Conservation of Biodiversity and Genetic Resources for Sustainable Agriculture

Mehmet Karaca and Ayse Gul Ince

Abstract The aim of conservation of biodiversity and genetic resources is to secure existing genetic biodiversity while allowing evolution and to build a wide base of genetic resources that meet demands of present and future uses not only for human kind but also for all livings forms on earth. Genetic resources for sustainable agriculture are irreplaceable natural sources for food, spice, medicine, fuel, fodder and building materials. Genetic diversity is an essential natural resource, like soil, water and the sun, without it life may not exist. Unfortunately, the most dramatic decline in the genetic diversity occurred with dramatic yield improvement of modern crops due to the development of hybrid technologies, synthetic fertilizers, irrigation, pest managements and farm machinery. Among 500,000 land species on earth, 100,000–160,000 are estimated to be under threats or about to enter the red list. It is estimated that today 15% of the earth land surface is protected for conservation, however, coverage varies widely among ecosystems and countries. Today approximately 7.4 million germplasm accessions representing more than 16,500 plant species are conserved in approximately 1750 gene banks worldwide, and more than two million accessions are estimated to be added soon. However, most gene banks around the world lack facilities, sufficient funds and staff to successful regeneration of gene bank collections and maintenances. Conservation of biodiversity and genetic resources is needed more than ever, given the cumulative effects of exploitation and destruction that is compounded by climate change. This chapter focuses on a brief history of public awareness on biodiversity and genetic resources for sustainable agriculture with specific highlights on next generational high-throughput techniques. The application of high-throughput phenotyping genomics and phenomics

M. Karaca (✉)
Department of Field Crops, Akdeniz University, Antalya, Turkey
e-mail: mkaraca@akdeniz.edu.tr

A. G. Ince
Vocational School of Technical Sciences, Akdeniz University, Antalya, Turkey
e-mail: ainceakdeniz.edu.tr

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opens new ways for a substantial enhancement of plant conservation activities for sustainable agriculture. Monitoring tools utilizing machine and deep learning approaches coupled with traditional plant breeding could not only inform us about the risk of genetic erosion through genetic drift and nonrandom viability selection within gene banks, but could help us to fight current pest and disease outbreaks, would also have the dual effects of contributing to enhanced food production and to the conservation of plant genetic resources.

Keywords Crop biodiversity · Gene discovery · Next generation genotyping · Phenotyping · Sources of diversity · Threats

Abbreviations

2b-RAD	Type IIB Restriction Enzyme Digestion Restriction Site Associated DNA
2D	Two Dimensional
3'-UTRs	3'-Untranslated Regions
3D	Three Dimensional
5'-UTRs	5'-Untranslated Regions
CBD	The Convention on Biological Diversity
CCD	Charge-coupled Device
cDNA	Complementary DNA
CFI	Chlorophyll Fluorescence Imaging
CGIAR	Consultative Group on International Agricultural Research
CGRFA	The Commission on Plant Genetic Resources for Food and Agriculture
CMOS	Complementary Metal Oxide Semiconductor
CNVs	Copy Number Variations
CPC	Centre for Plant Conservation
CRoPS	Complexity Reduction of Polymorphic Sequences
ddRAD	Double-digest RAD Sequencing
ECOSOC	The United Nations Economic and Social Council
FAO	Food and Agriculture Organization
GBS	Genotyping by Sequencing
GPS	The Global Positioning System
GRs	Genetic Resources
GT-seq	Genotyping-in-Thousands by Sequencing
IARCs	International Agricultural Research Centres
IBP	International Biological Program
IBPGR	International Board for Plant Genetic Resources
ICARDA	The International Center for Agricultural Research in the Dry Areas
INDELs	Insertions or Deletions

IPGRI	International Plant Genetic Resources Institute
IR	Infrared
iRRL	Improved RRLs
ITPGR	International Treaty for Plant Genetic Resources
IUCN	The World Conservation Union
LiDAR	Light Detection and Ranging
LMOs	Living Modified Organisms
loRNA	Long RNA
MIG-seq	Multiplexed Inter-SSR Genotyping by Sequencing
miRNA	Micro RNA
MSG	Multiplexed Shotgun Genotyping
NGS	Next Generation Sequencing
NIR	Near-infrared
NMR	Nuclear Magnetic Resonance
NRC/NAS	National Research Council/National Academy of Sciences
PAV	Presence/Absence Variations
PCR	Polymerase Chain Reaction
pERPLs	Paired-end Reduced Representation Libraries
RAD	Restriction Site-associated DNA
RAD-seq	Restriction Site-associated DNA Sequencing
RAM-seq	Random Amplicon Sequencing
Rapture	RAD Capture
RESTseq	Restriction Fragment Sequencing
RF	Radio Frequency
RNA-seq	High-throughput RNA Sequencing Analysis
RRL	Reduced-representation Library
RRS	Reduced Representation Shotgun Sequencing
SAR	Synthetic Aperture Radar
SBG	Sequence-based Genotyping
SEQ	Sequencing
sGBS	Spiked Genotyping-by-sequencing
SkimGBS	Skim Genotyping-by-sequencing
SLAF-Seq	Specific Length Amplified Fragment Sequencing
SMRT	Single Molecule Real-Time
SMTA	Standard Material Transfer Agreement
SNP	Single Nucleotide Polymorphism
sRNA	Small RNA
SSRs	Simple Sequence Repeats
tGBS	Tunable Genotyping-By-Sequencing
ToF	Time of Flight
UAV	Unmanned Aerial Vehicles
UN	United Nations
UNCED	The United Nations Conference on Environment and Development
UNCSD	United Nations Conference on Sustainable Development
UNFCCC	United Nations Framework Convention on Climate Change

UV	Ultraviolet
VIS	Visible
WES	Whole Genome Exome Sequencing
WGR	Whole Genome Resequencing
WGS	Whole Genome Sequencing
X-ray CT	X-ray Computed Tomography

1 Introduction

There exist approximately 500,000 land plant species on earth. Although 10–20% of earth's land plant species are not scientifically studied, about 100,000–160,000 land species are estimated to be threatened. About 15% of the earth land surface is protected for conservation, however, coverage varies widely within ecosystem (Sharrock et al. 2014; Coetzee et al. 2014; Geldmann et al. 2015; Sajjadi et al. 2015; Corlett 2016; Yousef et al. 2018). Conservation of plant biodiversity and genetic resources probably begun some 13,000–15,000 years ago when hunter-gatherers started to collect plants. Gatherers turned into farmers and discovered to save crop seeds they found easiest to process or tasted the best (Zhang et al. 2017). During the history of crop domestication, more than 7000 plant species have been cultivated. However, today in modern agriculture no more than 500 plant species are being grown, among the most widely cultivated crop species include cereals such as wheat, maize, rice (Duvick 2005), fiber crops such as cotton, flax, hemp (Uygur Gocer and Karaca 2016a), oil crops such as sunflower, canola, soybean (Edgerton 2009), pulses such as chickpea, lentil, bean (Ince and Karaca 2011a), forage crops such as bermudagrass, alfalfa, common vetch (Cakmakci et al. 2006; Karaca and Ince 2008), vegetables such as tomato, pepper, lettuce, mushroom, carrot, asparagus, celery, turmeric, artichoke, (Ince et al. 2009a, b, 2010a, b, c; Polat et al. 2010; Ince and Karaca 2011b), starch and sugar plants such as potato, sugar beet, cassava (Peroni and Hanazaki 2002), medicinal and aromatic plants such as sage, oregano, thyme, ginger, jojoba, chicory (Karaca et al. 2008, 2015; Ince et al. 2010d, e, 2011a; Ince 2012), ornamental crops such as carnation, rose, lily (Ince et al. 2009c; Ince and Karaca 2015a).

During the cultivation periods, genomes of the most cultivated species mentioned above experienced intense selection for desirable characteristics, many of which are not found in wild and crop wild relatives. On the other hand, wild species and crop wild relatives are rich in genes against biotic and abiotic stress factors and secondary metabolites such as phenolic compound many of which have economic importance (Elmasulu et al. 2009; Ince and Karaca 2009; Karaca et al. 2011). During the domestication periods cultivated crop species received long-standing events include the domestication bottleneck (occurs when a subset of the wild populations is brought into cultivation), directional selection (diversity can subsequently be lost through selective breeding for desirable traits during crop improvement), dispersal bottlenecks and gradual increase of genetic diversity as a consequence of gene transfer

within and between the domesticated species or crop wild relatives. Also cultivated species gained or lost allelic combinations via mutations and recombination events which could affect conservation of biodiversity and genetic resources for sustainable agriculture (Shepherd et al. 2016; Zhang et al. 2017; Kopnina et al. 2018).

In the agricultural history, the most dramatic decline in the genetic diversity occurred with dramatic yield improvement due to the development and widespread use of new farming technologies such as hybrid technologies, synthetic fertilizers, irrigation, pest managements and farm machinery (especially during the period of Green Revolution). Modernization of agriculture started in the middle of the nineteenth century in Europe and North America leading to the irreversible loss of innumerable heterogeneous landraces and other genetic materials. In Asia and other developing countries, the Green Revolution started in the beginning of the twentieth century and gained momentum in the 1960s. It is important to note that modernization of agriculture was evident in Europe and North America long before World War II indicating that Green Revolution started during nineteenth century in today's most developed countries (Baur 1914; Harlan 1975).

Biodiversity and genetic resources of plant species and their wild relatives are not equally and evenly distributed on earth. Significant amounts of *in situ* genetic resources are within the developing countries while developed countries have *ex situ* genetic resources. It is known that significant amounts of landraces in North America and northwestern Europe were lost due to genetic erosion. It is also interesting to note that loss in genetic variations due to genetic erosion has been less intensive in remote areas where these traditional varieties are still grown in small cultivation and patches of land (Evenson and Gollin 2003; Ince et al. 2009d; Karaca and Ince 2011a, b).

Landraces, heirlooms and traditional varieties are old cultivars selected by farmers over hundreds years to best fit their needs. These genetic resources generally display greater diversity and many desired metabolomic traits than modern cultivars as they have been selected to adapt to local, sometimes hostile environments. Metabolomic traits especially taste and health-promoting related traits are contained in landraces, heirloom and traditional varieties and thus serve as a good source of the best alleles for organoleptic quality improvement (Ince and Karaca 2011a; Gascuel et al. 2017; Vlk and Repkova 2017). Therefore, conservation of biodiversity and genetic resources of these valuable resources are very important for sustainable agriculture.

Landraces, heirloom and traditional varieties are also beneficial crops for soil fertility characteristics that save soil's organic matter and protect it from soil erosion. Also many of heirloom and traditional varieties contribute to healthy human nutrition and potential sources of resistance to abiotic and biotic stresses. However, they are less suited for new agricultural technologies and do not provide high yields as high as modern cultivars. Due to their less productivity in terms of yield, they are not widely cultivated in most parts of the world at present, thus, genetic diversity within landraces, heirloom and traditional varieties is seriously reduced. The rapid expansion of plant breeding applications during the second half of the twentieth century brought the introduction of a big number of improved varieties, which pro-

gressively replaced old landraces, especially in developing countries resulted in genetic erosion. Also the release of a large number of commercial varieties into traditional farming systems caused a reduction in the number of varieties cultivated in a given area. Unfortunately, almost due to the same reasons biodiversity in other valuable genetic materials such breeding lines, genetic stocks, obsolete cultivars, landraces, accessions, heirlooms, traditional or heritage varieties of crops along with wild species and crop wild relatives (please see the glossary at the end of this chapter for short descriptions) is narrowed. In addition, due to the climatic changes and monoculture agricultural practices genetic diversity on earth is being eroded (Elmasulu et al. 2011; Ince et al. 2010f). Therefore, conservation of biodiversity and genetic resources for sustainable agriculture is required to secure existing genetic biodiversity and to build a wide base of genetic resources that meet demands of present and future utilizations (Evenson and Gollin 2003; Ince et al. 2009e; Karaca et al. 2013).

According to the central dogma of conservation genetics, genetic variability is beneficial and thus it is worth preserving to the greatest extent (Pertoldi et al. 2007). Therefore, conservation of biodiversity is important for maintaining the adaptive potential of species and populations for sustainable agriculture. In turn, conservation of biodiversity ultimately depends on the conservation of genetic diversity and increasing genetic variation enhances the probability of population survival (Aravanopoulos 2016). Almost everybody on earth agrees that biodiversity is at risk from multiple threats including increasing human population and the genetic diversity contained within plant genetic resources needs to be conserved.

