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Application of Advanced Genomics for Conservation and Utilization of Plant Genetic Resources

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Abstract: The core activities that have been undertaken in gene bank include conservation and utilization of genetic resources. For a large number of germplasm, these activities require a huge financial and human resources efficient conservation within a short period of time. Researcher should give equal emphasis for both germplasm conservation and its utilization because conservation without utilization is useless. However, greater efforts have been focused on conservation then the utilization, indicating existing research gap for the germplasm utilization. Current paper reviews the role of genomics for plant germplasm conservation and its sustainable use in four specific activities: next generation sequencing, Quick DNA based disease diagnosis, DNA barcoding for species identification, and allelic mining. This review work provides overview of recent techniques and approaches of genomic tools for crop germplasm management and exploitation for various breeding programs.

Keywords: biotechnology, genetic resources, germplasm conservation, genetic diversity, microsatellite markers, population structure.

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1 Introduction

According to National Research Council (1991) "plant genetic resources (PGR) includes older and current crop varieties, specialized breeding lines used to develop new varieties and hybrids, landraces of crops that have emerged over centuries of selection by farmers, wild plants related to individual crops, and mutant genetic stocks maintained for research, particularly when gathered together in organized collections of plants, seeds, or tissues". PGR has a great potential to play for achieving food security, a major challenge for developing countries (Malik and Chaudhary, 2019; Roa et al., 2016. Plant biodiversity will be a key for sustainable development that would guarantee food security for the fast-growing population (Newton et al., 2010; Rabara et al., 2015; Yaldiz et al., 2018). The strategic importance of PGR for agriculture and industry, and the danger of genetic erosion have been recognized earlier (Harlan, 1975).

Exploring and collection of vanishing genetic legacy and subsequent classification are required for the genetic resources conservation. Moreover, their evaluation and documentation will benefit mankind for generations to come (Castaneda-Alvarez et al., 2016). Collection of genetic resources and their conservation activities have been initiated in many countries and conserved considerable accessions of plant genetic resources in gene bank (Rao, 2004).

According to International Plant Genetic Resource Centre (IPGRC) technical report, the goal of gene bank is the use of plant genetic resources that must support sustainable agriculture development without affecting or damaging the wealth of plant genetic resources as well as its habitats and ecosystems (Karp et al., 1997). In order to achieve this goal proper germplasm collection, regeneration or multiplication, characterization, evaluation, disease indexing and elimination, and distribution are required (Tandon et al., 2009). Long term conservation is the main activity in germplasm conservation. It can be practiced using ex situ and in situ conservation strategic method. However, it is advisable to employ both ex situ and in situ conservation methods at a time which insure the

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conservation of as much of genetic diversity as possible (Ayad et al., 1997; Samah et al., 2017).

In situ conservation is maintenance of plant genetic resource in their natural habitats that they exist. It includes a wild crop relative species or old farmers' cultivars in farmer's field (Meilleur and Hodgkin, 2004). In situ conservation methods provide opportunities for the process evolution to be taken place which is the sources of variability in the population and increase the level of diversity (Phillips et al., 2016). However, this approach is exposed to natural calamities like fire, drought, disease and insect damage etc. and has to be complemented with ex-situ conservation through different approaches (Karp et al., 1997; Phillips et al., 2017).

Ex situ conservation is performed outside the original habitat of plants. This approach gives a better protection of germplasm from manmade or natural calamities than in situ (Fu, 2017; Khoury et al., 2010). Its main objective is to maintain the accessions without changing their genetic constitution through minimizing the evolution process to happen (Frankel et al., 1995). Ex situ conservation uses different approaches which include storage of seed, DNA and pollens, in vitro and conservation methods for the field and botanical garden (Withers, 1992; Tandon et al., 2009).

PGR are the raw materials on which breeders depend for developing improved varieties and they are the sources for resistance to diseases, pests, and stress conditions, and other important values not yet identified (Cruz-Cruz et al., 2013; Rao, 2004). Because of this a considerable number of gene banks have been established in many countries so far. The total number of accessions that has been conserved in 1400 gene banks in the world reaches more than six million (FAO, 1998). Ethiopian Biodiversity Institute has been conserved more than 160000 accessions till 2016. However, the number of accessions is growing faster than they can be effectively maintained characterized, evaluated and utilized because of lack of adequate capital resources, gene bank and limited use of advanced technology. Advances in genomics sciences can give an important approach in order to improve the conservation and management of plant genetic resources. Therefore, in this review work it summarizes the application of the recent genomic advancement for plant genetic resources diversity analysis and to identify patterns of genetic diversity, quick disease diagnosis techniques, rapid species identification and mine novel alleles from wide variety of genetic resources.

