Review Article

Applications of Biotechnology for Characterization of Plants and Pests as the Key Components of Plant Protection and Production Strategies: A Review

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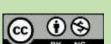
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Abstract

The second Sustainable Developmental Goals (SDG), among the seventeen SDG, is concerned with the pursuit of global food security and agricultural sustainability, which become the key to the success of the entire SDG. Whereas agricultural production and productivity are heavily threatened by the incidence of pests that inflict huge losses in various forms. This calls for prompt applications of biotechnology for the fast, accurate and reliable means for characterization of plant generic resources and pests as it is the pre-requisite and gateway for designing appropriate plant protection and production strategies. It is imperative that pests be identified properly so that judicious use of the literature can be made and sustainable management strategies can be implemented at the right stage. To this end, the application of biotechnology has made significant advances for reliable characterization of plant genetic resources as well as accurate diagnosis of pests, study of their genetic diversity and variability, detailed mechanisms of their transmission and evolutionary relationships. Accordingly, this review article covers the comprehensive account of the various molecular techniques, genome mapping and OMICS sciences utilized for characterization plants and pests that ultimately allow the detailed study of the biology and epidemiology of pests at any stage of their life cycle. The resulting data are eventually employed for enhancing successful implementation of sustainable plant protection and production strategies. In conclusion, the increasing projections of transboundary pests, environmental and abiotic factors together with the continuous scientific advancements and breakthroughs have made biotechnology to be an important engine of bioeconomy for generating invaluable products, processes and services.

The Need for Characterizations of Plant Genetic Resources and Their Pests

The Sustainable Developmental Goals (SDG), installed in 2015 by leaders of 193 countries, aim to secure immediate human needs, such as adequate food supply and healthcare and provision of clean, affordable, and accessible energy (Sach *et al.,* 2020). One of the seventeen agenda, second SDG-2, is concerned with the pursuit of global food security and agricultural sustainability. Thus SDG-2 aims to "End hunger, achieve food security and improved nutrition and promote sustainable agriculture". Since it is intrinsically related to society, economy, and the environment, SDG-2 is key to the success of the entire SDG agenda (Gil *et al.,* 2019). The main factors that are very instrumental towards attaining SDG-2 are highlighted as follows.

Challenges and Constraints of Agriculture in Attaining SDG

It is now well recognized that agriculture is one of the pillars and the engine of the overall growth of the bioeconomy in many countries which play the major role of delivering the

required biomass for food, feed, fiber, and other industry so as to ultimately attain SDG-2 (FAO, 2018a, Van Montagu 2020). However, agriculture in the 21st century will continue to face multiple, inter-connected challenges all over the world (FAO, 2018b; Aguilar and Patermann, 2020). Whereas, it must produce more food to feed a growing population characterized by changing consumption patterns, and dietary and nutritional preferences (FAO *et al.,* 2018b; Payumo *et al.*, 2018). Among many challenges and constraints, agricultural production and productivity are always heavily affected and threatened by the incidence of biotic factors (pests: diseases, insects and weeds) that inflict huge losses in various forms, both in qualitative traits (morphology, health and nutrition) and quantitative parameters (production and productivity). Moreover, transboundary pests can easily spread to several countries and reach epidemic proportions at a time (FAO, 2017). The adverse impacts of pest are also exacerbated and aggravated by several factors such as climate change, globalization, environmental factors and abiotic stresses (Meybeck *et al.,* 2018). To this end, FAO (2018a) produced the guidelines for Integrated pest management (IPM) and Emergency Prevention System for transboundary pests.

The Significance of Characterization of Plant Genetic Resources

In order to address these challenges, biotechnology is now considered as a series of enabling technologies, which each involve the genetic elucidation and manipulation of living organisms or their subcellular components to develop useful products, processes and services. Plant Biotechnology, in particular, encompasses applications of a wide range of scientific discoveries involving the explanation and exploitation of genetic potential and developmental systems in plants (Lebeda and Pokorný, 2012; Halford, 2012; Van Montagu, 2020). Biotechnological tools have been extensively utilized for the characterization and conservation of plant genetic resources for ensuring their sustainable conservation and utilization for enhancing the bioeconomy (Ruane and Sonnino, 2006; Laurentin 2009; Misra and Srivastava, 2016; Singh, 2016; Liu *et al.,* 2017). The review of Cano *et al.* (2017) revealed that the increasing recognition of biotechnology as an economic and social growth factor has stimulated governments in several countries to provide financial support to their local biotechnology institutions/companies to foster research, development, and commercialization of products and services. On the other hand, projections of malnutrition, famine, unmanageable pests (regular, invasive, migratory, transboundary) and unresolved environmental problems, climate changes, abiotic factors [water (drought/flooding), temperature (chlling/frost or heat), salts (high salinity or mineral deficiency)] have boosted biotechnological innovations and breakthroughs that resulted in the improvement of the quality of products and services provided by R and D institutions/companies.

Molecular Diversity/Variability of Plant Genetic Resources

Genetic diversity can be defined as any quantitative measure of the variability of a population, which reflects the equilibrium between mutation and the loss of genetic variation. Characterization of germplasm with respect to genetic diversity and variability is essential to provide information on the traits of accessions assuring the maximum utilization of the germplasm collection to the final users as well as long term conservation (Arif *et al.,* 2010). Accordingly, the genetic basis and molecular mechanisms underlying the evolution, diversity, reproduction, functions of traits and relationships with the environments have been studied that helped safeguarding the germplasms/collections. This ultimately generate the foundation for inferring genetic diversity and variability to engage in both breeding and conservation strategies. The development of molecular markers for plants, provided access to the genetic variability found in the accessions, which was useful for characterizing the germoplasm and for genetic improvement, based on specific markers (Carvalho *et al.,* 2019). Genetic variability is critical for a species to adapt to environmental changes and survive in the long term. To this end, characterization of plant diversity using molecular methods have been successfully employed with the final goal to track and tap various traits of interest (Arif, 2010; Idrees and Irshad, 2014; Govindaraj *et al.,* 2015; Bhandari *et al.,* 2017). Diversity analysis is attained upon applications of molecular taxonomy (Singh *et al.*, 2012) and molecular systematics (Marhold and Stuessy, 2011). There are three types of traits of plants that need to be characterized aiming at determination of the diversity and designing subsequent conservation and utilization strategies. The first is characterization of the resistance/tolerance of plants to biotic factors (pests), which is well addressed in this article (Tables 2-4). The second category is characterization of tolerance/resistance to environmental and abiotic factors such as temperature (chlling/frost or heat), water (drought or lodging) and salts (high salinity or mineral deficiency). The third type is characterization of parameters of productivity and quality traits such as (i) agronomic traits, (ii) yield and yield components, (iii) nutritional values: macronutrients (carbohydrates, fats, proteins, fiber) and micronutrients (minerals and vitamins), (iv) other quality parameters enhancement and post-harvest quality (storage/shelf life, processing conformity). The second and third category of biotechnological applications will be treated in detail elsewhere.

Molecular Evolution of Plants

Recent advances in molecular genetics have ushered in a new and exciting age for investigating where, when, and how crop plants arose. Progress in understanding crop evolution began with morphological studies and archeological finds of early domesticates (Lenser and

Theißen, 2013). In plants, interdisciplinary approaches combining phylogenetic and molecular genetic perspectives have enhanced our awareness of the myriad genetic interactions made possible by polyploidy (Burger *et al.,* 2008; Wendel, 2010). A large number of domestication genes (or domestication-related genes) have been identified for the characterization of the genetic architecture of crop plant domestication and investigate the evolutionary genomics (Lenser and Theißen, 2013; Meyer and Purugganan, 2013)

Molecular Phenology of Plants

Plant phenological events are defined as points in the life cycle of a plant that have been regarded as highly plastic traits, reflecting flexible responses to various environmental cues (Davies *et al.,* 2013; Yang *et al.,* 2020). Molecular phenology is defined here as the study of the seasonal patterns of organisms captured by molecular biology techniques (Kudoh, 2016). Closely related species tend to resemble each other in the timing of their life-history events, a likely product of evolutionarily conserved responses to environmental cues. The search for the underlying drivers of phenology must therefore account for species' shared evolutionary histories (Davies *et al.,* 2013). The technologies used for the determination of crop phenotypes have been compared for the integration of multiscale physiological phenotyping into a phenomics approach. Accordingly, several method have been proved to be valuable in the functional characterization of genomic information both in controlled environments and under field conditions. (Großkinsky *et al.,* 2015).

DUS Testing and Assessment

DNA based methods are applied to speed up plant breeding and release of varieties. To this end, after developing an elite variety, one of the requirements for variety release called Distinct, Uniform and Stable (DUS) can be determined unequivocally using biotechnological tools (Tommasini *et al.,* 2003; Gunjaca *et al.,* 2007; ISAAA, 2008). The process to grant plant variety rights is still based on the principles that applied when the International Union for the Protection of New Varieties of Plants (UPOV) was created in 1961; the morphological description of a set of pre-defined characteristics, and a new variety needs to be Distinct (whether a newly bred variety differs from existing varieties within the same species), Uniform (whether the characteristics used to establish Distinctness are expressed uniformly) and Stable (that these characteristics do not change over subsequent generations) (DUS) (UPOV, 2002). Molecular techniques are useful and required to keep the quality of the DUS decision high, and keep the costs of a DUS test reasonable. Therefore, UPOV has already established principles to guide the use of molecular techniques. Accordingly, molecular markers have been used for DUS testing in discriminating varieties of rape (Tommasini *et al.,* 2003) and maize (Gunjaca *et al.,* 2007).

Conservation and Preservation of Plant Genetic Resources

Analysis of genetic diversity and population structure of the existing population is required for the purpose of conservation and reintroduction of rare and endangered species. After the characterization steps, plant genetic resources need to be conserved and preserved by the national gene banks using appropriate methods. Endemic plant species are usually more vulnerable to anthropogenic threats and natural changes and, therefore, hold a higher extinction risk. To this effect, various conservation strategies need to be practiced such as *ex-situ* conservation exercised in botanic gardens (Mounce *et al.,* 2017), i*n-situ* conservation (Heywood, 2015; Whitlock *et al.,* 2016), cryopreservation (Kaviani, 2011; Streczynski *et al.,* 2019; Coelho *et al*., 2020) and biotechnological methods such as *in-vitro* preservation in TC collection including short-, medium-, and long-term strategies (Rao, 2004; Rajasekharan and Sahijram, 2015; Yohannes and Woldesemaya, 2017; Salgotra *et al.,* 2019; Coelho *et al.,* 2020). The DNA bank is an efficient, simple and long-term method used in conserving genetic resource for biodiversity. Compared to traditional seed or field gene banks, DNA banks lessen the risk of exposing genetic information in natural surroundings (ISAAA, 2013).

The Importance and Implications of Characterization of Pests

The applications of Biotechnology in plant protection can be broadly divided in to two categories namely (i) characterization of pests and (ii) management of pests. In this regard, the comprehensive and exhaustive account of the trends of biotechnology applications in pest management (i.e. the second application) has been recently reviewed by the same author of this article (Alemu, 2020). Whereas, the first part of the application is the subject of this review article which is, therefore, intended to complete the other half of the circle (i.e. characterization and implications to all other aspects of plant protection services) and show the whole range of applications of biotechnology in plant protection and production. To this end, it is well understood that early detection and identification of pests are the main challenges in protecting plants and limiting the epidemics and outbreaks (Lee *et al.* 2020). Thus, the availability of fast, accurate and reliable means for detecting plant pests is one of the main pre-requisite or gateways for designing sustainable management strategies. It is imperative that pests be identified properly so that judicious and contextualized use of the literature can be made and management strategies can be designed and customized as quickly as possible. Normally, the accurate and rapid diagnosis of pest plays an important role in controlling the pest, since useful protection measures are often implemented after correct diagnosis. In fact, effective pest

management requires enhanced skills and most costly inputs due the complex interaction of pests with environment and natural changes, weather factors or climate changes and anthropogenic pressure (Juroszek and Von Tiedemann, 2013). This is due to the fact that these pests are difficult to control as their populations are variable in time, space, and genotypes and new aggressive strains are likely to occur leading to invasiveness. outbreaks, upsurges and global spread.