There have been two different concepts on what types of biodiversity and genetic resources should be conserved. Also important concepts on how biodiversity and genetic resources are conserved and how genetic erosion is reduced, and what kinds of technologies can be implemented to enhance their conservation and use still need attentions. During the second half of twentieth century germplasm collection expeditions adopted an approach called "mission-oriented approach" (Dulloo et al. 2013; Buse et al. 2015; Kopnina et al. 2018). This approach focuses on targets specific to plant breeding projects. Therefore, collected genetic materials are mainly used in plant breeding stations. However, this approach, while responding to immediate individual or organizational needs, have limited effects on reducing the genetic erosion. Opposite to the mission-oriented approach is the "generalist approach" that directs towards collecting and conservation of much possible genetic materials in plant centers of origin (Bayerl et al. 2017; Fu 2017).

Today approximately 7.4 million germplasm resources representing more than 16,500 plant species are being conserved in 1750 gene banks worldwide, and more than two million accessions are estimated to be added (Shepherd et al. 2016; Fu 2017). Conservation of these genetic resources uses *in situ* strategy and *ex situ* strategy or both strategies. Although *ex situ* and *in situ* conservations are two main strategies for conserving genetic resources for sustainable agriculture, they are equally important and should be utilized at the same time as complementary approaches (Dulloo et al. 2013; van Kleunen et al. 2015).

Ex situ conservation is the conservation of genetic resources outside their natural habitats and it is generally used to conserve populations that potentially under threat. *Ex situ* conservations in gene banks are in the form of seeds, live plants, tissues, cells and/or DNA materials. On the other hand, *in situ* conservation is the conservation of populations of species at their natural habitats or close their gene centers including maintenance and recovery of viable populations of species. *In situ* conservation can be either on farm, requiring the maintenance of the agro-ecosystem along with the cultivation and selection processes on local varieties and landraces, or in the wild, which involves the maintenance of the ecological functions that allow species to evolve under natural conditions (Ince and Karaca 2011a; Korun et al. 2013; Buse et al. 2015; Hernandez-Suarez 2018; Kopnina et al. 2018; Manhaes et al. 2018).

We know that identification of genetic resources is also as important as conservation of biodiversity for sustainable agriculture. In this chapter, we also discuss the novel, and emerging approaches such as next generation phenotyping and next generation genotyping systems for detecting and conservation of biodiversity and genetic resources for sustainable agriculture after a brief historical view on global biodiversity and genetic resources, values and current status of genetic resources and diversity.

2 A Brief Historical View on Global Biodiversity and Genetic Resources

During 1845 and 1849, Irish Potato Famine caused about one million people death and a million more migrated from Ireland. One of the main causes of famine was potato blight disease that ravaged potato throughout the Europe during 1840s. Probably this was the first well know indication of the result of genetic erosions in cultivated crops. Second indication of genetic erosion was noted in 1970 with considerable yield loss in United States of America corn production caused by fungus *Helminthosporium maydis* race T, known as the southern corn leaf blight. These two events are good examples showing the consequences of the lack of genetic diversity and the use of monoculture modern varieties instead of landraces (Fu 2017).

Accumulated scientific, political and public awareness on conservation of biodiversity and genetic resources for sustainable agriculture were internationally sounded in 1960s and the landmark conferences sponsored by international organizations such as the Food and Agriculture Organization of the United Nations (FAO), the International Biological Program (IBP) and World Bank were held. The IBP and FAO in 1967 helped to lay the foundation for modern genetic resources conservation efforts (Goulart et al. 2018; Li et al. 2018). Table 1 summarizes some events, conferences and establishments concerned conservation of biodiversity and genetic resources of the world.

Table 1 Events relevant to the establishment and evolution of international instruments related to the conservation and sustainable utilization of plant genetic resources during the period 1961–2018

Event	Some underpinning principle(s)/agreements
1961–1973	Rising concern about formulating criteria for the conservation, diversity and genetic resources for sustainable agriculture
FAO technical meeting on plant exploration and introduction, (Rome, 1961)	The use of e*x situ* and *in situ* conservation strategies as a complementary strategy to conserve gene erosion of landraces and wild relatives
FAO/IBP technical conference plant exploration, utilization and conservation of plant genetic resources, (Rome, 1967)	Priority geographic areas for exploration and conservation of plant genetic resources.
	Establishment of the technical advisory committee for conservation of plant genetic resources
	Establishment of the world network of genetic resources centers
Third session of the FAO panel of experts on plant exploration and introduction, (Rome, 1969)	Establishment of a coordinating center support to gene banks already existing in international agricultural research centers (IARCs) of the consultative group on international agricultural research (CGIAR)
Founding meeting of the consultative group on international agricultural research (CGIAR), (Washington, DC, 1971)	
UN conference on human environment, (Stockholm, 1972)	
Establishment of the international board for plant genetic resources (IBPGR) group, (Beltsville, 1972)	
FAO/IBP technical conference on genetic resources, (Rome, 1973)	
1981–1991	Suggested clarity regarding the legal situation of the *ex situ* collections
FAO/IBP technical conference on genetic resources, (Rome, 1981)	Suggesting the need for an international agreement to ensure the conservation, maintenance and free exchange of plant genetic resources
21st session of the FAO conference, (Rome, 1981)	Adoption of the international undertaking on plant genetic resources establishment of the commission on plant genetic resources for food and agriculture (CGRFA) and of the global system on plant genetic resources
22nd session of the FAO conference, (Rome, 1983)	
	Plant breeders' rights are not inconsistent with the international undertaking, recognition of farmers' rights

(continued)

Table 1 (continued)

Event	Some underpinning principle(s)/agreements
National forum on biodiversity (Washington 1986)	Requested FAO a code of conduct for biotechnology to be used in conservation of genetic resources
25th session of the FAO conference, (Rome, 1989)	International board for plant genetic resources (IBPGR) was transformed into the international plant genetic resources institute (IPGRI)
3rd regular session of CGRFA, (Rome, 1989)	
26th session of the FAO conference, (Rome, 1991)	
1992–1994	Plant genetic resources of nations are recognized the sovereign rights of nations
The United Nations conference on environment and development (UNCED) (Rio de Janeiro, 1992)	Agreement on the development of the 1st state of the world's plant genetic resources and global plan of action on plant genetic resources
1st extraordinary session of the CGRFA, (Rome, 1994)	Agreement on risk assessment and management of all aspects of biotechnology
The convention on biological diversity (CBD, 1992)	Agreement on international policy framework for the conservation of plant genetic diversity
Establishment of the Scarascia Mugnozza community genetic resources center, (Chennai, 1994)	Agreement to hold the designated germplasm in trust for the benefit of the international community
1995–1999	Stating that biodiversity loss is not only an important environmental problem, but also a socio-economic, political and ethical problem
28th session of the FAO conference, (Rome, 1995)	
Science academies summit at the M. S. Swami Nathan research foundation, (Madras, 1996)	*Ex situ* conservation of plant genetic resources are essential but must be integrated by in situ, on farm, a community level conservation strategy
4th international technical conference on plant genetic resources, (Leipzig, 1996)	Genetic resources should be conserved and made available to scientists and farmers, but access should be regulated by international agreements
World Food Summit, (Rome, 1996)	
1st extraordinary meeting of the conference of the parties to the CBD, (Cartagena, 1999)	

(continued)

Table 1 (continued)

Event	Some underpinning principle(s)/agreements
2000–2004	Protecting biological diversity from the potential risks posed by living modified organisms (LMOs).
Resumed session of the conference of the parties to the CBD, (Montreal, 2000)	Recognition of farmers' rights access to plant genetic resources and equitable sharing of the benefits derived from their use
31st session of the FAO conference, (Rome, 2001)	
6th ordinary meeting of the conference of the parties to the convention on biological diversity, (Hague, 2002)	The rate of loss is still accelerating and the threats must be addressed
UN world summit on sustainable development, (Johannesburg, 2002)	Ensuring an absolutely requirement of funding for the conservation of plant genetic resources
Convention on biological diversity (CBD, Paris 2002)	The world conservation union (IUCN) red list categories and criteria were preliminary constructed to assess the threatened status of species or lower taxa on a global scale. Red data lists can play a crucial role by focusing attention on species most in need of conservation action
Establishment of the global crop diversity trust (now renamed crop trust) (2004)	
2005–2009	Stated that over the past 50 years, humans have changed ecosystems and have substantial net gains for their well-being, but at growing environmental costs
Publication of the millennium ecosystem assessment (2005)	Degradation of ecosystem services could grow significantly worse during the first half of this century
1st meeting of the governing body of international treaty for plant genetic resources (ITPGR), (Madrid, 2006)	Standard material transfer agreement (SMTA) is the legal instrument through which the multilateral system of access and benefit sharing operates
Establishment of the Svalbard global seed vault, (Svalbard, 2008)	Recognition of the crop trust as an essential element of the treaty's funding strategy, in regard to *ex situ* conservation and availability of plant genetic resources
12th Regular session of the CGRFA, (Rome, 2009)	
36th session of the FAO conference, (Rome, 2009)	*Ex situ* gene bank collections are put under the international treaty for plant genetic resources (ITPGR)
2010–2012	Establishing more predictable conditions for access to genetic resources, helping to ensure benefit-sharing when genetic resources leave the country providing the genetic resources
International technical FAO Conference on agricultural biotechnologies in developing countries (Guadalajara, 2010)	Target for 2020, establishment of an online flora of all known plants
10th meeting of the conference of the parties to the convention on biological diversity, (Nagoya, 2010)	Status and trends of biotechnologies applied to the conservation and utilization of genetic resources for food and agriculture and matters relevant for their future development
13rd regular session of the CGRFA, (Rome, 2011)	Biotechnologies largely used for conservation and use of plant genetic resources in developed countries but many developing countries do not have biotechnological capacities
143rd session of the FAO council, (Rome, 2011)	Need for a roadmap on climate change and genetic resources for food and agriculture

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Table 1 (continued)

Event	Some underpinning principle(s)/agreements
2012–2018 United nations conference on sustainable development (UNCSD), (Rio de Janeiro, 2012)	Need to promote, enhance and support more sustainable agriculture that improves food security, eradicates hunger
14th regular session of the CGRFA (Rome, 2013)	Conserving land, water, and genetic resources, biodiversity and ecosystems and enhancing resilience to climate change and natural disasters
International symposium on forest biotechnology for smallholders, (Foz do Iguacu, 2015)	Importance of genetic resources for food and agriculture for coping with climate change
39th session of the FAO conference, (Rome, 2015)	Biotechnologies can be used in production systems, based on agro-ecological principles, to enhance productivity while ensuring sustainability, conservation of genetic resources and use of indigenous knowledge
FAO international symposium on the role of agricultural biotechnologies in sustainable food systems and nutrition, (Rome, 2016)	Although in June 2017, United States announced the withdrawal for the Paris agreement, in May 2018, 195 UNFCCC members have signed the Paris agreement, and 177 have become party to it
United Nations Framework Convention on Climate Change (May 2018)	The Paris agreement aims long-term goal of keeping the increase in global average temperature to well below 2 °C above pre-industrial levels, and to aim to limit the increase to 1.5 °C, since this would significantly reduce risks and the impacts of climate change

During the period of 1961 to 1991, a large number of scientific and technical conferences, and workshops were held mainly in Europe and United States of America. These activities were among the initiatives on rising the concern about conservation of biodiversity, sustainable use of its components and a fair and equitable sharing of its benefits. For instance, the term genetic erosion was coined at the Technical Conference Plant Exploration, Utilization and Conservation of Plant Genetic Resources of FAO/International Biological Program, held in Rome in 1967 to describe this loss of individual genes and combinations of genes. The concept of biodiversity was first conceived by Walter G. Rosen from the National Research Council/National Academy of Sciences (NRC/NAS) in 1985, while planning to conduct a forum on biological diversity (Sonnino 2017; Kopnina et al. 2018).

During the last decades of twentieth century, plant genetic resources-related activities were primarily focused on the collection and *ex situ* conservation of germplasm. E*x situ* conservation strategy, which was suggested in 1973, was reinforced in 1981 by the FAO/IBP Technical Conference on Genetic Resources. However, several scholars expressed concern that the storage of seeds in gene banks (*ex situ* conservation) not allowed natural evolution to proceed (Brown and Hodgkin 2015). Centre for Plant Conservation (CPC, 1991) set *ex situ* guidelines range from relatively small targets (e.g. collection of seed from 10 individuals in each of five populations) to comprehensive collections of germplasm. Furthermore, in 1996, the Leipzig Declaration appropriated both the *ex situ* and *in situ* approaches, consider-

ing them as not mutually exclusive, but complementary components of conservation programs. The Convention on Biological Diversity (CBD) promotes *ex situ* conservation, via the establishment of protected areas and natural parks. In addition, on-farm conservation is often adopted to grow, utilize and conserve landraces, native varieties and other local materials, within their original landscapes and traditional farming systems (Kopnina et al. 2018).