2. Assessment of Genetic Diversity

The knowledge of the genetic structure of a plant species and its eco-geographic distribution is very important for effective conservation. This necessarily involves the measure of genetic variation which will help to conserve as much of genetic diversity as possible as well as it avoids the possibility of conserving duplicate accessions. In addition, understanding the level of diversity of conserved accessions is very important for planning new collection and germplasm exchange strategies to broaden the gene pool.

In earlier time, plant genetic resources were characterized based phenotypic traits and also pedigree and geographical distribution analyses were used for measuring genetic diversity (Hollington et al., 2011). However, later on due to development of molecular markers, DNA based diversity analysis came into practice to supplement phenotypic characterization (Ahmad et al., 2017; Tanksley and McCouch, 1997). Currently, there are various kinds of molecular markers have been developed and used for diversity studies for different plant species (Gupta et al., 2001, Moose and Mumm, 2008). Plant genetic diversity can be assessed using various techniques, namely (i) morphological (ii) biochemical characterization/ evaluation and (iii) DNA (or molecular) marker analysis like single nucleotide polymorphism (SNPs) (Govindaraj et al., 2015).

2.1. Morphological markers

They are based on visually observable characters which can be qualitative or quantitative traits. These traits include plant height, number of tillers, maturity days, flower color, seed shape, growth habits, pigmentation, etc. It requires an intensive works in the field for recording or measuring the phenotypic data. The accuracy of phenotypic data is essentials and it can be improved through various techniques like by replicating the trials across locations and over years, by using appropriate experimental design and blocking, on data recording and using appropriate statistical methods for data analysis (Zhu et al., 2008; Govindaraj et al., 2015).

2.2. Biochemical markers

Storage proteins and allelic variant of enzymes (isozymes) have been used to assess the genetic diversity of plant species. The variability can be examined using electrophoresis and specific staining chemicals. These markers are codominant in nature. They detect diversity at functional gene level and have simple inheritance. It requires only small

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amounts of plant material for its detection. However, only a limited number of enzymes markers are available and the resolution power for genetic diversity analysis is minimal (Govindaraj et al., 2015).

2.3. Genetic Markers

Genotyping has been practiced using different kinds of DNA marker to analyze the genetic and molecular variation among and within populations. Molecular markers are located near to genes that control the traits and does not affect the phenotypic expression of the given traits.These markers are inherited both in dominant and codominant patterns (Govindaraj et al., 2015). DNA markers are abundant in number and are not affected by environmental factors and/or the developmental stage of the plant (Winter and Kahl, 1995).

DNA-based molecular marker (for example, RFLP, RAPD, SSRs, and AFLPs polymorphisms, random amplified polymorphic DNAs, simple sequence repeat (SSRs) and amplified fragment length polymorphisms (AFLPs)) have been widely used for molecular characterization studies (Baloch et al., 2015; Collard et al., 2005; Wang et al., 2015). However, their procedure for analysis is tedious and time consuming for large number of accessions and some of them have reproducibility problem among the laboratories (Bansal et al., 2013). These called a new approach called next-generation sequencing (NGS) and the data generated from NGS do not suffer from the above shortcomings.

2.4. Next Generation Sequencing (NGS)

The analysis of complete or partial DNA sequence has been one of the most transformative influences on biological studies. It is helpful in understanding the roles, network, and evolutionary relationships of genes (Bevan and Uauy, 2013). Genome sequencing cost has been reduced by one-million-fold in the past several years. It is now inexpensive to gather genome sequence information in large numbers of individuals in short period of time, even shorter than any crop's life cycle (Graner and Killian, 2012). The advancement in genome sequencing technology gives us an opportunity to assess the genetic makeup of an organism, down to base pair resolution with fast and cheap methods. These days the genomic sequence data have been generated for most crops which become readily available in public databases for free use and also reference genome sequences have been developed for key species (Edwards et al., 2012) and the reference genome sequences can be used to study sequence variation within species. In addition to this, de novo assembly of sequence data can be used to detect all differences at nucleotide level (Henry, 2014). Therefore, the opportunities that genomic characterization will bring to the conservation and use of Plant Genetic Resources have been reported by various scientists in the last 15 years (McCouch et al., 2013).

