashmiri, Basooti, Sota, Kala Kola, Real Gala, Samret, Gala Mass, Mondi Gala, Gajra, Red Chips, Malochi and Watani are widely grown while mixed varieties are grown in Gilgit baltistan and Azad Kashmir. According to (FAOSTAT, 2019) the total area harvested of apples covers an area of 73995 ha and yield about 67503 hg/ha whereas, the production is estimated at 499490 tonnes. Besides, it contains polyphenols and antioxidants which lowers

the risk of cardiovascular diseases. The antioxidant benefits of apple can help lower the risk of asthma by controlling the release of free radicals from inflamed cells in the airways and oxygen-rich blood (Rehman *et al.*, 2018b). Besides this, a significant decline in its productivity is examined due to various factors like insect attacks and the selection of pollinizers (Klein *et al.*, 2015).

Mango (*Mangifera indica* L.): Mango cultivars are produced on a larger scale after citrus in tropical areas of Pakistan mainly in Sindh and Punjab provinces. There are more than two hundred varieties of mango cultivars that have been cultivated and among these varieties, chunsa and sindhri are most popular and cultivated because of their high economic value. Moreover, mango is the national fruit of Pakistan, due to its mouth-watering taste, it is called the “king of fruits” in Pakistan. Presently, the cultivation of mango varieties and their outcome is not enough to fulfill the requirement of mango consumers due to a lack of proper management techniques (leads to poor production and decline in the export), improper protective measures (fails to control the malformation results in decline in yield) and other climatic factors like (floods, the virulence of diseases and wilting of the mango orchards (Usman *et al.*, 2003). Like other fruits, due to various abiotic and biotic factors such as diseases caused by some new strains of microbes like mango malformation, powdery mildew and anthracnose. The causal organism of mango malformation is *Fusarium mangiferae* (Ploetz, 2002). A survey was conducted by (Malik *et al.*, 2018) about the virulence of mango malformation disease in five districts of Southern Punjab. The results of the study confirmed the severity of 41.44% in Layyah, Multan was recorded the second with 35.22%, 30.11% in Lodhran followed by Muzzaffargarh and Khanewal with 27.11% and 23.33% respectively. Moreover, the causal organism of mango powdery mildew disease is *Oidium mangiferae* Berthet. Powdery mildew of mango was recorded in Indo-Pak-Sub-Continent before 1874 (Johnson, 1994). Additionally, it is a serious disease of mangoes in Pakistan and other mango-growing regions of the world with important economical constraints. Anthracnose is another significant and serious disease of mango in Pakistan and alternatively called blossom blight as well. Their causal organism is (*Colletotrichum gloeosporioides*). A survey was conducted by (Naqvi *et al.*, 2014) and results confirmed the maximum disease incidence in Band Bosan (43.66 %) whereas minimum

severity was recorded in Nawab Pur (34.66 %) respectively. On the other hand, several insects and pests destroy the mango orchards but the most destructive are including fruit fly, mango hopper, stem borer, mango nut weevil and mealybugs are responsible for the significant decline in its productivity. Furthermore, the lack of proper fertilizers and manure for the improvement of soil texture and improper technologies of harvesting and packaging also affect their quality badly. For mango orchards, the recommended dose comprised of 70% Nitrogen, 100% phosphorous and 50% Potash provided immediately after fruit harvest while the remaining amount of N dose is applied at flowering and K dose after the fruit setting stage. The germplasm of mango cultivars is vulnerable to extinction so selection and cloning techniques (molecular / homology) must be adopted with regular breeding programs to obtain improved varieties of mango fruit and to better understand the molecular basis of flowering regulation in mango (Usman *et al.*, 2003; Wei *et al.*, 2016).

Guava (*Psidium guajava*): Guava is also known as the apple of the tropics and one of the most commonly produced fruit. This cultivar is nutritionally important for having many desirable nutrients like vitamin A, B, C, calcium and phosphorus. Vitamin C content in Guava is far greater than oranges and papaya. Guava is also very beneficial for strengthening the immune system and reducing cholesterol levels to prevent heart diseases (Khushk *et al.*, 2009). Cultivation of guava in Pakistan is adversely affected by the virulence of pests, insects, inadequate cultivation methods, immature fruit harvesting and poor management practices. By adopting improved management practices and modern technologies yield of this fruit crop can be elevated up to 30-40 % (Khushk *et al.*, 2009).

Banana (*Musa spp*): Banana is an important nutritional fruit crop that belongs to the family Musaceae. Two species *M. acuminata* and *M. balbisiana*, are considered the progenitors of modern edible bananas (Wang *et al.*, 2019). Bananas are usually cultivated in areas with great variations in temperature and average seasonal rainfall specifically in tropical and subtropical regions. More than 120 countries of the world including America, Asia, Africa; and Australia with humid tropics and subtropics support the cultivation of bananas. The total yield of bananas in Pakistan is 44,972,00 hg/ha over the area of 30,031,00

ha (FAOSTAT, 2018). The main contributor of the production in Pakistan is the Sindh (90%) and Punjab (10%) province respectively. Many factors such as human activities, growing of specific fruit cultivars of consumers choice along with many biotic and abiotic stresses are gradually causing a depletion in *Musa* germplasm (Sipen *et al.*, 2011). Likewise, banana is facing a decline in its yields because of bacterial, fungal and nematode infections. According to (Rao *et al.*, 2002) a survey was conducted in the five banana production areas of Southern Sindh. It was found that banana bunchy top virus (BBTV) showed high virulence in Tandojam (90%), and minimum virulence was recorded in Tando-Mohammad Khan (50%) respectively. Musacearum disease is the greatest threat to banana production in African regions (Tripathi *et al.*, 2009). Panama disease caused by a fungus (*Fusarium oxysporum*) also named as Fusarium wilt disease is also affecting the yield of many banana varieties and is a global threat for the banana production (Ganga and Chezhiyan, 2002). It is reported that the total post-harvest of bananas amounted to Pakistan has been 154,825 tons from 34, 830 hectares per year (Ahmada *et al.*, 2021).