Thus, this review article deals the whole range of state-of-the art modern biotechnological pest characterization methods which is found to be a very crucial prerequisite and gateway for the development and deployment of rapid, reliable and sustainable pest management strategies. Having such enabling capacity will tremendously help realized fast and reliable plant protection services. Furthermore, this review article appears to be unique as it covers brief account of the conventional methods and exhaustive application of molecular biotechnology techniques employed for detailed characterization of the pests which are referenced across plants and three pests (diseases, insects and weeds) that ultimately aid easier understanding of application for the better deployment options simultaneously.

Types of Methods of Characterization of Plants and Pests

The are several conventional methods that were used in the earlier times for characterization of plants and pests such as visual inspection and phenotypic observation of the morphological structure and anatomical features. However, using conventional methods of diagnosis early detection of the pest, before causing observable symptom damage, is not possible. This is due to the fact that these conventional methods are subjected to change in the environment and they are also culture dependent (season of specimen collection, age of culture). In general diagnostic and characterization methods used for pests include (i) Symptomatology and Phenotyping, (ii) Microscopy, (iii) Remote sensing Drones and Sensors, (iv) Imaging devices, (v) Spectroscopy, (vi) Biochemical and Physiological (vii) Chemotaxonomy and Chemosystematics, (viii) Serological and Immunological , (ix) Nucleic acid (molecular) techniques) and (x) polyphasic approach (a combine consensus application of these techniques) which are briefly described as follows

Symptomatology and Phenotyping

Visual inspection of the presence of a pest is normally suspected only when the pest has produced a symptom. Symptoms are abnormal features of the plant that indicate something is wrong which include (i) changes in the color, shape and texture, (ii) death of tissue, (iii) abnormal growth +/-, (iii) wilting, (iv) defoliation/fruit drop and (v). replacement of plant tissue. Traditionally, the most commonly used approach to identify plant pests relied with

visual inspection of symptoms involving three major steps (i) look: define the problem by describing signs and patterns of symptoms), (ii) Read: refer to reference materials describing similar signs and symptoms. (iii) compare-determine probable cause(s) through comparison and elimination. The problem with visual assessment is that, being a subjective task, it is prone to psychological and cognitive phenomena that may lead to bias, optical illusions and, ultimately, to error. (Barbedo, 2016). Furthermore, closely related organisms may be difficult to discriminate on morphological characters alone, symptoms are not always specific. By the time the symptom is visually observed, the concentration of pest is already very high in the plant and started causing damage of various magnitudes. On the contrary, very low concentration of a pest (at a time when the symptoms are not yet visible) can be detected by amplification of their chemical and sub-cellular components such as nucleic acids and proteins by advanced diagnostic techniques. For several years, the phenotypic classification was the only identification approach, although this methodology always resulted in uncertainties and difficulties with the analysis (Agrios, 2005).

Microscopy

Symptomatogical identification is usually followed by laboratory analyses based on morphological identification using microscopy. This method required isolation and culturing of the organisms, which sometimes encounter serious challenges as not all the microorganisms are cultivable *in vitro* (Tedeschi, 2012). There are several types of microscopy such Phase contrast Microscope, Stereoscopic Microscope, Electron Microscope (Scanning and Transmission), Fluorescence Microscope, Luminescence Microscope and Immunofluorescence microscopy. Microscopy images enable analysis of shape, tracking of motion, and classification of biological objects. The microscope-based observation is still frequently applied for the identification of microorganisms to define the morphological differences (spores, mycelium, fruiting bodies, colony arrangements...etc) that can also be compare with standard speciation keys (Mahlein, 2016; Franco-Duarte *et al.,* 2019). The availability of improved magnifying lenses and of microscopes made possible the detection and description of many plant diseases (Agrios, 2005; Goldsmithand and Miller, (2009). Similarly, microscopes allowed taxonomists to separate closely related parasitoid species on the basis of minor morphological variations (Gurr *et al*., 2004). The presence of a parasite in the vector insect (which can be determined by dissection and microscopy and/or biochemical means) generally appears not to harm the insect (Gullan and Cranstone, 2014).

Remote Sensing Devises, Drones and Optical Sensors

Remote sensing refers to noncontact measurements of radiation reflected or emitted from agricultural fields, which

are based on the interaction of electromagnetic radiation with soil or plant material. Remote sensing is an outgrowth of aerial photography which can be conducted through satellites, aircraft, or ground-based platforms. In recent years, remote sensing has become popular in pest monitoring, yield forecasting, and early warning to crop growers for timely management of potential pests in agriculture (Acharya and Thapa, 2015; Sudha Rani *et al.*, 2018). The use of drones or other remote-sensing devices, more systematic, routine monitoring and reporting of pests would facilitate awareness and implementation of timely and site specific management. Such alert systems are much more common for insect pests (CAST, 2017). Geospatial technology includes Geographic Information System (GIS), Remote Sensing (RS) and Global Navigation Satellite System (GNSS). These systems have been used in collecting, mapping, analyzing the distribution and predicting the scenario of pests and crop yield (Sabtu *et al.*, 2018; Anwer and Singh, 2019; Gebeyehu, 2019). With the development of information technology, Internet-of-Things (IoT) and low-altitude remote-sensing technology represented by Unmanned Aerial Vehicles (UAVs) are widely used in environmental monitoring fields (Gao *et al.*, 2020).

Imaging Devices

Machine vision systems are widely used for inspection of growing plants to recognize their diseases. The image processing can be used in various agriculture applications such as to detect diseased leaf and stem, to identify the affected area, to determine color of affected area etc. Automatic detection of plant diseases with the help of image processing technique provides more accurate pest detection and guidance for disease management. Various techniques have been used, including image processing for detection of objects, extraction of features and identification of pests based on various parameters such as color, boundary, background color, foreground color, intensity of pixels etc (Rajan and Radhakrishnan, 2016). There are some digital imagery softwares to combine multiple high-resolution images into a single, in-focus specimen image. Various image processing techniques have been used for identification of pathogens (Barbedo, 2016; Sun *et al.*, 2018), insects (Keszthely *et al.,* 2020) and weeds (Lin *et al.,* 2017).

Spectroscopy

Spectroscopic techniques reveal hidden information regarding the sample without destruction (Kandpal and Cho, 2014; Wang *et al*., 2018). Various types of Spectroscopy are described by (Chavan *et al*. (2017). The first group that belong to Atomic Spectroscopy includes (i) Atomic absorption spectroscopy (ii) Atomic fluorescence spectroscopy (iii) Atomic mass spectroscopy (iv) Atomic X-ray spectroscopy (v) Atomic emission spectroscopy. The second group that belong to Molecular Spectroscopy include *(*i) UV-Visible spectroscopy (ii) Molecular luminescence spectroscopy, (iii) Infrared spectroscopy (iv) Raman spectroscopy(v) Nuclear magnetic resonance spectroscopy (vi) Mass spectroscopy. These spectroscopic techniques have been extensively used for characterization of plants (Chavan *et al.,* 2017;), pathogens (Sankaran *et al*., 2010; Kandpal and Cho, 2014; Wang K *et al.,* 2018), insects (Dowell *et al.,* 1999; Ahmad *et al.,* 2018) and weeds (Shapira *et al.,* 2013).

Biochemical and Physiological Tests

Biochemical identification methods usually incorporate reactions of the pest (mostly pathogens) to different chemicals and reagents. One of the traditional methods most used is a simple visual detection of growth of the tested organism in the presence of a substrate by increased turbidity and color changes, after which the results are determined by comparing the microbe under analysis with a control test (Franco-Duarte *et al.,* 2019)**.** The various biochemical tests used for bacteria were reviewed by Hemraj *et al*., (2013). In order to speed-up the identification procedure, biochemical kits for detection, diagnosis, indexing and identification are commercially available for bacteria (Martinelli *et al.,* 2015). Some of the commercial kits include API (Analytical Profile Index) system such as (i) API 20E (20 substrates for demonstration of enzymatic activity or fermentation), (ii) API 50CH (49 Carbon source fermentation pattern), (iii) APIzym (substrates substituted with naphthyl- or naphthol containing groups) and (iv) BIOLOG test kit (95 carbon source assimilation test: colour change detected by ELISA reader)

Chemotaxonomy and Chemosystematics

Some macromolecules and cellular components carry enough information in their sequences/structures and thus allow a simple uniform approach for diagnosis, indexing and characterization of plants and pests. Thus, chemotaxonomy and chemosystematics are based on metabolites and cellular components of plants and pests as diagnostic characters. These methods involve tests for screening particular metabolic activities as well as production of characteristic primary or secondary metabolites. It is mostly utilized for the characterization of plants (Misra and Srivastava 2016; Singh, 2016; Liu *et al.,* 2017). This method is also utilized for the characterization of pathogens (Martinelli *et al.,* 2015; Franco-Duarte *et al*., 2019) and insects (Braga *et al.,* 2013; Snart *et al.,* 2015) and weeds (Dahiya *et al.,* 2017; Giri and Dhanalakshmi, 2018; Kaliyadasa and Jayasinghe, 2018).

Serological and Immunological Methods

Serological and Immunological methods are protein-based diagnostic techniques. Infection by pathogen can be detected by the presence of antigens (proteins, glycoproteins, etc.). The Immunoassays such as the

enzyme-linked immunosorbent assay (ELISA) is based on the principle of antigen-antibody interaction and thus makes use of this detection system, and forms the basis of some protein-based diagnostic kits. There are numerous commercial ELISA test kits available in the market for detection plant diseases (ISAAA, 2008; Martinelli *et al.,* 2015).

Nucleic Acids (DNA/RNA) (Molecular) Based Techniques

In recent years, nucleic-acids (DNA/RNA) (the universal code or blue print for inheritance of all living things) based methods have helped understand genetics of complex traits in plant systems and their pests as they are neutral to environmental influence and abundance. DNA-based or molecular methods allow reliable and rapid detection, diagnosis, identification and characterization of plants and their pests. Consequently, elucidation of the composition and structure or sequence of DNA has been routinely used

as the most preferred methods of characterization for plant genetic resources and pests, as described later in Table 2. Similarly, DNA-based markers are powerful for many diagnostic applications for typing biological samples in determining the identity of unknown samples, sample mixtures, criminal justice system, and curation of biological collections, to name a few. Interestingly, DNA-based kits for rapid detection, diagnosis, indexing and identification of pests are also commercially available (ISAAA, 2008; Martinelli *et al.,* 2015; Pusz-Bochenska *et al*., 2020).

Polyphasic Taxonomy

Polyphasic taxonomy employ the consensus approach (integration of different kinds of data and information: phenotypic, genotypic, and phylogenetic information) that is mostly used for the characterization of microorganism (Vandamme *et al.,* 1996; Gillis *et al.,* 2001; Das *et al.,* 2014; Vandamme and Peeters, 2014). The overview of this integrated approach is depicted in Fig. 1.
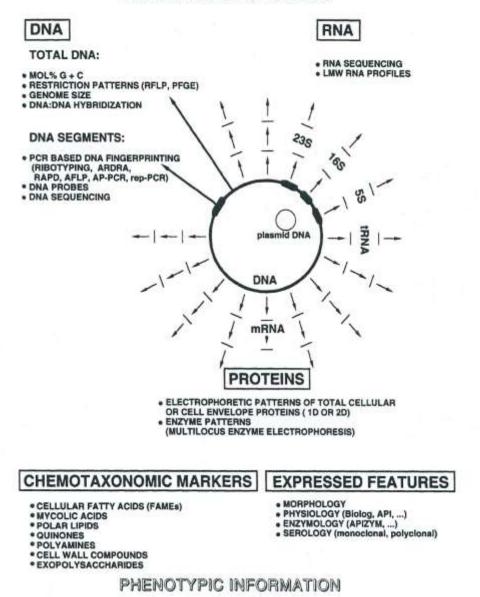

Fig. 1: Schematic overview of polyphasic taxonomy (Source: Vandamme *et al.,* 1996)

Comparison or Combined Use of Characterization Methods and Selection Criteria

In many cases, more than one types of characterization methods are used. Accordingly, some literatures indicate a brief comparison of the advantages and disadvantages or the combined use of some of the characterization methods, which are briefly described as follows.

