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Dilip Nandwani *Editor*

Genetic Diversity in Horticultural Plants

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Editor

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Foreword

This book analyses Genetic Diversity in Horticultural Plants and presents chapters revealing the fundamental importance of genetic diversity and origin of species, uncovering the extent of genetic variation existing in plant populations. The book discusses continuity of a species as it provides the necessary adaptation to the environment. The book presented link fields of biology, agrobiodiversity, plant genetics, and ecological and evolutionary studies. Modern tools of molecular biology, PCR-based technology, and markers are powerful tool to analyse in detail the evaluation of genetic diversity and important traits. This edition provides a glimpse into the vibrant process of genetic variation by presenting the point of views of researchers engaged in the generation of new ideas and techniques employed for the assessment of genetic diversity. Genetic adaptation of the plants depend on the availability of a wide range of plant varieties and the genetic resources, crops acclimatization to pests, diseases and climatic change pressures. The authors have spent great efforts in reviewing and addressing the opportunities of this significant area of agriculture.

The book contains key reviews from global experts in 10 chapters in three sections to cover wide range of case studies in fruits, vegetables, and root crops from Greece, Italy, Spain, Hawaii, Japan, India, Africa, Middle East, and North America. Researchers have shed some light onto the botany, origin of species, and molecular biology in selected horticultural crops.

The editor, Prof. (Dr.) Dilip Nandwani, is a researcher at the Tennessee State University College of Agriculture working to promote the organic agriculture and use of genetic diversity in fruits and vegetable crops. He has interdisciplinary background in botany, horticulture, and organic agriculture which enabled him to meticulously assemble and edit this book. Dr. Nandwani is an outstanding talented scientist, well-respected educator, and promoter of sustainable agriculture at the College of Agriculture. Dr. Nandwani is valuable colleague and asset to the University. His accomplishments in 6 years at TSU speaks volume, achievements,

quality of work for scientific community, students, and stakeholders, and prestige he brought to the institution.

The book is useful to students, scientists, professors, growers, and experts in the areas of genetic diversity, horticulture, and molecular biology.

Nashville, TN, USA
October 2019

Chandra Reddy
Dean, College of Agriculture

Acknowledgements

This is my fourth edited volume in the book series "Sustainable Development and Biodiversity". This volume was not an easy task to complete without support, valuable suggestions and encouragement received from Prof. K. G. Ramawat (Series Editor), contributions from the authors, and friendly support from my undergraduate and graduate students, faculty, colleagues, and Dean, College of Agriculture, Tennessee State University. Special thanks to my family members, wife Varsha, children (Gayatri and Rahul) and parents for their love and patience which enabled me to complete this project successfully.

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Dilip Nandwani, Ph.D., CPH

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About the Editor

Dilip Nandwani, Ph.D. is Distinguished University Professor of organic agriculture at the Tennessee State University and Certified Professional Horticulturist (CPH) from the American Society for Horticultural Science (ASHS). After completing his education at the Jai Narain Vyas University, Jodhpur, (M.Sc., 1987, and Ph.D., 1991), he joined M. L. Sukhadia University, Udaipur (1993–1995) as CSIR Research Associate and served as Research Scientist at the Maharashtra Hybrid Seeds Company (MAHYCO), Jalna (1995–1999). He joined the Tennessee State University as Research Associate Professor (Organic Agriculture) in 2014. Prior to joining Tennessee State University (TSU), he served University of the Virgin Islands as Research Associate Professor (Horticulture). He has been working with land-grant institutions for two decades in agricultural research, extension, and education in the American Pacific and American Caribbean. He had stint working for the United Nations Environment Programme (UNEP-GEF) as Regional Advisor. He has widely published in the area of production agriculture (organic and conventional) and horticultural sciences. His research and extension work has led over 200 articles (peer-reviewed, extension booklets, abstracts, and conference proceedings). He is editor of three books, serving on the boards of Tennessee Organic Growers Association (TOGA), Certified Professional Horticulturist (CPH) of American Society for Horticultural Science (ASHS), Southern Cover Crops Council (SCCC), Associate Editor of Organic Agriculture journal, and several others. He teaches courses Principles of Organic Agriculture (AGSC 4555/5015), Principles of Crop Science (AGSC 3010), Sustainable Crop Production (AGSC 3260) and Organic Certification course (CEU approved) at the TSU College of Agriculture. He received nine honours and awards, obtained over

$3M competitive grants and agreements from regional, national, and international organizations, and widely travelled for conferences, consultation, and training in international agriculture development.

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Abbreviations

AFLP	Amplified Fragment Length Polymorphism
ALV	African Leafy Vegetables
AMOVA	Analysis of Molecular Variance
ANSUB	Forest Producers Association of Sado Valley (Portugal)
APFC	Forest Producers Association of Coruche and Surroundings (Portugal)
B.O.E.	Boletín Oficial del Estado, the Spanish Official State Gazette
BCE	Before Common Era, a secular alternative for BC ("Before Christ")
BIOGECO	MR1202 BIOdiversity, GEnes & COmmunities (INRA, University of Bordeaux)
BP	Before Present (before 1950), a geological timescale
c.	Century
CBDV	Colocasia Bobone Disease Virus
CGIAR	Consultative Group on International Agricultural Research
cpDNA	Chloroplast DNA
CRN	National Research Council (Italy)
CTA	Technical Centre for Agricultural and Rural Cooperation
CWR	Crop Wild Relatives
DAMD	Directed Amplification of Minisatellite DNA
DNA	Deoxyribose Nucleic Acid
e.g.	Lat. *exempli gratia*, "for example"
EST	Expressed Sequence Tag
FAO	Food and Agriculture Organization
FAOSTAT	Food and Agriculture Organization, Statistics
F_{st}	Genetic Differentiation
g	Gram
GA	Gibberellic Acid
GBS	Genotyping by Sequencing
GEBV	Genomic Estimated Breeding Values
GS	Genomic Selection

GWAS	Genome-wide Association Studies
ha	Hectares, a unit of surface area equal to 10,000 square metres
H_E	Heterozygosity
INIA	National Institute for Agricultural and Food Research and Technology (Spain)
INIA-CIFOR	Forest Research Centre (Madrid, Spain)
INRA	National Institute for Agricultural Research (France)
ISSR	Inter Simple Sequence Repeats
iuFOR	Sustainable Forest Management Research Institute, University of Valladolid/INIA (Spain)
kg	Kilogram
km	Kilometre (0.6214 miles)
kyr	Thousand years
LD	Linkage Disequilibrium
LGM	Last Glacial Maximum (27–18 kyr BP)
m	Metre
MAS	Marker-Assisted Selection
mm	Millimetre
MSAP	Methylation Sensitive Amplified Polymorphism
nSSR	Nuclear microsatellite, nuclear simple sequence repeat (SSR)
PCA	Principal Component Analysis
PCR	Polymerase Chain Reaction
PGR	Plant Genetic Resources
PGRFA	Plant Genetic Resources for Food and Agriculture
PIC	Polymorphic Information Content
PNG	Papua New Guinea
PROTA	Plant Resource of Tropical Africa
QTL	Quantitative Trait Loci
r	Pearson's Correlation Coefficient
RAD	Restriction-associated DNA
RAM	Random Amplified Microsatellite Markers
RAPD	Random Amplified Polymorphic DNA
RFLP	Restriction Fragment Length Polymorphisms
SCoT	Start Codon Targeted Polymorphism
SLAF	Specific Length Amplified Fragment Sequencing
SNP	Single-Nucleotide Polymorphism
SPCSV	Sweet Potato Chlorotic Stunt Virus
SPFMV	Sweet Potato Feathery Mottle Virus
SPVD	Sweet Potato Virus Disease
SRAP	Sequence-related Amplified Polymorphism
SSR	Simple Sequence Repeat
t	Ton, metric ton (thousand kg)
TaBV	Taro Bacilliform Virus
TANSAO	Taro Network for Southeast Asia and Oceania
TaroGen	Taro Genetic Resources: Conservation and Utilization

TaVCV	Taro Vein Chlorosis virus
TLB	Taro Leaf Blight
UPGMA	Unweighted Pair-Group Method with Averages
USA	United States of America
WVC	World Vegetable Centre
yr	Year
π	Nucleotide Diversity at Gene Level

Part I
Biology and Phylogenetic Diversity

Chapter 1
Augmentation of Plant Genetic Diversity in Synecoculture: Theory and Practice in Temperate and Tropical Zones

Masatoshi Funabashi

Abstract Natural vegetation forms a complex fractal structure of ecological niche distribution, in contrast to human-managed monoculture landscape. For the sustainable management of diverse plant genetic resources, including crop and wild species, the introduction of such ecologically optimum formation is important to compensate for the biodiversity loss and achieve higher ecological state that can provide sufficient ecosystem services for increasing human population. In this chapter, we first develop a conceptual and theoretical framework for the implementation and management of self-organized niche structures and develop an adaptive strategy of sustainable food production resulting from the statistical nature of ecosystem dynamics called power law. Second, we construct the integrative measures for the management of plant genetic resources for food and agriculture in ecological optimum that incorporate both phylogenic and phase diversities as important functional indicators of plant communities. This formalization leads to the extension of conventional concepts of biodiversity and ecosystem services toward human-assisted operational ecological diversity and utility and provides the definition and property of potentially realizable and utilizable plant genetic resources in the augmented ecosystems beyond natural preservation state. Finally, case studies from the synecoculture project in temperate and tropical zones are reported in reference to the developed framework, which draws out legislative requirements for future protection and propagation of plant genetic resources. The necessity of supportive information and communication technologies is also demonstrated. This article contains theoretical foundation and the results of the proof of concept experiments that are essential to establish a novel developmental and legislative framework for the sustainable use of plant genetic resources, overarching the protection of the natural environment and agricultural production mainstreaming biodiversity.

Keywords Plant genetic resources (PGR) · Ecological optimum · Power-law distribution · Synecoculture · Anthropogenic augmentation of ecosystems ·

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Operational species diversity · Adaptive diversification · Ecological recapitulation principles · Open complex systems · Complexity measure · Information and communication technologies (ICT) · Traditional knowledge of indigenous peoples and local communities · Aichi biodiversity targets · United Nations sustainable development goals (SDGs) · The Nagoya Protocol on Access and Benefit-Sharing

1.1 Introduction

Human activities, especially agriculture, are considered to have exceeded planetary boundaries of natural resources and their cycles for the maintenance of the Earth System (ES) out of the Holocene epoch where human civilizations have developed (Steffen et al. 2016). It may lead the ES to an unprecedented shift to Anthropocene with various risks and uncertainty on the life support of the growing population (Crutzen 2002).

Among natural resources, material resources are estimated to peak and deplete around the middle of the twenty-first century, both for fossil fuels (Turner 2008) and rare metals (UNEP 2013). Not only the remained exploitable amount, but mining costs are approaching the efficiency bound. Significant parts of these material resources are non-renewable, and it is not yet technically possible to completely substitute with other resources at the level of sustaining the current rate of economic growth.

Biological resources such as biodiversity and plant genetic resources (PGR) are also incurring severe damage by the inappropriate practice of agriculture. The extinction rate of vascular plants is 500–1000 times higher than the natural background rate (Pereira et al. 2010), and the global collapse of the ecological state is anticipated (Barnosky et al. 2012).

Nevertheless, biological resources are renewable and have survived several massive extinction events during the evolution of ES (Rohde and Muller 2005). PGR itself can be considered as long-term renewable and sustainable resources that contain the self-adaptive capacity to climate change and other global forcings, whether natural or human-caused.

In responding to urgent needs for the transition to sustainable social–ecological systems, the in situ conservation of PGR is an essential framework both for primary food production and conservation of ecosystems (NRC 1993). It implies both on-farm and field management of PGR at the plant community level with its evolutionary context, in which there is importance on the mixed vegetation established by ecological optimum (Putman and Wratten 1984), in contrast to the physiologically controlled culture of a single crop.

Based on ecological incentives, there has been lots of attempts to partially introduce ecological optimization in farming method, such as organic farming, natural farming, and in its extreme case synecological farming (synecoculture) that totally relies on the formation of ecological optimum at the community level for the production (Funabashi 2016a, 2017a).

As the material resources are approaching its mining limit and biological diversity threatened, the in situ management of plant genetic diversity through low-input food production system becomes of primary importance for reconciling between development and sustainability. In this chapter, we first analyze the geometrical and statistical properties of plant communities realized by ecological optimum and develop a series of strategies to make use of these distributions in the context of synecoculture. Secondly, we report the results of field experiments of synecoculture in accordance with the developed theory, as a strategic augmentation of plant genetic diversity on the plots in Japan, Taiwan, and Burkina Faso, ranging over temperate and tropical zones.

1.2 Power-Law Distribution of Ecological Niches

Typical surface distribution of natural vegetation with symbiotic interaction in ecological optimum is known to follow a power law (Scanlon et al. 2007; Seuront 2010; Farrior et al. 2016), which is also observed in synecoculture (Funabashi 2017a). Here, we examine the invariant properties of the power law in naturally organized vegetation with respect to the measurement parameters, in order to build a theoretical framework for the interpretation of field data.

As a simple assumption, actual measurement of vegetation surface is confined by two parameters: total surface of measurement spot and spatial resolution of the measurement. We assume a general model of power-law distribution as Pareto distribution, whose probability density function $f(x)$ on $x > b > 0$ is defined as follows:

$$f(x) = \frac{ab^a}{x^{a+1}},$$

where $a > 0$ is the scale-free parameter of the power law.

Suppose we measure the vegetation surface with the resolution of percentage, from 1 to 100%. Then, the bottom limit of the surface x should be confined by more than 1% of the total surface, which fixes b to be 0.01 times total surface. We call this assumption as "%-measurable" property of vegetation. It means that species surface less than 1% of the total surface is ignored from the observation.

The gradient of a power law, a, varies according to vegetation types and resolution of observation. Therefore, we numerically change the value of a with respect to the total surface and examine acceptable species diversity as the number of surface divisions that follow a power-law distribution. Actually, it suffices to fix an arbitrary total surface and generate a range of a where samplings from the Pareto distribution are confined within the range of 1–100% of the total surface. Other sampling parameters that produce data with more than 100% values of total surface mean the observation scale is too small compared to the actual niche surface, which should be eliminated as an inappropriate observation. The result is shown in Fig. 1.1.

The simulation shows that the appropriate measurement of species diversity defined as the number of different partitions following power law should be situated

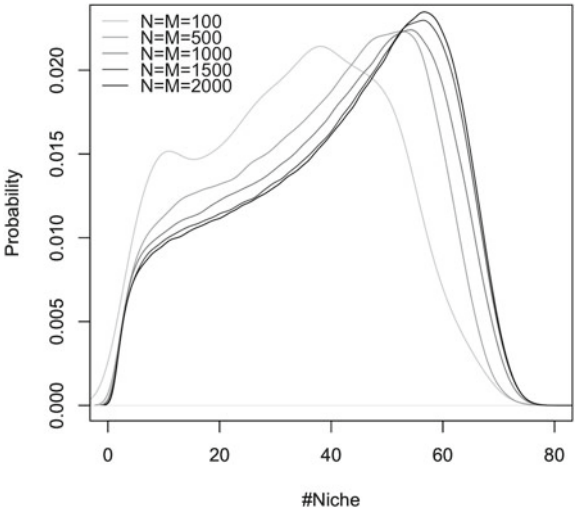

Fig. 1.1 Invariant distribution of species diversity following power-law surface distribution. As a simulation of %-measurable vegetation surface distribution, the total surface was set to 100, the parameters of Pareto distribution $b = 1$, and $0 < a \leq 3$. The range of a was divided by parameter N that ranges from 100 to 2000, which numerically encapsulated the range of %-measurable distribution. X-axis represents the number of partitions generated from Pareto distributions, which represents a maximum acceptable number of ecological niche or species diversity of plants, and Y-axis shows the estimated probability density after M times of sampling. As N and M increase, convergence to an invariant distribution is observed under random sampling assumption. All simulations and visualizations in this article were performed using R version 3.2.0 (R 2015)

somewhere between 10 and 65 species, most plausibly between 40 and 65 species, if based on the %-measurable assumption and choosing an appropriate scale with respect to actual power-law surface distribution. This model fixes the total surface and changes the characteristic parameter a, while in actual measurement we need to choose appropriate measurement scale in order to validate the count of species diversity in effective power-law range and estimate a (see Sect. 1.7).