Date palm (*Phoenix dactylifera* L.): Date palm is cultivated in every province of the country approximately covering an area of about 90,000 ha (Abul-Soad *et al.*, 2015). Major complications are associated with date palm cultivation in Pakistan are Red palm weevil (RPW) is a pest that has affected the palm orchards in many districts of Sindh province. Other common pests include stem borers, date palm lesser moths and termites are the prime source of causing the decline in date palm yield almost around the country. Furthermore, the scarcity of water and pest dusk bugs are important threats to the productivity of date palms in Balochistan province. Moreover, rodents are also causing damage to the date palms trees by destroying their offshoots. Furthermore, irregular raining patterns in Punjab and KPK are another damaging factor for date palms in these regions (Abul-Soad *et al.*, 2015).

Major constraints towards the cultivation of the fruit in Pakistan

In recent years, the agricultural sector of Pakistan has come across various hurdles and challenges which are adversely affected by irregular environmental fluctuations mainly due to climatic change. As a consequence, a fruit crop has undergone a Shortening of Growing Season Length (GSL), along with this,

experienced heat stress at critical reproductive stages and elevated water requirements of fruit crops ultimately leads to disrupt food availability and affect the food quality. As the agriculture fruit sector is highly sensitive to it and fruits like grapes /wine grapes, cranberries, peaches, bananas, avocados, plums, apricot, sweet cherries, mangoes, etc are being highly affected (Ullah *et al.*, 2018). Pakistan is among the highly affected countries and has been ranked 12th in terms of climatic changes from the year 1993 to 2012 (Abid *et al.*, 2015). In addition to this, soil salinity, drought, elevated amount of salt deposition, insects, pests, pathogens and the virulence of novel pandemic COVID-19 are the major contributors to the decline of yield and quality of fruit crops. The novel pandemic has generated a serious economic loss due to uncertain lockdowns, scarcity of labor occurred and long-distance distribution have been interrupted. As a result, elevation in the prices occurred and damages the storability of perishable fruits like berries, bananas, avocados, etc. (Bulgari *et al.*, 2021). In developing countries like Pakistan where the cultivation of fruit cultivars and other crops is declining due to the scarcity of proper knowledge and poor management practices of fruits. In addition to this, farmers are not fully equipped and aware of novel techniques and methodologies of sustainable farming systems. Pakistan is at higher risk of facing food insecurity and possibly a major decline in agricultural productivity due to the low adaptation level of modern technology (Ahmad and Farooq, 2010).

Agricultural productivity is greatly affected by biotic and abiotic stress factors (Krasensky and Jonak, 2012). Abiotic stresses are the prime reason for the decline in productivity of fruit crops around the world (Waqas *et al.*, 2019). On the contrary, biotic stresses contain a large number of microbes like bacteria and viruses that have a more drastic effect on the biochemistry of fruit crops. Nevertheless, they also affect other physiological parameters as well like phenology, ontogeny, etc (Ochoa-Velasco *et al.*, 2017).

On the contrary, to counter the challenges, Pakistan's environmental conditions are suitable for the cultivation of many tropical, subtropical and temperate fruits including citrus, apple, mango, banana, guava, dates, peach, melons, etc. These fruits are rich sources of vitamins and minerals accompanied by their unique taste and aroma, their demand is continuously increasing with regards to population growth and

consumption demands. Improvement in the cultivation of fruit crops would be a better option in this respect. As compared to other developed countries of the world, the yield and productivity of fruit crops are very low in Pakistan like scarcity of water, high prices of fertilizers, lack of technological innovations and shortage of fruit storage facilities. It is important to examine the reasons behind this low productivity of fruits (Aujla *et al.*, 2007). For strengthening the economy of Pakistan cultivation of high-quality food crops especially fruits can play a remarkable role to boost the country's economy, by increasing the exports of fruits (Akhtar *et al.*, 2013). Development of high-yielding and improved quality fruits with uniform size, shape, resistance against pathogens, fewer and soft seeds are, suitable for processing and longer shelf life is the need of the hour. Although conventional breeding methods are being applied for making improvements in fruit cultivars but have helped to a limited extent so they are unable to keep pace with the increased population demand. To meet the demand of consumers it is rite high time to exploit the biotechnological innovations, such as molecular marker techniques for the identification of genes with commercial importance and genetic diversity studies.

In the future, there is an ultimate need of increasing agricultural productivity at the national and international level to fulfill the requirement of the growing population (Wiebe *et al.*, 2019). To meet the demands of continuously increasing population expansion of fruit crop production is a great challenge for of the present world (Corte *et al.*, 2019). The ultimate and collective measures should be taken by training the farmers and fruit breeders to prevent and minimize the post-harvest losses (Akhtar *et al.*, 2013). In the present scenario, the need of the hour is to bring some major changes in our traditional agriculture system and tackle the challenges that would confront Pakistan and other developing countries in the future. Along with this, the cultivation of improved fruit cultivars or resistant fruit cultivars having resistance genes can also play a vital role in ensuring food security especially in developing countries (Tadele, 2019). To counter the major constraints and challenges towards the cultivation of the fruit in Pakistan, molecular markers have a full potential to address those obstacles particularly for fruit crop improvement program.