Comparison or Combined Use of Conventional and Molecular Methods of Characterization

The ability to detect plant pests varies with the sensitivity and specificity of the detection tools used. To satisfy this demand, molecular methods are developed very fast and new dimensions are being added every day. It is to be noted that most of the methods are developed due for application on microorganisms and that is why microorganisms are said to be the fundamental elements and building blocks of biotechnology. Accordingly, various molecular methods of

characterization for microbes are extensively described in several review articles and books (Dickinson, 2005; Tsiamis *et al.* 2014; Awasthi, 2015). The combined use of various conventional and molecular methods have been described mostly for the characterization of microorganism (Spiegelman *et al.,* 2004; Martinelli *et al.,* 2015; Cui *et al.* 2018, Franco-Duarte *et al.,* 2019) and insects (Farrokhzadeh *et al.,* 2017).

There are some reports that compare various methods of pest diagnostics and characterization.

The findings described on such comparison can be generalized which can be presented in tabular forms for easier understanding and cross reference on each method with respect to each pest type. To this end, Table 1 describes the generalized findings on brief comparison of the advantages and disadvantages of some of the conventional and molecular methods of diagnosis and characterization of pests. It is to be noted that some of the methods are still applicable for characterization of plant genetic resources.

Table 1: Comparison of conventional and molecular methods used for characterization of pests

Method	Advantage	Disadvantage	References
Symptomatology and Phynotyping	• Quick for distinct symptoms	• prior knowledge required • Symptom versus syndrome confused	• pathogens (Pernezny *et al.,* 2014; Martinelli *et al.,* 2015) • insect-pests (Moczek, 2010; Ahamad *et al.,* 2015) • weeds (Booth *et al.,* 2003; Naidu, 2012)
Microscopy	• morphological characters • EM for viruses & Bacteria	• Stains for fungi lacking • pathogen/saprophyte	• pathogens (Goldsmith and Miller, 2009; Mahlein, 2016; Jadczak and Pizoń, 2017; Franco-Duarte *et al.,* 2019), • insects (Castner, 2000); Parasitoides (Gurr *et al.,* 2004) • Weeds (Hani *et al*., 2017)
Remote sensing devises, Drones and Optical Sensors	• no need of physical presence of expertise in the field • area-wide pest management • early warnings and forecasting	• fairly expensive • image interfered by other phenomena	• pathogens (Martinelli *et al.,* 2015; Mahlein, 2016; Gogoi *et al.,* 2018) • insect-pests (Acharya and Thapa, 2015; Lima *et al*., 2020) • weeds (Herrera *et al.,* 2014)
Imaging devices	• non-destructive • detection of pests in almost all the cases	• boundaries of the symptoms often are not well defined • symptoms produced by different diseases may be very similar	• pathogens (Martinelli *et al.,* 2015; Rajan P and Radhakrishnan, 2016; Barbedo, 2016; Sun *et al.,*2018;) • insect-pests (Keszthelyi *et al.,* 2020) • weeds ((Herrera *et al.,* 2014; Lin *et al.,* 2017)
Spectroscopy	• require minimal sample preparation	• sample preparation/ extraction facility required	• pathogens (Sankaran *et al.,* 2010; Martinelli *et al.,* 2015; Wang *et al*, 2018; Franco-

Table 1: Comparison of conventional and molecular methods used for characterization of pests

Method	Advantage	Disadvantage	References
	and provide non-destructive measurements		Duarte *et al.,* 2019; Yue *et al* 2019; • insect-pests (Ahmad *et al.,* 2018)
Biochemical and Physiological tests	• documented for bacteria • may explain morphology	• not widely used for fungi • Quantity required for PAGE • limited profiles to compare	• pathogens (Martinelli *et al.,* 2015; Franco-Duarte *et al.,* 2019)
Chemotaxonomy and Chemosystematics	• analyze phenotypic character • protein bands as immunogens	• no genotypic information • Laborious, time-consuming • Requires database search	• pathogens (Zwickel *et al.,* 2018; Gokdemir and Aras 2019) • insects (Braga *et al.,* 2013; Snart *et al.,* 2015) • weeds (Giri and Dhanalakshmi, 2018; Dahiya *et al.,* 2017; Kaliyadasa and Jayasinghe, 2018) • Plants (Misra and Srivastava, 2016; Singh, 2016; Liu *et al.,* 2017)
Serological and Immunological	• Kits, specific to strain, accurate, quick, little expertise	• not developed. for most diseases • raising specific Monoclonal antibodies	• pathogens (Jeong *et al.,* 2014; Martinelli *et al.,* 2015; Abd El-Aziz 2019; Franco-Duarte *et al.,* 2019)
Nucleic acid (molecular) techniques	• Rapid, Sensitive, Reliable • probes can detect any part of DNA • specific to genus, species, strains, biotypes /serotypes	• extraction of DNA • some are laboratory based • higher cost and skills	• pathogens (Spiegelman *et al.,* 2005; Jeong *et al.,* 2014; Kumar *et al.,* 2015; Martinelli *et al.,* 2015; Belete and Boyraz 2019; Franco-Duarte *et al.,* 2019) • insect-pests (Haymer, 2016; Franck *et al.,* 2017; Rasool *et al.,* 2018; El Sheikha, 2019) • weeds (Darling and Blum, 2007; Stewart *et al.,* 2009; Tranel and Horvath, 2009; Božić *et al.,* 2019) • any species (Pereira *et al.,* 2008; Singh, 2012)
Polyphasic taxonomy	• correlation of various information	• cumbersome to conduct for all types of strains/biotypes of pests • depend on physicochemical parameters, are not cumulative,	• pathogens (Vandamme *et al.,* 1996; Gillis *et al.,* 2001; Das *et al.,* 2014; Vandamme and Peeters, 2014)

Comparison and Combined use of Phenotypic and Genotypic Methods of Characterization

The phenotypic information is derived from the proteins and their functions, different chemotaxonomic markers and a wide range of other expressed features, Whereas Genotypic information is derived from elucidation of DNA/RNA composition and structure or sequence. The comparison or combined use of phenotypic and genotypic methods of characterization have been described for several plants (Ene *et al.,* 2016; Shikha *et al.,* 2019; Wang *et al.,* 2019), pathogens (Popović *et al.,* 2013; Abdellatif *et al.,*

2017), insects (Kharrat *et al.,* 2012; Dutta *et al.,* 2014) and weeds (Banerjee *et al.,* 2019; Kerr *et al*., 2019).

Criteria for Choosing an Ideal Diagnostic and Characterization Method

A criterion for choosing an ideal diagnostic method has been developed that ultimately aid an accurate diagnosis and characterization of the pest. An ideal method must meet several criteria to be broadly useful such as (i) discrimination power: recognize a reasonable number. of types, (ii) reproducibility: reliable, accurate, over a long period of time, (iii) rapidity, (iv) sensitivity, (v). costs involved in setting up and using the method, (vi) shorter time to obtain the results), (vii). ease of use/complexity of the method, (viii) ease of interpretation of the results…etc. The choice will depend upon the needs, skill level and resource of the laboratory in the R & D institutions.

Strategies Employed in Pest Characterization

The strategic applications of biotechnology in pest characterization ranges all the way from generating information about the pest up to deployment of sustainable plant protection products and services. In this regard, the International Plant Protection Convention (IPPC) strive to guarantee effective action to prevent the spread and introduction of pests of plants and plant products and to promote appropriate measures for their control. To this end several guidelines are issued by IPPC to be adopted with similar standards across the globe. The first step in pest management strategy is generating detailed information about the pest by the research system, for which some of the strategies required for this purpose are briefly described as follows:

Resources Required for Characterization of Pests

There are three major intertwined forces that are the basis of modern civilization and shape the modern world namely science (advanced skills and state of the art facilities), society (educated and energetic personnel to manipulate the science) and economy (the financial power of the society to manipulate the science and then produce novel products and services (Van Montagu, 2020). Accordingly, effective pest characterization requires full-fledged resources (skilled and knowledgeable personnel, advanced systems and most costly inputs) to characterize plant and pests whose identification is complex due their interaction with environment and weather factors or climate changes (Juroszek and Von Tiedemann, 2013). Thus, the necessary human resources (diagnostic expertise with sub-specialization for each type of pest), physical resources [infrastructure/facilities/equipments/tools/utensils and consumable resources (chemicals and reagents) and full-fledged organizational structure should be put in place to characterize the pest. This will promptly help manage the risk of regular, new and emerging pests to safeguard genetic resources. To this effect, international standards require adequate training for personnel involved in phytosanitary surveillance, identification, sampling methodology, data management, record-keeping, and preservation and transportation of samples (Augustin *et al.,* 2012). Comprehensive research facilities are required to perform an exhaustive taxonomy and biosystematics services for new pests. Similarly, in the development firms or extension system and plant health clinics need to be equipped with the state-of-the-art infrastructure and the necessary expertise to expedite all routine and regular services as per the international standards. In general, continuous capacity building programs need to be strategically implemented together with adequate financial investment in both research and extension sectors (Flood and Day, 2016).

Survey, Surveillance and Monitoring of Pest

Pest survey is the act of surveying while pest surveillance is the close observation, collection, analysis, and timely dissemination of information about the presence/occurrence, incidence, severity prevalence, geospatial distribution and mapping of pests and the plants they affect. IPPC has also adopted 'Guidelines for surveillance' (ISPM No. 6) (ISPM, 2020) which aim to support National Plant Protection Organizations' (NPPO) declarations of pest freedom, aiding early detection of new pests, and compiling host and commodity pest lists and distribution records (Augustin *et al.,* 2012). Furthermore, FAO (2016) guide provides information to support the surveillance activities that NPPOs need to undertake as part of national phytosanitary systems and as obligations under the IPPC. In other words, "good surveillance practice" is an integral component and pre-requisite for IPM practices, to identify whether the pest is invasive, regular, migratory, quarantine or non-quarantine or pesticides-resistant pest species (CAST, 2017). To this end, automated molecular diagnostic kits can be used to detect the pests in the field at different stages of the life cycle following appropriate pest surveillance techniques (Augustin *et al.,* 2012; Kalaris *et al*., 2014). Parnell *et al.,* (2015) described early detection surveillance as a rule of thumb to predict prevalence at first discovery. Standardized ethics need to be implemented while conducting the surveillance as described by Devitt *et al.* (2019).

Diagnostics, Taxonomy and Biosystematics

Once the pest specimen is collected, the next task would be to conduct accurate identification of the pests as is the pre-requisite to take appropriate subsequent actions such as refer literature reports on previous similar/related finding and devise contextualized/appropriate suitable pest management strategy. Plant pest diagnostics is based on the use of a range of different biological principles and methods (ISAAA, 2008), some of them being highly specific and others more generic. In 2016, the Commission on Phytosanitary Measures adopted a recommendation recognizing that 'pest diagnosis is a cross-cutting issue that underpins most IPPC activities. To enable safe trade, pest diagnosis must further be completed quickly and to a high

level of confidence (FAO, 2016). NPPOs routinely perform pest diagnosis to support export certification, import inspections, pest surveillance and eradication programmes. The field of biology that deals with the theory and practice of classification of organism is called as taxonomy. It is the field of science in which organisms and systematic are classified (Singh, 2012). Biosystematics is the scientific study that deals with the discovery, identification, description, naming, classification and catalogue of forms; keeping in view their record of diversity, life histories, living habits, ecosystem, spatial and geographical distributions relationships among them (Braby and Williams, 2016). Molecular techniques, now an integral part of biosystematic studies, are used to clarify genetic affinities (phylogenies) among related species and are specifically applied in biosafety evaluation to determine the closest wild relatives of crop plants and validate traditional taxonomic circumscriptions. To this end, molecular data have been used in the biosystematics of plants (Crawford *et al.,* 2005; Marhold and Stuessy, 2011), pathogens (Crous *et al.,* 2015), insects (Brites-Neto *et al.,* 2014; Braby and Williams, 2016) and weeds (Les *et al.,* 2002).