Let us introduce another perspective on the origin of the power-law distribution in the spatial distribution of vegetation. It is known in general that a stochastic process including both additive and multiplicative noises has a property of converging to power-law distribution (Takayasu et al. 1997). Specifically, when the growth of niche surface is expressed by $p(t)$ with time parameter t, the development of niche surface distribution is expressed in discrete time as

$$p(t+1) = \mathrm{GR}p(t) + p'(t)$$

Here, $\mathrm{GR} > 1$ is a stochastically fluctuating growth rate, and $p'(t)$ is a fusion with another niche of the same species. Then, since $\mathrm{GR} > 1$ is multiplicative and $p'(t)$ is additive, the developed niche structure follows a power law.

1.3 Measuring Yield on Power-Law Vegetation

In the physiological optimization of a single crop, the average value of the environmental parameter giving the maximum production amount is fixed to a specific value. However, when niche division occurs in ecological optimization, the environmental condition giving the locally maximum amount of production could divide into two or more, with different distributional areas (Putman and Wratten 1984). In addition to the physicochemical parameters such as climate and soil conditions, other ecological parameters such as the type of competing vegetation become important in the set of environmental parameters; therefore, the parameters that define the niche condition cause a combination explosion. Assuming that there are n types of matrix vegetation that can grow under a given environmental condition, $\sum_{i=1}^{k} {}_{n}C_i$ types of combinations arise only by selecting any set of $k \leq n$ types or less therefrom. This combinatorics defines the upper limit of possible variations of niche diversification on these crops.

Specifically, let us schematically consider the diversification of niches on the three main factors of plant growth, temperature, humidity, and amount of sunlight, with a set of countless competing vegetation. Temperature, humidity, and sunlight are defined as x-, y-, z-coordinates, respectively, and competing vegetation grows in this environment as dominant species. Now, if you add another competing vegetation so that the single niche space is divided into two for each of the x-, y-, z-axes, the remaining cultivation niche is equal to the phase structure of fractal figure called Menger sponge.[1] Indeed, it is known that vegetation in a mixed state of many species organizes a fractal arrangement (Seuront 2010), and the fractal dimension is also applied to satellite image analysis that provides the proxies of vegetation type (Nayak 2008). The modeling approaches include cellular automata (Scanlon et al. 2007) and recursive formal grammar such as Lindenmayer system (L-system) (Prusinkiewicz and Lindenmayer 2012). Rigorous mathematical analyses of the models, such as the generative mechanism of the power law and numerical convergence of geometric measures such as fractal dimension, require the functional analysis of fractal figure. In the ecologically optimized state, if we cannot handle such a complex niche structure comprehensively, we cannot discuss the yield based on the vast combination of competing crops.[2]

[1] A concrete example in one dimension is given in Appendix 3.

[2] In measuring the surface on a fractal figure like Menger sponge, the usual Riemann integral is extremely difficult to handle. Originally, Riemann integral is defined based on infinite series, but a fractal figure is a function defined on the limit operation of infinite iteration of a map. Therefore, when attempting to perform Riemann integration of a fractal figure, it is necessary to calculate the "limit value of the function defined by the limit value," which becomes analytically difficult. In reality, although the actual vegetation distribution has fractal feature, the lowest resolution is fixed to a finite value in actual data, but it is still complex to analytically calculate Riemann integral of long finite series on an iterative model. Besides a simple surface area, to calculate essential characteristics of a fractal figure such as fractal dimension, one needs to be based on the measure theory such as Hausdorff measure and related numerical implementation such as the box-counting method. Furthermore, to integrate on qualitative variables contained in ecosystem data, a method of

The Lebesgue integral (or in a more general setting, measure integral) can build an integral over such a complex set.[3] In the Lebesgue integral, the area and the volume of a complex set can be collectively calculated by constructing a measure on the set theory. Besides length, area, and volume, the measure can be configured as the number of events, probability, and any other objective functions. In actual data analysis, Lebesgue integration can be programmed with a database search algorithm. Even if you do not solve it analytically, you can numerically calculate the objective functions based on the search conditions. The set-theoretic operation can be replaced with computation (Funabashi 2017b).

Let us see a concrete example. In the niche division, as shown in Fig. 1.2, the region of the environmental parameter where the vegetation gives a yield equal to or more than the lower bound α is given by $X|_{Y(X)\geq\alpha}$, where X is the space of the environmental parameter, and $Y(X)$ is the average yield distribution of the vegetation. This means to choose only the set that satisfies $Y(X) \geq \alpha$ out of the subsets of X, and it does not matter how complex it is in X. If multidimensional Lebesgue measure m is constructed on X, Lebesgue integration can be performed even in case $X|_{Y(X)\geq\alpha}$, where niche(s) above the yield α is a fractal figure. Specifically, the yield $Y|_{Y\geq\alpha}$ at niches above the yield α is calculated as the Lebesgue integral

$$Y|_{Y\geq\alpha} = \int_{X|_{Y(X)\geq\alpha}} Y \mathrm{d}m.$$

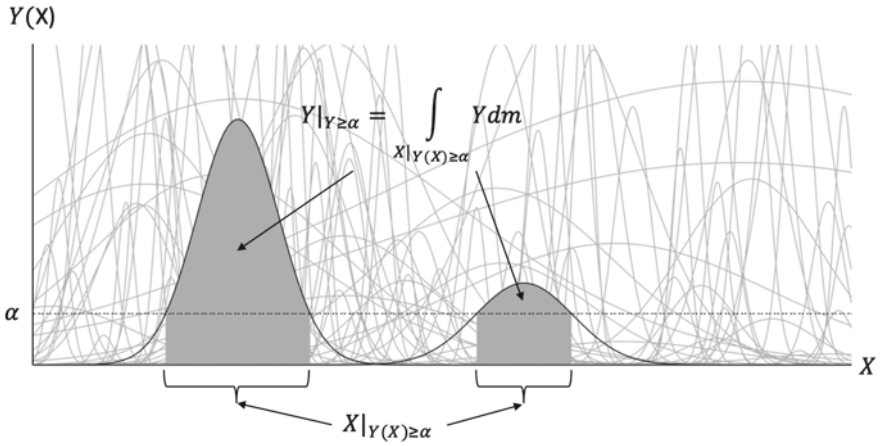

Fig. 1.2 Definition of yield on simulated examples of mixed power-law vegetation with multiple species. Examples of divided niches are depicted with the yield $Y|_{Y\geq\alpha}$ (gray area) as defined in Sect. 1.3. See Sect. 1.4 for the model description

counting qualitative variables must be set separately, which requires the formalization of measure integral.

[3]Basic formalization of Lebesgue integral for vegetation data is detailed in Appendix 1.

In general, the yield data such as biomass is challenging to obtain exhaustively, but the distribution map of vegetation can be more easily created from the field survey and is released from various research institutes. When dealing with a vegetation distribution map, the coverage area per vegetation can be calculated with the above formulation, if α is interpreted as a threshold for determining the presence or absence of vegetation.

In vegetation survey, when a map is prepared as a distribution site of a particular vegetation where the density $Y(X)$ is not less than α, the distribution function of the vegetation is given by the definition function $\mathbf{1}_{X|_{Y(X)\geq\alpha}}(X)$ of the distribution area $X|_{Y(X)\geq\alpha}$. The covering area S of the vegetation, if X is the two-dimensional coordinate of the map, is given as follows in the same way as the yield,

$$S|_{Y(X)\geq\alpha} = \int_X \mathbf{1}_{X|_{Y(X)\geq\alpha}} \mathrm{d}m.$$

This corresponds to the niche area of the vegetation in ecological optimum.

From this, the average yield per surface $E\left[Y|_{Y\geq\alpha}\right]$ in niches defined by the lower bound of yield α is given by

$$E\left[Y|_{Y\geq\alpha}\right] = \left.\frac{Y}{S}\right|_{Y(X)\geq\alpha} = \frac{\int_{X|_{Y(X)\geq\alpha}} Y \mathrm{d}m}{\int_X \mathbf{1}_{X|_{Y(X)\geq\alpha}} \mathrm{d}m}.$$

1.4 Adaptive Diversification: Management of PGR in Ecological Optimum

Let us think about the design of agriculture based on the ecological optimum, using the previously developed theoretical format that uniformly handles the yield and surface of the vegetation distribution with a complex fractal structure. In physiological optimization prevalent in conventional monoculture systems, it was better to eliminate competing vegetation and control the environment to realize the optimal physiological range of cultivated crops. The underlying concern was the cost of material resources necessary for the monoculture optimization in one place.

On the other hand, in ecological optimization, if we interfere too much with the environment, it hinders the elaboration of symbiotic effects that support niche formation (Funabashi 2016a). Instead, it is necessary to search for the spots with favorable environmental and vegetation conditions for target crops, from various ecological situations at each moment. The exploration of a better optimal condition for ecological niche formation that changes dynamically in spatiotemporal patterns becomes an essential management cost in ever-changing open complex systems.

In the absence of prior knowledge, the search cost of the environment that meets the condition can be measured by the amount of selected information in the information theory. Let α^{max} be the maximum yield in an ecologically optimized state of particular vegetation, such as $\alpha^{max} = \max_X(Y(X))$. The area of the niche whose yield range is $[\alpha, \alpha^{max}]$ is calculated using the definition function $\mathbf{1}_X$ across X as

$$\int_{X|_{Y(X)\in[\alpha,\alpha^{max}]}} \mathbf{1}_X \mathrm{d}m.$$

Dividing it by per unit area, its occurrence probability $p(\alpha)$ is given by

$$p(\alpha) = \frac{\int_{X|_{Y(X)\in[\alpha,\alpha^{max}]}} \mathbf{1}_X \mathrm{d}m}{\int_X \mathbf{1}_X \mathrm{d}m}.$$

Usually, each ecological niche is expressed as a unimodal distribution of growth rate on space, and harvesting can be interpreted as taking the sum of random sampling, which means the productivity from a niche is supposed to converge to a normal distribution through the central limit theorem. Based on the normal distribution of yield in each niche, from the property of its cumulative distribution function, we can assume that the lower yield bound α and mean yield $E\left[Y|_{Y\geq\alpha}\right]$ form sigmoidal function with respect to the selective information $-\log p(\alpha)$ of $p(\alpha)$. This means that the effective range of minimum and mean yield (which corresponds to the straight section of sigmoid) could be approximated as linear functions of selective information representing the search cost of niche condition.

Taking this assumption further, we construct a simulation of mixed power-law vegetation with multiple species. We define the yield distribution Y_k of niches $k = 1, 2, \ldots k^{max}$ as a series of normal distributions with random weights as follows:

$$Y_k(X) \sim \frac{w_u \sigma_k}{k^{max}\mu_p} N\left(\mu_k, \frac{\sigma_k}{\mu_p}\right),$$

$$w_u \sim U([0, 2]).$$

where $U([0, 2])$ is a uniform distribution on the interval $[0, 2]$ with the mean value 1, and $N(\mu_k, \sigma_k)$ represents normal distribution with the mean μ_k and the standard deviation σ_k that follows Pareto distribution,

$$\sigma_k \sim f(x) = \frac{ab^a}{x^{a+1}}.$$

μ_p is the mean value of the Pareto distribution, and k^{max} is the number of simulated niches (allowing the overlap of not more than k^{max} species), both serve as the regularization factors to satisfy the normalization condition of a probability distribution, $\lim_{k^{max}\to\infty} \sum_{k=1}^{k^{max}} \int_X Y_k(X)\mathrm{d}m = 1$. For simplicity, X was taken on one-dimensional

real value $\boldsymbol{R}$:$(-\infty, \infty)$, which can be interpreted as a projection from the actual two-dimensional surface to one-dimensional section. A simulated example is depicted in Fig. 1.2. Note that the randomness introduced to $Y_k(X)$ will converge to the normal distribution of occurrence frequency $E\left[Y_k|_{Y\geq\alpha}\right]$, by the central limit theorem, which is depicted in Fig. 1.4 (right graphs).

This sampling means to realize a power-law distribution of niche surface on X for a given threshold α, by taking the distribution width and height from a Pareto distribution. Since we do not generally know the yield rate of a species with respect to the niche surface, the yield is multiplied by w_u to introduce a uniform range of variation.

In this article, we only simulate exhaustive global search with a qualitative perspective, which is not concerned by the distribution of μ_k, nor by the chosen value of the parameters and spatial configuration. This means that we are interested in the general form of function that describes the qualitative relationship between the search cost of a niche and its yield. Further quantitative analyses need precise adjustment of parameters from measurement, with an extended model in higher dimensional space according to the number of variables that affect niche condition. Note that the constraint of local search will be affected by μ_k.

Concerning the yield level α, the selective information of k^{max} niches giving yields $Y_k(X)$ more than α is given by $-\sum_{k=1}^{k^{max}} \log p_k(\alpha)$, where

$$\rho_k(\alpha) = \frac{\int_{X|_{Y_k(X)\in\left[\alpha,\alpha_k^{max}\right]}} \mathbf{1}_X \mathrm{d}m}{\int_X \mathbf{1}_X \mathrm{d}m}.$$

We calculated the yield per surface $E\left[Y_k|_{Y_k\geq\alpha}\right]$ and regularized minimum yield α_k^{reg} defined as follows:

$$\alpha_k^{\mathrm{reg}} = \begin{cases} \frac{\alpha}{\alpha_k^{max}} \text{ if } \alpha \leq \alpha_k^{max} \\ \mathrm{NA} \text{ else} \end{cases},$$

where $\alpha_k^{max} = \max_X(Y_k(X))$ and NA signify not assigned. α_k^{reg} represents the ratio between the minimum harvest line and maximum potential yield, which means the degree of preservation or the inverse degree of exploitation of each niche. If α_k^{reg} is close to 0, the whole niche will be harvested, while completely preserved at $\alpha_k^{\mathrm{reg}} = 1$.

We numerically investigated the qualitative relation between the selective information versus yield per surface and regularized minimum yield, with respect to the yield level α. The results are shown in Fig. 1.3. As examined with a single niche, multiple species power-law configuration also accepts linear fitting between search cost and yield. This consistency is due to the diversity introduced with random variables in the model, which would become less plausible in case of small and biased sampling. This numerical relationship leads to a hypothesis that in managing harvest over power-law vegetation structure with sufficiently high species diversity, the search cost could be linearly scalable with respect to the profit from yield.

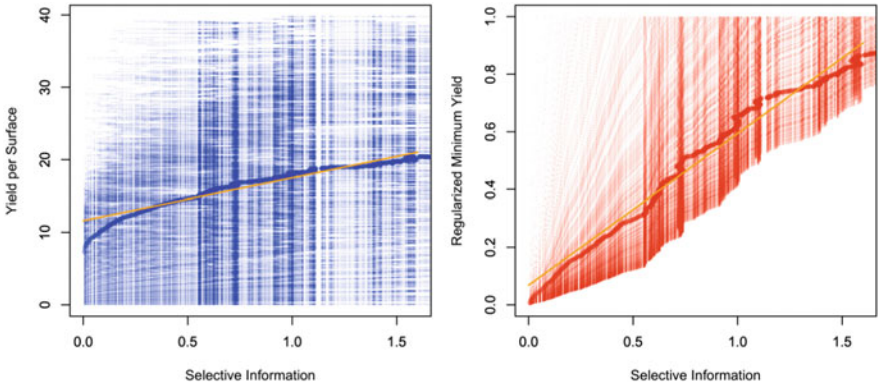

Fig. 1.3 Qualitative relation between selective information of niche $-\sum_{k=1}^{k^{max}} \log p_k(\alpha)$ versus minimum and mean yield. **Left** Selective information versus Yield per surface. Blue dots are the yield per surface $E\left[Y_k|_{Y_k \geq \alpha}\right]$, and blue circles are the mean value over all k^{max} niches for each α. **Right** Selective information versus Regularized minimum yield. Red dots are the regularized minimum yield a_k^{reg}, and red circles are the mean value over all k^{max} niches for each α. Orange lines in both figures are the linear regression in the range [0, 1.6] of the horizontal axis before the saturation of simulated yield, which qualitatively defines the efficiency bound of search energy cost. Parameters: $a = 2$ and $b = 0.5$, which results in $\mu_p = 1$ ($\mu_p = \frac{ab}{a-1}$ in case of $a > 1$). Yield level α was divided into 1000 steps from 0 to $\underset{k}{max}\left(\underset{X}{max}(Y_k(X))\right)$. In total, $k^{max} = 1000$ niches were simulated.