The United Nations Conference on Environment and Development (UNCED) (also known as the Rio de Janeiro Earth Summit) was a major United Nations conference held in Rio de Janeiro from 3 to 14 June 1992. In 2012, the United Nations Conference on Sustainable Development was also held in Rio de Janeiro from 13 to 22 June, and is commonly called Rio + 20 or Rio Earth Summit 2012. Among issues addressed in these two conferences included systematic scrutiny of patterns of production of toxic components, such as lead in gasoline, or poisonous waste including radioactive chemicals, alternative sources of energy to replace the use of fossil fuels which delegate linked to global climate change, new reliance on public transportation systems in order to reduce vehicle emissions, congestion in cities and the health problems caused by polluted air and smoke and the growing usage and limited supply of water (Ogwu et al. 2014; Kopnina et al. 2018). The Convention on Biological Diversity was opened for signature at the Earth Summit, and made a start towards redefinition of measures that did not inherently encourage destruction of natural eco-regions and so called uneconomic growth. USA failed to sign the proposed Convention on Biological Diversity. In order to ensure compliance to the agreements at Rio delegates to the Earth Summit established the Commission on Sustainable Development. In 2013, the Commission on Sustainable Development was replaced by the High-level Political Forum on Sustainable Development that meets every year as part of the United Nations Economic and Social Council (ECOSOC) meetings, and every fourth year as part of the General Assembly meetings. Critics point that many of the agreements made in Rio have not been realized regarding such fundamental issues as fighting poverty and cleaning up the environment (Sonnino 2017; Kopnina et al. 2018).

3 Genetic Resources, Conservation and Values

3.1 Genetic Resources

Genetic resources are genetic materials of actual or potential values that are used in the future improvement of crops utilized in food, spice, medicine, fuel, fodder and building material production. Genetic resources consist of genotypes or population of landraces, advanced cultivars, domestically bred cultivars, old local cultivars, genetic stocks, wild relatives and weedy species which are maintained in the form of seeds, plants, tissues etc. Genetic resources should be properly monitored in order to reduce the risk of within-gene bank erosion or *in situ* conservations. In the

absence of such monitoring, some unique germplasm accessions are lost and this reduces the biodiversity coverage present in a gene bank collection or *in situ* conservations (Krishnan et al. 2013; Brown and Hodgkin 2015; Kopnina et al. 2018).

Genetic resources of crop wild relatives on earth are not evenly distributed geographically, therefore, there exist great differences among the gene banks in terms of number and biodiversity. Due to global warming conditions efforts to collect germplasm of crop wild relatives have gradually increased (Castaneda-Alvarez et al. 2016). However, gene bank capacities and low funding limit the success of these efforts. Crop wild relatives usually have low germination rate, and require taxonomic evaluation, specialized pollinators and different life cycle. Many gene banks have insufficient capacity to maintain both old and newly acquired germplasm, affecting the efficacy of long-term germplasm conservation (Ogwu et al. 2014; Kopnina et al. 2018).

Conservation of genetic resources for sustainable agriculture is the art and science for the benefit of genetic improvement of crops in present and future generations. Researchers and staff involved in germplasm conservation through *ex situ* and *in situ* methods are expected to have knowledge and experiences in a variety of fields including biology, molecular biology, molecular genetics, plant systematics, population genetics, plant pathology, plant physiology, plant ecology, biochemistry, computer science, legal science, economics, and political science. This indicates that conservation teams should have special training, however, there is no specific institutes providing comprehensive professional training in the germplasm conservation. In the developing countries many researchers and staff working in gene banks since the 1970s have retired or will retire soon (Fu et al. 2015). That means some useful knowledge and experience in germplasm conservation for sustainable agriculture are being lost without replacing young and dynamic researchers due to restricted financial supports.

Gene banks in different parts of the world may suffer catastrophic events and can collapse. For instance, the N. I. Vavilov Institute of Plant Genetic Resources was damaged during World War II. Genetic resources in gene banks of Guinea-Bissau, Liberia and Sierra Leone have been damaged due to civil wars. Afghanistan's gene bank in Kabul and Iraq's Abu Ghraib national gene bank in Baghdad were looted. Syria's gene bank at the International Center for Agricultural Research in the Dry Areas (ICARDA) in Aleppo was probably damaged during civil war although the center has been relocated to Terbol, Lebanon (Bhattacharya 2016). Some gene banks have been destroyed due to natural disasters too. For instance, national seed bank of Nicaragua was lost in the 1971 earthquake. The national seed bank of Honduras was demolished by hurricane Mitch in 1998. The Thai gene bank was flooded and some of the 20,000 unique rice accessions were lost forever in 2011. The typhoon Milenyo damaged the Philippines' national gene bank in 2006 and was again destroyed by fire in January 2012. Destruction due to increased frequency of flooding, typhoons and civil war disasters rationalized for constructing the Svalbard Global Seed Vault in 2006 for long-term safety backup of valuable germplasm (Fu et al. 2015; Fu 2017).

3.2 Conservation of Genetic Resources

Genetic resources can be conserved either *in situ* (in their natural setting) or *ex situ* (outside their natural setting). *Ex situ* conservation is the dominant method of conserving natural ecosystem. On the other hand, *ex situ* conservation is commonly used by plant breeders. However, agricultural resources can also be held *in situ.* Many farmers developed landraces contain significant diversity and encouraging use of these varieties is one method to conserve agro-biodiversity *in situ*. Wild relatives of cultivated varieties may also be conserved *in situ* on wild land. More recent approaches view the *ex situ* and *in situ* forms of conservation as complimentary, rather than as substitutes (Ogwu et al. 2014; Kopnina et al. 2018).

The *ex situ* conservation method needs to obtain genetic materials from their ecological environment and grow them in different environment for long-term conservation. *Ex situ* examples include gene banks, national parks, botanic gardens, arboretums, museums, zoos and protected areas. Compared to *in situ* conservation certain methods of *ex situ* conservation can be used to store large amounts of genetic material at relatively low cost. Gene banks can hold a large amount of germplasm resources, for instance, the world's gene banks presently hold more than four million accessions, or specific samples of certain crop varieties (Ogwu et al. 2014). On the other hand, the *in situ* conservation method does not remove the genetic materials from their environment, instead genetic materials remain in their natural habitats. Most of the world's genetic diversity is found *in situ*. For agriculturally important species, the greatest diversity in landraces and in wild relatives may be found in or near their primer and seconder centers of origin, or the places in which they were first domesticated (Uygur Gocer and Karaca 2016b; Kopnina et al. 2018; Li et al. 2018).

Conservation of *ex situ* genetic resources is an efficient and economical way by seed conservation (seed bank). Seed bank represents the most cost-effective *ex situ* conservation strategy. Although most crop seeds can be stored for long periods under low relative humidity and low temperature conditions, it is not feasible for some species that cannot stand desiccation below a relatively high critical water content value (10–12% or 20%). Therefore, seed bank conservation is for the storage of predominantly orthodox seeds to maintain the allelic integrity and identity of a sample (Chandrakant et al. 2017). For those non-orthodox seeds gene bank requires essential infrastructure for short- and long-term seed storage, but also the efficient management of germplasm from safety backup to regeneration and characterization, germplasm distribution, and data management (Hernandez-Suarez 2018; Li et al. 2018).

Conservation of *ex situ* genetic resources may consist of following procedures; (i) collection, identification and characterization, (ii) regeneration, (iii) conservation, (iv) data management, (v), distribution, (vi) evaluation for subsequent use, (vii) acquisition, (viii) characterization, (ix) re-evaluation for supportive research and germplasm enhancement. All newly arrived plant seed samples are controlled for health and purity. Primarily a seed viability and some other related tests are

made and seed samples having the required standards are dried and stored. Seed drying is an important step to assure a long viability of seeds and it is carried out gently using temperatures below 25 °C. Seeds are dried to 4–8% moisture, filled into glass containers with vapor proof covers and placed into moving shelves in cooled chambers. The storage temperature is below −18 °C for the base collections. Seed viability as well as seed supply are regularly monitored during long-term storage. Regeneration of multiplied accessions is initiated when one of these parameters drops below the standard level. Recent years witnessed dramatic development in freezer technologies and thus ultra-low temperature freezers became much cheaper for preserving excised embryos, embryonic axes, or dormant buds of many non-tropical and some tropical species, avoiding the need to replace liquid nitrogen as it evaporates. It is likely that cryopreservation will become cheaper and easier and, become more widespread in next decades (Ogwu et al. 2014; Fu et al. 2015; Fu 2017; Li et al. 2018).

The information about the level of allelic diversity of a species is very important to capture a high proportion of the total genetic diversity in *ex situ* conservation such as gene bank. Genetic screening studies would provide information about the genetic diversity of the population. It is possible that a large number of individuals can be represented by a few genotypes or a few individuals can represent a large number of genotypes. Because genetic drift in gene banks is caused by the use of inadequate sample sizes, greater numbers of accessions are stored to guarantee that a particular proportion of possible genotypes has been preserved, but this can lead to prohibitively large sample sizes. Therefore, coverage of a sample is very important for conservation of biodiversity for sustainable agriculture. In simpler terms, the coverage of a sample can be defined as the fraction of individuals in the population that is represented in the sample. Clearly, the goal of conservation of biodiversity and genetic resources for sustainable agriculture is to achieve high coverage at all loci and accurately estimate the proportion of existing alleles in a genome that is included in an accession. The use of next generation phenotyping and genotyping method could be very useful on screening of accessions (monitoring) for high coverage and high level conservation of biodiversity in *ex situ* approaches (Truong et al. 2012; Karaca and Ince 2017).

3.3 Values of Genetic Resources

Today the agriculture of virtually every country is heavily dependent on a supply of genetic resources from other parts of the world. The United States of America and Australia, for example, place considerable reliance on many species originating in other regions of the world for their major food and industrial crops. Sub-Saharan Africa is estimated to be 87% dependent upon other parts of the world for the plant genetic resources, and the figure is estimated to be about 90% in Europe and 62% in East and Southeast Asia. Many countries hold a significant amount of plant genetic diversity in their gene banks, farmers' fields and natural habitats for food and

agriculture. In the medium- to long-term, however, these countries are likely to require additional genetic resources from the crop species' centers of diversity, the majority of which are restricted to eight crop diversity hotspots identified early in the last century (Vavilov 1926). Although developing countries contain many of the Vavilov centers of crop diversity and therefore have much of the world's genetic resources. However, many of developing countries struggle to conserve genetic resources, and they have limited technologies of advanced molecular and genomic tools and the corresponding expertise to use the genetic wealth for their own benefit. The onus is on the developed countries to work with those developing countries to conserve agricultural plant genetic resources, including diversity of crop wild relatives for sustainable agriculture (Phelps and Webb 2015; Castaneda-Alvarez et al. 2016; Vavilov 1926; Karaca and Ince 2017).

It is not possible to determine an organism's value if it is not used in direct or indirect by humans. However, if the organism is valued in agriculture (including all types of activities such as landscaping, forestry, arboriculture, horticulture, floriculture, viticulture, aquaculture etc.), its value arises from the direct use of genetic resources for sustainable agriculture. Direct use values include the use of genetic resources to produce food and fiber, or to help create new varieties of crops. Otherwise the value of genetic resources is not typically revealed by markets, because genetic resources are not directly traded in the markets. Conserved genetic resources could have more economic value in the future even if the resources are not currently being used or known. Therefore, an organism that is not presently economically valued, may have considerable value in the future, though this value is difficult to measure at the present. For instance, prior to 1980s, the economic value of bacterium *Thermus aquaticus* that lives in hot springs and hydrothermal vents was not known. *Taq* DNA polymerase of this bacterium and polymerase chain reaction brought $2 billion in royalties (Polat et al. 2010; Korun et al. 2013; Timmermann and Robaey 2016; Pavan et al. 2017).