2.4.1. NGS for Plant Genetic Resources Management

Holding germplasm with high degree of redundancy, in both in situ and ex situ collections, is common problem faced by most of the gene banks. It causes additional cost and efforts for the long-term maintenance of the redundant accessions (van Treuren and van Hintum, 2014; Varshney et al., 2010). This problem can be solved using DNA-based molecular markers; however DNA-based molecular markers have its own limitations to use it for large number of germplasm collection because of its tedious and time-consuming procedures. However, due to advancement of next-generation sequencing technology, the shortcomings of DNA based markers have been solved. Application of DNA sequencing in addressing the issue of redundancy after establishing a core collection which represents the entire collection of the germplasm (Bansal et al., 2014; Hawkins et al., 2010).

2.4.2. NGS genome sequencing to the discovery of genome wide variation

Crop genetic diversity in a narrow sense means that it is the variability of genes that exists within and among crop species (Gallusci et al., 2017; Huang and Han, 2014; Qi et al., 2013). Diversity describes the survival rate and adaptability of the crop species, as it determines resilience to changing environments, insect pest damage, and disease infestation, and responds to natural selection (Lin, 2011; Meyer and and Purugganan, 2013; Scheffers et al., 2016). There are different techniques are available to measure level of diversity among which DNA sequencing using NGS technologies is the most accepted one. And DNA sequencing is the most powerful tools to detect genetic variation at nucleotide level within and among germplasm (Hyten et al., 2010). Wide range of plant species has been sequenced and databases are made publically available (Itoh et al., 2018; Karakülah et al., 2016; NCBI Resource Coordinators, 2016; Sayers and Karsch-Mizrachi, 2016). These DNA sequence data can serve as a reference genome for studying genetic resources of the same species or related species to detect genetic variations for large number of accessions within short period of time (Bansal et al., 2014; You et al., 2011).

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NGS technology is an important tool for identifying SNPs (Single nucleotide polymorphisms) through sequencing and comparing with reference sequence data and these SNPs will be used as DNA marker which is a choice of most geneticists /breeders (Jackson et al., 2011; Kwok et al., 1996; Bernardo, 2008). SNPs are the DNA sequence difference by a single base. The variation of a single base between /among genotype may result in specific phenotypic change, or neutral effect. Recently, SNPs are the most widely used for diversity analysis or germplasm evaluation because it is abundant and uniformly distributed in the genome of plant species, amenable to automation, efficient, and increasingly cost-effective (Edwards and Batley, 2010).

3. Species Identification

Species identification fundamentally important to monitor the status of species diversity which is alarmingly affected by manmade and natural disaster, and to design planning to preserve the endangered species in the face of accelerating habitat destruction (Bell et al., 2016; Waldchen, J. and P. Mader, 2018; Waldchen et al., 2018). Most of the gene bank use morphological diagnosis method which has its own limitations. These limitations include requirement of large number of highly trained taxonomists and careful collection of specimens to preserve their distinguishing features (Hebert et al., 2003). Moreover it rarely helps to identify species that are the closest relative of each other and have not been distinguished from one another taxonomically. Its identification procedure is tedious and time consuming which makes it difficult to catalog biological diversity before it disappears

Subsequently these limitations forced to search alternative approach for taxon identification. Modern taxonomy using DNA barcoding is the most efficient and effective tools (Meusnier et al., 2008). It is becoming the most preferable by taxonomist and one of the novel approach to the diagnosis of biological diversity (Zeng et al., 2018). This technique minimizes the shortcoming of morphological based identification methods. Moreover, it provides easy solution for the identification of taxon using sample taken from small, damaged, or industrially processed material (Coissac et al., 2016).

DNA barcode is a short DNA segment from selected region of genome of species which is conserved within species and used to identify species (Hebert et al., 2003). It is rapid method identify the already existing species or new species. It allows us to discriminate life through the analysis of small segment of genome(Hebert et al., 2003) and it is also relatively cheap method for species identification (Candek, and Kuntner, 2016).DNA barcoding uses a segment of DNA which is specific to a given species, which are conserved at the species levels (Kress and Garcia-Robledo, 2014). It can be exemplified for animal, plant, fungi and bacteria identification that DNA fragments belonging to the mitochondrial, chloroplast genomes, ITS region and 16S ribosomal gene will be used respectively.