Molecular markers at a glance

A molecular marker is a sequence of DNA, which is located on the chromosome with a known position or it can also be defined as a gene whose phenotypic expression is easily distinguished and further used to detect an individual as a probe to mark a chromosome, nucleus, or locus (Semagn *et al.*, 2006). DNA Markers are mainly used to detect the differences among and within the individual organisms or species at the genetic level (Collard and Mackill, 2008). This detection is possible due to the phenomenon called polymorphism found in DNA or protein which may arise due to the alteration of nucleotides or mutation in the genome (Semagn *et al.*, 2006) and this phenomenon is highly beneficial to study and investigate the research in the field of plant breeding, taxonomy, ecology, phylogeny and genetics. Moreover, the molecular marker is a powerful tool to assess the genetic diversity, detection of mutant genes, marker-assisted crop breeding, cultivars identification, epidemiology and population studies in the field of agriculture biotechnology (Idrees and Irshad, 2014). A genetic or molecular marker is a segment or part of DNA that exists in correlation with a certain position in the genome and is helpful for the identification of specific DNA sequences within an unknown DNA pool. These are often linked with some of the important heritable traits controlled by polygenes (Collard *et al.*, 2005). Molecular markers are of great help in the identification of fruit cultivars, genetic variation, and diagnosis of sex at early stages in dioecious fruits for and improving fruit qualities and tolerance to biotic and abiotic stresses. Mysteries about many genes and phylogenetic histories of sequenced fruit crops have been unveiled through genome sequencing techniques. Molecular markers have diverse applications in the area of genetic diversity, varietal identification studies, hybrid detection, disease diagnostics and marker-assisted selection (MAS) (Collard and Mackill, 2008).

Types of molecular markers

Classical markers and DNA markers are two broad categories of molecular markers whereas classical markers are categorized as morphological, cytological and biochemical markers.

Classical markers: Classical markers can be observed visually and their inheritance can be judged through the naked eye and hence known as morphological markers. Heritable traits which are measurable appar-

ently through simple vision include the color of fruits, the height of trees, shape and color of flowers and seeds are referred to as classical markers. Morphological markers used in breeding practices of plants may cause certain changes in the phenotypes which are not required in the breeding programs and also hide the expression of small linked genes and thus making the identification of required combinations very uncertain for analysis (Bhat *et al.*, 2010).

Cytological markers: Cytological markers are mainly associated with alterations in number, shape, position and banding patterns of chromosomes and thus provides information about heterochromatin and euchromatin distributional differences *e.g* G and Q band are produced by giemsa stain and quinacrine hydrochloride respectively, and R bands are the opposite of G bands. Such type of information about chromosomes is useful to differentiate the mutated chromosomes and also helps in physical mapping etc (Jiang, 2013).

Biochemical markers: For more accurate identification of cultivars, biochemical markers would be a much better option because they are very stable and accurate for analyzing many characteristics of cultivars like germplasm/geographical origins and phylogenetic relations. The genetic impurity of plant cultivars can also be obtained by the application of biochemical markers (Sammour, 2014). Isozymes, allozymes and phytochemicals are commonly used examples of biochemical markers (Kumar *et al.*, 2018). Biochemical markers are protein-based markers and the key limitation of the biochemical marker is the exhibition of a low level of polymorphism, limited in number and influenced by the environment (Badaya *et al.*, 2016).

Functional and non-functional markers: Functional markers abbreviated as (FMs, are also called as perfect markers) that can be used as an alternative to random DNA markers. FMs are made up of polymorphic sites found in genes that generate phenotypic trait variation (Andersen and Lübberstedt, 2003). Unlike random DNA markers, FMs are directly connected to the allele having a trait of interest (Varshney *et al.*, 2005). As a result, FMs are outperforming random DNA markers, particularly in marker-assisted breeding (MAB). There are certain methods through which FM are identified like Association mapping and studies, QTL mapping, map-based cloning, comparing isogenic lines, RNA interference (RNAi) and by transformation technique (Lau *et al.*, 2015).

DNA markers: Unique DNA sequences located on a certain position on the chromosome having the ability to be transmitted into the new generations are termed DNA markers. These markers depend on the information stored in DNA, unlike other markers which are dependent on protein products *e.g* morphological and biochemical markers (Mondini *et al.*, 2009). Advanced study of nucleic acids in an organism used for the development whereas, DNA markers are used in agricultural breeding practices like the selection of desired traits and; genetic mapping (Wünsch and Hormaza, 2002).

Genes are the basic heredity component of an organism's genetic makeup and are made up of the DNA thus, controlling all the features and traits. The information about the selection of desired traits can be obtained from the genes responsible for these traits and this knowledge plays a significant role in the formation of DNA markers. DNA markers can be used as tags to identify the genes of particular traits in breeding practices. The development of DNA-based markers has revolutionized the breeding techniques for the selection-resistant varieties of fruits and other crop cultivars and made the selection of traits easy and quite fast (Dar *et al.*, 2019).

An ideal and efficient DNA molecular marker must be genome-specific, highly polymorphic which can identify the difference in allelic pattern with ease, cost-effective, reproducible and easily detectable (Bhat *et al.*, 2010). DNA markers include RAPDs (randomly amplified polymorphic DNA) used for gene tagging, AFLPs (amplified fragment length polymorphism) used for gene tagging, RFLPs (restriction fragment length polymorphism) used for physical mapping, SNPs (single nucleotide polymorphism) used for identification and construction of genetic maps, SSRs (simple sequence repeats) used for genetic diversity and ISSRs (inter-simple sequence repeats) used for gene tagging and identification and DarT (diversity array technology markers) used for assessing the genetic diversity.

Sequence and restriction based genomic markers: Sequencing is a technique used for the identification of nucleotide bases along with a DNA strand (França *et al.*, 2002) and sequence-based markers are molecular markers that are based on the identification of a specific sequence of DNA in a pool of unknown DNA. High-throughput sequence-based techniques

have been adopted in recent years particularly in fruit breeding. Simple sequence repeats (SSRs; also known as microsatellites) and single nucleotide polymorphisms (SNPs) are two types of sequence-based markers that currently dominate applications in modern genetic research (Gupta *et al.*, 2014). Furthermore, sequence-based genomic markers are essential for fruit breeding, mapping, varietal identification and identification of SNPs for any region of the genome (Sahu *et al.*, 2012). As far as restriction site associated DNA (RAD) markers are concerned a kind of genetic marker that is used to perform association mapping, QTL mapping, population genetics, ecological genetics, and evolutionary genetics. Polymorphisms that are detected and genotyped by isolating and examining RAD tags are known as RAD markers (Miller *et al.*, 2007).