From the viewpoint of a search model, the numerical result conforms to a theoretical assumption that the energy required for search E_s can be defined as the inverse of occurrence probability $\prod_{k=1}^{k^{max}} p_k(\alpha)$, with scale merit s and coefficient c, such as

$$E_s = c\left(\prod_{k=1}^{k^{max}} p_k(\alpha)\right)^{-s},$$

which qualitatively coincides with linear fitting in Fig. 1.3 (orange lines) in logarithmic scale,

$$\log E_s = \log c - s\sum_{k=1}^{k^{max}} \log p_k(\alpha).$$

This means that if the logarithmic increase of search energy remained less than the direct proportion of the yield increase, the management of harvest from power-law distribution of ecological niches is scalable in terms of cost-benefit ratio. The exact profitability could be defined depending on the actual conversion rate between search energy cost and yield profit, scale merit s, and initial cost c.

Let us think in more details for a concrete management strategy. The occurrence probability $p_k(\alpha)$ is given by the exponential function of selective information

$-\log p_k(\alpha)$. As the amount of selective information increases, the probability of such an environment appearing decreases exponentially. The appearance probability of the environment is an exponential distribution with respect to the selective information. This means that the place of the environmental condition that is convenient for the cultivation of the target crops is going to be rare as the demand of minimum yield level α becomes higher. Favorable environments are not necessarily abundant, so finding a suitable environment for a crop will require search cost. However, if we can find and use an ideal environment, we can get a higher yield than cultivating in most other places.

If we can formulate the search method for rare favorable conditions, we can significantly increase the yield in harvesting from ecological optimum. Even if a large yield cannot be obtained at the experimental stage where the search condition is not well understood, it is possible to develop a method of shifting the practical environment to favorable conditions, or introduce crop species that are more adaptive to the current environment, then the average yield can be greatly increased. Actually, among useful plants of wild vegetables and native species, some varieties occupy a wide range of ecological niches in the natural state. If those can be utilized, it is possible to raise agricultural production with little cultivation cost. This strategy is also compatible with the introduction of neglected and underutilized species (Jaenicke et al. 2009).

The fact that the probability of occurrence forms a power-law distribution implies that the mean value of yield in ecological optimum is not practically meaningful. The mean value has significance when the yield is distributed symmetrically and occurs most frequently around the mean value as in the normal distribution. In the power-law distribution, the mean value is not the most frequent occurrence. If we increase the number of samples, at the moment when a rare event occurs, it may change to a drastically higher value than the mean value so far. This fluctuation becomes more apparent when dealing with a small sample in a practical situation. To make it a concrete and practical indicator, the fluctuation is too large with the mean value, since the mean value can potentially diverge to infinity as a mathematical definition of the power law.

It must be remembered that the evaluation of the yield centered on the mean value is a concept based on the conventional farming method presuming artificial control to the physiological optimum state of monoculture. In ecological optimization, the cultivation niches diversify, and they follow a complicated fractal structure and power-law distribution with colossal fluctuation. Indeed, such habitat heterogeneity is a key factor in community processes in the reconstruction of ecosystems (Larkin et al. 2016). Self-sustaining ecosystems require appropriate heterogeneity as an outcome of self-organization over molecular to landscape levels. It is impossible to manage the structure and dynamics inherent in that diversity with only the mean value.

Then, when considering the cultivation method in ecological optimization, what is the index that corresponds to the yield average of the conventional farming method? It should be contextualized in the process of **adaptive diversification** that augments symbiosis among suitable crops using complex niche structures. Rather than targeting

a specific crop and asking how much the single yield in that environment will be, we need to shift the focus to a vegetation strategy at the plant community level and secures the lower bound of total yield, by exploring and utilizing various crops suitable for the given environmental conditions in many ways. In the power-law distribution, the mean value of the whole greatly fluctuates, but it is statistically easier to guarantee the lower bound based on the expectation value of frequently occurring small events. In this sense, the convergent average of the regularized minimum yield α_k^{reg} in Fig. 1.3 is more practically important and reliable in actual management than the yield per surface $E\left[Y_k|_{Y_k \geq \alpha}\right]$; the latter mean is only convergent in simulation [with large samples on Pareto distribution with finite mean value ($a > 1$)]. The regularization in α_k^{reg} suppresses the effect of rare big events of α_k^{max} $\left(\alpha_k^{max} \gg \alpha\right)$ to zero and weighs more on highly frequent small yield above α $\left(\alpha_k^{max} > \alpha\right)$ in an inversely proportional relationship. Such numerical behavior is supported by the mathematical property of power-law distribution, where the harmonic mean converges to a finite value even in case the arithmetic mean diverges to infinity.[4] By taking the minimum level of yield as a measure of reliable productivity, it reflects the property of harmonic mean as the average of rates between harvest and cost: The harmonic mean of the benefit-cost ratios of unit harvest represents the mean benefit-cost ratio of the total unit productivity, which converges to a finite value with heavier weight on frequently occurring small niches than rare big ones.

Generally, the environment that can be dominated by a single species is rare, and there are many more chances of realization for mixed communities with diverse small niches structure. If we try to introduce over-yielding as symbiotic gain, it is easier to mix small niches to better exploit the statistical nature of ecological optimum (Funabashi 2016a). This strategy supports the compatibility between the self-organization process in natural vegetation and the mixed formation of small niches through the artificial introduction of seeds and seedlings in synecoculture. In contrast, ecological vulnerability and contradiction to multiple ecosystem services are pointed out in conventional methods, which imply that the power-law niche distribution is necessary for ecosystem resilience and represents a functional proxy of adaptive diversification (Funabashi 2017a).

In actual management, even if we cannot predict the high-yielding dominant colony formation of a single crop, the expectation value of yield from small niches in a mixed state of multiple species will stabilize, because the risk of total extinction of community decreases as the number of species increases. As a consequence, the average yield of a specific time and area only comes out as a resulting figure, and it is not directly possible to predict environmental change and future fluctuation of yield based on the average yield.

The overall process of adaptive diversification of crop communities with ecological optimum is schematized in Fig. 1.4. The prediction of future yield from the average at a certain point is a kind of pseudo-correlation that is limited to conventional farming methods, since it posits constant control of the environmental condition under a

[4]Mathematical proof is given in Appendix 3, and a numerical example is simulated in Fig. 1.16 (right).

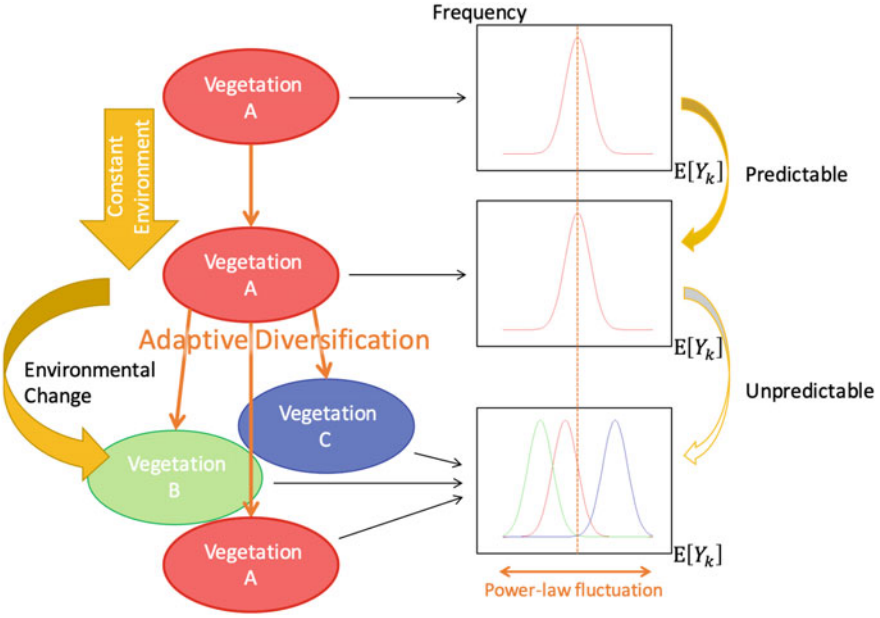

Fig. 1.4 Schematic representation of adaptive diversification of crop communities with respect to environmental fluctuation. The left diagram shows the development of vegetation portfolio from single vegetation A during the constant environment to multiple species A, B, and C to cope with environmental change. The right graphs show the yield $E[Y_k]$ of each vegetation versus its frequency of occurrence, in which red, green, and blue lines correspond to the distribution of vegetation A, B, and C, respectively. The red-dashed line corresponds to the most frequent yield of species A in the past environment, which is difficult to maintain through unpredictable environmental change that induces power-law fluctuation. However, the total yield from all vegetation can be secured by adaptive diversification of niche structure and its consequent productivity

small fluctuation.[5] If the environment fluctuates, the underlying conditions of niche formation change and then we cannot predict the causal relationship with the yield change unless the response of the vegetation to the environment is wholly known. Ecological niches are continually changing in response to environmental variation. In managing such open complex systems, we need to construct a broad strategy taking into account the diversity of responses to harness latent risks. In order to secure the lower limit of the average yield in a changing environment, it is not sufficient to only discover and rely on successful cases. We also need to consider other strategies in preparation for new contingencies. Adaptive diversification to a wide variation of the scenario by reinforcing the vegetation strategy is necessary, beyond the limitation of the past environment. With this respect, current efforts of transformational adaptation of key crop species to climate change are limited in the diversity of alternative crops and remain in monoculture framework (e.g., Rippke et al. 2016).

[5] Artificially controlled stable monoculture can be described as a dynamical system, as derived in Appendix 1.

The new management strategy based on the self-organized mixed polyculture is similar with the diversified investment for risk hedging in stock trading.[6] In a changing environment of the market, the unit price at a point in time has only a transient meaning. It is more important to obtain overall comprehensive profits while avoiding risks in long-term changes. With the same idea, in order to ensure a sufficient amount of agricultural production by adaptive diversification, we must take a vegetation portfolio and the field scale that can buffer sufficiently against environmental fluctuations (Funabashi 2016b). Even for crops that one cannot expect much yield at the present stage, we need to invest broadly in considering the possibility of becoming dominant in the future by the transition of niche structure. Besides, it is necessary to design a field with sufficient margin according to the scale of the change. If agricultural land sections decided by humans are not suitable, it will be necessary to revise the scale and topography that can structurally accept adaptive diversification.

Accurate forecasts of productivity based on rigorous measurements are effective only in the short term because environmental changes are known to involve chaotic instability both in climate and ecological dynamics (Cushing et al. 2005). However, plants living with symbiotic effects in a natural ecosystem have been surviving environmental change over more than a billion years and have continued to cover the earth surface repeatedly by constant evolution. In natural plant communities, the power-law distribution is observed in both vegetation-wise and the niche-wise surfaces (Funabashi 2017a). It represents the self-organized state of various niches from dominant to weak species as an outcome of the equilibrium between competition and symbiosis. Consequently, multiple buffering interactions are potentially prepared to cope with environmental changes, in a way that secures a certain amount of biomass by increasing some partial vegetation newly adaptive to the environment. In the land with necessary sunshine and rainfall, the absence of desertification is a manifestation that plants adequately buffer environmental changes due to naturally occurring adaptive diversification. The primary production does not deplete in highly established ecological optimum. It is the matter of replacing natural vegetation with useful plants to apply these dynamics into agricultural production.

It is in principle impossible to keep environmental conditions fixed, in the natural environment where there exist overlaps of multi-scale fluctuations in daily microscale, seasonal mesoscale, and macroscopic climate change. There is no guarantee that crops cultivated in a physiologically optimal range in past decades can grow at the same cost in the future. Recently, it has been reported in various places that the influence of global warming is reshuffling the wildlife (Pecl et al. 2017), which will call for a strategic mobilization of suitable crops in the global scale. Production areas that have relied on a small number of products based on conventional farming must fail at the moment when control cost of the environment exceeds productivity. Especially in terms of soil fertility, rock weathering is reported to amount

[6]Other than stock trading, adaptive diversification is similar to recently prominent e-commerce strategy that is based on power-law distribution. Sales of Internet shopping sites such as Amazon.com is known to follow the power-law distribution, which is also called as “the long tail” (Anderson 2008).

to ¼ of total soil nitrogen source worldwide (Houlton et al. 2018), which calls for the importance of the vegetation based on ecological optimum (i.e., natural vegetation and self-organized mixed polyculture such as in synecoculture) to prevent nitrogen runoff and to store more atmospheric nitrogen and carbon locally at each area.

In order to secure food production in the face of population growth, a viewpoint of adaptive diversification with the perspective of open systems management becomes necessary (Funabashi 2016b; 2017c). This approach can be translated to making use of the evolutionary mechanism in which plant communities in the natural state have been thriving and constructed the topsoil (Funabashi 2016a). It is to deliver the benefit from, while reinforcing the functioning of, the most highly elaborate buffer system in the evolutionary history of the Earth System.

1.5 Ecological Recapitulation Theory: Parallelism Between Phylogenic and Phase Diversities

Ecological interactions at community scale play essential roles in the evolution of the maintenance mechanism of biodiversity (Guimarães et al. 2017) and consequent primary productivity (Funabashi 2016a). Recently, toward the elucidation of the relationship between biodiversity and ecosystem functions, functional diversity that takes into account the intra and interspecific trait variability and phylogenic diversity that incorporates evolutionary relationship between species is widely investigated (Cadotte et al. 2011; de Bello et al. 2011; Albert et al. 2012; Carmona et al. 2017). The effect of functional diversity and phylogenic diversity on the aboveground biomass has been verified in field experiments in grasslands (Flynn et al. 2011). Functional and phylogenic diversities were found to be effective indicators to explain the changes in ecosystem function, which implied the importance of the evolutionary process that created mutations among species traits.

The integrative approach between the evolutionary process and ecological succession is also underway. Long-term increases in the effect of biodiversity on ecosystem functions have been reported in field experiments (Reich et al. 2012). A part of such temporally cumulative effect was revealed as the improvement of ecosystem functions through the promotion of niche division across generations in highly diversified communities (Zuppinger-Dingley et al. 2014).

In order to apply this evidence from community ecology to the food production in ecological optimum, we need to establish a complexity measure for interactive management model taking both phylogenic and succession phase diversity into account. The complexity measures in the context of open complex systems are defined as a set of low-dimensional proxies that incorporate useful features for the development of the system's diversity and effective management strategy (Funabashi 2017b). In the case of the natural ecosystem, succession stages of soil and land ecosystems are integrated and proposed as a complexity measure for the management of material cycles in ecological optimum (Funabashi 2016c). As an example of food production,

synecological farming adopts the complexity of evolutionary traits of land plants as an index of assessment and control of vegetation, which is also associated with the development of species traits through succession stage (Funabashi 2016b).

The similarity and correspondence of the developed traits between ecological succession and evolution of land plants are useful for the actual management of mixed plant communities, in a way that integrates both functional and phylogenic diversity. This accordance can be termed as "**ecological recapitulation principles**," taking after the historical notion of "ontogeny recapitulates phylogeny" in embryological parallelism. The ecological recapitulation principles can be listed as follows, as mutual characteristics of plant species that have been observed to develop both in ecological succession and phylogenic evolution of plants:

- Growth height (biomass)
- Vascular development and lignification (tree structure, an increase of lignin)
- Complexity and resilience of ecological network based on the diversity of competition and symbiosis, its spatiotemporal scale such as history dependence
- Diversity of secondary metabolites
- Complexity of food chains
- Buffering and retention time of trace elements in soil such as nitrogen, carbon, water, oxygen, mineral, and phosphorus
- Utilization of fauna (e.g., development of fruit pulp and seed strategy) and its spatiotemporal scale (e.g., Synchronization of bird migration and fruiting phenology)
- Water supply capacity of the root system.