Pest References Collections

Well characterized pest specimens need to be preserved as they are an essential part of the plant health system. The knowledge and information from the specimens collected are indispensable for conserving biodiversity. Reference collection provides baseline information on how the range, abundance and form of species may change over time. Thus, collections need to include physical specimens of plant pests and the information on where, when and how they were collected. In this regard, microbial culture collections also known as Biobanks or Microbial Resource Centers are crucial resource centers providing microbial materials that remain the most important scientific infrastructure. They act as repositories for microbial strains as part of patent deposits, confidential services to store key organisms for research, industry and society (Çaktü and Türkoğlu, 2011; Sharma *et al.,* 2017). For preservation and curation of insects, adult insects are pinned or mounted and stored dry, although the adults of some orders and all soft-bodied immature insects (eggs, larvae, nymphs, pupae or puparia) are preserved. Voucher specimens need to be deposited in a well-maintained museum collection (Gullan and Cranstone, 2014). Weeds herbarium and digital database are very important to preserve weed species diversity in the face of accelerating habitat destruction. Herbarium specimens are among the most accessible and verifiable sources of data on distribution and habitat (Hanan *et al.,* 2016; Randall, 2017).

National List of Pests in the Country and Neighboring Countries

National plant pest lists need to be developed and well maintained so as to fulfill international obligations for trading countries. The list need to focus on pests of greatest significance and well characterized in order to provides a foundation for national biosecurity systems (Van Klinken *et al.,* 2018). It is extremely mandatory to get the national pest list of the neighboring countries so as to predict and get prepared for managing the transmission of transboudary pests.

Elucidating Factors that Trigger Pest Occurrence and Exacerbate the Adverse Impacts

Some factors such environmental factors, abiotic stress and climate change affect the incidence, epidemics, outbreaks and upsurges of plant pests (Xu, 2016; FAO, 2019a; Raza *et al.,* 2019). To this end, climate-smart agriculture actions are needed to reorient entire agricultural systems in order to support development and ensure food security in a changing climate (Heeb *et al.,* 2019). Thus, elucidation of these factors is very crucial to predict and duly manage the impacts ahead of time before exacerbated (Dukes *et al.,* 2009; Shaw, 2009; Juroszek and Von Tiedemann, 2013).

Pest Information Management Platform

In order to monitor pests in real-time, an automated and ICT based information system and database need to be developed (Isard *et al.,* 2006). In this regard, IPM-Information Platform for Extension and Education (ipmPIPE) was developed as a web-based database of current observations for pests (Huang and Wang, 2010). The automation of pest management data analysis will continue to improve as more systems are developed for handling larger data sets (CAST 2017).

Modelling and Forecasting the Occurrence and Impacts of Pests

Reliable model and timey forecast are critical aids that provide foresighted and informed planning for management of pests in the uncertainty ridden agriculture (Chapman *et al.,* 2015; Donatelli *et al.*, 2017; Newlands, 2018). There are abundant literatures on development and validation of models to forecast risk to crops from pests, among which weather-based forecast models are commonly used (Magarey and Isard, 2017). Weather monitoring systems are developed to more accurately predict pest occurrence and spread as a result of increasing temperature, frontal patterns, and other atmospheric conditions as well as anthropogenic factors (CAST, 2017). In summary, several quantitative models to determine the spread of plant pests for use in pest risk assessment has been implemented such as for pathogens (Dalal and Singh, 2017), insects (Tonnang *et al.,* 2017; Balikai *et al.,* 2019) and weeds (Hanzlik and Gerowitt, 2016; Somerville *et al.,* 2020)**.**

Collaboration, Coordination, Linkage and Networking of R & D Institutions

There is an international consensus that the world's greatest threats or challenges are beyond the ability of any one nation to confront them alone, needing to join international efforts to address them effectively (Aguilar and

Patermannb, 2020). Furthermore, Van Montagu (2020) indicates that science, society and economy are the three intertwined factors that shape our modern world and seriously demand an integrated network between likeminded nations. Thus, collaboration, cooperation and networking at local, regional and international levels are mandatory for any nation to achieve sustainable management of pests and enhance the bioeconomy (Flood and Day, 2016). In this regard, FAO (2019a) issued a guide for collaboration on Climate-Smart Agriculture. One of the required networks is, real-time pest diagnostic systems that should be networked so as to share the resources (technical and facilities) and promptly prevent the establishment of new invasive pest in a concerted manner. This will be accomplished by increasing the speed, accuracy, and effectiveness of local pest scouting and identification and minimizing the time required to implement control measures (CAST, 2017). Thus, a national plant diagnostic network should be put in place (Stack *et al.,* 2006; Miller *et al.,* 2009). A summary of some of the national and international plant disease diagnosis networks is described by Vakilian (2017). BioNet International and ASEANET are some of the networks in biosystematics that involve several counties.

Molecular Characterization Techniques Used for Plants and Their Pests

Overview of Molecular Techniques

Nucleic-Acids (DNA/RNA)-based (molecular) methods of characterization can be broadly divided into two namely those methods that require prior amplification of the DNA/RNA regions or genes and those that proceed on direct detection without the need of prior amplification. Pusz-Bochenska *et al.,* (2020) described one of the field adaptable diagnostic methods. For more precision purpose and accuracy, the majority of methods of diagnosis and characterization utilize the power of amplification of the DNA/RNA regions or genes using polymerase chain reaction (PCR). Thus, genetic fingerprinting is one of the DNA-based techniques that have permeated a wide gamut of biological research, beginning with forensic biology and medicine and now extending to agriculture (Pereira *et al.*, 2008; Romero *et al.*, 2009). The advent of PCR ushered a revolutionary approach in producing genetic fingerprints, supplanting hybridization-based techniques (Archak 2000; Weising *et al.,* 2005; Romero *et al.*, 2009), which are briefly described as follows:

Polymerase Chain Reaction (PCR) Based Methods

By virtue of the speed, sensitivity, specificity and inherent simplicity, PCR has been extensively used for many kinds of applications, particularly revolutionized the field of diagnostics (Singh *et al.,* 2014; Rajalakshmi 2017; Belete and Boyraz, 2019). PCR-based Method of Diagnosis/Identification can be broadly dived into two

namely (a) when the sequence of nucleotide is known and (b) when it is unknown. In this regard, PCR Methods used when targeted nucleotide sequence is known include (i) Standard PCR: phytoplasmas (MLO), bacteria, fungi, nematodes, (ii) RT-PCR: detection of RNA plant viruses or viroids, (iii) Multiplex PCR: amplify multiple viruses from a single reaction (iv) Immunocapture PCR: using virus-specific antiserum to concentrate, (v) Quantitative PCR: amount of start. template & yield of final product. On the other hand PCR Methods used when nucleotide sequence is limited or unknown include (i) RAPD-PCR: a single short primer of arbitrary sequence, (ii) Inverse/Inverted- PCR: DNA flanking an area known sequence 3'-ends face away from each other, (iii) Anchor-PCR: when 1 end of the sequence is known/variable add a universal primer to the missing end (iv) Real-Time PCR: to quantify gene expression cf. DNA array tech DD-PCR: amplify and display many cDNA derived from mRNA. There are different variants of PCR-based DNA-fingerprinting methods which include (i) DGGE/TGGE (Denaturing/Thermal Gradient Gel Electrophoresis), (ii) SSCP (Single Strand Conformation Polymorphism), (iii) CFLP (Cleavase Fragment Length Polymorphism), (iv) T-RFLP (Terminal-Restriction Fragment Length Polymorphism), (v) LH-PCR (Length Heterogeneity-PCR) (vi) REP-PCR (Repetitive Sequence Elements-Based PCR, (vii) AFLP (Adaptor/Amplified Fragment Length Polymorphism), (viii) ARDRA (Amplified Ribosomal DNA Restriction Analysis), (ix) RISA (Ribosomal Internal Spacer Analysis), (x) RAPD (Randomly Amplified Polymorphic DNA.

DNA Barcoding

DNA Barcoding is a technique for identifying organisms based on one or few relatively short gene sequences present in the genome which is unique enough to identify species. Since its formal introduction in 2003, DNA barcoding has become a well-accepted and popular tool for the identification species and the detection of cryptic taxonomic diversity (Nagy *et al.,* 2013). The standardized sequence region is called a DNA barcode because it is like a barcode tag for each taxon. In plant protection, DNA barcoding is a useful tool for fast and accurate taxonomic classification and identification of species by sequencing a very short standardized DNA sequence in a well-defined gene. The essential aim of DNA barcoding is to use a large-scale screening of one or more reference genes in order to assign unknown individuals to species, and to enhance discovery of new species. In this technique, complete information of the species can be obtained from a single specimen irrespective to morphological or life stage characters which will make ecological system more accessible (Kaur, 2015; Purty and Chatterjee, 2016).

DNA Hybridization

One of the molecular methods that doesn't involve PCR is Nucleic acid Hybridization. It requires short segment of DNA called probe to hybridize with complementary sequence of the sample under investigation Some of the hybridization techniques include (i) Colony (In situ), (ii) Spot/dot-blot/slot, (iii) Southern (DNA), (iv) Northern (RNA), (v) FISH (Fang and Ramasamy, 2015).

DNA Markers

DNA-based genetic markers rely on differences in DNA sequences (polymorphisms) between two parental lines. Polymorphisms can result from various factors that lead to either nucleotide changes or differences in DNA segment lengths such as mutations, errors in DNA replication, and insertions, inversions, and deletions of DNA fragments. DNA Markers have been used for diagnosis, diversity analysis, fingerprinting of genetic resources. In recent years, DNA-based marker systems have been increasingly employed in diverse areas of biology including phylogenetic studies, evolution, ecology, population genetics, population dynamics and genetics of complex traits. This has been possible because of the rapid advances in molecular biological methods and bench-level protocols for wider application (Fakrudin, *et al., 2006*). Some of the DNA-based marker systems RFLP, RAPD, AFLP, microsatellites/simple sequence repeats (SSRs), expressed sequence tag (EST) based marker system, Cleaved Amplified Polymorphic DNA (CAP), single nucleotide polymorphisms (SNPs). The marker systems that are now being progressively developed and also has shifted from the first- and second-generation marker systems including RFLPs, RAPDs, SSRs and AFLPs to the third and the fourth-generation marker systems, which include SNPs, KASper, DArT assays, and Genotyping by Sequencing (GBS) (Chauhan and Kumar. 2015; Lateef, 2015). Darling and Blum (2007) summarized the potentially useful DNA-based technologies for monitoring of invasive pests. These include PCR/RFLP, SSP (species-specific PCR), SSCP and DGGE, DNA barcoding, qPCR (quantitative PCR), Shotgun barcoding, T-RFLP, POA (phylogenetic oligonucleotide microArray). In summary, a diverse set of molecular markers techniques have been developed over the last 40 years and used with success for breeding a number of major crops (Rasmussen 2020).