Plant genetic resources could be used by breeders to develop new and improved varieties for agricultural production. This process of genetic enhancement has produced substantial economic benefits for the producers and plant breeders. Plant breeders are requested to provide genetic diversity to farmers' fields in order farmers produce the agricultural products. Therefore, well conserved genetic diversity and genetic resources such as crop wild relatives and landraces have replicable values in the development of new varieties resistant to biotic and abiotic stresses, as they are drought, insect, pest, disease tolerant and resistant to biotic and abiotic stresses (Sharrock et al. 2014; Timmermann and Robaey 2016; Li et al. 2018).

Due to the genetic recombination, gene flow, mutations and the intensities of pest pressure in the field farmers need to replace their seeds used for production. Although depending on many factors it was estimated that new varieties are resistant for an average of about 5 years, while it generally takes about 10 years to breed new varieties (Karaca 2001; Ince and Karaca 2011c). Breeders often rely on landraces, old cultivars or crop wild relatives as a last resort to gain alleles of interest from these materials (Ince et al. 2010g). These genetic resources are constantly required as repertories into the continuing process of enhancement through selective breeding

(Ince et al. 2010h). Because pests and diseases evolve over time, new alleles and epialleles are continually needed to transfer from outside the utilized stock, landraces, and wild relatives to maintain or improve yields (Ince and Karaca 2011a, 2016). Genetic resources are not only utilized to transfer resistance to pests and diseases, and tolerance to non-biological stresses, such as drought but also include rapid and simultaneous germination, flowering, and maturation of crops (Ince et al. 2011b; Ogwu et al. 2014; Ince and Karaca 2015a, b; van Kleunen et al. 2015; Uygur Gocer and Karaca 2016b).

4 Biodiversity

Biodiversity refers to variation in number and frequency within the natural system. In another word it refers to the variety of all forms of life in the world. Reduction or decline in genetic diversity, also called genetic erosion, in many commercially important plant species has been observed (Ince and Karaca 2012; Aavik et al. 2017; Dorey and Walker 2018). One reason for this decline in diversity has been the loss of genetic resources such as landraces and wild relatives of cultivated crops. It is a widely held belief that modern agriculture, particularly the transition from landraces to modern varieties as exemplified in the twentieth century's green revolution, has profoundly narrowed the genetic base of modern crop varieties. In the broadest sense, however, genetic alteration and narrowing began with the first domestication of wild plants (Karaca and Ince 2008, 2016; Ince et al. 2010i; Ogwu et al. 2014; Aavik et al. 2017; Manhaes et al. 2018).

Principle threats causing adverse effects on the status or sustainable use of any component of biological diversity include habitat alteration or destruction, overharvesting or over-exploitation of biological resources, weather, water or soil or biological pollution, introduced or invading species, climatic changes, and expanding human population (Karaca et al. 2015). Increased demand for resources results to land use changes hence loss to genetic diversity, species reduction and increased ecosystem changes such as random population changes, habitat fragmentation and many others resulted in biodiversity losses. Population size and habitat fragmentation differ in response to inbreeding depression and environmental adaptations. Levels of genetic diversity are affected from population size along with habitat fragmentation. Therefore, if not well conserved, *ex situ* populations can become genetically different from their original source populations, may lose adaptation to their source environment, and may become inbred (Karaca et al. 2015; Aavik et al. 2017; Li et al. 2018).

Major sources of threats for genetic diversity include (i) plant loss, fragmentation, and degradation, (ii) over-exploitation (including over-collection and overgrazing), (iii) invasive species, (iv) increased air, soil water or biological pollution and nitrogen deposition, (v) severe climate change and (vi) wrong land uses and urbanization (Shearman et al. 2012; Ogwu et al. 2014; Sharrock et al. 2014; Goettsch et al. 2015; Phelps and Webb 2015; Specht et al. 2015; ter Steege et al. 2015;

Thomas and Palmer 2015; Corlett 2016; Timmermann and Robaey 2016; Dorey and Walker 2018). Plant loss, fragmentation, and degradation are among most important threats to plant diversity particularly in the tropics. Tropical forests have been replaced with monoculture of oil palm, rubber, soybean (ter Steege et al. 2015). Also logging, fire, and other impacts, including fuelwood harvesting in densely populated areas are among the main threats in developing countries and regions (Specht et al. 2015). Furthermore, mining of stone, construction of wide roads in the regions of endemic plant species are among other threats (Ince et al. 2014; Ogwu et al. 2014; Dorey and Walker 2018).

Over-exploitation (including over-collection, over-harvesting and over-grazing) of the whole plant, seeds or reproduction systems reduces the chance of survival. Over-harvesting results when individuals of a particular species are harvested at a higher rate than that they can be sustained by the natural reproductive. This could lead to extinction of the biological resources, eventually leading to loss of species. It is known that over-exploitation is usually species-specific and it is well correlated with its value. Horticultural trade for private collections is important threat to some plant species such as cacti, orchids as well as cycads and ornamental species (Ogwu et al. 2014; Sharrock et al. 2014; Goettsch et al. 2015; Phelps and Webb 2015; Dorey and Walker 2018; Li et al. 2018). In some other plant species overexploitation of animals may also threaten plant species in the long term, by restricting seed dispersal or pollination. Also in the tropical forests, it is known that over-logging is the main threat factor (Shearman et al. 2012; Dorey and Walker 2018).

Species that are not habitats but were introduced in an ecosystem may cause changes in the host (existing) ecosystem. Introduced species are those species arising in areas/habitats in which they were previously not native. Such some introduced species could refer to as biological pollutants. These kinds are also called as invasive alien species that have potentially threat to native species. A study of van Kleunen et al. (2015) showed that more than 13,000 species of the world's vascular plant flora naturalized somewhere outside their native range as a result of human activity. It is known that invasive plant species can reduce native plant diversity by changing hybridization, out competition, disruption of original ecosystem, plant pathogenic influences, disease transmission, fire regimes, nutrient cycling, pollen transfer and some other physiological requirements, disruption of food webs and to some situations extinction (Thomas and Palmer 2015; Dorey and Walker 2018; Manhaes et al. 2018).

Any chemical, thermal, air, soil, water or biological pollution is a threat to biodiversity. Species in habitats are increasingly being harmed by industrial activities and pollution from excessive use of chemicals such as dichlorodiphenyltrichloroethane (DDT), oil spills, acid precipitation etc. Due to human activities the concentration of the major greenhouse gases (CO_2, CH_4, N_2O) changed, thus, plants on earth today are exposed to an atmosphere that differed significantly in composition from any that their ancestors experienced. Burning of fossil fuels is the major source of air pollution and primary pollutants are SO_2 and NO. Ozone produced from hydrocarbons and nitrogen oxides in the presence of sunlight is the most important secondary pollutant. According to Corlett (2016) air pollution is declining in Europe

and other developed regions, but increasing in much of Asia. Zhu et al. (2015) informed that wet and dry deposition of nitrogen compounds dramatically changed nutrient cycles in southern China due to its acidifying effects on the soils.

Impacts of climate change caused by humans are complex and mostly unpredictable, and even more pervasive. The rate of evolution of plant species is driven by their genetic makeup and mainly climate. When the climate changes in a local area in where plant populations adapt, a plant can either adjust physiologically within the lifetime (acclimation), or evolves by evolutionary changes over multiple generations (evolution), or move to some other places where with a more suitable climate (migration), or vanish (extinction). Although the problem is not directly attributable to climate change, it has been reported that crop yields of wheat, barley, and canola have been reduced by over 40% in Australia due to drought (Hijioka et al. 2014). It has been estimated that rising temperatures and reduced precipitation would affect semiarid regions and reduce yields of maize, wheat, and rice over the next 20 years. It is known that extinctions of some species at local have occurred at the climatic margins of species ranges (Buse et al. 2015) and some species have extended their ranges escaping regions where the high temperature and water are limiting factors (Hijioka et al. 2014; ter Steege et al. 2015; Castaneda-Alvarez et al. 2016).

Land uses, urbanization, hydroelectric dam construction, road construction for transportation and competitions in global market economies do strongly contribute indirect negative effects on biodiversity. Also the use of alien species and chronic weed infestation have increased the number of threatened species. A significant of damage for the biodiversity is also caused by collection for local and global markets, often by professional collectors. Many countries have laws against inappropriate collections, but often commercial collection makes use of legal loopholes which urgently need to be closed (Corlett 2016). Furthermore, it is known that many protected areas fail to prevent over-exploitation of valuable plants. In these regions *ex situ* conservation requires effective monitoring to ensure that viable plant populations of threatened species persist within protected areas. Unfortunately, in many developing countries many protected areas are subject to encroachment by farmers or their fires. For instance, it has been reported that the expansion of rubber plantations and the promotion of biofuel crops such as physic nut (*Jatropha curcas*) in southern Yunnan's Xishuangbanna region in China was reduced two-thirds of a unique rainforest (Heywood 2015; Corlett 2016; Chandrakant et al. 2017).

5 Next Generation High-Throughput Phenotyping

Next generation high-throughput phenotyping consists of collection of huge quantities of image data and safe storage, fast and well-organized working flow, economical and time-saving analysis procedures, and dissection of objective data (without influence of human perception). The lack of accession-level information on conserved germplasm is one of the major limitations to wider germplasm utilization and conservation for sustainable agriculture. Varying among the gene banks around the

world a majority of the conserved genetic resources show only with basic germplasm description records such as passport data. However, due to a lack of detail in the passport data, factors influencing genetic diversity like sampling strategy, regeneration procedures and selection during regeneration could not be well reconstructed, fortunately next generation phenotyping approaches could be used to add valuable and detailed information to passport data that could be used in sustainable agriculture (Cobb et al. 2013; Song et al. 2015; Afonnikov et al. 2016; Walter et al. 2018).

Many activities may be classified as genomic research or simply genomics, including mapping the genome of an organism; sequencing a single individual or several individuals from a given species; identifying a large number of genes; studying genetic variability within species; studying genetic similarities and differences within and among species; discovering a large number of genes' function, and the relationship between gene structure, protein synthesis, and metabolic pathways; studying gene regulation, including gene activation and silencing; studying gene interaction and phenomena dependent on many genes (Ogwu et al. 2014; Karaca and Ince 2017).

Genomics is expected to provide a comprehensive view of genetic capacity, however, the information it contains is cryptic and does not directly explain the differences between cells and all plant phenotypes. On the other hand, some phenotypic traits provide more direct information about plant production and health than genomic data. The recent improvement in phenotyping methods enable us to broaden the concept of phenotyping and include both molecular mechanisms (proteomics and metabolomics) and all intermediate layers that result in macroscopic, physiological and phenological traits (Ubbens and Stavness 2017).

Phenotyping can be performed at different depth scales such as high or low resolution, and high or low throughput volumes. High-throughput techniques in general involve the analysis of the whole plant with medium-low resolution, therefore, it is suitable for conservation of biodiversity and genetic resources for sustainable agriculture. High-throughput techniques could be used in *ex situ* and *in situ* conservation fields for phenotyping and crop monitoring which could allow the screening of hundreds of accessions per day in a nondestructive manner with automated systems. The integration of genomics and phenomics has promised to revolutionize the field of plant breeding indicating that these high-throughput methods can be used in conservation of biodiversity and genetic resources for sustainable agriculture (Breccia and Nestares 2014; Lobos et al. 2017; Sun et al. 2017.

Phenomics is driven by large-scale and economical generation of phenotype differences coupled with increasingly sophisticated and comprehensive sensors and cameras called high-throughput phenotyping (phenomics). The aim of plant phenomics is to characterize all the possible phenotypes under different environmental conditions of a given genotype or species. For that purpose, phenomics includes phenotyping at multiple levels of organization (ranging from cellular components to whole plant and canopy level) and comprises structural, physiological, and performance-related traits (Busemeyer et al. 2013; Breccia and Nestares 2014; Lobos et al. 2017; Sun et al. 2017; Crain et al. 2018; Thompson et al. 2018; Walter et al. 2018).

Satellite imaging technologies have become an extremely useful tool for collecting data for various agricultural applications including conservation of biodiversity and genetic resources. However, the major limitations of using the currently available satellite sensors include the high cost, the lack of spatial resolution for the identification of desirable traits, the risk of cloudy scenes and the long revisit periods. To data many field-based high-throughput phenotyping methods and platforms have been developed for high-throughput phenotyping. Some of the platforms use push carts, tractor mounted systems and aerial vehicles (Crain et al. 2018). Advanced plant phenotyping platforms include phenomobiles, phenotowers and blimps equipped with a global positioning system, navigation device and sensors, however, although performing well, they are quite costly, data acquisition and handling needs for specialized personnel (Araus and Cairns 2014; Lausch et al. 2017; Habib et al. 2018; Tripodi et al. 2018). Therefore, these platforms are not easily affordable in many developing countries where they are needed most. Although still often used in practical phenotyping, manual measurements of crop traits have significant limitations and drawbacks such as they are time-consuming, labor intensive and subject to human error due to fatigue and distractions during data collection (Arend et al. 2016; Yang et al. 2017; Jimenez-Berni et al. 2018).