These common features can be formalized as "vegetation succession recapitulates evolution," though not in the sense of a scientifically rigorous description. It is rather for a practical matching between succession stage and phylogenic diversity to develop an integrated proxy for the management of plant communities.

One of the simplest ways to construct such a measure can be achieved with a geometrical mean between phylogenic and succession stage diversity.[7] Figure 1.5 schematically represents an example. The geometrical mean C_g between phylogenic diversity d_p and succession stage diversity d_s can be formalized as

$$C_g := \sqrt{d_p d_s}.$$

This measure coincides with the adoption of gamma (γ) diversity that describes the total species diversity in target ecosystem, with the considerations on alpha (α) diversity in phylogeny d_p and beta (β) diversity in succession stage d_s (Whittaker 1960).

As an alternative perspective, different vegetation types are reported to correlate with different fractal dimensions in satellite image analyses (Nayak 2008), which

[7]Note that we can find other characteristics of geometrical mean that are compatible with the nature of biodiversity and ecosystems functioning. Another example of application in the context of food security concerning product diversity is developed in Appendix 2.

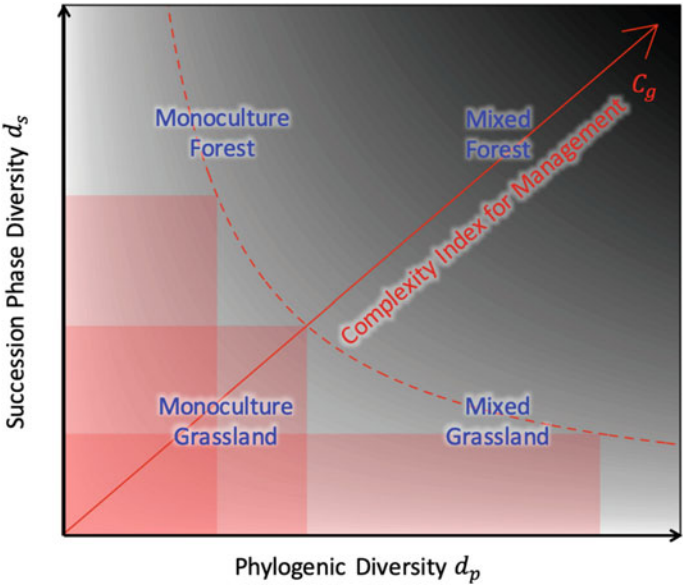

Fig. 1.5 A typical example of complexity measures that integrate phylogenic and succession phase diversity. The value of geometric mean C_g between d_p and d_s is shown with a grayscale gradient, with a contour line example with a red-dashed line. The surface of red rectangles corresponds to the value of C_g^2 at the upper-right corners, which are conserved along the red-dashed line. Typical vegetation physiognomy of the highest succession stage with different combinations of d_p and d_s is described in blue. The overall complexity of evolutionary traits that support primary production of naturally organizing ecosystem develops qualitatively along the red arrow and grayscale, as an integrated diversity of phylogeny and succession stage

provide another source of complexity measure that should work in a complementary way with the ecological recapitulation principles.

Actual management requires an intensive operation on each scale of species diversity for global optimization of utility (Funabashi 2016a). Beyond existing biodiversity, operational diversity with newly introduced species becomes central when considering food production based on the diversity of plant genetic resources.

1.6 Operational Ecological Diversity and Extended Notions of Biodiversity and Ecosystem Services

There are more than 30,000 edible plant species recorded in the world, out of which we depend only on 30 crops for 90% of calories in our food. While there are 7000 plants used for food, commonly cultivated species in agriculture are limited to 120 (Yong et al. 2006). This highly homogenous situation of crop diversity distribution wipes out natural biodiversity through agricultural land conversion, which is consid-

ered as a principal driver of the sixth massive extinction in life history (Pereira et al. 2010).

To overcome the fundamental trade-off between biodiversity and productivity in conventional agriculture, synecological farming (synecoculture) sets a series of inclusive strategies to intensively augment species diversity in the farming plot and surrounding ecosystems (Funabashi 2016a, b, 2017a). Such human-driven introduction of new species for multiple socio-ecological purposes and total enhancement of biodiversity and ecosystem functions stems from the conception of operational ecological diversity (Fig. 1.6), which extends the notion of biodiversity and ecosystem services beyond the context of conventional agriculture and ecology (Fig. 1.7).

Operational ecological diversity in Fig. 1.6 includes all sorts of human activity possible in relation to biodiversity, except transgenetic technologies for the risk con-

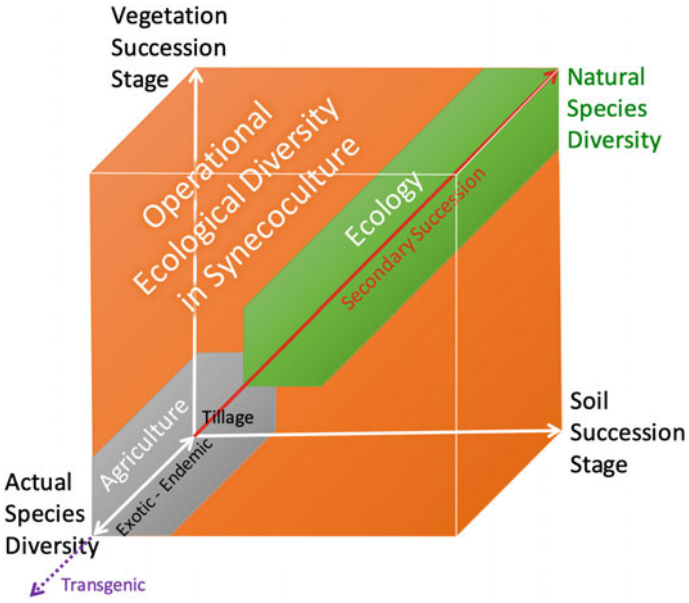

Fig. 1.6 Operational ecological diversity with respect to species diversity, succession stages vegetation (aboveground), and soil (underground). Tillage-based agriculture only treats crop species diversity without elaborate soil ecosystem that can only be formed through long-term vegetation succession (gray area). The formation of soil structure that develops over the years to decades in ecological optimum critically lacks even in advanced conservation of agriculture practices, thus remaining at the stage of reduced tillage and fertilization. On the other hand, studies in ecology mainly treat secondary succession of natural vegetation (red arrow) over a long period, with more importance on endemic species and natural species diversity for the conservation value (green area). In contrast, operational ecological diversity in synecoculture can explore all combinations of endemic and exotic species through intensive introduction in various soil and vegetation succession stages in three-dimensional space (orange volume). Under the concern of interspecies gene transfer problem, transgenic technologies are refrained from the concept of operational ecological diversity in synecoculture

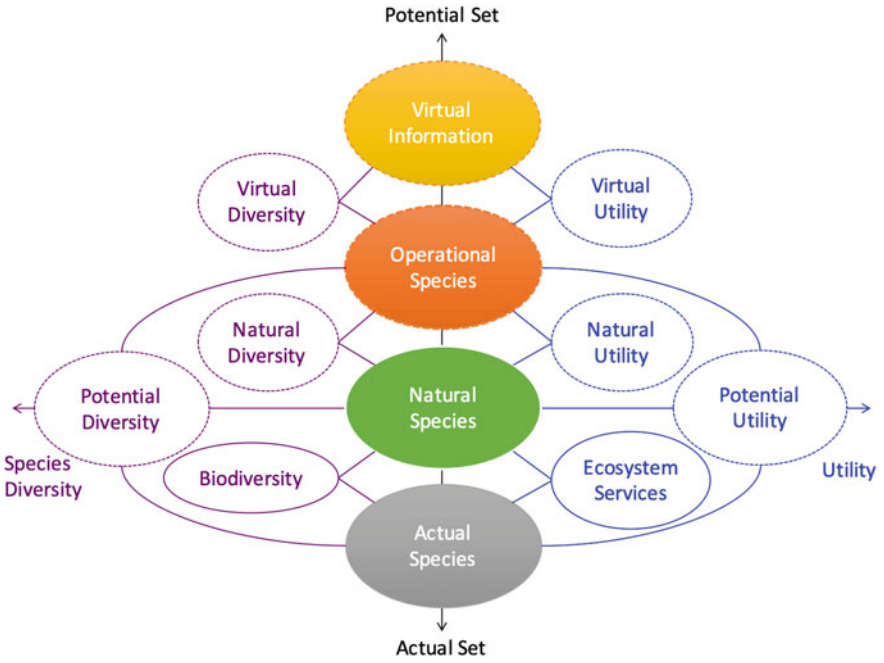

Fig. 1.7 Extended notion of biodiversity and utility in synecoculture. Usually, the loss and recovery of biodiversity are discussed in a comparison between naturally occurring maximum species diversity (green circle) and actually observed species diversity (gray circle), on which various utilities including economic benefit are termed as ecosystem services. With operational species diversity (orange circle) and virtual information of other ecosystems (yellow circle), we can think of the extension of these concepts (dashed circles) as listed in Sect. 1.6. Colors of circles conform to those of divided area in Fig. 1.6

cerned on the interspecies gene transfer. Since conventional agriculture produces under the constraint of physiological optimization, it ignores most of the ecological succession. On the other hand, ecological studies focus on the natural succession of vegetation under varying degrees of genetic and ecological disturbance, but a wide range of introducible species remains out of concern. Current food industry and academic disciplines only occupy a limited area compared to all possible ecosystems realizable by human assistance.

By considering the possibilities of operational ecological diversity, it is possible to extend the concept of biodiversity and ecosystem services as schematized in Fig. 1.7. Here, we take neutral terms as "species diversity" and its "utility" to deconstruct and expand the concepts. Newly derived concepts (dashed circles in Fig. 1.7) concerning the anthropogenic augmentation of ecosystems are as follows:

- Operational species: It describes a set of possibly introducible species, whether wild plants or crops, in a given environment by human assistance. The introduc-

tion should be based on the open-field environment under coexistence of various ecological niches.

- Virtual information: It represents digitalized information on biodiversity and other related information to the ecosystem, such as meteorological data, in all parts of the world. It includes the global databases of biodiversity (e.g., GLoBI 2017) and recorded traditional knowledge of indigenous peoples and local communities (DGM 2017). It is a potential resource, e.g., for future climate change adaptation, which could be reflected in the set of operational species in the future.
- Natural diversity/utility: Taking a reference to the set of operational species, what is realized in natural state ecosystem is only a subset of what is possible in social–ecological interaction in, e.g., preservation state. Therefore, the species diversity realized by natural process is termed as "natural diversity" to describe the species diversity that nature has chosen from the set of operational species and its utility as "natural utility." As natural ecosystems have only limited and partial economic profit for industrial activity, it only reflects a part of possible realization under natural selection.
- Potential diversity/utility: By comparing the sets of "operational species" and "actual species," one can estimate the true potential of biodiversity and its utility realizable with plant communities managed by humans in ecological optimum.
- Virtual diversity/utility[8]: Information and Communication Technologies (ICT) can store, search, and share various kinds of information relevant to biodiversity and its management, which will serve for the comprehensive exploration of "operational species" set and potential utilities for future adaptation to changing environment, which can be described as "virtual diversity" and "virtual utility," respectively. It is important to consider the use of virtual diversity to provide essential supports for the exploration of non-monetary benefit-sharing, such as open databases and research uses.

These concepts provide a basic framework in designing concrete strategies of the adaptive diversification theoretically explored in Sect. 1.4, such as the implementation of vegetation portfolio in synecoculture.

1.7 Experimenting Anthropogenic Megadiversity

We have experimented an extreme case of adaptive diversification with a small-scale synecoculture farm in Tokyo, Japan. During April 2011–March 2017, we have intro-

[8]Virtual diversity does not exist in real ecosystems but only in human-prepared databases, which serves as the reservoir of resilience in the future adaptation of ecosystems. In stable ecosystems with saturated species diversity, virtual diversity does not make any significant contribution to ecosystem functions, just like redundant species in the redundancy hypothesis. It can, however, be a source of compensation for the loss of ecosystem functions under the rivet hypothesis in dynamical change and important accelerator of ecological transition. In either case, the virtual utility can contribute to enhancing ecosystem services for human purposes, such as the adaptation of the product portfolio to market value.

duced commercially available 379 varieties of crops from 81 families that comprise more than 1000 cultivars, as listed in Table 1.1. Spatial configuration was chosen randomly following the general principle of management in synecoculture (Funabashi 2016b). The field view is shown in Fig. 1.8. This experiment aimed to realize anthropogenic megadiversity as an augmented ecosystem with respect to biodiversity and self-organized ecosystem functions (Funabashi 2016a), through the intensive introduction of operational species diversity and utility defined in Sect. 1.6 (Fig. 1.7).

In consistency with Fig. 1.6, ecological diversity realized with synecoculture extended beyond conventional agriculture practice and field experiments in ecology: In terms of vegetation succession, secondary succession from bare land during six years with introduced and naturally occurring species was observed, which deviates from the culture environment under the control method of conventional farming (such as tillage, fertilizer, and chemicals), also involving much wider varieties of exotic crop species than natural succession.

In terms of the succession of the soil ecosystem, short-term species control experiment was performed to elucidate the relationship between management strategies of synecoculture and self-organized ecosystem functions (Funabashi 2017a). Water retention and permeability were found to correlate with the aboveground development of vegetation following ecological optimum. Soil microbial diversity and activity were also found to correlate with the operational species diversity, attaining highest grade of the production and regulating services compared to other reported examples of farmland.

These ecological successions have led to the phase transition of acceptable crop species in no-tillage and no-fertilizer conditions, as well as sufficient regulation of pest through self-organized food chain under no-chemical condition.

We also investigated the relationship between operational species diversity for different surface scales, based on the experiment data in (Funabashi 2017a). The results are summarized in Fig. 1.9: Fig. 1.9a shows the diversity of species and its taxonomical family with respect to the measuring surface generated by the $n = \{1, 2, 4, 8, 16, 32\}$ tuples from 36 spot measurements. At the smallest scale ($n = 1$), it corresponds to the α-diversity of observation spots, which are merged to the γ-diversity of the whole plot at the largest limit ($n = 36$). The species–area relationship shows typical power-law relation observed in the natural ecosystem (Arrhenius 1921), with slight saturation toward the whole plot scale, possibly due to the spatial constraint of the experiment. Plant species diversity ranges between 10 and 70 species, which fits well with the most probable niche diversity distribution by %-measurable sampling from various power-law vegetation, as simulated in Fig. 1.1. Notably, the diversity of herbaceous species in Fig. 1.9c ranges over 10–50+ species, which coincides with the small sampling case $N = M = 100$ in Fig. 1.1. It implies that the measurement covers sufficiently appropriate spatial scales for the actual power-law gradient parameter a.