DNA barcoding of plant identification uses chloroplast gene regions which are conserved across species. These are Maturase K (matK) and ribulose 1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL-RuBisCo) gene. It involves in carbon fixation as catalyzer (Hollingsworth et al., 2011) and also the spacer between tRNA-His and photosystem II protein D1 (trnHpsbA spacer) and the nuclear internal transcribed spacer 2 (ITS2) (Fu et al., 2011), which are the common and widely used.

DNA barcoding is based on the amplification of selected short DNA region and sequencing of the amplified product and matching sequence data (a query sample which is unknown specimen) to a reference sequence using the DNA BOLD and NBCI databases (Ferri et al., 2009; Kress et al., 2005; Kress and Erickson, 2008). There are a number of public database for free use such as The International Barcode of Life (iBOL). It is established by more than 150 countries and all members participate in species identification for several species (Taylor and Harris. 2012; Ugochukwu et al., 2018).

4. Disease Monitoring

Disease monitoring is one of the core activities that have been undertaken by gene bank creators to protect the germplasm from plant disease damage as well as to deliver healthy planting materials to the users to reduce the spread of plant diseases (Krattinger and Keller. 2016; Zhang et al., 2018). It is very important to detect the pathogen as early as possible from any plant parts including seeds in order to control the spread of new pathogens in a growing area where it is not present yet (Ray et al., 2017).

Disease monitoring of seed can be done through examining the symptoms produced in the host due to specific pathogen, or by growing pathogen in the culture media and examine its morphological characteristics, through chemical induction and see pathogenic characteristics, or cultural conditions for growth of the pathogen. However, these methods are laborious and time consuming and also sometimes the

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disease diagnosis is imprecise. In addition, especially in the gene bank where there is a large number of accession needs to be tested, this cultural-based morphological or disease symptoms approaches is not efficient since it is time-consuming and laborious. Because of these limitations another alternative method which are effective, reliable, rapid and early detection of pathogens should be employed and molecular-based one is a novel approach to be used because the technique can offer greater sensitivity, specificity, reliability and may be quicker than many conventional methods used to detect plant-pathogens in different plant hosts and environments (Puri et al., 2015).

4.1. Polymerase Chain Reaction (PCR) Based Methods

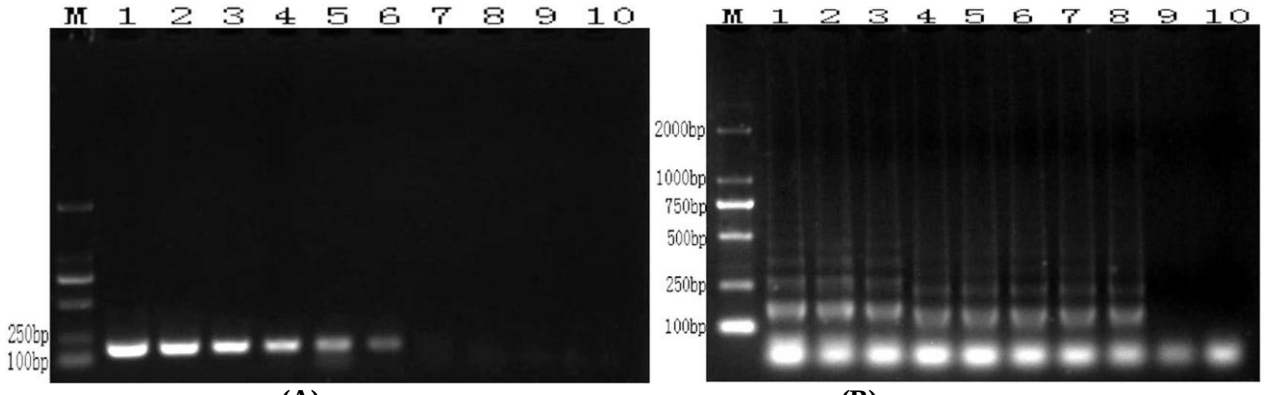
PCR plays a significant role for disease diagnosis with high sensitivity and better pathogen detection accuracy. Scientists have developed molecular techniques to detect pathogenic fungi using PCR and designed primers and these techniques are undertaken by PCR amplification of ITS region followed by either restriction analysis (Braun and Takamatsu, 2000) or direct sequencing and BLAST searching against GenBank or other databases (White et al.,1990). Puri and his colleges demonstrated that the application of genomic tools for accurate and quick pathogen detection at molecular level for plant (Puri et al., 2015). For example, Bioneer (http://www.bioneer.com/) designed forward primer (ITS5:GGAAGTAAAAGTCGTAACAAGG) and Reverse primer (PINF2: CTCGCTACAATAGCAGCGTC) that can amplify the *Phytophthora infestans* rRNAgene using DNA extracted from infected dried leaf samples. These designed primers amplify selectively the *Phytophthora infestans* gene only with the amplicon size of ~600 kb.