DNA Sequencing

The recent development of high-throughput sequencing (HTS) technologies, also called next-generation sequencing (NGS) or deep sequencing, has revolutionized the research on plant-associated organisms. DNA sequence information has been used in a wide range of applications and for addressing different biological questions (Singer and Hajibabaei, 2009) from development to evolution and biodiversity. The improvement of the sequencing technology and in particular the advent of NGS together with the evolution of bioinformatics enable large-scale sequencing projects permitting a new comparative genomics approach and thus facilitate the identification of candidate genes to be used in plant protection. The possibility to down-regulate or over express genes in plants or in plant pathogenic organisms is a very powerful tool, within pest management and agriculture in general, to meet farmer and consumer demands (Tedeschi, 2012; Huq *et al.*, 2016). The advent of NGS technologies has led to the development of rapid genome-wide SNP detection applications in various plant species (Peterson *et al.*, 2014; Chung *et al.*, 2017). A large number of genomes have been wholly sequenced in the last decades in order to better understand their biology and to identify genes that could represent a potential target for their control in the field. Genome sequencing represented a landmark in the development of biological sciences and the methodologies of approaching biological problems. It enhanced our grasp of biological systems by examining the base of life (i.e. DNA) and allowed an enriched understanding of gene structures and functions (Mosa *et al.,* 2017). The availability of genome sequences for major crop plants have opened up new possibilities for combining genotyping and phenotyping to make crop improvements, while more powerful statistical methods are being developed that allow for the identification of the underlying genes of quantitative traits. The low-cost and easy-to-use PCR-based simple sequence repeat (SSR) makers showed its efficiency in the study of genetic diversity (Rasmussen 2020)

Microarray

The application of DNA microarray assay has entered a new era owing to recent innovations in omics technologies. DNA microarrays are used to detect and monitor alterations in genomic DNA and mRNA and to monitor genes and their expressions associated with various functions; thus, they have been widely used in basic research and industrial R and D (Galbraith and Edwards, 2010; Kiyama2017)

Biosensors and Nanobiosensors (Bionanotechnology Tools

DNA-based biosensor allows rapid, simple and economical testing of genetic and infectious diseases. The most commonly adopted DNA probe is single stranded DNA (ssDNA) on electrodes with electroactive indicators to measure hybridization between probe DNA and the complementary DNA (Fang and Ramasamy. 2015).

In summary, the major DNA fingerprinting methods such DNA barcoding and molecular markers are applied to determine the diversity and variability of the pest population. Full characterizations of pests are achieved through DNA/RNA sequencing (partial and whole genome) and Phylogenetic Analysis to depict their evolutionary relationships. Table 2 describe the review of major molecular methods used for characterization of plants' traits and pests with continuous advancements on biosystematics

and DNA Fingerprinting of genetic resources. These molecular methods are routinely used for detection, diagnosis, indexing of pests such as (i) DNA Barcoding (ii) DNA hybridization (iii) DNA Markers: diagnosis, diversity analysis, fingerprinting, (iv) DNA sequencing, (v) Microarray, (vii) Biosensors and Nanobiosensors (Bionanotechnology tools, diagnosis, indexing,

Table 2: Molecular methods used for characterization of plants and their pests

DNA Based method	Plants	Pathogens	Insect-pests	Weeds
DNA Barcoding	Identify and preserve the biodiversity (Astrin *et al.,* 2013; Kress *et al.,* 2015; Purty and Chatterjee, 2016)	Identification of microbial communities (Kaur, 2015; Miller *et al.,* 2016; Chaudhary and Dahal, 2017)	Agriculturally important insects (Jalali *et al.,* 2015; Rasool *et al.,* 2018); Arthropods (Ashfaq and Heber. 2016)	Invasive grasses (Wang *et al.,* 2010; Wang *et al.,* 2017)
DNA hybridization	study of hybrid lineages, genetic improvement and evolution (Silva and Souza, 2013; López-Caamal and Tovar-Sánchez, 2014; Ramzan *et al.,* 2017)	Detection of pathogens using serological and DNA-hybridization (*Martinelli et al.,* 2015; Fang and Ramasamy, 2015; Patel *et al.,* 2016; Lau and Botella, 2017)	Hybridization markers for detection of insects (Singh *et al.,* 2017)	
DNA Markers: diagnosis, diversity analysis, fingerprinting	Markers in plant improvement (Semagn *et al.,* 2006a; Agarwal *et al.,* 2008; Johan *et al.,* 2011; Kaur *et al.,* 2013; Chauhan and Kumar, 2015; Lateef, 2015), DUS test (Tommasini *et al.,* 2003; Gunjaca *et al.,* 2007)	Diagnostics and management (Awasthi, 2015); plant disease detection (Fang and Ramasamy, 2015; Martinelli *et al.,* 2015)	Utility in entomology (Behura 2006; Fakrudin *et al.,* 2006; Gariepy *et al.,* 2007; Jain *et al.,* 2010; Singh *et al.,* 2017), fruit fly management (Jenkins *et al.,* 2012)	Wild oat seed dormancy (Fennimore *et al.,* 1999); discrimination of parasitic weed (Pineda-Marto *et al.,* 2010), monitor biological control of weeds (Gaskin *et al.,* 2011)
DNA sequencing	Genotyping by sequencing (GBS) for plant genetic diversity analysis (Peterson *et al.,* 2014; He *et al.,* 2014; Huq*et al.,* 2016; Thottathil *et al.,* 2016; Chung *et al.,* 2017; Rasheed *et al.,* 2017)	16S rRNA, 23S rRNA gene and ITS region Sequencing (Tadesch, 2012; De Boer and Lopez, 2012, Patel *et al.,* 2016)	16S and 12S subunits of mitochondrial DNA as well as the (ITS1 and ITS2), the 18S and 28S subunits of the rDNA (Fakrudin *et al.,* 2006; Tedeschi, 2012; Wang *et al,* 2016)	DNA Sequencing (Shaik*et al.,* 2016); ITS region (Du, *et al.,* 2010). Chloroplast DNA sequencing (Wallinger *et al.,* 2012)
Microarray	Diversity analysis (Galbraith and Edwards, 2010; Kiyama, 2017)	Identification of pathogens (Rodrigo *et al.,* 2014); Plant–Pathogen Interactions (Lodha and Basak, 2012)	Identification of insect diapause development (Kostal *et al.,* 2017)	Identification of weeds (Horvath, 2010; Lai *et al.,* 2012)
Biosensors and Nanobiosensors (Bionanotechnology tools)	Nanoencapsulated material delivery to plants and benefits in precision farming (Duhan*et al.,* 2017; Kumar *et al.,* 2017a; Mishra *et al.,* 2017; Prasad *et al.,* 2017; Abobatta 2018; Thaku *et al.,* 2018)	plant disease detection (Fang and Ramasamy. 2015; Martinelli *et al.,* 2015; Lau and Botella, 2017; Kumar *et al.,* 2017a; Cui *et al.,* 2018), Diagnosis of plant diseases (Hussain 2017; Kumar *et al.,* 2017a; Veeranna *et al.,* 2017);	Detection of insect-pest (Liu *et al.,* 2017a; Cui *et al.,* 2018; Khandelwal and Joshi, 2018), detection insects semiochemicals (Brezolin *et al.,* 2018)	detection and measurement of weeds (Khandelwal and Joshi, 2018)

Genome Mapping Methods for Characterization of Plants and Their Pests

Overview of Genome/Gene Mapping Methods

Genome mapping is used to assign short DNA sequences (molecular markers) or specific genes to particular regions of chromosomes and to determine their relative linear orders and distances A genome map, like any other map, defines and pinpoint the relative locations/positions of features that are of interest, or which can serve as reference points for navigation across the genome (Dear, 2000). Almost any identifiable feature of the genome can serve as a marker in mapping. Genome maps are essential tools in identifying genes responsible for pests or traits, for comparing the genomes of different species, and for complete genome sequencing (Fay, 2006; Tiwari *et al*., 2006). Mapping genome of an organism is an important tool to provide a guide for the sequencing experiments by showing the exact positions of genes and other distinctive features in the chromosomal DNA (Dixit *et al*., 2014). There are different kinds of genome maps, which differ in techniques used to construct them and in the degree of resolution that is, the ability to measure the separation of elements that are close together. Some of the most common types of genome mapping approaches are briefly described as follows:

Gene Mapping

Genetic mapping (also known as linkage mapping or meiotic mapping) refers to the determination of the relative position and distances between markers along chromosomes. Genetic map distances between two markers are defined as the mean number of recombination events, involving a given chromatid, in that region per meiosis. Genetic maps are based on the recombination frequency between molecular markers which are population specific. Genetic map construction requires that the researcher develop appropriate mapping population, decide the sample size and type of molecular marker(s) for genotyping, genotype the mapping population with sufficient number of markers, and perform linkage analyses using statistical programs (Semagn, *et al*., 2006).

Linkage Mapping

Linkage mapping has been a key tool for identifying the genetic basis of quantitative traits in plants. However, for linkage studies, suitable crosses, sometimes limited by low polymorphism or small population size, are required (Braulio *at al*., 2012). High density genetic linkage maps facilitate map-based cloning, quantitative trait mapping, marker-assisted breeding, and comparative genome evolution. Linkage mapping based on a segregating population from a cross between two parents displaying maximally different phenotypes is a well-known approach to locate quantitative trait loci (QTL). They are statistically inferred, generally via linear regression and maximum

likelihood estimate methods and based on a genetic linkage map (Xiao *et al.,* 2017)

Association Mapping

Association mapping (AM) has the potential to identify a single polymorphism within a gene that is responsible for phenotypic differences. AM involves searching for genotype-phenotype correlations among unrelated individuals. Its high resolution is accounted for by the historical recombination accumulated in natural populations and collections of landraces, breeding materials and varieties. By exploiting broader genetic diversity, AM offers three main advantages over linkage mapping: mapping resolution, allele number and time saving in establishing a marker-trait association and its application in a breeding program (Flint-Garcia *et al*., 2003). Although AM presents clear advantages over linkage mapping, they are often applied in conjunction, especially to validate the associations identified by AM, thus reducing spurious associations (Braulio *et al.,* 2012; Kloth *et al.,* 2012).

Fine-Mapping

Fine mapping of QTL to a more narrowly precise genetic position and cloning of the underlying gene, as large secondary populations are generally required to achieve sufficient map resolution, are particularly resource- and time-consuming processes (Xiao *et al*., 2017).

Physical Mapping

A physical map of an organism is a representation of its entire genome as a set of overlapping cloned DNA fragments that make up a genome and ordered with respect to a reference map (such as genetic map) (Dixit *et al*., 2014). Physical maps are an alignment of DNA sequences, with distance between markers measured in base pairs.

Genetic and Physical Mapping

Genetic and physical maps illustrate the arrangement of genes and DNA markers on a chromosome. The relative distances between positions on a genetic map are calculated using recombination frequencies, whereas a physical map is based on the actual number of nucleotide pairs between loci.

QTL Mapping

Quantitative traits loci (QTL) is defined as "a region of the genome that is associated with an effect of a quantitative trait." So, a QTL can be a single gene, or it may be a cluster of linked genes that affect the traits (Veeresha *et al*., 2015; Mulualem and Bekeko, 2016). The most economically important traits are usually inherited in a quantitative manner, and the genetic basis is attributed to polygenes and interaction effects between genes and/or genes and the environment (Xiao *et al*., 2017). QTL mapping is process of locating genes with effects on quantitative traits using molecular markers. There are two types of traits, one type is quantitative type and another type is qualitative type. Here, quantitative types show continuous variation and qualitative type show discontinuous variation. Qualitative

type is generally governed by few genes or single genes and fall into a few distinct phenotypic classes called as discrete classes (Khan, 2015; Yang *et al*., 2017).

Genome-Wide Association Mapping

Genome-wide association study (GWAS) has become a widely accepted strategy for decoding genotype-phenotype associations in many species thanks to advances in NGS technologies GWAS using diverse populations provides another strategy to effectively fine map QTL due to a large number of historical recombination events that lead to the rapid decay of linkage disequilibrium (LD) The general goal of GWAS is to link genotypic variations to corresponding differences in phenotype using the most appropriate statistical model in a given population (Xiao *et al*., 2017). Thus, GWAS over- comes several limitations of traditional gene mapping by (i) providing higher resolution, often to the gene level, and (ii) using samples from previously well-studied populations in which commonly occurring genetic variations can be associated with phenotypic variation. The advent of high-density SNP typing allowed whole-genome scans to identify often small haplotype blocks that are significantly correlated with quantitative trait variation (Brachi *et al*., 2011).