The variance of species and family diversity in Fig. 1.9b, d corresponds to the measures of β-diversity within the plot. In Fig. 1.9b, interspecies diversity increases as the scale of measurement expands (except the combinatorial saturation as it approaches the whole plot scale at $n > 18$). Therefore, the functional diversity that is supported

Table 1.1 List of 81 taxonomical families comprising 379 varieties introduced in 250 m^2 at Todoroki synecoculture farm in Tokyo during April 2011–March 2017

Family	#Varieties	Cannabaceae	1	Juglandaceae	2	Polygonaceae	5
Actinidiaceae	1	Caprifoliaceae	9	Lamiaceae	45	Primulaceae	2
Adoxaceae	2	Caryophyllaceae	4	Lardizabalaceae	2	Ranunculaceae	1
Aizoaceae	1	Chenopodiaceae	1	Lauraceae	2	Rosaceae	28
Alismataceae	1	Commelinaceae	1	Malvaceae	7	Rubiaceae	2
Alliaceae	9	Convolvulaceae	4	Meliaceae	1	Ruscaceae	1
Amaranthaceae	4	Cornaceae	1	Moraceae	2	Rutaceae	7
Amaryllidaceae	4	Crassulaceae	1	Myricaceae	1	Sapindaceae	1
Apiaceae	13	Cucurbitaceae	10	Myrtaceae	9	Saururaceae	1
Apocynaceae	2	Cupressaceae	1	Oleaceae	1	Scrophulariaceae	1
Araceae	1	Dioscoreaceae	1	Onagraceae	2	Solanaceae	8
Araliaceae	5	Dipsacaceae	3	Orchidaceae	1	Theaceae	2
Asparagaceae	1	Elaeagnaceae	2	Oxalidaceae	1	Urticaceae	1
Asphodelaceae	3	Equisetaceae	1	Papaveraceae	1	Valerianaceae	3
Asteraceae	42	Ericaceae	3	Passifloraceae	1	Verbenaceae	3
Basellaceae	2	Euphorbiaceae	1	Pedaliaceae	1	Violaceae	1
Berberidaceae	1	Fabaceae	17	Phytolaccaceae	1	Vitaceae	2
Betulaceae	3	Fagaceae	2	Plantaginaceae	4	Xanthorrhoeaceae	1
Boraginaceae	3	Geraniaceae	4	Poaceae	15	Zingiberaceae	3
Brassicaceae	36	Hypericaceae	1	Podocarpaceae	2	#Total	#Total
Campanulaceae	1	Iridaceae	1	Polemoniaceae	1	81	379

Fig. 1.8 View of Todoroki synecoculture farm in Tokyo, Japan, that introduced 379 varieties (more than 1000 commercial cultivars) in 250 m^2 during six years as listed in Table 1.1. Picture taken on May 21, 2016

by interspecies variability may enhance ecosystem functions within this scale. On the other hand, taxonomical diversity at the family level shows consistency or slight decrease with respect to scale change in Fig. 1.9b. It may imply the stability of ecosystem functions that are supported by phylogenic diversity. Such inferred difference of functional contribution between interspecies and phylogenic diversity may arise from the characteristics of the operational species diversity introduction specific to this experiment.

Figure 1.9c shows the breakdown of species diversity between herbaceous and arboreous plants, introduced and naturally occurring or spontaneous species, and edible and non-edible species (see Funabashi 2017a, for definition). In terms of power-law niche formation, it is the introduced and/or edible species that are contributing to such formation, more than spontaneous and/or non-edible species that remain in the exponential distribution (differences shown with red and blue arrows, respectively, in Fig. 1.9c).

As shown in Fig. 1.9d, the contribution to β-diversity also shows a similar difference between introduced/edible and spontaneous/non-edible species. The β-diversity within the plot was principally augmented by the introduced and/or edible species (except the combinatorial saturation as it approached the whole plot scale at $n > 18$), compared to the spontaneous and/or non-edible species that did not show an increase by scale.

The overall results imply that even with limited small areas, the human-driven introduction of operational edible species diversity with synecoculture strategies is able to establish the species–area relationship that follows qualitatively similar distribution as a natural ecosystem. The whole process can be formalized as a replication of ecological optimum by replacing secondary succession with introduced species. As the operational species set contains larger areas of diversity and utility than nat-

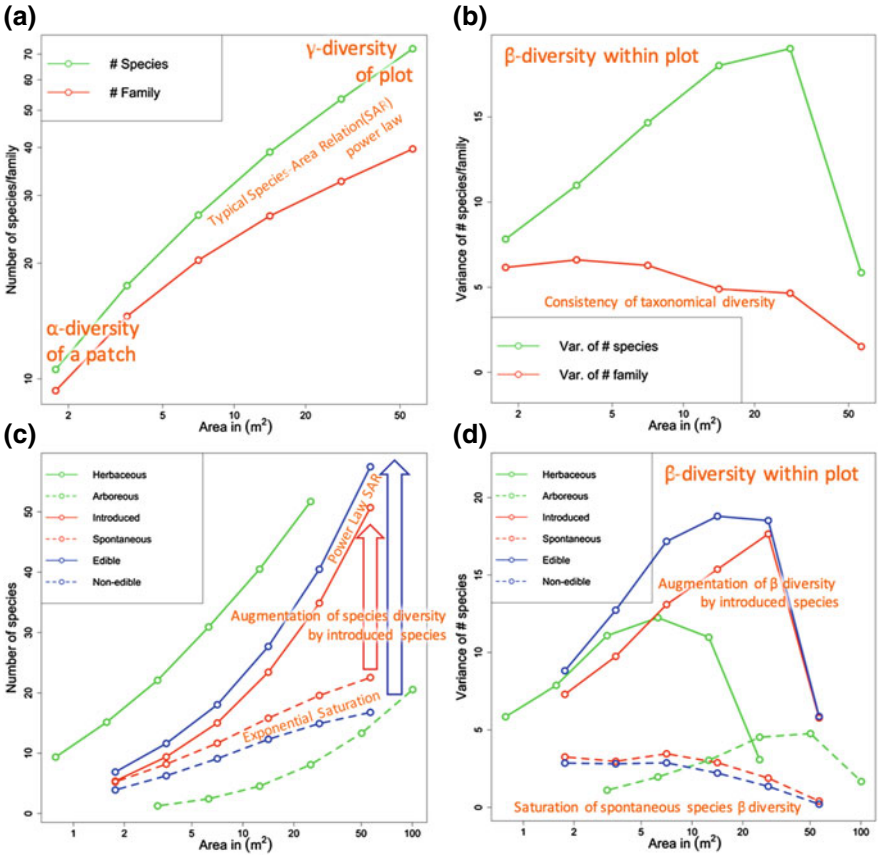

Fig. 1.9 Relationship between species diversity and surface in Todoroki synecoculture farm in June 2015. For experimental design and measurement methods, see Funabashi (2017a). In **a**, **b**, **c**, and **d**, based on the smallest sampling area for herbaceous species (circle with 0.5 m radius) and arboreous species (circle with 1 m radius), species and family diversity was counted on average surface (circle with 0.75 m radius), and higher surface scales were randomly generated by combining $n = \{2, 4, 8, 16, 32\}$ sets from 36 sampling spots. The variance was calculated from 1000 random numerical sampling for each surface scale. **a** Sampling area versus the number of plant species (green) and taxonomical family (red) in double logarithmic scale. **b** Sampling area in logarithmic scale versus variance of plant species diversity (green) and taxonomical family diversity (red) in linear-log scale. **c** Sampling area in logarithmic scale versus number of herbaceous species (green solid line), arboreous species (green-dashed line), introduced plant species (red solid line), naturally occurring plant species (red-dashed line), and edible species that were utilized in synecoculture (blue solid line) and non-edible species that were not yet utilized in synecoculture (blue-dashed line). **d** Sampling area in logarithmic scale versus variance of the number of herbaceous species (green solid line), arboreous species (green-dashed line), introduced plant species (red solid line), naturally occurring plant species (red-dashed line), and edible species that were utilized in synecoculture (blue solid line) and non-edible species that were not yet utilized in synecoculture (blue-dashed line). See Sect. 1.7 for the explanation of the texts in orange

ural ones in Fig. 1.7, there is a possibility of increasing biodiversity and associated ecosystem functions with synecoculture, beyond natural preservation state.

In the context of the argument between single large or several small reserves of equal area (SLOSS) (Laurance 2009), it may be effective to allocate synecoculture in a fragile small area such as forest edge. As species with narrow distribution areas are generally harder to be included in protected areas, local extinction is likely to occur for those species without strategic inclusion (Akasaka et al. 2017), and synecoculture can provide a modality to integrate such long-tail conservation efforts and local economic activities.[9] Globally, it is of future expectation whether the anthropogenic introduction of operational species diversity could accelerate both evolutionary adaptation and evasion from extinction in endangered zones, such as overly disturbed tropical rainforest by forest fragmentation (Laurance et al. 2004). This perspective involves the alteration of ecosystem with newly introduced species, which should take into consideration the trade-off between development and conservation in the context of population growth, legal preparation on access and benefit-sharing of plant genetic resources (CBD 2010a; Paroda et al. 2017), and consider how to design sustainable social–ecological system as an alternative stable state other than the global collapse of biodiversity (Barnosky et al. 2012).

1.8 Virtual Diversity that Supports the Extension of PGR

In order to assess operational diversity comprehensively, one needs supportive virtual information as wide as possible to explore the possibility of operational species set (Fig. 1.7). Such relevant ecological information can be obtained from global and commercial databases and other field experiments.

Figure 1.10 shows a part of virtual information from field experiments in Japan that supported the megadiversity experiment of Todoroki synecoculture farm in Sect. 1.7. In order to attain the maximally possible operational diversity, virtual information should be elaborated ahead of the future ecological succession and environmental change. Not only the possibility of species diversity but the management knowledge should also be shared as virtual information, which helps knowledge transfer in citizen science practice (Funabashi 2013).

Such a strategy of information investment becomes particularly crucial in zones sensitive to climate change and experiencing ecological regime shift such as desertification. From synecoculture experiment in Burkina Faso, it has been reported that it is possible to reverse the regime shift from bare desert to mixed forest formation, with all types of species that can be found in mature forest, covering a wide range of the complexity measure in Sect. 1.5 (Tindano and Funabashi 2016; Funabashi 2017a). The field proof conforms to the theory developed in Sect. 1.4 that reproduces and makes use of the Earth System's buffering function through adaptive diversification of plant community with operational species diversity. As a drastic transition between

[9]More theoretical details in Appendix 3.

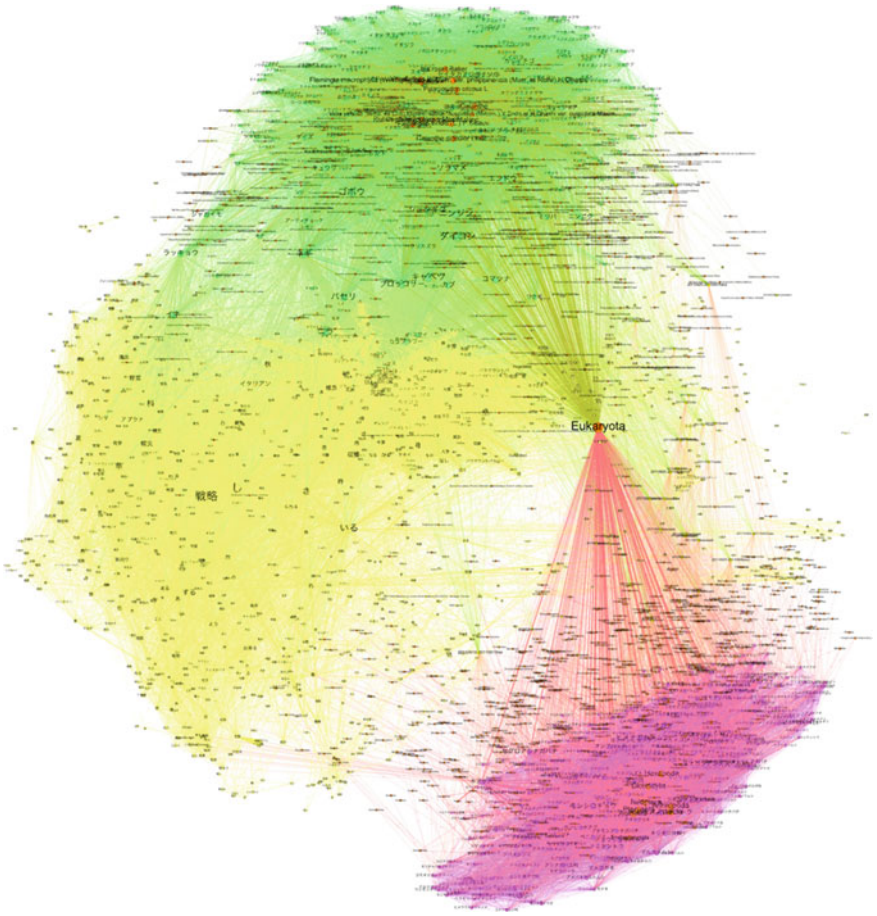

Fig. 1.10 Graph visualization of the virtual information for the management of operational species diversity in Todoroki synecoculture farm. Based on ten experimental plots of synecoculture in Japan during April 2011–March 2012, possibly introducible or naturally occurring 1130 plant and insect species and related knowledge are depicted as a graph based on the association of observation. Green nodes correspond to the cluster of virtual plant species diversity, magenta that of insect species, and yellow that of morpheme (nouns, verbs, and adjectives) extracted from the management knowledge written as Japanese texts. The morphological analysis of Japanese texts was performed using RMeCab library (version 0.996) on R (MeCab 2017). Graph visualization with Gephi (Bastian et al. 2009)

different ecological states occurs in a short period, potential diversity and utility also change to a totally different capacity; therefore, the virtual information that incorporates a future extension of operational diversity becomes crucially important for the sustainable management.

Figure 1.11 shows an example of virtual information in relation to actual species diversity in the experiment. For real-time processing and use of ecological big data such as with the combination of actual environmental and virtual information, computing technologies such as machine learning and artificial intelligence will be required to scale up the management (Funabashi 2017c; Funabashi et al. 2017). This information can be efficiently and interactively treated with emerging human—computer interfaces such as augmented reality (AR) as represented in Fig. 1.12.

With the scenario of adaptive diversification of plant genetic resources, the Information and Communication Technologies (ICT) will become an essential infrastructure for the achievement of global sustainability goal such as Aichi biodiversity targets (CBD 2010b) and UN Sustainable Development Goals (UN 2015), together with the institutional need of developing access and benefit-sharing clearing-house for the equitable implementation of operational species diversity (CBD 2010a, 2017).

1.9 Adaptive Diversification Experiments in International Level

Climate change is triggering worldwide geographical redistribution of plant and animal species (Pecl et al. 2017). Conservation activities beyond national jurisdiction are essential for the sustainable use of biodiversity at large scale (UNEP 2017). Adaptive diversification across national boundaries will increase its importance to secure biodiversity and food production.[10] Under this perspective, we extended the operational diversity toward the international exchange of plant genetic resources (PGR) between synecoculture experimental farms in Japan, Taiwan, and Burkina Faso.

The number of exchanged commercial varieties is shown in Fig. 1.13. Since Japan is situated in a temperate zone, Burkina Faso in semi-arid tropics and Taiwan between temperate and tropical zones, main overlaps of introducible species can be found between Japan–Taiwan and Taiwan–Burkina Faso. Especially, Taiwan can serve as an integrated validation site in both temperate and tropical conditions, as most of the species are overlapped with the other countries. As soil humidity can be controlled by irrigation and density of vegetation, and the sunlight is consistently available in open-filed culture, the temperature is the limiting factor among other environmental parameters. The geographical distributions of these countries can encompass a wide range of operational species diversity introducible to similar climate condition, which serves as a screening for the introduction to megadiverse countries such as

[10] A simple simulation on food security grounded on product diversity is shown in Appendix 2.