4.2. PCR-RFLP Based Detection Method

Restriction Fragment Length Polymorphism (RFLP) marker is the most common and widely used for plant pathogens identification (Martínez-García et al., 2011; Mondal et al., 2004). It employs the amplification of a target region of a pathogen gene with designed primes and followed by restriction enzymes digestion. And finally, the digested PCR product will be separated by electrophoresis in agarose or polyacrylamide gels to detect differences in the size of DNA fragments and the difference sizes of DNA fragments will be analyzed to identify the specific pathogens causing that particular disease. Drenth et al. (2006) used this approach in order to distinguish Phytophthora spp and identified 27 different species using specific primers called “A2” (forward) and “I2” (reverse) and then PCR product digested by Msp I restriction enzyme which produced RFLP band patterns for 17 species of phytophthora pathogen.

4.3. Isothermal amplification method (LAMP)

LAMP technique was discovered by Notomi and his collogues (Notomiet al., 2000) and the technique amplify target DNA having with few copies to 10^9 times within 45 minutes using single temperature (45 ^{o}c). After the discovery of LAMP, it is becoming an innovative technique that has been utilized in the development of detection assays for multiple plant pathogens.



(A) **(B)**

Fig. 1. Sensitivity test of conventional PCR (A) and LAMP (B) using different concentration of *A. rabiei*. Where; 1=6.01 × 101 ng/µl; 2= 6.01 × 100 ng/ µl; 3= 6.01 × 10−1 ng/µl; 4= 6.01 × 10−2 ng/µl; 5= 6.01 × 10−3 ng/µl; 6= 6.01 × 10−4 ng/µl: 7=6.01 × 10−5 ng/µl; 8= 6.01 × 10−6 ng/µl; 9=6.01 × 10−7 ng/µl; and 10= 6.01 × 10−8 ng/µl) (Adopted from Chen et al., 2016).

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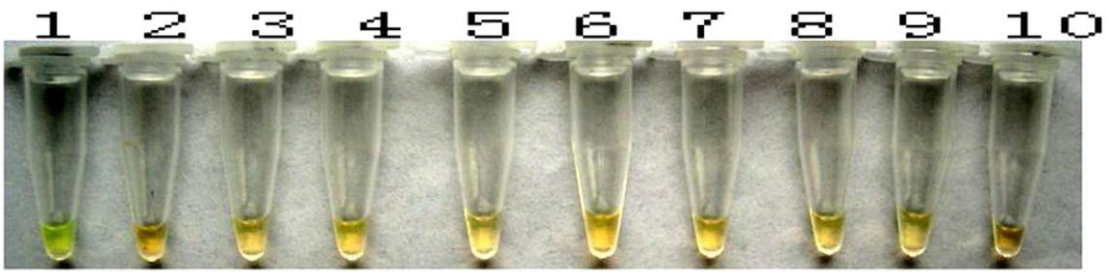

Fig. 2. Necked eye visualization of LAMP using SYBR Green I dye. Where 1= *A. rabiei*; 2=Rhizoctoniasolani; 3=Alternaria alternate; 4=Penicilliumsp.; 5=Aspergillus sp.; 6=Nectriasp.; 7= Chaetomiumsp.; 8=Bionectriasp.; 9=Fusarium sp.; and 10= negative control) (Adopted from Chen et al., 2016).

This technique has three detection methods; 1) SYBR Green I: The amplification product that has the pathogen DNA, shows a color changes upon the addition of SYBR Green 1 which can be detected directly by visual inspection in vials. 2) Turbidity Change: the pathogen DNA can be detected by measuring the increased turbidity this happened because of the production of large amounts of magnesium pyrophosphate. 3) Gel electrophoresis and see the expected band size of the pathogen DNA.