Advanced DNA markers: Latest DNA markers techniques include anchored microsatellite primed PCR (AMP-PCR), fluorescence-based PCR-SSCP (F-SSCP), anchored simple sequence repeats (ASSR), arbitrarily primed polymerase chain reaction (AP-PCR), cleaved amplified polymorphic sequence (CAPS), DNA amplification fingerprinting (DAF), expressed sequence tags (EST), inverse PCR (I-PCR), inverse sequence tag repeated (ISTR), microsatellite primed PCR (MP-PCR), multiplexed allele-specific diagnostic assay (MASDA), random amplified microsatellite primed PCR (RAMP), random amplified PCR (RAM), sequence characterized amplified regions (SCAR), sequence-specific amplification polymorphism (S-SAP), sequence-tagged microsatellite site (STMS), sequence-tagged sites (STS), simple sequence length polymorphism (SSLP), single-strand conformation polymorphism (SSCP), single primer amplification reactions (SPAR), start codon targeted polymorphism (SCoT), target region amplification polymorphism (TRAP) and a novel micro RNA (miRNA) based molecular markers. These advanced DNA markers are used in fruits for genotyping, map-based cloning, accessing genetic diversity and gene flow, molecular identification studies but along with this they need high purified DNA, required a high developmental cost and are difficult to detect QTL.

Role of molecular markers for the improvement of fruit cultivars

In recent decades, rapid advancement in DNA technology for the improvement of fruit cultivation practices is observed. To increase the efficiency of culti-

vars and to understand the versatility in their genetics several factors like gene flow, developmental stages, the pattern of evolution, mating types and population density are playing a crucial role. In fruit crop breeding the role of molecular markers is vital and can never be denied (Jiang, 2013). The use of genetic markers for the complete genetic description of fruit crop varieties has changed the concept of traditional breeding practices by providing a huge amount of information about the genome sequences of fruit crops and the role of many genes in their physiology (Nadeem *et al.*, 2018).

Characterization of fruit cultivars by using molecular markers

Citrus (*Citrus spp*): For accurate identification of various species of a citrus large number of molecular techniques like restriction fragment length polymorphisms (RFLPs), randomly amplified polymorphic DNA (RAPDs), amplified fragment length polymorphisms (AFLPs), and microsatellites, *etc* are being applied. Simple sequence repeats (SSRs) are considered as an important source of molecular markers for fruit crops because of the polymorphic nature, specificity in locus and for being present in the whole genome of plants randomly (Lamine and Mliki, 2015). A research study was carried out by (Jiang *et al.*, 2006) to investigate the gene-derived SSR markers in six citrus cultivars. The results confirm the existence of polymorphism among six citrus cultivars and this polymorphism confirms the exploitation of the citrus EST database. Inter-simple sequence repeats (ISSRs) are used for the study of evolutionary relationships among various species of citrus. Likewise (Shahsavari *et al.*, 2007) conducted a study on 33 citrus genotypes in Iran to investigate the phylogenetic relationship. For this study, ISSR markers were used. The results clarified the relationships among the unknown genotypes of citrus with known varieties in the Fars Province of Iran, by this, they further conclude that the ISSR marker is a powerful tool to differentiate the closely related individuals. Similarly, RAPDs are being applied for the assessment of genetic similarities and detection of polymorphism in many citrus cultivars. RAPDs are also useful for the genetic mapping of citrus cultivars. Moreover, many significant traits in citrus including resistance against pathogens like nematodes, Citrus Tristeza virus also linked with RAPDs (Ahmed *et al.*, 2017). Hybrid selection in Polyembryonic varieties of citrus fruit can also be done efficiently through the analysis of inter-simple

sequence repeats (ISSRs). Some economically important cultivars of citrus fruit such as “Bakraee” were assessed to find out the phylogenetic associations among them with the help of simple-sequence repeats (SSRs) and PCR-based RFLP markers (Go-lein *et al.*, 2011). Likewise, a study was carried out by (Naz *et al.*, 2014) to investigate the genetic relationship among 17 citrus cultivars of Pakistan. They used RAPD markers for the study. The genetic similarity value was observed and results indicated the high genetic difference among the cultivars which means they did not belong to a similar origin.

Apple (*Malus spp*): The use of molecular markers empowers the breeder to improve the apple breeding practices through the selection of desired traits, genetic diversity analysis of germplasm, and by the construction of genetic maps. Genetic markers like RFLPs, RAPDs, SCARS, SSRs and isozymes are being utilized for linkage analysis in *Malus spp* (Liu *et al.*, 2016). In apple fruit, many genes coding for monogenic traits have been identified 76 genes coding for the traits like disease resistance and other morphological characters and 69 enzyme coding genes have been described so far (Marić *et al.*, 2010). Primarily molecular markers are used in *malus spp* for pyramiding of Scab resistant genes through marker-assisted selection (MAS) in progenies and also for the germplasm to find out the cause of resistance. Identification of fifteen genes of scab resistance and mapping of twelve have been done with the help of genetic markers in apple cultivars. Similarly, seven powdery mildew resistant genes are also identified and mapping of four is being done in apple fruits. The powdery mildew resistance genes PI-w from White Angel, PI-d from D12 and PI-m from MIS (James *et al.*, 2004). Whereas, the resistance genes PI-m and PI-2 have been mapped on LG11; PI-d has been mapped on LG12 and PI-w has been mapped on LG8 (James *et al.*, 2004). An increase in the use of molecular marker techniques is being observed for making advancements in apple genetics like never before (Marić *et al.*, 2010).