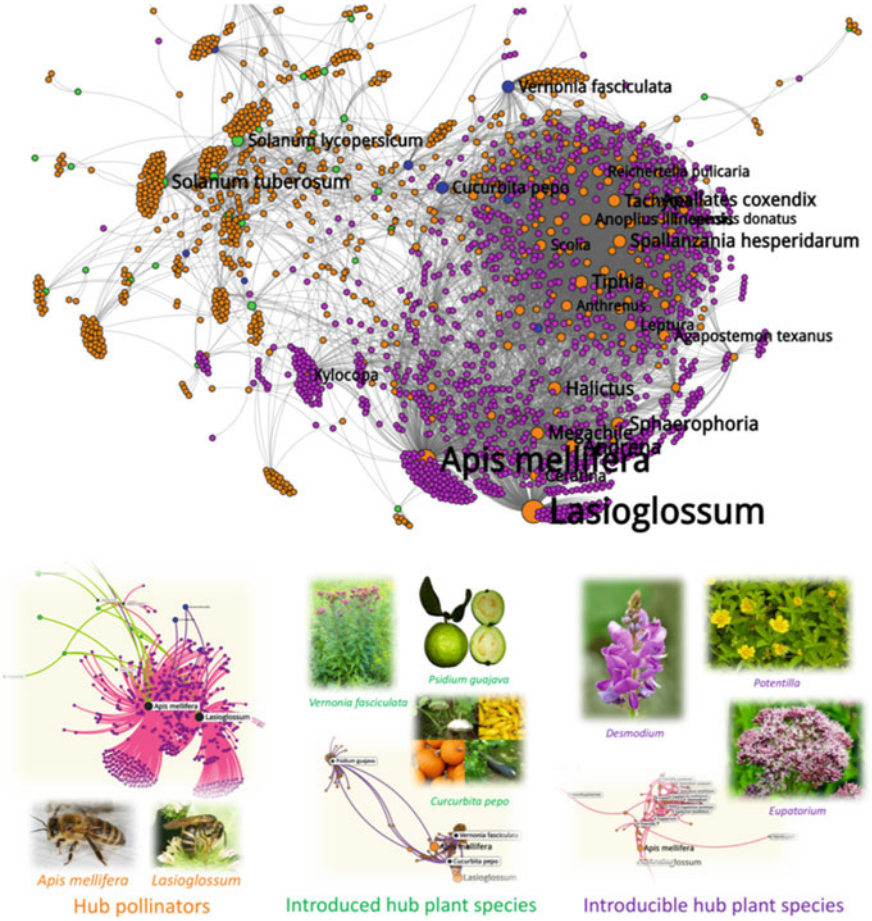

Fig. 1.11 Virtual species interaction graph in relation to introduced species with the synecoculture experiment at Mahadaga pilot farm, Tapoa province in Burkina Faso. **Top** Based on the 150 edible plant species introduced in the plot (Tindano and Funabashi 2016), 907 *Animalia* species with 1527 reported interactions were retrieved from a global database (GLoBI 2017) and shown as orange nodes. Further 1195 plant species that will be pollinated by this estimated acceptable fauna with 7259 plant–animal interactions were retrieved and added to the graph. The overall plant species were divided into three categories; plant species that were already introduced in the plot and can be pollinated by estimated fauna (blue nodes); plant species that were already introduced in the plot but that cannot be pollinated by estimated fauna (green nodes); and plant species that were not yet introduced in the plot and can be pollinated by estimated fauna (purple nodes). The node and label size of each species reflect the number of estimated interactions with other species, which means a larger node is more important as the network hub. **Bottom left** The most significant potential hubs in terms of ecological interactions, principally the pollination, revealed to be the European honey bee, *apis mellifera*, and the sweet bee genus, *lasioglossum*. **Bottom middle** Estimated hub plant species within introduced crops. **Bottom right** Estimated hub plant genera further introducible to the experimental farm. Pictures from Wikipedia. Graph visualization with Tinaweb (TINA 2017)

Fig. 1.12 Example of the management interface using augmented reality (AR) technology. Through portable and wearable devices with a camera such as a smartphone, tablet computer, and glass interface, one can view and manage virtual information related to actual vegetation on-site, using this information overlapped with the real world in a non-invasive way to the environment

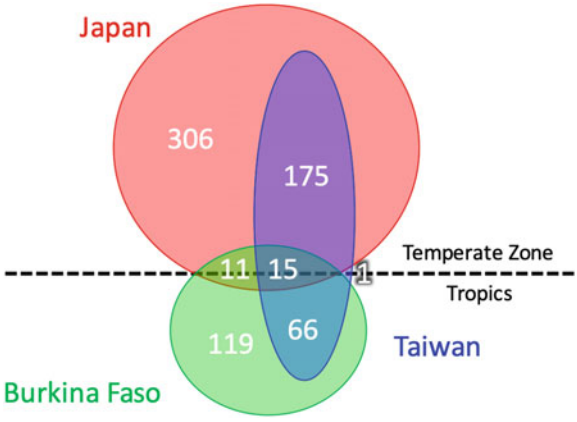

Fig. 1.13 Venn diagram on the number of commercial crop varieties exchanged between synecoculture experimental fields in Japan (red), Taiwan (blue), and Burkina Faso (green). The dashed line shows the separation between the temperate zone (above) and tropics (below)

India (Funabashi 2017a) and small tropical countries vulnerable to climate change (Petherick 2012).

The choice of exchanged varieties at this stage depends only on heuristics based on commercial availability and not yet exhaustive in terms of potential operational diversity. The exchange was conducted with solely dry commercial seed package, following the direction of local authorities such as quarantine and with consideration to the international agreement such as the Cartagena Protocol on Biosafety (CBD 2000) and the Nagoya Protocol on access and benefit-sharing (CBD 2010a).

Considerations on population and ecological genetics of native and introduced flora were also taken into account (Richards et al. 2016). Locally restricted varieties such as those of *glycine max* in Taiwan were refrained from export. The experiment in Burkina Faso followed the FAO guideline for mainstreaming biodiversity (FAO 2016; Tindano and Funabashi 2017) and respected access and benefit-sharing (ABS) guideline in African Union (UA 2015).

Exhaustive realization in diversity and quantity of operational plant genetic resources should wait for the preparation of national ABS laws with proper Prior Informed Consent (PIC) and Mutually Agreed Terms (MAT) formats, which are still absent in many countries including Japan, Taiwan, and Burkina Faso.

At the current stage, the international exchange of PGR in synecoculture is limited in a small quantity of commercially available and commonly cultivable species. Traditional knowledge on these PGR was occasionally shared under the open-source principle based on the provider's will. In order to maximize the resilience and adaptation capacity to climate change, it is urgent to establish consistency between ABS-PIC/MAT, quarantine systems, and monetary/non-monetary benefit distribution for the large-scale implementation of adaptive diversification at international level.

1.10 Conclusion

We theoretically examined the power-law distribution of ecological niches in plant communities and developed a global strategy of adaptive diversification and complexity measure reflecting functional diversity for the management of food production in ecological optimum. We extended the notion of biodiversity and ecosystem services with operational and virtual ecological diversities and reported the synecoculture experiments constructed on this framework. Future development of ABS legislation compatible with the anthropogenic augmentation of ecosystems is required to maximize the resilience to climate change and compensate for the biodiversity loss with the operational ecological diversity.

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Appendix 1: Construction of Lebesgue Integral in an Ecosystem Data Set

This section summarizes the basics of Lebesgue integral, which gives the mathematical basis of analysis in ecosystem data with power-law configuration. The integration of the probability measure can be implemented by a search algorithm, and an IT platform such as content management system (CMS) is required for the analysis of massive data (Funabashi 2017b).

Lebesgue Integral as Probability Integral Over Real Number and Integration of the Probability Measure on a Set

In order to give the calculation of the probability by Lebesgue integral on the real parameter space, consider Lebesgue measurable space $(X, \boldsymbol{B}, \mu)$.

The normalization condition by the Riemann integral of the probability density function $p(x)$ on $X = \boldsymbol{R}:(-\infty, \infty), \boldsymbol{x} \in X$ is given by

$$\int_{-\infty}^{\infty} p(x)\mathrm{d}x = 1.$$

The Riemann integral is defined by the limit value of infinite series. Therefore, fractal figure, etc., which involves the limit operation in the definition of the function $p(x)$ itself, will encounter double limit operations, and the analysis becomes extremely difficult. Since vegetation pattern has the property of fractal figure, the calculation of analytical solution is difficult when extrapolating measurement value to fractal figure model.

In such a case, it can be calculated using the Lebesgue integral. Let μ be the Lebesgue measure on $\boldsymbol{R}$, then the normalization condition of probability by Lebesgue integral is

$$\int_{X} p(x)\mathrm{d}\mu = 1.$$

Then, the Lebesgue integral of the probability satisfying $p(x) \geq \alpha$ is given by

$$\int_{X|_{p(x)\geq\alpha}} p(x)\mathrm{d}\mu.$$

This can be calculated for the distribution of $p(x)$ with a complex configuration, such as fractal distribution on $\boldsymbol{R}$, by the Lebesgue convergence theorem. Intuitively, since the phase structure of the completely additive class $\boldsymbol{B}$ is defined, the convergence of the infinite sequence can be treated in a topologically simple manner, by putting

the limit operation that defines the fractal function outside of the integration. The probability density function $p(x)$ on $X = \boldsymbol{R}^{\boldsymbol{n}}:(-\infty, \infty)^{\boldsymbol{n}}$ is also given in the same way.

In order to calculate the probability on a symbolic set S, not on $\boldsymbol{R}^{\boldsymbol{n}}$, it is necessary to determine the completely additive class $\boldsymbol{E}$ on S and the probability measure P. In the measurable space $(S, \boldsymbol{E}, P)$, let 1_S be the definition function of S, then the normalization condition of the probability measure is

$$\int_S 1_S \mathrm{d}P = 1.$$

The occurrence probability that is not less than α when measured with P is given as

$$\int_{S|_{P(S)\geq\alpha}} 1_S \mathrm{d}P.$$

Mean Information

The mean information of all events of S is

$$\int_S -\log(P)\mathrm{d}P.$$

When P is binarized with a certain threshold value with respect to the occurrence probability $P(s)$ of the element $s \in S$, the mean information is redefined as follows, on a newly binarized probability measure P' on the measurable space $\left(S, \boldsymbol{E}, P'\right)$:

$$\int_S -\log\left(P'\right)\mathrm{d}P'.$$

Mean Yield Per Surface

The mean yield per surface on S can be defined by constituting a yield function $Y{:}S \to \boldsymbol{R}$, such as

$$\int_S Y \mathrm{d}P.$$

The mean yield of vegetation with a yield above a certain value β, such as $Y \geq \beta$, is given as

$$\int_{S|_{Y(S)\geq\beta}} Y\mathrm{d}P.$$

If $Y(S)$ is a skewed distribution with respect to S, such as power-law distribution, attention should be paid to interpretation since the fluctuation of mean yield could be enormous in actual data.

Occurrence Probability of Objective Function

To obtain the occurrence probability of vegetation having an objective function $O{:}S \to \boldsymbol{R}$ greater than or equal to the constant value β, the measure space $(S, \boldsymbol{E}, O)$ of the objective function should be constructed on the same completely additive class $\boldsymbol{E}$ as $(S, \boldsymbol{E}, P)$, and given by

$$\int_{S|_{O(S)\geq\beta}} 1_S\mathrm{d}P.$$

Example: For tagged ecosystem data, the occurrence probability of various combinations of tags can be calculated. In order to handle co-occurrence of tags, it is necessary to construct a completely additive class $\boldsymbol{E}$ of resolution that satisfies $s_i \cap s_j = \emptyset\big(s_i, s_j \in S\big)$.

Conditional Probability

If the two objective functions O_1 and O_2 are constructed as the measure $O_1{:}S \to \boldsymbol{R}$ and $O_2{:}S \to \boldsymbol{R}$ on the completely additive class $\boldsymbol{E}$ of S, the integral of the target measure O_2 under the condition $A = \{s \in S | O_1(s) \geq \beta\}$ is given by

$$\int_A 1_S\mathrm{d}O_2.$$

Calculation of Expected Value of an Objective Function by Non-uniform Probability Density Function

The expected value of the objective function $O(s)$ with respect to the non-uniform probability density function $P(s)$ on $s \in S$ is given as

$$\int_S O(s)\mathrm{d}P = \int_S O(s)f(s)\mathrm{d}\mu(s).$$

where $f{:}S \to \boldsymbol{R}$ is the probability density function of P for μ, which is called the Radon–Nikodym derivative. The set measure μ is, for example, $\mu(s) = \#\{s\}$ in case of a symbolic set.

On Vegetation Succession: Perron–Frobenius Operator and Climax Community

The vegetation transition can be represented as a symbolic dynamical system that is the temporal change of the direct product space $\boldsymbol{R}^{\boldsymbol{m}} \times \mathbf{Str}^{\boldsymbol{n}}$ of numerical ($\boldsymbol{R}$) and symbolic (**Str**) data of soil and vegetation variables. Then, as an example, the climax community can be described using the Perron–Frobenius operator of the symbolic dynamical system. For simplicity, we consider a symbolic dynamical system $\boldsymbol{T}$ of n kinds of plant species on a two-dimensional map, as a model of vegetation succession:

$$\boldsymbol{T}:\boldsymbol{R}^2 \times \mathbf{Str}^{\boldsymbol{n}} \to \boldsymbol{R}^2 \times \mathbf{Str}^{\boldsymbol{n}},$$

where $\boldsymbol{T}$ is a non-singular map. If we want to include other numerical data $\boldsymbol{R}^{\boldsymbol{m}}$ such as environmental parameters, this extends to

$$\boldsymbol{T}:\boldsymbol{R}^2 \times \boldsymbol{R}^{\boldsymbol{m}} \times \mathbf{Str}^{\boldsymbol{n}} \to \boldsymbol{R}^2 \times \boldsymbol{R}^{\boldsymbol{m}} \times \mathbf{Str}^{\boldsymbol{n}}.$$

Let $\boldsymbol{V}$ be a completely additive class on $X:\boldsymbol{R}^2$. $\boldsymbol{V}$ corresponds to a list of every niche structure of vegetation with real-value (infinite) resolution. Let $g(X)$ be the density function on X (Radon–Nikodym derivative). Practically, in case of a map which displays only the presence or absence of vegetation in practice, $g(X)$ is a binary function.

Here, we call the PF that is defined as follows for the Lebesgue measure m on X as the Perron–Frobenius operator of vegetation succession $\boldsymbol{T}$.

$$\int_V \mathrm{PF} \cdot g(x)\mathrm{d}m = \int_{T^{-1}(V)} \mathrm{PF} \cdot g(x)\mathrm{d}m.$$

The stationary density function satisfying $\mathrm{PF} \cdot g = g$ gives the area of each species that potential natural vegetation (climax community) comprises.

Practically, in consideration of intrinsic fluctuations, if the following relation holds with respect to the function norm of $\mathrm{PF} \cdot g = g$ and the upper bound δ of fluctuation, the vegetation can be judged as climax community for a time scale t and inherent fluctuation δ:

$$\left|\mathrm{PF}^t \cdot g - g\right| \leq \delta.$$

This relationship can also represent a stabilized monoculture method by human control, as detailed in Sect. 1.4.

If Perron–Frobenius operator PF for vegetation succession $\boldsymbol{T}$ can be completely determined, the potential natural vegetation and the response to the vegetation strategy of synecological farming can be uniformly described. In practice, since the real ecosystem is too complex to model as a dynamical system, the stationary density function that determines the Perron–Frobenius operator can only be numerically

approximated by the convergence of the function norm under inevitable fluctuation. Therefore, it can be described as a dynamical system of conditional probability, and it becomes necessary to connect to probabilistic analysis such as information geometry (Funabashi 2017b). By interpreting vegetation succession $\boldsymbol{T}$ and corresponding Perron–Frobenius operator PF as a probability map, finding deterministic structure as much as possible from these can be considered as the primary direction of model refinement.

Appendix 2: Food Self-sufficiency Measure with a Geometric Mean

The conventional definition of food self-sufficiency rate (SSR) is based on the arithmetic mean, whether it be calorie-based or production-based (FAO 2001). As an application of the concept of selective information developed in Sect. 1.4, we simulate a novel food self-sufficiency rate related to the geometric mean.

The problem of arithmetic means is that it does not correctly reflect the notion of self-sufficiency with respect to the diversity of food products: Suppose there exist food items that cannot be produced in a social community but crucial for the survival. Then, the community could not survive when the importation is prohibited, even if the total SSR over the whole food products is superior to 100%. This is typically the case with food production in limited geographical scale such as in small island (Hashiguchi 2005).