Studies of conservation of biodiversity and genetic resources for sustainable agriculture could utilize phenotyping and monitoring technologies. These technologies include spectral laboratory and phenomics facilities, close-range, airborne and satellite approaches (Lausch et al. 2017). For phenotyping purposes, images obtained from satellite, manned and unmanned aerial vehicles typically have a low spatial resolution (in the context of *ex situ* and *in situ* conservation), poor sensitivity under cloudy conditions, and slow data transmission and expensive. Most long-distance remote sensing technologies could sufficiently capture the fine data suitable for the studies of conservation of biodiversity and genetic resources for sustainable agriculture. On the other hand, spectral laboratory and plant phenomics facilities provide biochemical-biophysical, structural variables in organs (roots, leaf, stem) and whole plants. These close-range remote sensing methods or platforms include field spectrometers, wireless sensor networks, towers and next generation unmanned aerial vehicles provide taxonomic, phylogenetic, genetic, epigenetic or morphological-functional features. Therefore, spectral laboratory and phenomics facilities could be used to detect biochemical-biophysical and morphological traits (Lausch et al. 2017; Jimenez-Berni et al. 2018; Thompson et al. 2018).

Among the phenotyping and monitoring technologies proximal sensing carts and unmanned aerial vehicles (UAV), both can be called as phenomobiles, are suitable in the application of conservation of biodiversity and genetic resources for sustainable agriculture. UAVs are equipped with multiple sensors [some of visible light imaging sensors, spectral sensors, infrared thermal sensors, fluorescence sensors, digital camera (RGB), multispectral camera, infrared thermal imager, hyperspectral camera, Light Detection and Ranging (LiDAR), three-dimensional camera and synthetic aperture radar (SAR)], using communication technology and GPS positioning technology to rapidly and non-destructively acquire high-resolution images (Table 2). The typical UAVs used for phenomics include multi-rotors, helicopters,

Table 2 Some sensing and imaging techniques used next generation phenotyping

Technique	Short description of the technique
Magnetic resonance imaging (MRI)	It is based on nuclear magnetic resonance (NMR) analysis, allows measuring resonance signals produced from H, C, and N isotopes. With this technology, 3D acquisition can be accomplished to acquire information on plant phenotype with high resolution
Tomography imaging	It uses radio frequency (RF) magnetic fields to construct tomographic images. It produces imagines by sections or sectioning, through the use of any kind of penetrating wave. For instance, x-ray computed tomography (x-ray CT) employs x-rays to produce tomographic images of specific areas of the scanned object. The process of attenuation of rays together with a rotation and axial movement over objects produces 3D images
Light detection and ranging (LiDAR)	It uses short pulses of laser light distributed from a scanning device across a wide area and their reflections from different objects are recorded by the sensor. It produces set of 3D points, which represent the scanned surfaces from where the pulses were reflected. LiDAR provides an alternative approach for 3D plant model reconstruction.
Synthetic aperture RADAR (SAR)	SAR uses a receiver (an antenna) to transmit microwave pulses in a specific waveband (or frequency) at an oblique angle to the target area. Radio waves that are reflected off the object back (from the target area) to the source can be acquired in a variety of modes
The time of flight camera (ToF camera)	It is one of the recent imaging devices to be incorporated into automatic plant phenotyping. ToF has as a general principle the measurement of the distance between the objective of the camera and each pixel. This is achieved by measuring the time it takes for a signal emitted in near infrared (NIR) to come back, reflected by the object. This allows a precision 3D reconstruction.
Multispectral imaging sensor	Multispectral imaging sensors are defined as hardware that are capable of sensing and recording radiation from invisible as well as visible parts of the electromagnetic spectrum, which have been widely used for crop phenotyping due to the advantages of low cost, fast frame imaging and high work efficiency; however, they are limited by the low number of bands, low spectral resolution, and discontinuous spectrum. Hyperspectral imaging sensors are cameras that can obtain a large number of very narrow bands and continuous spectra. Compared with multispectral imagers, hyperspectral imagers have the advantages of more band information and higher spectral resolution and can accurately reflect the spectral characteristics of the crop in the field and the spectral differences between crops
Hyperspectral imaging sensor	
Thermography imaging/Thermal imaging	Thermographic cameras are able to acquire images at wavelengths ranging from 300 to 14,000 nm allowing the conversion of the irradiated energy into temperature values once the environmental temperature is assessed. Thermal imaging uses infrared detectors and an optical imaging lens to receive infrared radiation and produces time series or single-time-point analysis based data
Fluorescence imaging	It belongs to spectroscopy but differs greatly from reflectance, absorbance, and transmittance measurements in the way by which plant tissues interact with electromagnetic radiation. It uses a low-light camera/sensor and appropriate filters to collect fluorescence emission light from samples

fixed-wing, blimps and flying wing. Among this one or more are selected based on the purpose and budget (Busemeyer et al. 2013; Sun et al. 2017; Virlet et al. 2017; Thompson et al. 2018).

Past three decades witnessed the development of large number of phenotyping technologies. Those phenotyping techniques using high-throughput and high resolution are called next generation phenotyping methods that contain more than one sensing (multi-sensor) approaches or platforms. It has been shown that sensor characteristics (spatial, radiometric, spectral, temporal or angular resolution) and the sensing approaches (hyperspectral, multispectral, digital (RGB), LiDAR, SAR and passive microwave sensors) show different level of discriminate between certain plant species, populations, communities, habitats. Advanced phenotyping methods and platforms based on multi-sensor remote sensing would be able to discriminate and monitor threatened plant species or invasive species, bio-pollutants, the pattern and spatial distribution and diversity of plant species and communities as well as natural disasters and disturbance regimes, i.e., volcano eruptions, wildfires, beetle infestations, and the global carbon cycles (Perez-Sanz et al. 2017; Sun et al. 2017; Virlet et al. 2017; Thompson et al. 2018).

Various studies have shown that the implementation of multi-sensor approaches improves the discrimination of plant properties over time and thus the accuracy of estimation of population indicators. Multi-sensor systems on single platform enable the simultaneous acquisition of information related to different spectral traits and ensure the same illumination conditions, weather conditions and flight parameters for all mounted sensors. The package of a platform may include RGB camera, infrared thermometers, active spectral reflectance, and light or ultrasonic sensors. Next generation phenotyping platforms can be classified considering many different characteristics. For instance, they can roughly be divided into the categories of point sensors (spectro-radiometers and fluorimeters) and imaging sensors that allow the acquisition of spatial information of the detected data. We classified some phenotyping techniques based on the sensors and platform and depicted in Table 2.

Recent developments in remote and proximal sensing for high-throughput field phenotyping have led to proposed alternatives to the destructive sampling, including the use of digital photography and sensors, across multiple scales, using both aerial and ground platforms. High-throughput phenotyping spectral traits (Table 3) suitable for conservation of biodiversity and genetic resources for sustainable agriculture include plant structure and morphogenetic traits; abiotic and biotic stresses, adaptation to abiotic and biotic limiting conditions, metabolomics traits, quality traits and physiological traits (Perez-Sanz et al. 2017; Yang et al. 2017; Espina et al. 2018; Jimenez-Berni et al. 2018).

Next generation phenomobiles equipped with infrared thermal imagers can quickly and non-destructively acquire the crop canopy temperature, which can effectively identify the temperature differences in the crop canopy under different environmental conditions. The canopy temperature can be used to predict plant yield when a significant positive correlation between lower canopy temperature and higher yield under conditions of high temperature and drought exists. Leaf water potential could be estimated since the stomatal closure results in the leaf tempera-

Table 3 Spectral traits suitable for conservation of biodiversity and genetic resources

Category	Traits suitable for analysis
Structure and morphogenetic traits	These traits include plant height, chlorophyll content, biomass, yield, length of the growth period, flowering, crop canopy cover, canopy spectral texture
Plant physiological traits	These traits include chlorophyll, pigment content, carotenoids, pigment indices photosynthesis, protein content, malnutrition, crop vigor and water status
Plant yield and quality traits	These traits include total oil, protein, starch, moisture content, fatty acid and amino acid compositions. Yield prediction is defined as building the relationship between the canopy spectra and crop yield based on the biological characteristics of crops for yield prediction using spectral data at different crop growth stages
Plant geometric traits	These traits include crop height, vegetation cover fraction, fraction of intercepted radiation, leaf area, leaf area index, lodging, 3D structure, leaf angle distribution, tiller densities, and emergence
Plant biotic and abiotic stress	These traits include water stress and deficit, low temperature, high temperature, high salinity, environmental pollution, susceptibility to pests and diseases, stomatal conductance, canopy temperature difference, leaf water potential, senescence index
Metabolomic traits	These traits include flavor, phenolic, vitamins, sugars, organic acids, and volatile compounds. Metabolomics plays a remarkable role in assessing genotypic and phenotypic diversity in plants, in defining biochemical changes associated with developmental changes during plant growth and, increasingly, in compositional comparisons
Quality traits	These traits include fatty acid and amino acid compositions, fiber quality, nitrogen concentration and protein content, seed traits such as total oil, protein, starch, moisture content
Ground canopy cover	It is an important parameter related to the crop photosynthesis and transpiration. It is dynamic during the crop growth stages and is reduced as a result of leaf rolling or wilting under drought stress conditions, which can be used for studying the response of crop varieties under abiotic/biotic stress.
Qualification and selection	These traits include leaf/pod/fruit counting, vigor ratings, injury ratings, disease detection, age estimation, and mutant classification

ture increase under osmotic stress caused by excess salinity and high temperature. Also drought and salinity can induce the same effects on stomatal conductance and photosynthesis (Hoyos-Villegas et al. 2014; Tripodi et al. 2018).

Crop yield of conserved genetic resources could be estimated using next generation phenotyping approaches such as using phenomobiles. Since the crop canopy temperature is related to photosynthesis, the canopy air temperature difference, which is the ratio of the canopy temperature and air temperature, can be used to predict crop yield when there is a significant negative correlation between the air temperature difference and yield of plant as seen in sorghum. In wheat it has been seen that there existed a significant positive correlation between air temperature difference and wheat yield under water stress conditions. The water deficit index obtained from thermal imaging data can be used to determine the water status of crop

leaves and to estimate the stomatal conductance (Rascher et al. 2011; Ecarnot et al. 2013; Simko et al. 2016; Padilla et al. 2017; Crain et al. 2018; Tripodi et al. 2018).

Cell structures could be estimated using next generation phenotyping methods. The reflectance of plant leaves in visible light (about 390–700 nm) is affected by the contents of chlorophyll, carotene and lutein in the palisade tissue. The reflectance of plant leaves in the near-infrared (NIR) band is closely related to the cell structure and can be used to estimate several spectral traits including plant physiological trait, geometric traits and ground canopy cover (Perez-Sanz et al. 2017; Espina et al. 2018; Jimenez-Berni et al. 2018). Biodiversity and genetic resources could be validated, monitored or conserved using plant cell structures based on the next generation phenotyping techniques and platforms such as phenomobiles. Phenotypic information plays an important role in revealing the resistance of crops to stress, therefore, rapid phenotyping is also essential for conservation of biodiversity and genetic resources for sustainable agriculture. Infrared canopy temperatures provide an efficient method for rapid, non-destructive monitoring of whole plant or population response to water stress, which has been widely used to screen drought tolerance in domesticated plant species. Biotic and abiotic stress factors, including water deficit, low temperature, heavy metals, high temperature, high salinity, environmental pollution, pests and diseases, can have significantly adverse effects on plant growth and development. Abiotic stress during early canopy development can decrease plant biomass and height, reduce leaf area, and abbreviate green area duration. Effects of most of these biotic and abiotic stress factors affect plant's membrane permeability, the chlorophyll content, hormone and enzymatic activities under stress conditions, thus, they can be detected by spectroscopy at early growth stage if an effective correlation or regression method is available (Liebisch et al. 2015; Crain et al. 2017, 2018).