Mango (*Mangifera indica spp*): For the molecular characterization of cultivars in Pakistani mango analysis of genetic diversity is vital. PCR-based molecular markers including RAPDs, AFLPs, SSR and ISSR are being utilized to reveal the genetic diversities and thus help in the selection of traits. Among these markers, simple sequence repeats (SSRs) are most efficient

and gaining attention due to their unique properties like reproducible nature, high variability, codominance, abundance and relatively high distribution throughout the genome (Nazish *et al.*, 2017). Studies of genetic diversities in 254 cultivars of mango *Mangifera indica L.* and some other species from different geographical regions have been made with the help of 11 SSR genetic markers which shows that the average number of alleles (8.45) is relatively high in South Asian regions like Indonesia; and in Malaysia, but is very low in varieties from Philippines (2.55) (Dillon *et al.*, 2013). ScoT start codon-targeted polymorphism is a recent and novel molecular technique reported by (Collard and Mackill, 2009) which involves the use of an 18- mer single primer in a polymerase chain reaction and requires a temperature of 50 °C for annealing. The designing of primers depends upon the conserved regions present around the initiation codon ATG. Start codon-targeted polymorphism (SCoT) is successfully applied in mango for analysis of genetic diversities and identification of varieties. Based on (SCoT) data analysis three mango indigenous cultivars, Chappaniyo, Sopari and Jamrukhiyo found to be distinct from other 12 indigenous cultivars, and likewise cultivars includes Jamadar and Kesar were also found distinct from Alphonso, Dasherri, and Neelum (Gajera *et al.*, 2014).

Guava (*Psidium spp*): Identification of guava cultivars in a more precise way has been done with the help of various genetic markers. Amplified fragment length polymorphisms (AFLPs) are also being utilized for the analysis of 88 varieties of guava collected in 10 states of Brazil. In this study he identified two major groups one group contains guava accessions and the other contains Brazilian guava. The results revealed the high genetic variability ranging from 28 to 98 % among them (Corrêa *et al.*, 2011). Simple sequence repeats have been applied on 13 cultivars of guava (*Psidium guajava*) for the characterization of genome and genetic diversities. The results successfully depicted the genetic relation between the accessions and seven alleles were also identified. This study could be useful for the parental selection of guava fruit (Viji *et al.*, 2010). Studies of genotypic diversities in *P. guajava L.* from regions of different altitude has been done with the help of microsatellite genetic markers (Singh, 2014).

Banana (*Musa spp*): A large number of techniques like DNA fingerprinting which involves the use of

genetic markers like RFLPs, RAPDs, ISSRs; and AFLPs have been applied by molecular biologists to identify banana cultivars. Among these molecular tools, AFLPs are mostly preferred because they allow high-resolution genotyping by producing a large number of replicable markers in a very short time (Opara *et al.*, 2010). The role of molecular marker techniques has been neglected in bananas as far as the improvement of genetic traits are concerned, so there is a wide scope of improving the cultivar through the marker-assisted selection of traits and introgression of resistance to stress factors.

Date Palm (*Phoenix* spp): Traditionally, the characterization of germplasm in date palms is based on morphological characters but with the advent of molecular genetic techniques DNA based genetic markers are being widely applied for the genomic characterization of cultivars. Most of the studies demonstrate the use of AFLPs, RAPDs and SSRs for analysis of genetic diversities and genetic relationships in date palm varieties (Zhao *et al.*, 2012). RAPDs and ISSR marker have been utilized for characterization of genotypes in date palm cultivars of the Kutch region in India and the results depict that use of randomly amplified polymorphic DNAs (RAPDs) for detection of polymorphic traits is much more efficient than inter simple sequence repeats (ISSRs) *i.e* 39.77% and 23.07% respectively (Al-Qurainy *et al.*, 2015).

Peach (*Prunus* spp): Molecular marker techniques such as AFLPs, SSRs, AFLPs and RAMP have been regarded as a valuable tool for the characterization and assessment of genetic diversities in peach (*Prunus persica* L.) cultivars. Randomly amplified polymorphic DNAs (RAPDs) are tremendously applied for the identification of polymorphism in peach varieties for being more efficient and quick as compared to other genetic markers in this regard (Hidayatullah *et al.*, 2017). Genome-wide association studies (GWAS) is another significant technique used for the selection of domestication traits like the weight of fruit, soluble acid and sugar contents in the peach cultivar. GWAS is widely adopted for the identification of loci of various phenotypic traits in many species (Cao *et al.*, 2019). Although great progress has been made in diversity studies in peaches still there are certain limitations due to which a large amount of germplasm remained untouched for genetic diversity analysis in peach cultivars of china which is considered as the origin of peaches.

RFLP techniques play an important role in the genetic linkage mapping of fruit crops. Genetic mapping of peach is a successful example of the RFLP technique used in fruit cultivars (Williams *et al.*, 1990). QTL analysis of traits like flowering and fruiting in sour cherry is another application of RFLPs in fruit crops (Wang *et al.*, 2000). Successful applications of RAPDs in fruit cultivars involve the identification of peach cultivars, comparison of genetic diversity in peach and understanding of genetic relationships in almonds cultivars (Aranzana *et al.*, 2002). AFLP markers were used for the genetic analysis of olive cultivars, 121 polymorphic AFLPs were generated through the combination of five primers for this analysis. A comparison of 43 cultivars of olive involving 9 spp of *olea* and 30 wild cultivars was done to determine the genetic relation in *oleagenus*. Therefore, results depicted the confirmation of the genetic relation and molecular characterization among olive and wild olive cultivars with the aid of AFLPs (Besnard *et al.*, 2018). The 4 types of pears varieties in japan were also analyzed for studying the genetic similarities through AFLPs. They used seven primer combinations, and results revealed molecular polymorphisms of (87%), representing 418 total polymorphic bands. They successfully accessed the genetic relatedness among pear cultivars (Monte-Corvo *et al.*, 2000). The types of markers that have been used for the assessment of genetic diversity, characterization, genome mapping, and other significant parameters in various fruit cultivars are presented as follows in (Table 2).