To properly adopt the notion of self-sufficient "ability" with respect to the survival of a community in isolation, the following geometric mean I_{g} can provide a simple definition of risk that threatens self-sufficiency regarding the diversity of food products:

$$I_{\mathrm{g}} := \sqrt[k^{max}]{\prod_{k=1}^{k^{max}} l(SSR_k)},$$

where SSR_k is the SSR defined with the percentage of k-th food item ($k = 1, \ldots, k^{max}$), and to cut above 100% of each SSR_k,

$$l(x) := \begin{cases} 1 & \text{if x} \geq 100\% \\ 0.01 \cdot x & \text{else} \end{cases}.$$

It transforms to the mean selective information I_{s} if we measure the self-sufficiency risk as a mean information cost for the search, such as

$$I_{\mathrm{s}} := -\log\left(I_{\mathrm{g}}^{k^{max}}\right) \Big/ k^{max} = -\log\left(\prod_{k=1}^{k^{max}} l(\mathrm{SSR}_k)\right) \Big/ k^{max}$$

$$= -\sum_{k=1}^{k^{max}} \log l(\mathrm{SSR}_k)/k^{max}.$$

The mean selective information I_s represents the mean information cost to search all food products under given SSR. It diverges to infinity when there is a single food item with $\mathrm{SSR}_k = 0\%$, while coincides with 0 when all food items' $\mathrm{SSR}_k = 100\%$. It represents the situation that there exists sufficient food in the world, but the distribution is not equitable for the self-sufficiency of the global population (FAO 2011).

Actual trade of food products between and within social communities may substitute some items with others. We define a natural extension of the mean selective information I_s to the domain including $\mathrm{SSR}_k > 100\%$, as the extended mean selective information I'_s:

$$I'_s = -\sum_{k=1}^{k^{max}} \log l'(\mathrm{SSR}_k)/k^{max},$$

where

$$l'(x) := 0.01x.$$

This formulation is equivalent to define the exchange rate of a product with others according to the ratio of selective information representing the search cost. For the value $\mathrm{SSR}_k > 100\%$, the selective information turns into a negative value and can be interpreted as a search gain that cancels out the search cost of other products.

We simulated the I_s and I'_s for the different levels of production from power-law vegetation. The algorithm is defined as follows:

1. Define the parameters a and b of Pareto distribution (see Sect. 1.2).
2. Sample k^{max} values from the Pareto distribution and define them as $p_k (k = 1, \ldots, k^{max})$.
3. Create a new series $p'_{k_1 k_2}$ with respect to each value of p_k as a regularization factor, such as $p'_{k_1 k_2} := p_{k_1}/p_{k_2}$ $(k_1, k_2 = 1, \ldots, k^{max})$.
4. Calculate I_s and I'_s for each k_2 with respect to $k_1 = 1, \ldots, k^{max}$, with the use of $\mathrm{SSR}_{k_1 k_2} = 100 \cdot p'_{k_1 k_2}$. For each k_2, the number of $\mathrm{SSR}_{k_1 k_2}$ that exceeds 100% should be attributed as $\#\left\{\mathrm{SSR}_{k_1 k_2} | \mathrm{SSR}_{k_1 k_2} \geq 100\%, k_1 = 1, \ldots, k^{max}\right\}$, which can be simplified to a single parameter k such as $\#\{\mathrm{SSR}_k \geq 100\%\}$ $(k = 1, \ldots, k^{max})$.
5. Repeat from 2 to 4 for N_s times and take the mean values and standard deviations of I_s and I'_s for each k_2 with respect to N_s samplings.

The results are shown in Fig. 1.14. As a representative example, $k^{max} = 120$ was chosen to represent the commonly utilized crop species diversity in world agriculture (Yong et al. 2006). $N_s = 193$ corresponds to the number of member states of the United Nations (UN 2017). Naturally from the definition, the value of I_s converges

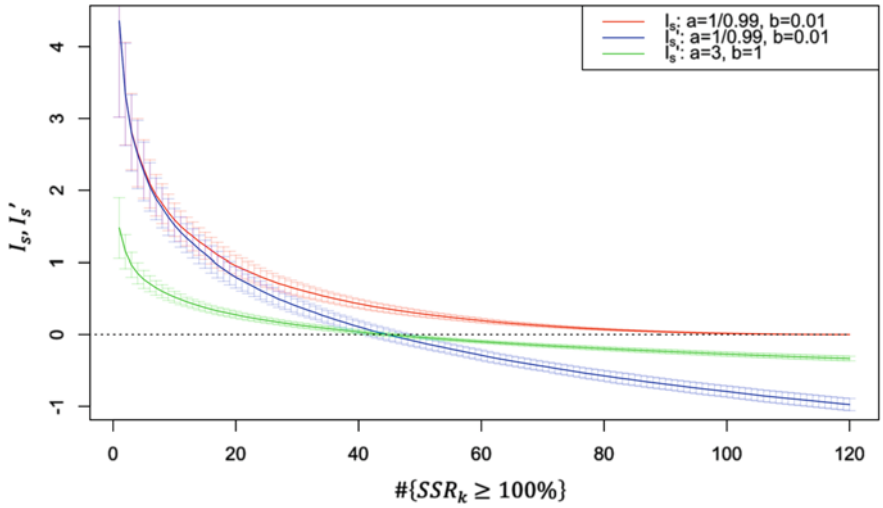

Fig. 1.14 Simulation of the mean selective information I_s and the extended mean selective information I_s' with respect to the number of self-sufficient food products $\#\{\mathrm{SSR}_k \geq 100\%\}$ ($k = 1, \ldots, k^{max}$). Mean $\pm$ standard deviation of I_s and I_s' is shown with the red and blue plot, respectively, and those of I_s' with the green plot. The value of $\#\{\mathrm{SSR}_k \geq 100\%\}$ where I_s' crosses zero (intersection between blue and green lines) is shown to be invariant with respect to different values of parameters a and b that define Pareto distribution

to zero as the number of self-sufficient crops approaches to k^{max}, since all food products need to achieve self-sufficiency for the survival. On the other hand, I_s' admits the compensation between crops by the extended definition of search cost, which achieves self-sufficiency risk zero around $\#\{\mathrm{SSR}_k \geq 100\%\} = 44$ to 45. This critical value is invariant with the parameters of power-law vegetation a and b, due to the logarithmic property of selective information and regularization process of the algorithm.

This simulation suggests that the self-sufficient ability defined with respect to the risk index I_s' can be achieved by securing SSR of about 37% of the necessary product diversity within a closed territory if we take on the strategies of adaptive diversification with power-law productivity. By exchanging k^{max} and N_s in the algorithm, we can simulate the case of international trades between N_s countries (following power-law productivity) with variance on k^{max} crop diversity, which derives qualitatively the same behavior of I_s': 37% of the member states need to achieve self-sufficiency for each crop, in order to attain global sufficiency under the exchange rate defined in I_s'.

Parameters: The values of a and b are shown in the figure legend. $k^{max} = 120$, $N_s = 193$.

Appendix 3: Ecological Scarcity and Land Utilization

How can we most efficiently allocate the land use distribution (e.g., between city, farmland, and protected area) for the protection of biodiversity? For that purpose, let us think about the ecological value of a species. The value of a species in ecosystems has multi-faceted criteria, over multiple ecosystem functions and services, on various spatial–temporal scales. There is an only limited amount of known value compared to the unknown part that cannot be measured advance. Take for example ecological resilience, there could be an infinite unknown possibility of ecological disturbance in the future in which each species may play constructive roles for the recovery and development of new ecosystems.

An indeterminable amount of our ignorance primarily limits the argument on resilience. If we are to formulate the total amount of functions and these values of a species with respect to the future resilience of ecosystems, an only expression such as "all species are equally invaluable at the largest limit of spatial–temporal scale" would be allowed to eliminate any specific bias. We call this premise as the "**value equivalence principle of species**," which mathematical formulations commonly adopt for multiple diversity indexes in ecological study. This standpoint is also vital in the management process of one-time-only events such as natural disasters, in which we need to select a fail-safe strategy without prior knowledge of future change (Funabashi 2017c).

Now, suppose that all species are equally invaluable for a whole ecosystem. The simplest ideal scenario of the conservation is to allocate each species an equal surface, or uniform distribution of habitat surface over species, which maximizes Shannon's diversity index measured on the proportion of habitats. The actual distribution of species would differ under multiple social and ecological factors, which can be quantified with the following measure of ecological scarcity R_k of a species k:

$$R_k := \frac{C}{X_k},$$

where X_k is the habitat surface of the species k, and C is the total surface taking arbitrary constant value. Here, ecological scarcity R_k represents how much a species deserves conservation value with respect to the smallness of actual habitat under the value equivalence principle of species.

Let us consider the fractal dynamics of niche differentiation. For simplicity, we consider the recurrence relation of one-dimensional Cantor set[11] f_c as follows, which divides the surface C represented as a line segment into two isometric parts:

$$C_n := f_c(C_{n-1}) := \frac{C_{n-1}}{3} \cup \left(\frac{2}{3} + \frac{C_{n-1}}{3}\right)$$

[11] Three-dimensional generalization of the Cantor set is the Menger sponge discussed in Sect. 1.3 as an essential model of niche differentiation.

for $n \geq 1$, and $C_0 = [0, C]$.

This means that by deleting the open middle third (C/3, C/3) from the interval [0, C], this transformation leaves two line segments: [0, C/3] and [$2C/3$, C].

After n iteration of f_c, the habitat X_k will divide into 2^n separated niches, and the ecological scarcity becomes

$$R_k = \left(\frac{3}{2}\right)^n.$$

To avoid parameter dependency, we normalize the niche surface ratio of a species k by dividing X_k with C as

$$\frac{X_k}{C} = \left(\frac{2}{3}\right)^n.$$

The relationship between the number of different niches, its surface ratio, and ecological scarcity of a species is depicted in Fig. 1.15. Starting from a monoculture situation, as niche differentiation progresses with n and increases the complexity of fractal configuration (Fig. 1.15 left), ecological scarcity increases in an exponential manner (Fig. 1.15 right).

Such situation is particularly familiar with rapidly expanding urban land use planning, where complex intersections with multi-fractal features between city, farmland, and natural ecosystems are often at the forefront of the development (e.g., Wu et al.

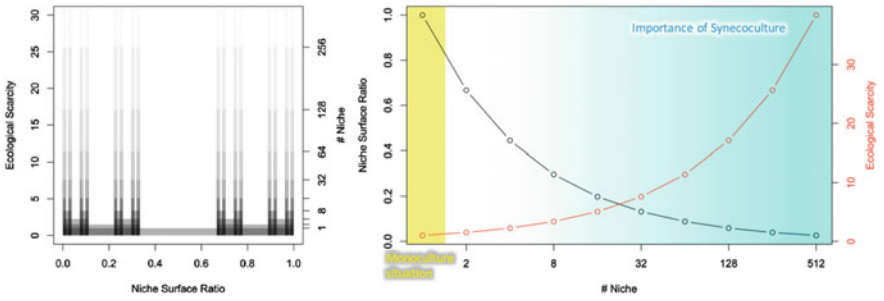

Fig. 1.15 Relationship between niche surface ratio X_k/C, ecological scarcity R_k, and niche number 2^n of a species k after n–time niche differentiation with recurrence relation f_c. **Left** Niche partitions on the scale of surface ratio versus ecological scarcity R_k. The gray rectangles that correspond to the direct product sets $\frac{C_n}{C} \times [0, R_k]$ are superimposed for different $n = \{0, 1, 2, \ldots, 9\}$. The intervals C_n/C correspond to the niche partitions after n-th iteration measured on the scale of niche surface ratio on X-axis. The number of differentiated niches is aligned at right Y-axis in correspondence with the value of R_k in left Y-axis. **Right** Number of niche partitions 2^n versus niche surface ratio X_k/C and ecological scarcity R_k. As the niche number increases with n, niche surface ratio decreases, and ecological scarcity increases in an exponential manner. Monoculture situation (yellow background) is dominated by a single species with low ecological scarcity, while highly differentiated mixed polyculture situations (cyan background) consist of niches with high ecological scarcity, where the role of synecoculture on the conservation efforts becomes increasingly important

2013). In finely fragmented lands with various heterogenic environments, conventional monoculture farming methods are inefficient to perform, while synecoculture can provide combined solutions by making use of the particularity of each niche condition with various vegetation portfolios, and combining with other modes of food production according to the adjacent land use that provides different accessibility for the distribution of the products. This strategy can harmonize the conservation of rare species with high ecological scarcity and small-scale local production of various food products in the burgeoning frontiers of urban development. To raise awareness and increase susceptibility to synecoculture, practices in small-scale fragmented land such as family gardens, abandoned farmland, and city greenbelt are important, which will strengthen the future option of environmental conservation in smart green cities. Figure 1.16 left shows a schematic diagram of the patterns of the combination of synecoculture with other adjacent land use. Not only the in-field strategy of adaptive diversification in synecoculture (Sect. 1.4, Fig. 1.4), we can also produce local food from surrounding practices and environments, such as conventional agriculture, urban farming, and hunting-gathering. In either case, coping with small-scale diversified practices are strongly influenced by power-law fluctuation, which needs to count on the stability of harmonic mean for the management (Fig. 1.16 right, see also Sect. 1.4). To realize human augmentation of ecosystems at the boundaries between city and natural environments, we need to deeply understand these properties of the

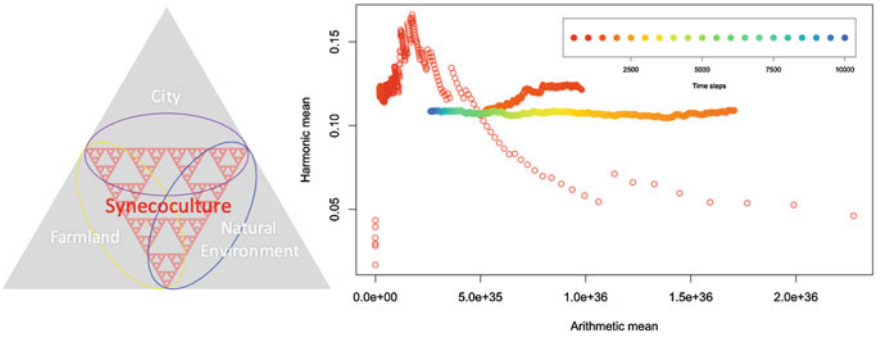

Fig. 1.16 **Left** Synecoculture as an interface between urban and natural environments. Conceptual diagram of growing margins at the intersections between city, farmland, and natural environment is depicted as self-organized fractal landscape (red triangles), where synecoculture can play an important role for both conservation of ecologically scarce species and local food production. According to the adjacent land use, synecoculture can be combined with 1. conventional agriculture (yellow circle); 2. urban farming (violet circle); and 3. hunting–gathering activities (blue circle) in the mixed topography. **Right** Convergence of harmonic mean and divergence of arithmetic mean in Pareto distribution. Ten thousand independent samplings were performed from a Pareto distribution with $a = 0.1$ (i.e., no finite arithmetic mean, see Sect. 1.2), $b = 0.01$. The dynamics of the arithmetic mean of previous samplings (horizontal axis) show huge discontinuous fluctuation several times triggered by rare big events, while that of the harmonic mean (vertical axis) is confined in a small stable range and converges through time. Color gradient represents the time step of the sampling

power law prevalent in urban and vegetation dynamics and presuppose the benefits to both sides in the formation of public opinions and policymaking.