The absorption and reflection characteristics differ between spectral bands in the crop leaves, with strong absorption in the visible band and strong reflection in the near-infrared band, providing the physical basis of crop growth monitoring by remote sensing suitable for conservation of biodiversity and genetic resources. Digital cameras in the range of visible spectrum (400–700 nm, VIS) allow capturing 2D images in which raw data are recorded in the red (about 600 nm), green (about 550 nm), and blue (about 450 nm) array using charge coupled device (CCD) or complementary metal oxide semiconductor (CMOS) silicon-made sensors. These kinds of 2D images show many limitations, especially when used for plants that have a high degree of structure complexity, therefore, 3D images are preferred for the estimation of plant biomass, leaf area and leaf area index, and plant morphology. The use of stereo cameras and computer programs produce 3D images taken by multiple angulations allow drawing sophisticated models for the reconstruction of plant structures. Also digital cameras offer further characteristics that deal with plant color analysis, however, to the specific purpose of plant structure and biomass analysis, the most widely adopted technologies are based on light detection and ranging (LiDAR) by using laser-scanner sensors. LiDAR provides direct measure-

ments of canopy architecture and organ distribution for the estimation of plant volume, leaf area index, and biomass. LiDAR allows plant growth analyses from the vegetative to reproductive stages (Jin et al. 2017; Tripodi et al. 2018).

Measurements for different data can be obtained in the range of ultraviolet (UV), visible (VIS), near-infrared (NIR), and infrared (IR) radiation using the electromagnetic spectrum. Instruments working in the hyperspectral range (from tens to hundreds of wavelengths) offer more flexibility analysis than multispectral analysis (from two to tens of wavelengths) or single-wavelength measurements since the broader the covered wavelength range and the number of measured wavelengths, the higher the detection capabilities are obtained. Crop growth rates based on changes in crop height could be used to assess the efficiency and effectiveness of management strategies. Ultrasonic sensors are most commonly used sensors to measure crop height in agriculture applications. However, the main disadvantage of an ultrasonic sensor is that the field of view becomes larger as the distance between the sensor and the object increases due to the sensor's relatively wide angle divergence of ultrasonic waves. This reduces the accuracy of ultrasonic measurements. Furthermore, the ultrasonic sensor is sensitive to temperature variations, which limits its outdoor use (Sun et al. 2017).

LiDAR equipped on an airborne vehicle could detect fallen dead trees and the remains of large branches on the ground in forests indicating that LiDAR and similar remote sensing techniques could be used in conservation of biodiversity and genetic resources. For instance, they provide opportunities to monitor endangered plant and animal species for conservation purposes. However, the application of LiDAR is costly because it is limited to airborne missions covering local to regional areas (Lausch et al. 2017). Aerial LiDAR has been successfully used to obtain forest structure attributes such as tree height, leaf area, and branch detection. However, aerial LiDAR was not as effective in annual crops phenotyping activities since it has limited capability to provide high resolution information for crops which are much smaller than trees. This indicates that aerial LiDAR is not suitable for conservation of biodiversity and genetic resources for sustainable agriculture. On the other hand, terrestrial LiDAR has the potential to provide denser point over a relatively small area, from which high resolution information could be extracted. Therefore, it has been increasingly used in field phenotyping. Comparison studies of ultrasonic sensors and LiDAR indicated that LiDAR was generally more precise than data obtained with ultrasonic sensors (Sun et al. 2017). One of major limitations of image based methods is that data quality can be significantly affected by the variable environment, since shadows and sunlight can result in under or over exposure and limit automatic data processing (Araus and Cairns 2014; Walter et al. 2018).

Fluorescence imaging has been used in a large number of experimental setups, as ultraviolet (UV) light in the range of 340–360 nm is reflected by different plant components as discrete wavelengths. Chlorophyll fluorescence emits in red and far-red (690–740 nm). Chlorophyll fluorescence imaging (CFI) is a step forward in fluorescence analysis, accomplished by the support of CCD cameras. In CFI, different lamps are used to induce fluorescence excitation while the plant response is monitored by the digital camera measuring fluorescence at different wavelengths in the

typical spectral ranges of blue (440 nm), green (520–550 nm), red (690 nm), far-red (740 nm), and NIR (800 nm). Fluorescence imaging can be utilized in phenotyping of crops to asses biotic and abiotic stresses, tissue chemical composition and characterization, and different plant physiological conditions (Zarco-Tejada et al. 2012; Hoffmann et al. 2015; Virlet et al. 2017; Yang et al. 2017; Tripodi et al. 2018).

Thermography is a widely used technology in plant phenotyping. Plants are induced to open stomata in response to environmental cues and circadian clock depending on the type of photosynthetic metabolism they have. With this imaging method the evapotranspiration can be assessed with thermography, and quantification can be made at different scales, such as a leaf, a tree, a field, or a complete region. Thermography imaging provides monitoring and detecting water stress, irrigation management and plant diseases where all the specimens are located under strict control conditions: However, temperature, wind velocity, irradiance, leaf angle, and canopy leaf structures are potential issues for quality image acquisition. Both thermographic and fluorescent images capture a single component, and images are in principle easy to analyze but require sophisticated data analysis methods to obtain quality data, but it is an emerging solution (Prashar and Jones 2014; Perez-Sanz et al. 2017; Tripodi et al. 2018).

Synthetic Aperture RADAR (SAR) is an imaging radar used for conducting coherent processing of the obtained echo in different fields or area locations to obtain high-resolution data. SAR is a type of active microwave sensor and high-resolution radar images can obtain in a fashion similar to optical sensor. RADAR data can be acquired in a variety of modes, including standard polarizations (horizontal (H)- vertical (V), HH, VV, VH), polar metric and interferometric way (two signals at slightly different incident angles). This technique can obtain images in very low visibility weather conditions and can work around the clock, which can be used for crop identification, crop acreage monitoring, key crop trait estimation and yield prediction, providing strong technical support for large-scale crop growth monitoring by remote sensing. It is suitable in in tropical areas where persistent cloud cover, or in northern boreal areas where low sun angle effects can reduce the quality of optical model estimates. SAR is very effective in the determination of above ground biomass, fire impacts and forest inundation. It is clear that forest removal, disturbance and degradation analysis and monitoring using RADAR is very important for conservation of forest biodiversity and genetic resources (Perez-Sanz et al. 2017; Thompson et al. 2018; Tripodi et al. 2018).

Thermal infrared imaging sensors equipped with infrared detectors and optical imaging lens receive infrared radiation energy and can produce time series or single-time-point analysis (Gonzalez-Dugo et al. 2015). As the stomatal conductance, photosynthetic characteristics and transpiration rate are closely related to canopy temperature. Canopy temperature in the infrared thermal imaging technology can be used to determine the response of crops under stress conditions, to estimate leaf water potential and stomatal conductance, the cell structure and can be used to estimate plant physiological trait, geometric traits and ground canopy cover (Thompson et al. 2018; Tripodi et al. 2018).

The digital camera, multispectral camera, hyperspectral camera, thermal infrared imager and LiDAR have been widely used to field-based phenotyping. The use of phenomobiles in the studies of conservation of biodiversity and genetic resources will enhance our ability to conserve and widen genetic resources on earth since they provide the advantages of high operation efficiency, low cost, suitability for complex field environments, and high resolution. The limiting factors for phenomobiles based phenotyping for conservation of biodiversity and genetic resources include the strict airspace regulations and higher costs in many countries, the lack of methods and researchers for fast data processing and models for estimating complex traits under different environmental conditions. Also low payload and short endurance in air are among disadvantages. Improving the phenomobiles with machine learning approaches, reducing the cost of sensors, speeding up data processing and developing strategies for analyzing crop phenotype by remote sensing are future trends to be used in conservation of biodiversity and genetic resources. Fortunately, it is expected that with the advancement of new technologies with larger payload and longer endurance, low-cost sensors, improved image processing methods and effective airspace regulations, phenomobiles will find wider applications in high-throughput phenotyping and would be very suitable in conservation of biodiversity and genetic resources for sustainable agriculture (Perez-Sanz et al. 2017; Thompson et al. 2018; Tripodi et al. 2018).

6 Next Generation High-Throughput Genotyping

A DNA marker may be defined as a DNA sequence or fragment that is detected and its inheritance can be monitored. A DNA marker can be as small as a single base or it can be as long as several hundred or more bases. A marker must show at least two different forms (polymorphism) so that genotype carrying a form can be distinguished from other genotype with the other forms. Following the first DNA marker technology developed in the 1980s, a larger number of polymerase chain reaction (PCR) based DNA markers were developed and acted as versatile tools in fingerprinting of varieties, mapping of genes and quantitative trait loci, marker assisted breeding, positional cloning of genes, identification of chromosomes or/and chromosome segments, inferring and establishing phylogenetic relationships among species, building and detection of gene pyramiding; and maintenance and utilization of genetic resources (Bostein et al. 1980; Jeffreys et al. 1985; Bilgen et al. 2004; Ince et al. 2008; Karaca et al. 2008; Wang et al. 2009a, b; Zhang et al. 2009; Ince and Karaca 2011a; Ince et al. 2011c; Jonah et al. 2011; Ince and Karaca 2012; Olarte et al. 2013; Saebnazar and Rahmani 2013; Erbano et al. 2015; Ince and Karaca 2015b; Will et al. 2015; Aydin and Karaca 2016; Karaca and Ince 2017; Song et al. 2017).

Traditional (Karaca et al. 2005a, b; Ince et al. 2007, 2010j; Karaca and Ince 2017) and next generation sequence (NGS)-based DNA markers (Ali et al. 2016; Jiang et al. 2016; Du et al. 2017; Karaca and Ince 2017) are single (such as single

nucleotide polymorphism, SNP) or larger nucleotide sequences (fragments) that are located within or between regulator sequences (promoters, enhancers and silencers) and gene bodies (5'-UTRs, exons, introns and 3'UTRs). DNA marker polymorphisms could result from substitutions, insertions or deletions (INDELs), variation in repeats (such as simple sequence repeats, SSRs) and copy number variations (CNVs). Those DNA markers associated with phenotypic/physiologic trait variations are called functional DNA markers, gene based markers or perfect markers. Functional DNA markers are divided into two main groups. Those functional markers that closely associated with the phenotypic trait variations are called direct functional markers whereas those functional markers that less or not directly associated with the phenotypic traits due to recombination and genetic interaction are called indirect functional markers (Karaca and Ince 2017). Functional DNA markers have advantages over general DNA markers including: (i) not lost due to the recombination between marker and gene of interest; (ii) more meaningful in plant breeding; (iii) more useful in determination of population dynamics, germplasm collections, and monitoring evolutionary changes (Ince et al. 2007, 2010j, 2011d; Salgotra et al. 2014; Michael and van Buren 2015; Kage et al. 2016; Karaca and Ince 2017).

High-throughput sequencing technologies opened new ways for development of novel types of DNA markers, increased our ability to genotype larger numbers of genomes and individuals, and dramatically improved our understanding of how evolutionary processes shape genetic variation across populations, species, and genomes of plant species. High-throughput approaches provide great help and monitor the transfer of genes from distantly related species into breeding programs. Wild species and crop wild relatives have already contributed significantly to improving food production using traditional DNA markers. For instance, Asian rice is one of the clearest examples on application of biotechnological techniques for the genetic improvement of crops. More than 7000 lines were screened to find one from wild *Oryza nivara* that possessed a resistance to the grassy stunt virus; this resistance can now be found in most rice crop germplasm (Li et al. 2018). It has been some time that plant breeding has been supplemented with newer processes involving chromosomal manipulation, embryo rescue, alien introgression lines, mapping populations, marker-assisted selection, and the use of doubled haploids to create inbred lines, allele mining, map-based cloning, the analysis of quantitative trait loci, gene isolation, and genetic modification. Many of these approaches can be used in conservation of biodiversity and genetic resources for sustainable agriculture (Ogwu et al. 2014; Li et al. 2018).

Sequencing of whole genomes involves considerable time, labor, and financial and other resources. In order to reduce time, labor and cost of whole genome sequencing, genotyping by sequencing methods have been developed (Huang et al. 2009; Rife et al. 2015; Rowan et al. 2015). Although the term genotyping by sequencing (GBS) method was first introduced to plant science by Elshire et al. (2011) it had been already available since the earliest form of GBS methods such as complexity reduction of polymorphic sequences (CRoPS), restriction site-associated DNA sequencing (RAD-seq) and reduced-representation library (RRL). Whole genome sequencing and resequencing (WGS and WGR) along with GBS methods

produce polymorphisms of SNPs, insertions/deletions (InDels), microsatellites (SSRs) and copy number variation (Kozarewa et al. 2009; Andolfatto et al. 2011; Mascher et al. 2013; He et al. 2014; Yang et al. 2015; Voss-Fels and Rod 2016; Zhu et al. 2016; Furuta et al. 2017; Scheben et al. 2017; Stetter and Schmid 2017).