Significant applications of molecular markers

Genetic diversity assessment: Genetic diversity analysis of a large number of plant species including fruits is one of the most significant applications of genetic molecular markers. The development of different molecular markers makes the study and assessment of variations in the genetic makeup of germplasm of species, phylogeny of plants, comparative and population genetics of species is convenient through the use of sequencing techniques like genome sequencing, DArT markers and SNP markers (Baloch *et al.*, 2016). Detailed genetic information about the germplasm of many plant species which is important for the selection of desired traits can be obtained through the use of molecular markers (Wünsch and Hormaza, 2002). The assessment of genetic diversity includes identification of QTLs, disease diagnostic, varietal identification, Pedigree analysis and detection of hybrids were performed in various fruits crops with

Table 2: *The effective use of different molecular markers in fruit cultivars.*

Name of fruit	Marker type	Work done	References
CITRUS (<i>Citrus</i> spp)	RFLPs, AFLPs, RAPDs, SSRs, ISSRs, SNPs and DArTs	assessment of genetic similarities and detection of polymorphism, Hybrid Identification, genome mapping, phylogenetic studies.	(Ahmed <i>et al.</i> , 2017)
APPLE (<i>Malus</i> spp)	RFLP, RAPD, AFLPs, SSRs, SNPs, SCARs, ISSRs and DArTs.	pyramiding of resistant genes, Estimation of genetic variability, QTLs identification, genome mapping, detection of resistance genes.	(Costa <i>et al.</i> , 2005; Bus <i>et al.</i> , 2008)
MANGO (<i>Mangifera Indica</i> L.)	RFLP, RAPDs, ISSR and SSR	Hybrids and cultivars identification.	(Nazish <i>et al.</i> , 2017)
GUAVA (<i>Eugenia</i> spp)	RAPDs, AFLPs and SSRs	Identification of guava cultivars, evaluation of genetic diversities among genetic variants.	(Pessanha <i>et al.</i> , 2011)
BANANA (<i>Musa</i> spp)	RFLPs, RAPDs, ISSRs, AFLPs	Identification of banana cultivars, high resolution genotyping, estimation of genetic variability, genetic breeding, disease resistance, increased productivity.	(Amorim <i>et al.</i> , 2009)
DATE PALM (<i>Phoenix dactyliferous</i>)	ISSR, RAPDs, AFLPs, RAPDs and SSRs	Studies of genetic variations among species.	(Zhao <i>et al.</i> , 2012).
PEACH (<i>Prunus</i> spp)	AFLPs, SSRs, AFLPs and RAMP	Characterization and assessment of genetic diversities Identification of disease-causing agents.	(Cao <i>et al.</i> , 2019).
GRAPES (<i>Vitis viifera</i>)	AFLPs, SSR, ISSR, SNPs, RAPDs, SCC8	Sex expression studies, identifying seedless parents, determine the association between downy mildew resistance and QTLs.	(Li <i>et al.</i> , 2015; Divilov <i>et al.</i> , 2018)

the aid of molecular markers like apple (*Malus domestica* L.) AFLP and RAPDs, guava (*Psidium guajava*) AFLP, citrus (*Citrus reticulata*) RFLP, banana (*Musa paradisiaca*) RAPDs, avocado (*Persea americana*) minisatellite DNA, mango (*Mangifera indica* L.) ISSR and RAPDs, grapes (*Vitis vinifera*) RFLP and SSRs, pistachio (*Pistacia vera* L.) minisatellite markers, and pears (*Pyrus* spp) SSRs and AFLP have been done (Bhat *et al.*, 2010).

DNA fingerprinting for identification of varieties

DNA fingerprinting is an important technique that utilizes the use of molecular markers like restriction fragment length polymorphism (RFLPs), randomly amplified polymorphic DNA (RAPD) and some other markers based on PCR to identify the crop varieties and to find out the versatility in their germplasm (Ahmad *et al.*, 2009; Hidayatullah *et al.*, 2017). Genetic markers can produce specific patterns for every genotype of the organisms either through polymerase chain reaction (PCR) or hybridization techniques. These patterns are simply known as genetic or DNA fingerprinting. High yielding varieties can be identified and differentiated by utilizing the simplest types of molecular markers like randomly amplified polymorphic DNA (RAPD), inter simple sequence repeats (ISSRs) to highly elaborated types such as (VNTRs) variable number of tandem

repeats (VNTRs), amplified fragment length polymorphism (AFLPs) and RAPDs (Arti *et al.*, 2018). Randomly amplified polymorphic DNA (RAPDs) have been used for the varietal identification of mango (Díaz-Matallana *et al.*, 2009) and SSR markers are utilized for the identification of grape varieties (This *et al.*, 2004). Varietal identification of apple cultivars is also being done through the use of RAPDs (Liebhard *et al.*, 2002).

Disease diagnostic

The development of techniques for the diagnosis of disease-causing microbes and pathogens with the help of molecular markers plays an important role in producing disease-resistant fruit cultivars. Diagnosis of downy mildew in grapevine (*Vitis vinifera*) has been done by using molecular markers like SSRs, SSCP and SCAR (Moreira *et al.*, 2011). Genes for sharka disease in apricot (Pdre x 54P455) and plum root nematode resistant genes are also diagnosed with the aid of AFLPs (Gosal *et al.*, 2010).

Genetic mapping

One of the primary aims of the Human Genome Project (HGP) was to provide new, better, and less expensive techniques for identifying novel genes and understanding their function. Genetic mapping is a type of genetic analysis and often refer as link-

age mapping. Genetic mapping is a method of determining which chromosome contains which gene and where that gene is located on that chromosome. They may also be used to predict which gene will most likely recombine depending on the distance between two genes. Genetic mapping is an important and most widely used technique in molecular genetic studies nowadays, which involves the significant role of molecular markers. Genetic markers which are adjacent to the desired genes located on one chromosome are said to be linked. Segregation of genes occurs at the meiosis stage which will result in chromosomal recombinations. These chromosomal recombinations are the principal basis for their genetic map construction. Genetic maps are of great help for the selection of markers to be used in breeding techniques. An ideal linkage map must consist of a suitable number of evenly distributed markers showing variability in populations to identify the desired gene or QTLs with accuracy (Ravi *et al.*, 2011). Among the fruit cultivars, the construction of a genetic map of watermelon involves two types of genetic markers RAPD and species-specific sequence-characterized amplified regions (SCARs) markers. This genetic map helps to locate the genes which are responsible for developing resistant properties against fusarium wilt disease and QTLs formation for fruit quality improvement (Zhang *et al.*, 2004). Likewise, (Blas *et al.*, 2012) developed the F₂ mapping population from two Papaya fruit varieties to locate the QTLs that influence and trigger the significant characters related to papaya's shape, length, diameter and weight. Furthermore, (Sánchez-Sevilla *et al.*, 2014) carried and analyzed the genetic mapping and genome-wide transcriptome analysis to detect the variation in γ -decalactone in strawberry fruit.