Table 3 describes the review of various Genome mapping techniques that are effectively used for crop improvement in characterization of plants and their resistance to pests such as (i) Gene mapping, (ii) Linkage mapping, (iii) Association mapping, (iv) Fine-mapping, (v) Physical mapping, (vi) Genetic and physical mapping, (vii) QTL Mapping, (viii) Genome-wide association mapping.

OMICS Sciences Used for Characterization of Plants and Their Pests

The suffix "omics" has been attached to many fields of study, instantly conferring buzzword status and attention. These include Genomics, transcriptomics, proteomics, proteogenomics and metabolomics which are modern methodologies and approaches that have been recently applied in the study of plant stress mechanism responses. They provide new insights and open new horizons for understanding stresses and responses as well as the improvement of plant responses and resistance to stresses (Gomez-Casati *et al*., 2016). Fig. 2, described below shows the interrelationships of omics disciplines.

Omic technologies allow the visualization or monitoring of all of the changes that take place when the genetics, nutritional state, or environment of an organism is altered, thus revealing an understanding of the alterations in plant metabolism resulting from environmental interactions (Agarwal and Narayan, 2015). These are briefly described as follows.

OMICS in general

Omics disciplines allow us to learn more about the biological cycle of the pests in addition to the identification of novel virulence factors in pests and their host targets. Both approaches become important to decipher the mechanism underlying pest attacks and to develop strategies for improving pest resistant plants. Using omics, the genes responsible for proteins that confer or block the desired traits can be determined. Once these genes have been identified, they can be silenced in a plant or introduced from one plant to another or from another species, making a transgenic plant . Thus, a transgenic plant contains a gene or genes (i.e., transgenes) that have been artificially inserted rather than the plant acquiring them through pollination. (Agarwal and Narayan, 2015).

Genomics

Genomics is the study of all the genes in a given genome including the identification of gene sequences, intragenic sequences, gene structures and annotations. It is the study of the organization, evolution, and function of the genes and non-coding regions of the genome. Next generation sequencing (NGS) is revolutionizing genomics and is providing novel insights into genome organization, evolution and function (Beyrouthy *et al.,* 2014; Doležel *et al.,* 2014).

Table 3: Genome/Gene mapping for crop improvement and pest management

Type of genome/gene mapping	General application in Plants	Resistance to Pathogens	Resistance to Insect-pests	Resistance to Weeds
Gene mapping	molecular breeding (Semagn *et al.,* 2006b; Cone and Coe, 2009)	late blight resistance in potato (Thakur *et al*., 2016)	understand the evolution (Mandrioli *et al.,* 2017); resistance to plant hopper (Kobayashi *et al.,* 2018), control of insect resistance in rice (Du *et al.,* 2020)	Orobanche race in sunflower (Tang *et al.,* 2003); Cowpea Resistance to parasitic weed (Kouakou *et al.,* 2009)
Linkage mapping	genetic analysis of important traits (Maughan *et al.,* 2012; Guo *et al.,* 2013)	late blight resistance in potato (Massa *et al.,* 2015)	diamond back moth resistance to insecticide (Baxter *et al.,* 2011)	ecologically important traits in switchgrass (Millano *et al.,* 2016), markers linked to striga resistance in

Table 3: Genome/Gene mapping for crop improvement and pest management

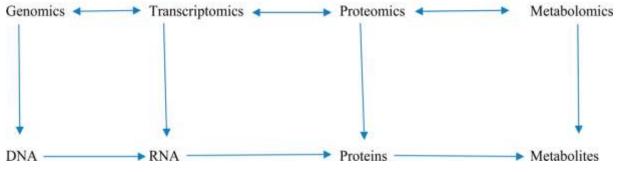| Type of genome/gene mapping	General application in Plants	Resistance to Pathogens	Resistance to Insect-pests	Resistance to Weeds
				sorghum (Mutengwa *et al.,* 2005)
Association mapping	molecular breeding (Lopes et al., 2012; Soto-Cerda, and Cloutier, 2012; Kushwaha *et al.,* 2017b)	Wheat rust resistance (Bajgain *et al.,* 2015); genes with resistance to late blight in Potato (Alvarez *et al.,* 2017)	plant resistance to insects (Kloth *et al.,* 2012)	Resistance to orobanche (Darvishzadeh, 2016)
Fine-mapping	grain productivity (Sasaki *et al.,* 2017; TC response (Salvo *et al.,* 2017)	Soybean resistance to root rot (Zhong *et al.,* 2018)	Soybean resistance to Insects (Zhu *et al.,* 2006)	
Physical mapping	phylogenetic studies and genome evolution (Dixit *et al*, 2014), visualize closely located genes (Kirov *et al.,* 2015)	leaf rust resistance (Jiang *et al.,* 2018)	Hessian fly in wheat (Aggarwal *et al.,* 2009)	herbicide resistance of amaranthus (Dillon *et al.,* 2017)
Genetic and physical mapping	Transgene Insertion Sites (Salvo-Garrido *et al.,* 2004)	Genetic and physical mapping (Simpson and Luna-Martinez, 2011)	Maize resistance against borer (Sadder and Weber, 2002)	
QTL Mapping	molecular breeding (Khan, 2015); Mulualem and Bekeko, 2015; Veeresha *et al.,* 2015; Sehgal *et al.,* 2016; Xu *et al.,* 2017)	resistance to multiple races of loose smut in wheat (Kumar *et al.,* 2018); resistance to late blight in tomato (Panthee *et al.,* 2017)	Planthopper resistance in rice (Ren *et al.,* 2004; Yang *et al.,* 2019), Strain identification of fruit fly (Sim *et al.,* 2017)	Sunflower resistance to orobanche (Louarn *et al.,* 2016)
Genome-wide association Study (GWAS) mapping	link genotypic and phenotypic variations (Brachi *et al.,* 2011; Xiao *et al.,* 2017)	plant–pathogen interactions (Bartoli and Roux, 2017; Genissel *et al.,* 2017); Wheat resistance to rust (Liu *et al.,* 2017b); Rice resistance to fungus (Mgonja *et al.,* 2016)	cork borer resistance in maize (Samayoa *et al.,* 2015); Earworm resistance in maize (Warburton *et al.,* 2017); soybean resistance to insects (Chang and Hartman, 2017; Hanson *et al.,* 2018)	cowpea tolerance to striga (Burridge *et al.,* 2016); plant-plant interaction (Subrahmaniam *et al.,* 2018)

Fig.2. The interrelationships of omics sciences (Source: Van Emon, 2016).

Transcriptomics

The transcriptome is the RNA expression profile of an organism. Unlike the genome which remains constant despite age, organ or growth conditions, the transcriptome is highly dynamic. Therefore, the term transcriptomics refers to the capturing of the RNA expression profile in spatial and temporal bases in certain plant organs, tissues

and cells within particular context. Transcriptomics mainly helps in finding genes that are associated with alterations in the plant phenotype under different conditions. For instance, transcriptomics can be used in finding candidate genes that contribute to stress tolerance through the comparison of transcriptomes of the same plant under optimal and stress conditions (Komatsu *et al*., 2013).

Proteomics

Proteomics is the study of the entire set of proteins produced or modified by an organism or system The proteome is the total expressed protein under certain conditions in a given organism, organ, cell, tissue or microorganism population. Similar to the transcriptome, the proteome is highly dynamic and changes based on temporal and environmental factors. Proteomics are the techniques used in capturing and measuring (or "profiling") the expressed proteins in a specific context. There are several types of proteomes that can be measured, and each of them can reveal particular information about the expressed proteins. The most common proteomes to be measured in plant stress tolerance and other plant related studies are the whole proteome and the phosphoproteome. The whole proteome is the quantitative and/or qualitative profiling of all the expressed proteins in a given sample, while the phosphoproteome is the quantitative and/or qualitative profiling of the phosphorylated proteins expressed in a given sample. Proteomic studies have identified numerous proteins that play crucial roles in plant growth and development. Proteomic analysis is expected to complement traditional molecular genetics approaches for studying the mechanisms by which this pathogen attacks cereal crops. (Komatsu *et al.,* 2013; Katam *et al*., 2015).

Metabolomics

Metabolomics is quantitative measurement of the complete set of small-molecule metabolites of specific cellular processes The metabolome is the complete set of metabolites that can be identified in a given organism, organ, tissue or biological fluid. Thus, metabolomics refers to techniques and methods used to study the metabolome. Due to differences in the chemical and physical properties of the metabolites, a combination of several analytical and separation techniques is required to obtain the metabolic profile of a plant or given sample. Metabolomics is fast emerging field in the world of omics, and generally used to scan all the metabolites present in sample using LC-MS, NMR-MS and GC-MS instruments. In plant we can find out metabolites in relation to numerous types of biological conditions such as treatments, tissues and genotypes (Agarwal1 and Narayan, 2010)**.** A plant's metabolome plays an important role in a wide range of physiological processes, and current research on plant stress responses greatly benefits from recent advances in metabolite profiling methods (Balmer *et al*., 2013).

Metagenomics

Metagenomics has risen as strong weapon that can be used to study microbial communities regardless of the ability of member organisms to be cultured in the laboratory using conventional isolation and also has offered the opportunity to describe the microbial diversity in environment as many of them cannot be yet cultured. Metagenomics is also described as environmental and community genomics which involves genomic analysis of microorganisms by direct extraction and cloning of DNA from an assemblage of microorganisms in most environments on earth such as water or the soil (Nazir, 2016).

Phenomics

With the advancement of omics technology, high-throughput and high-resolution phenomics tools have been evolving rapidly for the measurement of phenotypic traits of organisms in response to genetic mutation and external factors. Phenomics is a highly interdisciplinary field which needs co-ordinated expertise of non-invasive imaging, spectroscopy, image analysis, robotics, and high-performance computing. Significant work is in progress to develop technologies towards computerized video tracking, movement analysis, and behavior recognition in insects. High-throughput phenotyping methods have been developed to identify plants with an increased resistance against aphids in large plant collections (Barah and Bones, 2015;. In recent times, the role of phenomics-based breeding has become evident in improving the crop's performance, and similarly, genomics has made notable contribution in achieving higher genetic gains (Kumar *et al*., 2017).

Bioinformatics

Due to the large-scale nature of these approaches, bioinformatics and computational approaches are highly associated with the above for either developing new data analytical methods, better visualization or storage in sustainable online resource. The combination of high-throughput profiling techniques, bioinformatics tools, and the data from ecological studies will provide ways by which to achieve a comprehensive systems-level understanding of various aspects related to plant defence responses during insect attack at different hierarchical levels (Barah and Bones. 2015). In the following table, some of the contribution of OMICS in devising the strategies to obtain plants with increased resistance to pests are described. These approaches constitute important research tools in the development of new technologies for the protection against pests and increase plant production.

Table 4 describe the application of OMICS Sciences for generating various genetic information on the plants and their pests for subsequent use. These include (i) OMICS in general, (ii) Genomics (iii) Transcriptomics (iv) Proteomics, (v) Metabolomics, (vi) Metagenomics, (vii) Phenomics, (viii) Bioinformatics.