High-throughput sequencing methods could be mainly divided in two approaches; reduced representation sequencing (RRS) and whole-genome resequencing (WGR) approaches (Table 4). Although both RRS and WGR methods profit from prior genomic information, reference sequence is a prerequisite only for WGR methods. This relative independence from prior genomic information means that RRS shows particular promise for characterizing the genomes of non-model species. The sequencing read depth can be affected by some biological factors of a target species, including: genome size, genome complexity, ploidy, and expected heterozygosity. Read depth differs between RRS and WGR. Low read depth in WGR methods is typically less than 1x and this low read depth can cause problems when genotyping heterozygotes. On the other hands, read depth in most GBS methods is grater but varies from 1× to 15× depending on the type of GBS methods used (Table 4). Read depth in GBS methods can be increased by reduced numbers of genotypes per library, use of rare cutting restriction enzymes, double digestion, and multiple sequencing runs for a library (Deschamps et al. 2012; Stolle and Moritz 2013; Beissinger et al. 2013; Rife et al. 2015; Du et al. 2017; Karaca and Ince 2017).

GBS methods are derivatives or improvement of approaches that have mainly evolved from reduced representation library (RRL) or reduced representation sequencing (RRS). The use of RRL for single nucleotide polymorphism discovery was first based on Sanger sequencing (Altshuler et al. 2000). In this method, pools of DNA from multiple individuals are reduced in complexity by the type II DNA restriction enzyme digestion and fragments produced by complete digestion of enzymes are size selected. The use of restriction enzyme digestion has the advantages of reducing the fraction of the genome present in the RRL by one to two orders of magnitude and ensuring that independently constructed libraries contain nearly identical fragment populations. Other strategies for genome reduction such as multiplexed amplification of target sequences, molecular inversion probes or the use of probes to capture DNA fragments by direct hybridization prior to sequencing are available but in comparison to the use of restriction enzyme they can be labor intensive. RRS approach is suitable for simultaneous de novo discovery of high-quality SNPs and population characterization of allele frequencies of any species with at least a partially sequenced genome. RRS is a general category of techniques that sequence a subset of the genome following different strategies and can be obtained using restriction enzymes, mechanical shearing or amplification, or natural resources such mRNA populations. High-throughput sequencing RRS can be classified in three major approaches: Restriction site Associate DNA sequencing (RAD-seq) and related method collectively called genotyping by sequencing (GBS), sequencing of cDNA obtained from mRNA and other non-coding RNA (RNA-seq) and whole exome sequencing (WES) (van Orsouw et al. 2007; Baird et al. 2008; van Tassell et al. 2008; Ali et al. 2016; Karaca and Ince 2017).

Table 4 Some high-throughput sequencing (next generation) methods currently available, divided into reduced-representation sequencing (RRS) and whole genome resequencing (WGR)

Reduced-representation sequencing based methods	**References**
Reduced representation shotgun sequencing (RRS)	Altshuler et al. (2000)
Complexity reduction of polymorphic sequences (CRoPS)	van Orsouw et al. (2007)
Restriction site-associated DNA sequencing (RAD-seq)	Baird et al. (2008)
Reduced-representation library (RRL)	van Tassell et al. (2008)
Paired-end reduced representation libraries (pERPLs)	Kerstens et al. (2011)
Multiplexed shotgun genotyping (MSG)	Andolfatto et al. (2011)
Simple genotyping-by-sequencing (GBS)	Elshire et al. (2011)
Two-enzyme genotyping-by-sequencing (GBS)	Poland and Rife (2012)
Double-digest RAD sequencing (ddRAD)	Peterson et al. (2012)
Sequence-based genotyping (SBG)	Truong et al. (2012)
Paired-end reduced representation libraries	Deschamps et al. (2012)
Type IIB endonucleases restriction-site associated DNA (2b-RAD)	Wang et al. (2012)
ezRAD	Toonen et al. (2013)
Restriction fragment sequencing (RESTseq)	Stolle and Moritz (2013)
Specific length amplified fragment sequencing (SLAF-Seq)	Sun et al. (2013)
Scalable genotyping by sequencing (GBS)	Sonah et al. (2013)
Genotyping by genome reducing and sequencing	Chen et al. (2013)
GBS with one enzyme digest	Beissinger et al. (2013)
Ion torrent genotyping by sequencing	Mascher et al. (2013)
Flexible and scalable GBS	Heffelfinger et al. (2014)
GBS with two enzyme digests	Gardner et al. (2014)
Improved RRLs (iRRL)	Greminger et al. (2014)
Genotyping-in-thousands by sequencing (GT-seq)	Campbell et al. (2015)
Spiked genotyping-by-sequencing (sGBS),	Rife et al. (2015)
Multiplexed inter-SSR genotyping by sequencing (MIG-seq)	Suyama and Matsuki (2015)
RAD capture (Rapture)	Ali et al. (2016)
Tunable genotyping-by-sequencing (tGBS)	Ott et al. (2017)
Random amplicon sequencing (RAM-seq)	Bayerl et al. (2017)
Whole genome resequencing (WGR) methods	**References**
Sliding window WGR	Huang et al. (2009)
Parental inference WGR	Xie et al. (2010)
Parental inference WGR with individualized model	Rowan et al. (2015)
Skim genotyping-by-sequencing (SkimGBS)	Bayer et al. (2015)
Whole-genome shotgun (WGS) SMRT sequencing	Du et al. (2017)

The reduced representation sequencing approaches select a fraction of the whole genome for sequencing and reduce the cost and labor for high-throughput genotyping. For instance, hypo-methylated regions of a genome can be obtained (selected) for sequencing. The genomic DNA of the target individual is digested with a 5-methylcytosine-sensitive restriction enzyme and the digest is subjected to electrophoresis; fragments of 100–600 bp are separated and used for sequencing using a

suitable platform of NGS technologies. Alternatively, WES or RNA-seq (also called transcriptome sequences) could be used for genotyping studies. There are several different strategies or approaches for DNA and RNA studies such as sequence capture approach of NimbleGen SeqCap, Agilent SureSelect method and RainDance Targeted Sequencing System (Cui et al. 2011; Levy and Myers 2016; Karaca and Ince 2017).

Knowledge of the biologic system and genomic resources can assist in selecting among RRS (RAD-seq and other GBS methods), RNA-seq, WES or WGR. It is important to select correct high-throughput method to be used in conservation of biodiversity and genetic resources. Clearly it depends on the aim of study, biological system, genomic resources available, the genetic architecture of phenotypic traits, background of the researchers and ultimately on funding. For example, if selection is operating on a specific tissue, stage or development time, RNA-seq would be very appropriate for assessing genetic variation in the genomic regions expressed at time of sampling. On the other hand, if the genes of interest are already known, then GBS such as target capture could be the best strategy. However, if no candidate genes are known, a higher density screening methods such as WES or WGR could be preferable. When selection acts on protein-coding parts of the genome, the use of WES would be a cost-effective approach than WGR. On the other hand, if selection could be acting in regulatory elements or could be mediated by large structural variations and the research focus is the analysis of neutral processes, then WGR could be the best choice because it provides the highest DNA marker density. When, WGR would not be necessary as RRS methods would excel for an affordable price (Bayerl et al. 2017; Fuentes-Pardo and Ruzzante 2017; Karaca and Ince 2017).

In a typical high-throughput RRS method, different samples from the related organisms are pooled and pooled samples are then digested with a type II DNA restriction enzyme. Enzyme treated DNA samples are size selected and selected DNA fragments are ligated with adapters required for sequencing on a NGS platform. Ligated fragments are again size selected and purified. Purified DNA fragments are amplified and the PCR products are sequenced using an Illumina platform (van Tassell et al. 2008; Kerstens et al. 2011). One of the main limitations of RRS method is that it requires reference sequence of the species under study. A reference genome sequence is used to order SNPs within the sequence assembly. However, this challenge may be overcome by genotyping linkage mapping populations or by using comparative genomic information to infer likely or relative genome position (Elshire et al. 2011; Deschamps et al. 2012; Karaca and Ince 2017).

RAD-seq refers to a group of RRS methods such as RAD, ddRAD, ezRAD, RAD-cap that evaluate the genetic variation present within and at the restriction cut sites. The selection of frequent or rare cutter restriction enzyme determines marker density making these methods flexible and customizable. RAD-seq typically examine thousands of low-density genome wide SNPs located in neutral and putatively functional loci that can be genotyped by sequencing in multiple individuals and populations for a relatively low cost. A typical RAD-seq is performed as follows: genomic DNA samples are individually digested with a restriction enzyme and

adaptors with nucleotide barcodes for unique identification of each sample are ligated to DNA fragments. Fragments with 300–700 bp are size selected and different DNA samples are pooled. Pooled DNA fragments with adapters are randomly sheared by sonication, and ends are ligated with a second type adapters. Purified fragments are PCR amplified and sequenced using a high-throughput sequencing (NGS) such as reversible dideoxy based Illumina sequencing which uses either sequencing one (one read, single end) or both (two reads, paired end) ends of each fragment and currently gives reads of up to 300 bp in length (Karaca and Ince 2017).

High-throughput RNA sequencing analysis (RNA-seq) focuses on genetic variation of genome transcribed in a particular time/tissue. RNA-seq is able to reveal genes that are being actively expressed in specific tissue and species of interest, and facilitate the discovery of potential molecular marker of SNPs, microsatellites or InDels markers, some of which could be functional DNA markers. This type of analysis is useful in non-model organisms where the full genome data is still not available for comparison. Sequences that are targets for RNA-seq analysis do not contain repetitive genomic regions and rich in regulatory sequences 5'-UTR, 3'-UTR, miRNA and gene bodies. Furthermore, these regulatory sequences and genes are present in only those genes that are transcribed in a particular tissue/organ during the given developmental stage and under the environmental conditions. RNA-seq is mostly used as a cost-effective approach for gene expression quantification research (Li et al. 2010; Yan et al. 2010; Fuentes-Pardo and Ruzzante 2017; Yamanaka et al. 2018).

Although RNA-seq provides abundant information on gene expression, gene regulation and amino acid content of proteins, it is limited to only those genes that are transcribed in the concerned tissue/organ during the given developmental stage and under the environmental conditions prevailing at the time of sample collection. Therefore, a fair number of organs/tissues, developmental stages should be sampled to ensure the representation of most, if not all, of the genes present in the genome of the concerned species. For a typical RNA-seq analysis, mRNA, RNA with polyA tails is isolated from total RNA and reverse transcribed to cDNA with reverse transcriptase and polyT or polyU primers (Wang et al. 2009a, b; Hua et al. 2011; Du et al. 2015; Waiho et al. 2017). To isolate micro (miRNA), small (sRNA), and long (loRNA), these non-coding RNA molecules are selectively ligated to 3′ and 5′ adapters and reverse transcribed to cDNA (Li et al. 2010; Yan et al. 2010; Batovska et al. 2017; Waiho et al. 2017; Wei et al. 2017).

Whole genome exome sequencing (WES) provides a cost and time effective alternate to whole genome sequencing. The goal of WES is to determine DNA sequence information for regions of a genome that code for proteins. Target regions are referred to as exons. WES selects exonic regions of interest and separating them from non-exon regions of the genome. It is fast and cost effective approach to identification of variants (SNPs, copy number variations (CNVs), small InDels), linkage, association and conservation pedigree studies. WES is often chosen as a substitute for whole genome sequencing because of its lower cost, lower data storage and analysis requirements. RNA-seq and WES differ in the first steps of creating a sequencing library. WES uses genomic DNA regions while RNA-seq utilizes

RNA molecules. WES is a cost-effective alternative to RAD-seq, RNA-seq and whole genome resequencing (Elshire et al. 2011; Altmann et al. 2012; Krumm et al. 2012; He et al. 2014; Suyama and Matsuki 2015; Yamanaka et al. 2018).

Whole genome sequencing and resequencing (WGR) could produce complete or nearly complete genomic DNA sequences of an organism using and assembling numerous shotgun reads that cover the genome multiple times. WGR studies could use four different approaches such as the sequencing of individuals to a high depth of coverage with resolved haplotypes and unresolved haplotypes, the sequencing of population genomes to a high depth by pooling the same amounts of individual DNA, the sequencing of multiple individuals from a population to a low depth. WGR allows the discovery of a huge number of DNA markers such as SNPs, InDels, copy number variations, and presence/absence variations (PAV) in crops and provides deep insight into genome evolution. Moreover, the combination of WGR with bulked segregant analysis allows rapid identification of genes and causal mutations in crops (Huang et al. 2009; Xie et al. 2010; Bayer et al. 2015; Du et al. 2017; Fuentes-Pardo and Ruzzante 2017). Unfortunately, WGR is not currently cost-effective for particularly those species with large genomes, or for those studies requiring large numbers of individuals (Jamann et al. 2017; Karaca and Ince 2017; Vlk and Repkova 2017; Parchman et al. 2018).