QTL mapping

Most of the heritable traits of agricultural importance are under the control of various genes which are located on the same or different chromosomes. These genetic traits are polygenic or quantitative because of the influence of more than one gene. The position of these quantitative traits on chromosomes is termed as quantitative trait loci (QTL) and the technique which is applied to identify the genes responsible for these traits with the help of molecular markers is known as QTL mapping. There are two categories of these traits, one is quantitative traits that show constant variations while the other is qualitative traits having disruptive variations. Genetic markers play a vital role

in the study of quantitative trait loci (Angaji, 2009). For the construction of a QTL map, two distant parents having variability in genes responsible for desired traits must be selected and detailed genetic information is obtained with the help of polymorphic markers. After that genetic mapping is done to locate the genetic markers which are linked to the desired trait (Collard *et al.*, 2005). Approximately 100-200 molecular markers are being applied for the genetic mapping purpose and among them, restriction fragment length polymorphisms (RFLPs), amplified fragment length polymorphism (AFLPs), DarT, Inter-simple sequence repeats (ISSR), simple sequence repeats (SSR), ESTs and single nucleotide polymorphisms (SNP) are most popularly used. Variation in size of the genome in species influences the number of molecular markers because species having a large size genome must have the requirement of a greater number of genetic markers. Presently, next-generation sequencing (NGS) has made use of thousands of genetic markers for linkage mapping techniques with great resolution (Dhingani *et al.*, 2015). Analysis of genetic linkage is a crucial step for the detection of genes that are responsible for traits in QTL mapping, which depends upon the recombination of genetic material at the meiosis stage (Collard and Mackill, 2008). Different types of molecular markers associated with the polygenic traits of fruit cultivars have been identified for example SCAR and SSR markers are known to be associated with fire blight resistant trait in apple cultivars (Bonarska-Kujawa *et al.*, 2011), AFLP and RAPD are linked with citrus leprosis virus resistance in citrus fruit (Bastianel *et al.*, 2009) and RFLP shows association with sugar content in banana fruit cultivar (Ming *et al.*, 2001). There is some software used in fruits to increase the accuracy of QTL mappings like FlexQTL, PediHaplotyper and MapChart software (Rawandoozi *et al.*, 2020). The data sets of the various fruits can be found in the following Genome database (<https://www.rosaceae.org/publicationdatasets>) and (www.rosaceae.org) (Calle and Wünsch, 2020).

Marker-assisted selection (MAS)

Marker-assisted selection (MAS) is a technology that involves the use of DNA markers have revolutionized fruit crop breeding efforts dramatically all over the world (Jiang, 2013) by eliminating the crops having undesirable gene combinations and selecting the agriculturally significant traits. Marker-assisted selection is an important aspect of genetic markers which allows the selection and development of many

cultivars by considering only a few varieties. Some significant steps of marker-assisted selection can be seen in Figure 5. The selection of varieties through traditional breeding methods is a very time-consuming process because breeders have to introduce the desired trait into the cultivars by making crosses and then analyzing the results in the next generation. As the screening of genes has not been done in traditional methods, undesired traits can also travel into the next progeny along with the desired ones. By applying marker-assisted selection techniques in breeding practices breeders can locate the desired genes with the help of genetic markers by tagging the desired genes with specific molecular markers and thus can make the selection of the traits easy (Testolin, 2002). In addition to this, MAS allows the fruit breeder to make an early selection by examining the fewer plants. Currently, marker-assisted techniques have been used for many breeding practices including identification of cultivars, selection of hybrids, analysis of diversities among varieties which were previously based on traditional selection techniques like the assessment of morphology and other visual characters. Additionally, the more significant and widely adopted feature of marker-assisted selection is marker-assisted backcrossing (MABC) in which desired traits or QTLs from relatively inferior varieties are transferred into a better-quality cultivar which is considered as recurrent parent; for the improvement of the desired character (Jiang, 2013). In contrast to traditional methods, MABC involves the use of genetic markers which are linked with the desired genes or QTLs rather than a visual selection of phenotypes. The process of selecting genes for various traits at a time and combining them in a single genotype by utilizing DNA markers is known as marker-assisted gene pyramiding which has been used in crops for increasing resistance against several diseases. Molecular DNA markers are of great support in gene pyramiding because only a single sample of DNA is required to check the markers for many particular genes of interest hence limiting the need for phenotypic analysis and other conventional breeding practices (Collard and Mackill, 2009). SMAS has also been applied in many fruit cultivars for the selection of desired traits such as in apple for scab resistance, in papaya for fruit skin color, in citrus for Citrus Tristeza Virus and plum for root-knot nematodes (Bhat *et al.*, 2010).

Future prospects: The last three and half decades have witnessed a continuous improvement and the

developments in molecular marker technology from RFLP to SNPs and the diversity of array-technology-based markers (Figure 6). Advancements in sequencing technologies have led to the development of NGS platforms that are low cost with high throughput which has revolutionized quantitative genetics. The current study focuses on the applications of molecular marker techniques which are used for genetic diversity assessment in fruit crops. Molecular markers can reveal a piece of useful information regarding DNA polymorphism and can describe the genetic makeup of plants in depth. In the case of fruit breeding programs, more time is required as compared to other crops because of their long juvenile period, high heterozygosity level and incompatibilities among different cultivars.