Table 4. Application of OMICS sciences in characterization of plants and their pests

OMICS Sciences	Plants	Pathogens	Insect-pests	Weeds
OMICS in general	Study Plant Responses to Stress (Pérez-Clemente *et al*., 2013; Van Emon, 2015; Mosa *et al.*, 2017)	increased resistance of plants to pathogens (Gomez-Casati *et al.,* 2016), biological control of pathogens (Massart *et al.*, 2015),	Insect biology (Boerjan *et al.,* 2012); plant defense against insects (Barah and Bones, 2015)	weed science (Maroli *et al*., 2018)
Genomics	Genetic diversity (Beyrouthy *et al*., 2014; Doležel et al., 2014; Mosa *et al.,* 2017), effects of climate change (Kole *et al*., 2015; Chown *et al.,* 2014)	Management of diseases (Klosterman *et al.,* 2016; Tiwari *et al.,* 2017); Plant-microbe interactions (Imam *et al.,* 2016)	Trends in entomology (Roy 2013); insect-pest management (Haymer, 2015)	Weed science (Horvath, 2010), Weediness and Invasiveness (Stewart *et al.,* 2009):
Transcriptomics	molecular response to environmental challenges (Wan *et al.,* 2011; Alvarez *et al.,* 2015; Mosa *et al.,* 2017)	Mechanisms of Plant-pathogen interactions (Li and Zhang, 2016; Nibedita and Jolly, 2017) ,	Evolution of modern insects (Yila *et al.,* 2018), resistant to insecticides (Zhou *et al.,* 2018), mechanism of cotton resistance to white fly (Li *et al.,* 2016)	Genes in Parasitic plants (Ichihashi *et al.,* 2015), Ranjan *et al.,* 2014; Yang *et al.,* 2014); genes involved in the herbicide resistance (Zhou *et al.,* 2017)
Proteomics	Characterize stress response (Komatsu *et al.,* 2013; Aslam *et al.,* 2017; Mosa *et al.,* 2017)	Plant–pathogen interactions (Kav *et al.,* 2007; Mehta *et al.,* 2008)	insect pest defense mechanism (Kumar *et al.,* 2016b), Aphids effectors sets (Thorpe *et al.,* 2016)	*Partheniumhysterophorus* (Pablos *et al.,* 2017); Herbicide resistance (Tetard-Jone *et al.,* 2018
Metabolomics	Characterize massive metabolites responsible for various traits (Balmer *et al.,* 2013; Tian *et al.,* 2016a; Hong et al., 2016; Kumar *et al.,* 2017b; Mosa *et al.,* 2017; Tugizimana *et al.,* 2018)	plant defence compounds targeted by pathogens (Carere *et al.,* 2016)	resistant markers against Aphids (Undas *et al.,* 2018)	Allelochemicals (Beck *et al.,* 2014; Scognamiglio *et al*., 2015)
Metagenomics		Interactions of plants and multiple microbes (Lara-Victoriano *et al.,* 2011; Bashir *et al.*, 2014; Melcher *et al.,* 2014, Nazir 2016); functions of Soil rhizosphere (Ofalm *et al.,* 2017; Soni *et al.,* 2017; Jansson and Hofmockel, 2018)	Aphids endosymbionts (De Clerck *et al.,* 2015)	Bioherbcides for weed management (Kao-Kniffin *et al.,* 2013)
Phenomics	correlate with the genotypic data (Großkinsky *et al.,* 2015; Rahman *et al.,* 2015; Kumar et al., 2015; Bhanu *et al.,* 2016; Kudoh, 2016; Yang *et al.,* 2020)	Plant pathogen interaction (Simko *et al.,* 2017)	plant–insect interactions (Goggin *et al.,* 2015)	distinguishing weeds from crops (Kelly *et al.,* 2016)
Bioinformatics	extract useful genetic information from data produced by high-throughput biological techniques such as genome sequencing (Agarwal *et al*., 2015; Kushwaha *et al.,* 2017a; Shariatipour and Heidari, 2017)	Plant disease management (Alemu, 2015), web-based platforms for characterization of pathogens: pathosphere (Kilianski et al., 2015), PhytoPath (Pedro *et al.,* 2016)	techniques reliant upon bioinformatics (Roy, 2013); molecular evolution in insects (Tong *et al.,* 2017); structural feature differences across 24 insect species (Li *et al*., 2013)	Predicting species invasions (Peterson and Vieglais, 2001), distinguish plant parasite and host transcriptomes (Ikeue *et al.,* 2015)

Molecular Studies on the Biology and Epidemiology of Pests

The study of the biology of the pest is necessary for understanding the pest and its life cycle as it the pre-requisite and gateway to design subsequent actions. DNA based methods have been applied for the study of the biology (life cycle) and epidemiology (cause and effect or dynamics of the pests over time and space) (Agrios, 2005; Cooke *et al.,* 2006). These include studies such as diversity, mechanisms of transmission, and their interaction with plants with an ultimate aim of generating useful data that help determine the best time and specific type of control measures to be deployed. In addition to these characteristics, climate change (CC) inflict the occurrence of invasive pests and other unpredictable biotic factors. Thus, CC adaptation and mitigation strategies as related to pest management: will affect the patterns of pests through changes in host distribution and phenology, plant-pest interaction and may resulted in rapid evolvement of pests (Juroszek and von Tiedemann, 2013). To this effect control and management of pests become difficult and complex as

their populations are variable in time, space, and genotypes and new aggressive strains are likely to occur leading to invasiveness. outbreaks, upsurges and finally to global spread. The incidence and effects of pests are driven to a large extent by weather conditions and CC as well as environment and natural changes and anthropogenic pressure. Accordingly, biotechnology can help address issues of CC adaptation and mitigation strategies as related to pest management (Tesfahun, 2018).

Table 5 describes the review of molecular studies on the biology and epidemiology of pests (i) Diversity of pests: Race/strain/ pathovar/biovar, serotypes, biotypes, Host range, (ii) Mechanism of transmission of pests to plants: (Virulence/avirulence, pathogenicity, aggressiveness,, (iii) Life-cycle, prevalence, invasiveness; latency and dormancy, ((iv) Host-Plant-pest interaction , (v) Molecular evolution, (vi) Phylogentic relationship , (vii) Population dynamics , (viii) Molecular mechanisms plants Resistance or defence to pests and (ix) Climate Change (CC) adaptation and mitigation strategies as related to pest management

Table 5: Molecular studies on the biology and epidemiology of pests

Characteristic	Pathogens	Insect-pests	Weeds
Diversity of pests: Race/strain/ pathovar/biovar, serotypes, biotypes, Host range,	Microbial diversity (Tsiamis *et al.,* 2014), Pathovar of Plant Pathogen (Tian *et al.,* 2016b); genetic diversity of bacteria (Shishir *et al.,* 2015), host specificity of fungi (Borah *et al.,* 2018)	Biotypes of aphids (Wenger and Michel, 2013),	Genetic diversity and variation orobanche (Pineda-Martos *et al.,* 2014), Mikania (Geng *et al.,* 2017); Acacia (Vicente 2018), Striga (Unachukwu *et al.,* 2017)
Mechanism of transmission of pests to plants: (Virulence/avirulence,, pathogenicity, aggressiveness, Life-cycle, prevalence, Weediness and invasiveness; latency and dormancy)	Molecular mechanisms of pathogenicity (van Baarlen *et al.,* 2007; Discover prevalence by early detection (Parnell *et al.,* 2015)	Virulence and avirulence of aphids (Kanvil *et al.,* 2015); Prevalence of Sporadic Insect Pests (Sappington *et al.,* 2018); Long distance dispersal of insects (Kim and Sappington, 2013)	Evolution of weediness and Invasiveness (Stewart *et al.,* 2009; understanding invasiveness through the genes (Broz *et al.,* 2007; Guo *et al.,* 2017a); Seed dormancy mechanisms (Finch-Savage and Footitt, 2017)
Host Plant-pest interaction	Mechanism of interaction (Chisholm, at al., 2006; Arie *et al.,* 2007; Salvaudon *et al.,* 2008; Doughari 2015; Wille *et al.,* 2019); Post genomic era application (Imam *et al.,* 2016), microbes interaction with invasive weeds (Coats and Rumpho, 2014), plant microbiota (Li *et al.,* 2018)	Host and Parasitoid Species of fruit fly (Jenkins *et al.,* 2012), molecular ecology of aphids (Morales-Hojas, 2017)	Parasitic plants and interaction with host plants (Kaiser *et al.,* 2015; Westwood and Kim, 2017), microbes interaction with invasive weeds (Coats and Rumpho 2014)
Molecular evolution	molecular evolution of pathogen (Schulze-Lefert and Panstruga, 2011)	molecular evolution of insects (Condamine *et al.,* 2016; Dogantzis *et al.,* 2018)	Evolutionary genetics of invasive weeds (Lee 2002)
Phylogentic relationship	Signal in pathogen–host range (Gilbert and Webb, 2007); diseases ecological and evolutionary processes (Alexander, 2010)	Molecular systematics of some insect orders (Trautwein *et al.,* 2012; Brites-Neto *et al.,* 2014; Song *et al.,* 2016),	Native and Invasive Lineages of Phyla in the world (Gross *et al.,* 2017), genetic differentiation among subpopulations (Marshall *et al.,* 2011)
Population dynamics	Epidemiology and Population genetics (Milgroom et al., 2003; dos A. Marques and Samson,	Population History of white fly (Hadjistylli *et al.,* 2016); fruit fly (Arias *et*	Population genetic structure (Muller-Scharer and Fischer 2001; Chapman *et al.,* 2004;

Table 5: Molecular studies on the biology and epidemiology of pests

Characteristic	Pathogens	Insect-pests	Weeds
	2016), hyperparasite and co-infection effect on population dynamics of pathogen (Tollenaere *et al.,* 2014; Susi *et al.*, 2015)	*al.,* 2018); aphids (Guo *et al.,* 2017b)	Pramual *et al.,* 2011; Ramírez *et al.,* 2017)
Molecular mechanisms plants Resistance or defence to pests	Metabolomics of the plant defence (Mhlongo *et al.,* 2018), molecular mechanisms of resistance (Avila Mendez and Romero, 2017; Sahni *et al.,* 2018, Singh *et al.,* 2018); response of RNAs (Islam *et al.*, 2018)	Molecular Bases of Plant defence against insects (Smith and Clemens, 2012; War *et al.,* 2012; Barah and Bones. 2015; Belete, 2018)	Molecular mechanisms of plant competition (Pierik *et al.,* 2013), resistance to striga (Bardaro *et al.,* 2016), Allelopathic mechanisms (Cheng and Cheng, 2015; Trezzi *et al.,* 2016; Ashraf *et al.*, 2017)
Molecular mechanisms of pests resistance to pesticides	Gene duplication mechanism (Jugulam and Gill 2018); reversing resistance (Allen *et al.*, 2017)	Molecular genetics of insecticide resistance (IR) (Ffrench-Constant, 2013); Molecular detection of IR (Javed *et al.,* 2017)	Molecular mechanisms of herbicide resistance HR (Delye *et al.,* 2015; Alebrahim et al., 2017), Gene duplication mechanism (Koo *et al.,* 2018)
Molecular studies on Climate Change (CC) adaptation and mitigation strategies as related to pest management	Impacts of CC on plant diseases and implications on management (Dukes *et al.,* 2009; Shaw 2009; Chakraborty and Newton, 2011; Pautasso *et al.,* 2012; Juroszek and von Tiedemann, 2013;	Impacts of CC on insect-pests and implications on management (Dukes *et al.,* 2009; (DeLucia et al., 2012; Musolin and Saulich, 2012; Juroszek and von Tiedemann, 2013)	Impacts of CC on weeds and implications on management (Dukes et al., 2009; Juroszek and von Tiedemann, 2013, Clements *et al.,* 2014; Ramesh *et al.,* 2017

Plant Protection Services that Demand Applications of Biotechnology

Plant protection products (pest management strategies generated by the research system) are better implemented by the Plant Protection Services (Extension system) for reducing/eradicating the qualitative and quantities losses of plants incurred due to pests of all sort. As emphasized above biotechnological characterization of pests influences and impacts the design and implementation of plant protection products and services. Thus, it is to be understood that the capacity and capability of biotechnology need to be corroborated and augmented with appropriate plant protection strategies. International regulation of plant pests are installed that formally began in 1878 (MacLeod *et al.,* 2010; Devorshak, 2012). The NPPO, tasked with national plant protection services, are entrusted with an overall responsibility for law enforcement on plant protection issues for successful implementation of processes and products of the research system. The NPPO has the overall responsibilities for pest surveillance, prevention of the entry of exotic pests, conduct phytosanitary measures and issue of Phytosanitary Certificate for export of agricultural commodities and Plant Import Permit. Some of the plant protection services that need to be promptly implemented as part of the International Standards of Pest Prevention and Control System are briefly described as follows:

Plant Quarantine Services
Plant quarantine covers all aspects of the regulation of the movement of living plants, living plant parts/plant products

between politically defined territories or ecologically distinct parts of them. Intermediate quarantine and post entry quarantine are used respectively to denote the detention of plants in isolation for inspection during or after arrival at their final destination (Devorshak, 2012; FAO, 2014). Testing of planting material for freedom from pests is an important control method that immensely contribute to taking urgent action on the other sides. Thus, quarantine is the first line of defence, that operates at strategic points of entry and considered as an essential component of pest management and control measures (Janse and Wenneker, 2002; James *et al.,* 2004; Zhang *et al.,* 2011; Laxmi *et al.*, 2014). Improvements in molecular techniques have allowed the development of sensitive, highly specific tests for many important pests so as to instantly monitor their geospatial distribution and occurrence. Thus, the use of diagnostic testing, together with traditional visual inspections, is expected to reduce the quarantine impediment. To this end, biotechnological tools have been effectively utilized for quarantine services such as the prevention of alien pests and diseases (Chand *et al.,* 2017); diagnosis of quarantine thrips (De Grazia *et al.*, 2016), invasive pest identification at ports-of-entry (Madden *et al.,* 2019) and prevention of weed (Duary 2014).