LAMP method is preferable to field disease monitoring and it does not require PCR machine. And it is a cost-effective technique because it can be done using a water bath and it also avoids the need of using a thermocycler apparatus (Notomi et al., 2000). Chen et al. (2016) developed primers that can be used to identify the causal agent of blight disease in chickpea (*A. rabiei*) using sample taken from infected plant parts of seed by LAMP technique and they demonstrated also the LAMP detection efficiency compared with a conventional PCR method. And they discovered that LAMP method has shown better sensitivity and specificity in the detection of *A. rabiei* (Fig 1). They demonstrated also the use SYBR Green I dye to visualize the color change with the naked eye (Fig 2). The color change will be observed in vial that contains *A. rabiei* DNA (Fig 2-1).

5. Allele Mining of Individual Loci

One of the responsibilities of gene bank is to deliver germplasm with known and desirable characteristics to researchers. This is important for the researcher to save time because it gives chance to the researcher to work with accessions with known character which allow them to identify valuable traits and its associated gene very quickly (Hufnagel et al., 2018). In addition, it is important to gene bank also to save germplasm because it delivers the required accession for the requested objective only instead of giving the lamp sum of accession which finally the breeder discard the accessions not interested to work on. This activity creates burden to the gene bank for frequent seed multiplication which in turn brings genetic deterioration. Therefore, efficient and quick discovery of gene controlling economically important traits are required for variety development program to develop superior and high yielding crop varieties and it saves time and money for gene bank also (Leung et al., 2015).

It is known that wild crop relative and farmers' varieties are sources for desirable traits which include disease, insect pest and drought resistance, wide adaptation, stress tolerance etc. However, the utilization of these resources as sources of desirable traits are minimal because of lack of efficient strategies to characterize, identify important traits with its associated gene, and transfer important alleles to the target crops. Currently, these limitations have been solved through the application of genomic tools which untapped desirable genes of wild relatives and farmers' varieties for proper utilization for the development of agronomically superior variety (Reddy, 2016; Tranksley and McCouch, 1997; Wing et al., 2018). There are a considerable gene banks available in the world and these gene banks hold a huge number of accessions. Recently, because of the advancement in genomics tools, most gene bank have been given a priority for characterization activity in order to determine allele variation at nucleotide level among and within accessions through DNA sequencing technology. DNA sequencing of a representative collection of individuals is very effective to study allelic richness at a given locus (Kilian and Graner, 2012).

DNA sequencing technology brought the concept of allele mining which is defined as a technique used to identify alleles of a known gene that control for any given trait and their variants within or among genotypes (Ashkani et al., 2015).

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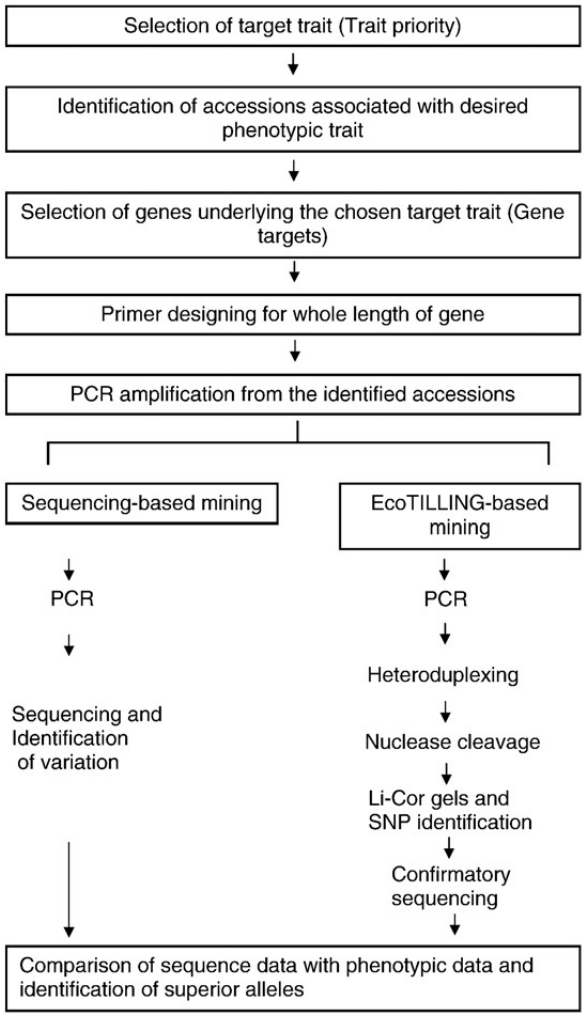

Fig. 3. Steps involved in allele mining adopted from Kumar et al., 2010.