A typical WGR method is performed as follows: genomic DNA is fragmented to about 500 bp by sonication and the fragments are end repaired before adding dATPs to generate a protruding 3′ A for ligating with the adaptor carrying a three-base index. Three based indexes are linked to adapters and the indexed DNA samples are run on 2% agarose gels to purify fragments of 150–180 bp. Each sample is amplified by PCR for about 18 cycles and DNA samples of individuals with different indexes are mixed in an equal molar concentration and are loaded into one lane of the Illumina GA for 36-cycle sequencing, with the Illumina PhiX sample used as control. Image analysis and base calling are performed using Illumina GA processing pipeline (Huang et al. 2009; Xie et al. 2010; Bayer et al. 2015; Du et al. 2017; Karaca and Ince 2017).

WGR methods based on NGS technologies and platforms are theoretically capable of identification all genetic variants among individuals of populations. WGR is more robust than WES for the detection of exome variants as it provides a more homogeneous sequence read coverage and a better sequencing quality overall. Another advantage of WGR approaches is that they examine multiple types of genetic variations simultaneously including structural variations (deletions, insertions, substitution, rearrangements, and copy number variation) and mutations in regulatory elements. In contrast, RRS techniques are mostly restricted to one base changes (i.e., SNPs), and RNA-seq and WES are for detection of variation within coding sequences (Fuentes-Pardo and Ruzzante 2017). Although WGR provides complete resolution of any genome it is cost-prohibitive for researchers in developing countries and indeed WGR may be unnecessary for many studies involving a large number of individuals. The parental genomes with high-quality sequences and a reference sequence are often required for WGR. It differs from RRS, in the lack of complexity reduction steps before sequencing. WGR is well suited to genotyping

biparental cross populations with complex, small and moderate sized genomes. It provides the lowest cost per marker data point. Compared to WGR methods, RRS approaches differ in their suitability for various tasks, but demonstrate similar costs per marker data point. However, RRS approaches are generally better suited for de novo applications and more cost-effective when genotyping populations with large genomes or high heterozygosity. On the other hands, WGR offers the greatest cost-efficiency per marker data point, and is particularly useful when recombination is high and many markers are needed for a well-resolved genetic map in a species with a small or moderate sized genome. WGR has the added benefit of increasing the chances of finding causative SNPs, InDels or genes, which allow development of "perfect" or "functional" markers. In the light of the decreasing costs of sequencing, the use of WGR to increase the resolution of mapping studies is likely to become more common in the future (Huang et al. 2009; Rife et al. 2015; Rowan et al. 2015).

WGR could be used in the detection of biodiversity, selection of genetic resources and the characterization of the genetic basis of phenotypic traits and diseases affecting survivor. RRS approaches can also be used for this purpose at the fraction of the genome screened, although their success may depend on the proportion of the genome covered. With the help of high resolution of high-throughput genotyping approaches (high-throughput sequencing) measures of nucleotide diversity and divergence can be estimated. For instance, deviation from neutrality can be readily tested, and identification of thousands of genes altered can be achieved. In typical genetic conservation studies about 10–50 variables are used but conservation genomics based on high-throughput sequencing involves tens of thousands of genes. Conservation genomics, in particular the availability of genome-wide sequences permits the simultaneous study of the effects of demographic history, migration and selection (Bayerl et al. 2017).

High-throughput sequencing based genotyping provides higher resolution for phylogenomics, hybridization and taxonomical studies, all of which relate with conservation of biodiversity and genetic resources. The successful implementation of conservation plans relies on the correct identification of the taxonomic status of organisms that are targeted for conservation of biodiversity and genetic resources. Whole or nearly whole-genome data provide a complete record of a species evolutionary process. However, more works are required to be done to resolve algorithm limitations associated with the analysis of such large amount of genomic data. In some cases, genome rearrangements, lateral gene transfer, incomplete lineage sorting make analyses more difficult (Aravanopoulos 2016; Karaca and Ince 2017; Yousef et al. 2018).

High-throughput sequencing based genotyping could provide data on species demographic history, migration patterns, range expansion and changes in historical effective population size. Such data also allow obtaining information regarding barriers to gene flow, anthropogenic disturbance, climate change, historical demographic processes, population structure and admixture. It is very important to maintain high genetic diversity in vulnerable species with lower population size for genetic conservation. Because most natural populations are structured in local subpopulations, genetic differences may occur among subpopulations over time as a

result of gene flow, genetic drift and local adaptation. Because high-throughput sequencing based GBS and WGS approaches provide the highest marker density, these methods allow the simultaneous evaluation of genome wide patterns in neutral and functional loci that act as a record of demographic and historical events, and adaptation. GBS and WGS provide data on the identification of genomic regions, which involved in adaptation to local environmental conditions. These data are crucial for conservation biology because of the importance of functional genetic diversity. Furthermore, these data provide connection between genotype, phenotype and fitness (Fuentes-Pardo and Ruzzante 2017; Karaca and Ince 2017).

High-throughput sequencing based genotyping provides valuable data that could be used assessment of genetic diversity, which is essential for the organization, conservation and use of genetic resources to develop strategies for optimal germplasm collection, evaluation and seed regeneration. Next generation genotyping methods have advantages for characterizing gene bank accessions such as a major advantage is their applicability to any species. These do not cost much per individual data, but provide sufficient power for genome-wide analyses of population structure and genetic relationships. The main disadvantage of high-throughput sequencing is the presence of a high proportion of missing data that may reduce the power for correct estimation of population parameters. Also, high cost of high-throughput sequencing and the elevated demand for computing resources limit their implementations in conservation of biodiversity and genetic resources (Aravanopoulos 2016; Yamanaka et al. 2018; Yousef et al. 2018).

Genomics provides an unprecedented level of resolution for population genetic studies since next-generation sequencing data will be more powerful and accurate, especially in cases where significant adaptive differentiation is expected among evolutionary significant units considered as candidates for gene conservation. Today because of high-throughput resequencing platforms, it is feasible to substantially increase the numbers of populations, individuals per population and loci per individual studied at a fraction of earlier experimental costs (Pertoldi et al. 2007; Karaca and Ince 2017). With the use of NGS based genotyping approaches genomics offers high precision estimates of genetic and demographic parameters and could result in high-resolution characterization of adaptive genetic variation in nature. Therefore, studies dealing with conservation of biodiversity and genetic resources would provide considerable benefits for humankind (Bayerl et al. 2017; Jamann et al. 2017; Scheben et al. 2017).

The use of genomics in genetic monitoring of biodiversity is very important since genetic monitoring provides valuable information regarding an early detection mechanism that leads to management decisions aimed to lessen potential harmful effects before permanent damage occurs. In another word, genetic monitoring is an effective prognostic tool to secure genetic diversity in natural populations. It could provide plenty information on natural selection, genetic drift, mating system, migration, gene flow and health of population. For instance, the effects of natural selection may lead to differentiation associated with local or regional adaptation, while genetic drift can lead to genetic erosion (Ali et al. 2016; Jiang et al. 2016; Du et al. 2017). Second generation based GBS technologies use DNA enrichment methods prior to

amplification, resulting in relatively short sequencing templates. Third generation sequencing platforms are capable of producing significantly larger read lengths and sequencing through traditionally difficult sequence templates with high GC content (Du et al. 2017). Third generation sequencing platforms seem best suitable method for conservation of biodiversity and genetic resources when several associated disadvantages are mitigated (Beissinger et al. 2013; Sun et al. 2013; Heffelfinger et al. 2014; Karaca and Ince 2017; Scheben et al. 2017; Elbasyoni et al. 2018).

7 Conclusions and Future Prospects

It is estimated that the global population is approaching to nine billion by 2050, and demand for food and fiber crops is expected to increase by about 60% (Sun et al. 2018). Although phenotypic, metabolomics, proteomics and genetic diversity are more heavily reduced in cultivated germplasm, fortunately international movement on conservation and sustainable use of biodiversity and genetic resources for agriculture have greatly sounded during the last 50–60 years. Today approximately 7.4 million germplasm accessions, representing more than 16,500 plant species are secured in 1750 gene banks worldwide. However, unfortunately conservation programs are chronically underfunded and the impact of climate change on crop genetic diversity is not completely understood. In many parts of the world, appropriate capacities and adequate infrastructures to explore biodiversity are still lacking and genetic erosion is far from being stopped (Sari et al. 2005; Davey et al. 2011; Fu et al. 2015).

The genetic drift in gene banks is caused by the use of inadequate sample sizes. Also regeneration delays cause genetic integrity loss for some cross-pollinating species in gene banks. Furthermore, gene bank conservation gets less strengthen political support in todays' capitalist world. In many developing countries there exist inadequate germplasm evaluation and characterization. Efficient conservation of genetic resources requires efficient and effective global networking of gene banks around the world. Effectively upgrading gene bank information systems is also important and required. In many countries there exist low diversity coverage and inadequate gene bank capacities. Unfortunately, private sectors not interested in conservation of biodiversity and there is inadequate gene bank support from stakeholders. Many stakeholders are mainly interested in germplasm for economic potential and do not provide supports for management of gene banks and establishment facilities for long-term conservation (Fu 2017).

Over-grazing, over-exploitation, urbanization, hydroelectric dam construction, roads and global market economies have caused the impoverishment of many native forests and grasslands. For instance, heavy collection of aromatic and medicinal plant species narrowed genetic diversity in the Mediterranean basin of Turkey. It is known that over-exploitation in some other parts of the world threatened genetic biodiversity of many plant species. Increasing water and air pollution along with deforestation and biologic pollution contribute to the genetic erosion of both cultivated and wild species. In turn, the unsustainable use of natural resources such as

forests and ponds has resulted into disturbed water balance and severe erosion. Also in many countries current legislations discourage the use of landraces and also have a strong negative impact on their conservation. For instance, Italy reports that out of 41 farms growing landraces of forage legumes in the 1970s only one now carries through this activity (Fu et al. 2015; Manhaes et al. 2018).

In order to conserve the biodiversity and genetic resources for sustainable agriculture next generation based genomic and phenomic monitoring should be considered and used simultaneously. NGS based phenotyping and genotyping could be effectively used in monitoring of genetic diversity during seed regeneration and plantation. These technologies would allow to manage diversity within accessions to mitigate some disadvantages of small population sizes of *ex situ* conservation (Davey et al. 2011; Poczai et al. 2013; Jia et al. 2016; Tsai et al. 2015). But most of these techniques are not yet widely available in developing countries where they are most needed such as in tropics regions including many threatened species (Fu et al. 2015; Kang et al. 2016; Manhaes et al. 2018). Most phenotypic traits involved in local adaptation survival are polygenic, and the importance of epistasis, transposable element activity or epigenetics plays significant roles. Since polygenic traits could be effectively analyzed using GBS technologies, genomic monitoring based on GBS is very suitable for conservation of biodiversity and genetic resources for sustainable agriculture. GBS could be used to estimate population parameters including allelic richness, expected heterozygosity and the total and the effective number of alleles, outcrossing and inbreeding rate, out coming gene flow and effective population size (Kang et al. 2016; Watanabe et al. 2017).

The presence of dramatic climate changes and the direct adverse anthropogenic influence and activity are two major issues that are driving the need for immediate, extensive and comprehensive conservation of genetic resources of world. It is expected that global temperature will rise about 1.8–4.0 °C during the twenty-first century and this will cause a shift of species spatial distributions more than 6 km towards the poles and 1 m in elevation, per decade. This may result in population spatial shifts, fragmentation, reduction of population size or even extinction in mountainous ranges (Aravanopoulos 2016). As a final sentence, we believe that a well-designed, genomic and phenomic tools-monitored, and well-managed systems coupled with *ex situ* and *in situ* conservation strategies (seed banks, cryogenic storage, living collections in botanical gardens, arboreta, and similar facilities where necessary) is enough to protect many endangered plant species and conserve biodiversity through the several decades of rapid global change. However, people on earth should learn to live with nature as a part of nature not against to nature for long-term conservation for themselves and for the nature surrounding them, and should listen the nature while it is still able to speak.

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