Phytosanitory Measures and Treatments; Phytosanitory Certificate
Ideally, pest-resistant planting material is desirable that requires implementation of strict phytosanitary measures and deliver the resulting certification (Janse and Wenneker, 2002; FAO 2014). To this effect, plant health clinics need

to be equipped with the state-of-the-art infrastructure and the necessary expertise to expedite all Phytosanitory measures as per the international standards. International Standards for Phytosanitary Measures (ISPM) are prepared by the Secretariat of the International Plant Protection Convention (IPPC) as part of the United Nations Food and Agriculture Organization's (FAO) global programme of policy and technical assistance in plant quarantine. ISPMs are standards, guidelines and recommendations to achieve international harmonization of phytosanitary measures, with the aim to facilitate trade and avoid the use of unjustifiable measures as barriers to trade. As of February 2020, there are 43 adopted ISPMs, 29 Diagnostic Protocols and 32 Phytosanitary Treatments. The purposes of these international standards are (i) protect sustainable agriculture and enhance global food security, (ii) protect the environment, forests and biodiversity, (iii) facilitate economic and trade development. Accordingly, effective plant quarantine and phytosanitory measures can be implemented to safeguard movement of planting materials as well as import/export of genetic resources. Some of the ISPMs related to this review articles can be referred for further information. These includes standards and guidelines on Phytosanitary issues (measures, treatments and certificates), pest risk analysis, establishment of pest free areas, pest reporting, listing of regulated pests, guidelines for surveillance and diagnostic protocols....etc (ISPM, 2020).

Pest Risk Analysis (PRA)

The procedure of identifying appropriate phytosanitary measures required to protect plant resources against new or emerging and regulated pests or plant products is called pest risk analysis (PRA) (Schrade *et al.,* 2010; Solima *et al.,* 2010). According to ISPM 2020 (IPPC-FAO). PRA is defined as "The process of evaluating biological or other scientific and economic evidence to determine whether an organism is a pest, whether it should be regulated and the strength of any phytosanitary (plant health) measures to be taken against it". PRAs may also be conducted on commodity imports to determine whether they provide a pathway for pests to enter the importing country or area. Risk analysis means the process that consists of three components (i) risk assessment; (ii) risk management; and (iii) risk communication; evolutionary tools have been used for phytosanitary risk analysis (Gilbert *et al.,* 2012). ISPM 02 describe the Framework for PRA and ISPM 11 describe PRA for quarantine pests (ISPM, 2020). Post-harvest quality assurance has been practiced using molecular methods. To this end genomics has been used for post-harvest quality analysis (Kader, 2002; Pech and Latché., 2013), improve nutritional quality and shelf life of fruits and vegetables (Aghdam *et al*., 2013; Abano and Buah, 2014).

Early Detection and Rapid Response Strategies

Early detection (ED) and a rapid response (RR) strategy are crucial to reduce the risk of entry and spread of plant pests into an area (Olmos *et al.,* 2018). EDRR is a proactive approach to managing invasive species that prevents establishment of new infestations to predict prevalence at first discovery (Parnell *et al*., 2015); diagnostics for early detection of alien pests (Morales-Rodríguez *et al.,* 2019), noxious weeds (Andreas *et al,* 2015). EDRR consists of 6 steps: (i) Early Detection, (ii) Identification, (iii) Alert Screening, (iv) Risk Assessment, (v) Rapid Response and (vi) Monitoring and reassessment. Parnell *et al.,* (2015) described that early detection surveillance as: a rule of thumb to predict prevalence at first discovery. Various molecular techniques are employed for monitoring the occurrence and dispersal of the pests at any situation to facilitate EDRR strategies. Consequently, effective plant quarantine and phytosanitory measures can be instantly implemented to safeguard movement of planting materials as well as import/export of genetic resources.

Early Warning and Alerting Systems

The early warning technology of pests is a strong guarantee to respond to the increasingly dire situation of major pests and diseases and ensure national food security. The combination of the IT provide a useful reference for the study of the early warning of crop pests. (Wang *et al.,* 2013). Early warning systems to predict and mitigate transboundary pant pests have been developed for desert locust (*Schistocerca gregaria* Forskål) (Cressman and Hodson (2009), Fall Army Worm (*Spodoptera frugiperda* JE Smith) (Prasanna *et al.,* 2018), wheat rust diseases (Allen-Sader *et al.,* 2019). The technology involves daily automated data flow and utilises expertise and environmental research infrastructures from within the cross-disciplinary spectrum of biology, agronomy, meteorology, computer science and telecommunications. These frameworks together with the underpinning technologies are transferable and adaptable for alerting and forecasting other pests.

Biosecurity and Agro-terrorism

Plant biosecurity is a strategic and integrated approach that encompasses the policy and regulatory framework to analyse and manage risks in the sectors of plant life and health, and related environmental concerns. It is the array of strategies aimed to assess and manage the risks of infectious diseases, quarantined pests, invasive alien species, living modified organisms, and biological weapons in natural and managed ecosystems. Thus, it covers the protection of a country from pests during transboundary movement of commodities, from emerging indigenous pests, from introduction and release of GMOs as well as from the use of pests/GMOs in biological warfare (Khetarpal and Gupta, 2007; Magarey *et al*, 2009). Although use of plant pests by criminals or terrorists is rare, their use could spread pests

more widely and new sources of potential threat have been identified through assessment of the use of pests for bioterrorism. Plant pests can be used as weapons and be deliberately introduced to reduce food security or have a more indirect impact on a nations' economy e.g. by denying access to lucrative export markets (MacLeod *et al.,* 2010). Thus, biosecurity is a holistic concept of direct relevance to the preventive measures to secure biological material and ensure sustainability of agriculture,, food safety, and the protection of the environment, including biodiversity (Khetarpal and Gupta, 2007). To this end molecular methods have been utilized to prevent biosecurity such DNA barcode for bisecurity (Armstrong and Ball, 2005; Khetarpal and Gupta, 2007; Hodgetts *et al.,* 2016). Similarly, other molecular biotechnological tools are routinely used to enforce and realize an efficient biosafety regulatory system that demand instant methods of characterization of the plants and pests (Cunningham, 2005; ISAAA, 2008),

Establishment and Demarcation of Pest Free Area

Pest free production site need to be demarcated in which a specific pest is absent, as demonstrated by scientific evidence, and in which, where appropriate, this condition is being officially maintained for a defined (FAO, 2016). Recognition of pest free areas and areas of low pest prevalence are described in ISPM 29 (ISPM, 2020). Furthermore, understanding the principal requirements for pest free areas, pest free places of production, pest free production sites and areas of low pest prevalence are described in the Guide for Establishing and Maintaining Pest Free Areas (FAO, 2019b).

Pest Risk Management (PRM)

Pest risk management (PRM) is the process of identifying ways to react to a perceived risk, evaluating the efficacy of these procedures, and recommending the most appropriate options. Thus, the conclusions from pest risk assessment, are used to decide whether pest risk management is required and the strength of measures to be used. Many of the elements and individual components of PRM are described in ISPM 2,11 and 21. In more details, the use of integrated measures in a systems approach for PRM are described in ISPM 14 (ISPM, 2020).

Management of Pest Invasions, Epidemics, Outbreaks and Upsurges

Plant pests can be managed most effectively when control measures are implemented at an early stage of infestation just before invasions and outbreaks. In this regard, invasion by exotic weed species is a serious threat to natural ecosystems, for which economical and sustainable methods need to be developed to reduce their abundance and dominance (Darling and Blum, 2007; Li *et al.,* 2015). According to FAO (2017) transboundary pests can easily spread to several countries and reach epidemic proportions at a time. Such pests have increased significantly in recent

years due to globalization, trade, climate change and the reduction in the resilience of production systems due to decades of agricultural intensification. The risk of transboundary epidemics, outbreaks and upsurges is increasing and can cause huge losses in crops, threatening the livelihoods of vulnerable farmers and the food and nutritional security of millions of people at a time (Lee *et al.,* 2020). Upsurges can be caused by crop management, evolution of new strains, and environment change that national systems must be responsive to detect problems and address them quickly (Flood and Day, 2016). To this end, sustainable plant protection strategies, that emphasize on the deployment of state-of-the-art IPM approach is critically needed so to produce higher quality plants with minimal loss and wastage of all sorts (Dara, 2019).

Emergency Management and Response Plan, Preparedness and Contingency Plan

The threats and/or the impacts from the introduction, establishment, and spread of plant pests can be prevented by developing Biosecurity emergency preparedness, response and recovery plan and mechanism for implementation to manage and mitigate pest invasions, epidemics, outbreaks and upsurges (TWG, 2008**;** SEMC, 2010; MPI, 2018**).** Similarly National Plant Health Emergency Management/Response Frameworks/Manuals are also implemented by several countries (CGAPS, 2013; BCPPAC, 2014; USDA, 2010; USDA, 2017). Similarly, FAO (2018a) produced the guidelines for IPM and Emergency Prevention System for transboundary pests.

In all of these plant protection services; the use of molecular techniques immensely contributes towards the successful implementation of judicious pest management strategies. As mentioned above, the use of fast and accurate methods for monitoring the occurrence and dispersal of the pests ultimately facilitate the implementation of sustainable pest management strategies.

Conclusion

In conclusion, the application of biotechnology has made significant advances in (i) characterization and conservation of plant genetic resources and (ii) rapid and accurate diagnosis of pests and characterization their biology, population dynamic and epidemiology. Consequently, the molecular data enabled elucidation and explanation of the pest and its impacts that serve as the gateway for development and timely deployment of sustainable plant protection and production strategies. The developments are very fast and new dimensions are being added every day. It is to be underscored that the increasing projections of transboundary pests and many other abiotic factors together with the continuous scientific advancements and breakthreouhs made biotechnology to be considered as an important engine of bioeconomy for generating invaluable products, processes and services. The author realized that no publication exist that covers the whole wider spectrum

and exhaustive account of the applications biotechnology in plant protection in two volumes. Thus, the combination of this review article (on characterization of plant genetic resources and pests) and the previous review article produced by the same author (on biotechnological applications for pest management) (Alemu 2020) believed to be the best references and guides for research and higher education curricula in both plant biotechnology and plant protection fields.

Conflict of Interest

The author declares that there is no conflict of interest with present publication.

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