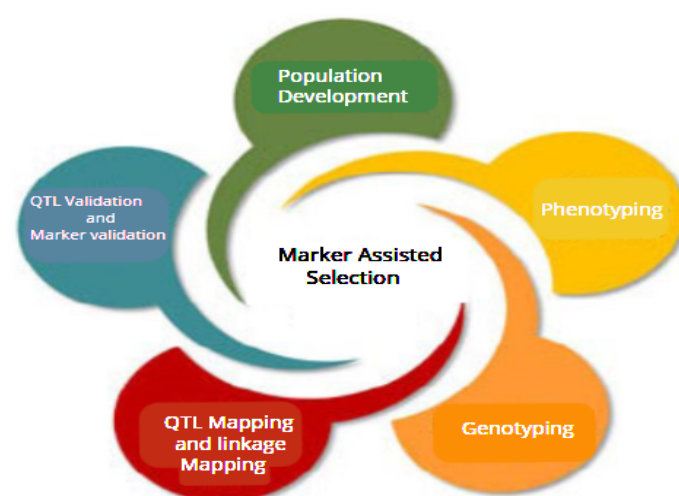


Figure 5: Some significant steps of marker-assisted selection (MAS).

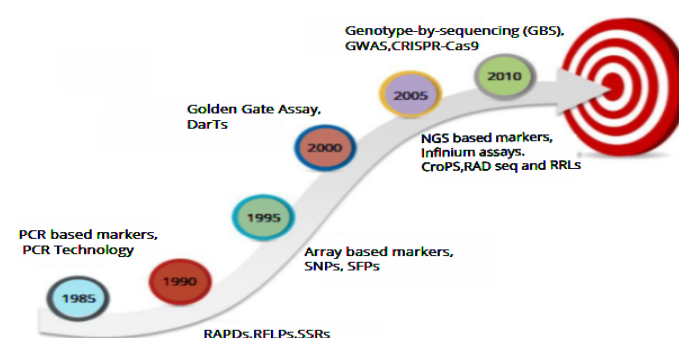


Figure 6: Advancements trends in molecular markers.

Additionally, advanced molecular genetics utilizes both conventional breeding and molecular tools such as Microarrays and Next-generation sequencing (NGS) to develop cost-effective sequencing along with obtaining the accurate analysis with high precision of data (M Perez-de-Castro *et al.*, 2012). Furthermore, genomic selection (GS) offers the opportunity to accelerate the selection time. Therefore, it is believed that

alternating progeny field testing with selection based only on markers should boost the total genetic gains per unit of time. The constant development in molecular marker technologies has panned out the evolution of ultra-high-throughput genotyping platforms.

The recently emerged NGS-based molecular marker system may substitute the array-based high-throughput marker systems in the coming future, especially when costs are decreasing and high throughput is increasing for the NGS technologies. The NGS marker system for various fruits is available on the website in the NCBI database (<http://www.ncbi.nlm.nih.gov/>) and a previously published review article (Tar-tarini, 2003). These future marker systems may prove handy for enhancing the deployment of modern genetics and various fruit breeding approaches such as genome-wide association studies (GWAS) and genome-wide significant (GWS) loci that are still in their early stage of development particularly in plant systems. Moreover, plant genetics and breeding programs have been revived by CRISPR-Cas9 technology in recent decades and the focus of research is now to edit the genome of all plants having economic importance. So, in the coming years, we can expect continuous innovations in molecular marker techniques to make it more effective and precise to explore various traits of interest in plant cultivars.

Despite the availability of all these effective and advanced techniques the goals set in this area of research have not been fully achieved yet and still there is a need of discovering highly appropriate and more effective molecular marker approaches. Genetic improvement of fruit cultivars and success of DNA marker technologies would depend on the interaction of breeders, fruit growers and biotechnologists, availability of skilled individuals and significant financial investment in this area of research. Conclusively, high-density molecular marker maps of fruit cultivars can be constructed with the help of Marker Assisted Selection (MAS) by applying various molecular markers such as ISSRs, SNPs, SSRs, RFLPs, DarT, SSCPs, and RAMPs which results from sequencing techniques. Despite all the improvements made in the traditional breeding methods for better yield of crops, modern biotechnological techniques can increase the success rate to a large extent. The use of genetic molecular markers in fruit breeding is a relatively new and highly adopted aspect of molecular breeding. On the contrary, the agriculture industry is

the backbone of Pakistan. However, the industry is not contributing to the economy to the extent that it might be expected. Whereas, all of the country's primary crops including fruits have low production. The use of biotechnology in the horticulture sector particularly in fruits of Pakistan has the potential to improve the overall agricultural output. The use of biotechnology by Pakistani farmers would not only increase fruit production but would also aid in solving the country's food security issues. Biotechnology offers a huge potential to improve the quality of all the cultivated Pakistani fruits to match the market standards and meet the exports criteria by improving color, aroma and postharvest behavior.

Ultimately, the various efficient and improved breeding efforts could be handy to ensure food security in the world. Nevertheless, the awaited years are likely to see continued novelties in molecular markers technology to make it more accurate, formative and cost-effective to examine the fundamental biology of various significant traits of interest in fruit species.

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Novelty Statement

This review study brings novelty by cumulating the applications, advancements and future implications of molecular markers in the context of their role in the genetic improvement of the germplasm of various important fruit cultivars cultivated in Pakistan. For this reason, this review article has given a road map by providing a piece of scientific knowledge and approach for fruit researchers, growers and students to improve the quality and obtain the high-yielding fruit varieties in Pakistan.

Author's Contribution

Ghassan Zahid and Sara iftikhar: Conceived the idea, designed the framework and wrote the manuscript.

Muhammad Umer Farooq: Assist in writing the manuscript.

Shakeel Ahmed Soomro: Part of paper writeup.

Conflict of Interest

The authors have declared no conflict of interest.

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