This technique identifies single nucleotide polymorphism at coding region of the particular gene (exons) and also non-coding region specifically 5' UTR, promoter, introns and 3′ UTR because the nucleotide change in these regions may have a significant change in protein structure and function which may result in alter the phenotypes expressions (Kumar et al., 2010). Allele mining can be done through two approaches. These are (i) Eco-Tilling and (ii) DNA sequencing. The steps involved in these approaches are indicated in Figure 3 (Kumar et al., 2010).

5.1. EcoTilling

Eco-Tilling is the modified form of TILLING (Targeting Induced Local Lesions in Genomes) procedure. Eco-TILLING is a technique used to detect polymorphism at nucleotide level from naturally induced mutations in a target gene through heteroduplex analysis (Khan et al., 2018; Okabe and Ariizumi, 2016; Szurman-Zubrzycka et al., 2017; Wang et al., 2010), while in TILLING mutation is artificially induced in a target gene.The method is essentially the same as TILLING except that the mutations are not induced artificially and are detected from naturally occurring alleles in the primary and secondary crop gene pools (Kumar et al., 2010).

5.2. Sequencing-based allele mining

Two major activities have been undertaken in this technique i.e. amplification of the target alleles from the diverse genotypes using PCR and followed by DNA sequencing. This technique helps to analyze individuals for haplotype structure and diversity to infer genetic association studies in plants (Kumar et al., 2010).

5.3. Bioinformatics Tools for Allele Mining

The progressive development of bioinformatics tools has assisted genomic science to improve the efficiency and use of genomic technology to detect allele variation and SNPs identification, and also to understand the association between the gene and the traits (Somers et al., 2003; Varshney et al., 2005). Currently, computer software applications or web based bioinformatics tools are freely available for public use which can analyze sequence polymorphisms, predict amino acid change, identify transcription factor binding sites, transcriptional regulatory elements and a database of plant promoter sequences. Different kinds of bioinformatics tools are used currently. The most widely used includes CARE, TRANSFAC, JASPAR, MEME, PlantCARE, DCPD, SCPD, Clustal-W and Bioedit (Ashkani et al., 2015).

6. Conclusion

Plant germplasms are vital sources for important agronomic traits potentially used for varietal development program. However, these genetic resources are consistently facing natural as well as human-caused challenges. Considering paramount importance of diverse plant genetic resources for survival of humankind serious efforts are required for their conservation. Consequently many countries have given priority for germplasm conservation through establishing their own gene banks, causing conservation of large number of accessions. Most gene banks use both ex situ and in situ conservation strategies. These gene banks have a responsibility to conserve the germplasm with safety and cost-effective manner. Moreover, genetic integrity should be maintained. Combination of *ex situ* and *in situ* conservation strategies is suitable for long-term conservation of germplasm, which can be efficiently utilized for future variety development programs

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through accurate phenotyping and genotyping. This review summarizes the significant contributions of recent advances in genomics for improved conservation and use of plant genetic resources, including development of high throughput molecular marker technologies which can speed up data generation and also improves the quality of data which helps to characterize larger number of germplasm with limited time and resources and reduces redundant accession to save cold room space and costs. DNA barcoding is useful technique for species identification, rapid disease diagnosis, and allelic mining, of individual loci of desirable traits. Therefore, this paper provides useful and contemporary information about the role of genomic for plant genetic resources conservation and sustainable use.

List of Abbreviations: PGRC: Plant Genetic Resources; SNPs: single nucleotide polymorphism; NGS: Next-Generation Sequencing; PCR: Polymerase Chain Reaction; LAMP: Loop mediated Isothermal Amplification.; PLACE: plant cis-acting regulatory DNA elements; TRANSFAC: transcription factors; JASPAR: Database of Transcription Factor Binding Site (TFBS); MEME: Multiple EM for Motif Elicitation; PlantCARE: A database of plant cis-acting regulatory elements; DCPD: Drosophila Core Promoter Database; SCPD: Saccharomyces cerevisiae promoter database; Clustal W: Multiple alignment of DNA and protein sequences/Multiple sequence alignment programs.

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