Mathematical proof for the convergence of the harmonic mean of Pareto distribution is given as follows:

For a harmonic mean $H(X)$ of $f(x) := \frac{ab^a}{x^{a+1}}$, $(x \in X := (b, \infty])$ in Sect. 1.2,

$$\begin{aligned}\frac{1}{H(X)} &:= \int_{-\infty}^{\infty} \frac{1}{x} f(x)dx \\ &= \int_{b}^{\infty} \frac{ab^a}{x^{a+2}} dx \\ &= \frac{ab^a}{a+1}\left[-x^{-(a+1)}\right]_b^{\infty} (a \neq -1).\end{aligned}$$

Then, the condition that the right side does not diverge to infinity is given by

$$-(a+1) < 0\,,$$

$$a > -1$$

By definition, $a > 0$, then for all $a > 0$, $1/H(X)$ converges to a finite value as follows:

$$\begin{aligned}\frac{1}{H(X)} &= \frac{ab^a}{a+1}\left\{0 - \left(-b^{-(a+1)}\right)\right\} \\ &= \frac{a}{(a+1)b},\end{aligned}$$

which gives

$$H(X) = \frac{b(a+1)}{a}\,.$$

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Chapter 2
Amplified Fragment Length Polymerase-Based Phylogenetic Relationships of Heirloom Tomato and Dark Green Vegetable Accessions

Ahmad Naseer Aziz

Abstract Dark green vegetables such as broccoli, lettuce, and spinach are documented for their nutritional importance. Similarly, heirloom varieties also have value-added traits such as the attractiveness of fruits, limited availability, unique flavor, or other characters not found in commercial types. Since heirloom tomato varieties may require a little more care during production or handling, compared to commercial hybrids, producers can obtain a higher price when appropriately marketed. In this report, six heirloom tomato (*Lycopersicon esculentum*) varieties as well as four varieties of broccoli (*Brassica oleracea*), three varieties of lettuce (*Lactuca sativa*), and two varieties of spinach (*Spinacia oleracea*) were selected for comparative DNA fingerprinting. On quality DNAs from the leaf tissues of these vegetables, amplified fragment length polymorphism (AFLP) analyses were performed with 64 different primer pair combinations. The AFLP primer pairs, that produce ample polymorphic markers and provided desirable polymorphisms, were identified for further analyses. AFLP-generated DNA fragments were then separated by sequencing gel electrophoresis using an automated DNA analyzer (4300S NEN DNA Analyzer and Sequencer, Li-Cor Inc., Lincoln, NE, USA) through Saga™ Generation 2- AFLP® Analysis (Li-Cor Inc.) software. AFLP profiles were analyzed to generate binary code (0/1) reports on markers' data, and then TreeCon-Dendrogram (Scanalytics Inc., Fairfax, VA, USA) software was used to deduce genetic relationships between accessions of selected vegetables. Although each accession had a distinctive DNA fingerprint, correlations between structurally similar plants and their DNA profiles were apparent enabling true to type identification and marker-assisted breeding programs.

Keywords *Lycopersicon esculentum* · *Brassica oleracea* · *Lactuca sativa* · *Spinacia oleracea* · Molecular markers · Sequencing gel electrophoresis · Dendrogram-cluster analysis

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Abbreviations

DNA Deoxyribose Nucleic Acid
USA United States of America

2.1 Introduction

An essentially derived cultivar conforms to the genotype of the initial variety while being clearly distinguishable from it by certain phenotypic characteristics. Such phenomena have increased in commercial breeding, since the development of various biotechnologies including gene-gun and tissue culture (Saunders et al. 2001) where it is possible to introduce a single gene into a variety. An heirloom vegetable refers to a cultivar that was commonly grown during earlier periods in human history, but could not be used in modern large-scale agriculture. However, many heirloom vegetables have kept their traits through open pollination. The trend of growing heirloom plants in gardens has been growing in the USA and Europe over the last couple of decades. Heirloom value-added traits include the attractiveness of fruits, limited availability and unique flavor, or other characters not found in commercial varieties (Whealy 1990). Likewise dark green vegetables are quality sources of vitamin-E which is a fat-soluble antioxidant (National Institute of Health 2016). Vitamin-E deficiency is rare and occurs mostly when a person has either inherited deficiency or impaired ability to absorb it (Manor and Morley 2007). However, its deficiency can cause blindness, cardiac arrhythmia (an irregularity in the normal rhythm of the force of the heart beat), dementia, muscle weakness, and decreased vibratory senses (Dawe and Unnevehr 2007). Thus, there is a growing interest for the identification of dark green vegetable varieties that are rich in vitamin-E content and thereby can be used as niche crops by small farmers. Such nutritive niche varieties as well as heirloom vegetables can provide underserved rural communities, a needed marketing advantage for sustainable farming operations (Tegegne et al. 2012). For many such niche varieties, genes and gene combinations selected in the past through nature and by humans will remain the vital source of germplasm improvement. Genomics can play a key role in conservation by determining genes and chromosome segments which are duplicated, unique, as well as easy to be recreated in various combinations for modern breeding programs (Lombard et al. 2002). DNA fingerprinting methods can be used to identify the genetic make-up of plants, and thus are very useful for true to type identification while reducing development costs and time required for the evaluation of crosses (Gutiérrez et al. 2009; Myers et al. 2008; Vos et al. 1995). Molecular markers associated or linked to desirable traits can be used in breeding protocols for developing new varieties.

Plant breeders can strengthen their claim for the protection of new cultivars by including molecular profiles as supplementary information to substantiate the distinctiveness of their products. Molecular markers have been successfully used to resolve

court dispute on the unauthorized seeds of cultivars marketed under the brand name of an elite variety (Kumar et al. 2001). Molecular markers may be particularly relevant for specially developed cultivars where little phenotypic differences exist between a new variety and an extant. Amplified fragment length polymorphism (AFLP) is an extremely powerful technique for cultivar identification (Powell et al. 1996) since it generates a large number of markers in a single analysis without requiring prior sequence information (Vos et al. 1995). AFLPs have been successfully applied for intraspecific genetic diversity analyses in many crops (Hu and Zhang 2005; Nguyen et al. 2004; Saini et al. 2004; Sauvé et al. 2005; Vosman et al. 2004; Xing et al. 2004). The key facilitation through the use of AFLP is the capacity to simultaneously screen many different DNA regions distributed randomly throughout the genome. The AFLP-based 'DNA-fingerprints' are simply (RFLPs) restriction fragment length polymorphisms visualized by selective polymerase chain reaction (PCR) amplification of DNA restriction fragments. During AFLP analyses, the genomic DNA is digested with two restriction enzymes (*Eco*RI and *Mse*I), generating *Mse*I-*Mse*I, *Mse*I-*Eco*RI, and *Eco*RI-*Eco*RI fragments. Specific double-stranded oligonucleotide adapters (~25–30 base pair) are ligated to the restricted *Mse*I-*Eco*RI DNA fragments and oligos homologous to the adapters, with extensions at the 3'-end, are used to selectively amplify a subset of these prepared DNA fragments (Thermo Fisher Scientific Inc. 2016). Later 1–3 base pair extensions of AFLP primers, which are not homologous to adapter sequence but are complementary to plant DNA fragments, are used in cycles of PCR to amplify AFLP markers. AFLP produced markers are reproducible, abundant, and typically highly polymorphic for assessing the genetic differences among individuals, populations, as well as independently evolving lineages in species (Debener et al. 2000). AFLPs have applications not only for identification of a cultivar, but also for variety protection and maintenance of cultivar uniformity (Parks and Moyer 2004). Thus, AFLP markers can be adopted for the granting of plant breeders' rights while making the protection of new cultivars more specific and effective (Bernet et al. 2003).

The use of AFLP has the potential for precisely characterizing and identifying particular plant varieties as well as the registration of new cultivars (Loh et al. 1999). In the International Union for the Protection of New Varieties of Plants Act of 1991, the mutation is mentioned as one of the mechanisms to obtain an essentially derived variety (EDV). For the implementation of the EDV concept, a newly released cultivar must be clearly distinct from all previously released varieties while the level of genetic relatedness between an initial variety and derived mutants can clearly be distinguished. A relationship between an original variety and its mutants can easily be identified and distinguished using AFLPs (Debener and Mattiesch 1999; Debener et al. 2000) which is of crucial importance in the registration and protection of cultivars as well as patents (Lombard et al. 2002). AFLP markers are well adapted for measuring genetic differences and to discern small genetic derivations (Lefebvre et al. 2001; Mitchell and Edwards 2001). The discriminating power of AFLP markers, which allows easy identification of cultivars, could also be very useful in checking the conformity of seed lots if the genetic relationships are consistent with their genetic origin (Krauss 1999). AFLPs, compared to other molecular marker, have

been reported to be more efficient for genetic diversity estimation, polymorphism detection, and cultivar identification (Rana and Bhat 2003; Sobotka et al. 2004). The costs of AFLP procedures are documented (Aziz et al. 2007), which can facilitate their use by price-conscious small farmers for niche crops where DNA sequence information is usually not available. AFLP is one of the popular genotyping tools (Nybom et al. 2014) but florescent primers used in automated protocols need to be standardized for each plant type (Aziz 2008). AFLP protocols were standardized, and these markers were used to genotype three dark green vegetable varieties, relevant to vitamin-E contents, as well as six heirloom tomato varieties. The four broccoli varieties analyzed included *B. oleracea* 'Broccoflower,' *B. oleracea* 'Packman,' *B. oleracea* 'Green Sprouting' and *B. oleracea* 'Premium Crop.' For lettuce, three varieties, i.e., *L. virosa* 'Oak Leaf,' *L. virosa* 'Bibb Forming' and *L. virosa* 'Deer Tongue' were genotyped through AFLPs. For very important dark green vegetable spinach, two varieties analyzed included *S. oleracea* 'Bloomsberg Long Standing' and *S. oleracea* 'Catalina.' Six heirloom tomato (*Lycopersicon esculentum*) varieties, i.e., Russian, Tidwell German, Marizol Red, Andrew Rahart Jumbo, Brimen and Brandy Wine were also genotyped via AFLP-based DNA markers.

2.2 Materials and Methods

2.2.1 Plant Materials

A collection of selected accessions used in this study was obtained from relevant sources. The plants were grown in a greenhouse using plastic pots (15 cm in diameter) containing peat, perlite, and pine bark, while being watered daily. DNAs were isolated from leaf tissues using a DNeasy Plant Mini Extraction Kit (QIAGEN, Santa Clara, CA) and a Bio 101 Fast Prep (Q. biogene, Irvine, CA) system. One percent agarose gel analyses were used to check for successful extractions, and the purified DNA samples were stored at −70 °C until use. Before analyses, sample DNA concentrations were determined through DyNA Quant 200 DNA Quick Assay fluorometer (Hoefer, San Francisco, CA).

2.2.2 AFLP Analysis

AFLP-based DNA fragments were amplified via PCR using an AFLP System-Analysis Kit (Invitrogen™ Life Technologies, Carlsbad, CA) in the presence of fluorescent dye IRD-800 labeled *Eco*R I primers (LI-COR, Lincoln, NE) designed for *Eco*RI and *Mse*I adaptors. AFLPs generated were then separated by sequencing gel electrophoresis using an automated DNA analyzer (4300S NEN DNA Analyzer and Sequencer, Li-Cor Inc.) controlled through Saga™ Generation 2- AFLP®

Analysis (Li-Cor Inc.) software to generate binary code (0/1) marker data reports. Complete AFLP protocol included restriction digestion of the sample DNAs, ligation of the adaptors (synthetic oligonucleotides), pre-amplification of adapter-ligated DNA fragments, and amplification of the molecular markers with selective AFLP primers. For the restriction digestion of the samples, 100 ng of sample DNA, 2 μl of *Eco*RI/*Mse*I restriction enzymes (1.0 units/μl), and 5 μl of 5X reaction buffer were combined in a 25 μL reaction mix and incubated at 37 °C for 6 h. The reaction mix was later heated at 70 °C for 15 min, for enzyme deactivation before adding an equal volume of the adapter ligation solution and 1 μl of T4 DNA ligase (1 unit/μl) and incubating at 20 °C for 2 h. This ligation mix was then diluted 1:10 with TE buffer (10 mM Tris-HCL, pH 7.5, 0.1 nM EDTA), and 5 μl of this mix was added into 40 μl of pre-amp primer mix, 5 μl of 10X PCR buffer plus Mg^{+2}, and 1 μl of Taq DNA polymerase (5 units/μl) in a 0.5 ml thin-walled microcentrifuge tube for pre-amplification. Twenty PCR cycles were performed at 94 °C for 30 s, 56 °C for 60 s, 72 °C for 60 s before 4 °C soak. Agarose gel (2%) analyses were conducted to visualize restriction digestion and pre-amplification of the DNA samples after staining with ethidium-bromide and then photographed through an AlphaImager 2000 System (Alpha Innotech, San Leandro, CA). Pre-amplification reaction solution was then diluted 1:50 with TE buffer (10 mM Tris-HCL, ph 8.0, 1 mM EDTA), before adding 1.1 μl of 10X PCR buffer plus Mg^{+2}, 0.06 μl of Taq DNA polymerase (5 units/1 μl), 3.85 μl of ultra-pure deionized water and one of the 64 AFLP primer pairs (0. 5 μl of *Eco*RI florescent-labeled primer and 2.4 μl of *Mse*I primer) to 2.5 μl of the diluted DNA template. The PCR program for selective amplification was one cycle of 94 °C for 30 s, 65 °C for 30 s, and 72 °C for 1 min, followed by 12 cycles of 94 °C for 30 s 65 °C for 30 s with decremental temperature lowering by 0.7 °C per cycle, and 72 °C for 1 min, and final completion by the 23 cycles of 94 °C for 30 s, 56 °C for 30 s, and 72 °C for 1 min before a 4 °C soak.

2.2.3 Polyacrylamide Sequencing Gel Electrophoresis

The Li-Cor glass plates (soda lime—25 cm^2) were prepared by thoroughly washing with 1:10 dilution of micro-90 (International Products Corporation, Burlington, NJ) detergent and then rinsing with deionized water. A 1:1 solution of bind-saline stock (50 μl γ-methacryloxypropyl tri-methoxysilane in 10 ml Ethanol) and 10% acetic acid was coated over the plate where the 64 shark-tooth wells comb was to be placed to prevent gels from sliding. The gel plates were then assembled 0.25 mm apart using appropriate spacers and clamps to pour 6.5% KB plus acrylamide solution (Li-Cor) containing 150 μl of a 10% ammonium per sulfate solution and 15 μl of TEMED. Polymerized gel in the plate assembly was placed in the DNA sequencer, and one liter of Tris-Borate-EDTA was added to the top and bottom gel trays for electrical conductivity. The gel manager window of the Saga™ Generation Client allowed the input for samples' information, while the electrophoresis conditions were 1500 V, 40 W, and 40 mA at 45 °C. Before analysis, 3X blue loading dye (Li-Cor) was added

to all AFLP reactions, and up to 0.6 μl of these prepared samples were loaded into sample wells after the gel's pre-run of 25 min. 0.6 μl of DNA florescent molecular sizing standards (50–700 bp) prepared in 3X loading dye were also loaded at the left and right lanes of each gel. The gel was simultaneously electrophoresed and analyzed for approximately 3.5 h, and AFLP profiles were obtained. Re-analyses of randomly selected samples were used to confirm the reproducibility of the results.

2.2.4 AFLP Markers' Scoring

All AFLP profiles were recorded using Saga™ Generation 2- AFLP® Analysis Software Version 3.1 (Li-Cor Inc.) as TIFF images. The gel manager tool of Saga™ was used and through lane mode, it was confirmed that the blue vertical lines are straight, spread equally apart while superimposed on the gel lanes. Later, calibration mode was selected to connect corresponding DNA bands for all molecular standards' gel lanes through horizontal red calibrating lines. Finally, orange-colored horizontal desmile line were added on the gel images on the basis of monomorphic DNA bands in the sample gel lanes. The re-analyzed gels were thus genotyped to be scored for the presence/absence of AFLP markers denoted by '+' and '−,' respectively. This marker data was subsequently added to an Oracle® database to generate reports, which were exported into Gene ImagIR™ Database Manager (Li-Cor). After the analysis, a (PAUP) phylogenetic analysis using parsimony phenetic report (Sinauer Associates Inc., Sunderland, MA) was generated and imported into TreeCon-Dendrogram (Scanalytics Inc., Fairfax, VA) software. The Dendrogram software was used to graph the genetic distances between each accession in a tree topology.

2.3 Results and Discussion

The discovery of marker-based gene tags, map-based clonings of agronomically valuable genes, variability studies, phylogenetic analyses, and markers'-assisted selections of desirable genotypes has given new dimensions to concerted efforts of breeding for developing new and better varieties. Because of the vast genetic difference between plants, it is becoming increasingly important to identify varieties from development to production and follow the cultivars from their origin to the consumer. A DNA fingerprint unique to a plant type can be used as a tool in the plant breeder's arsenal for intellectual property rights (IPR) protection. The EDV implementation for breeding endeavors requires polymorphism of genetic markers, which is defined as the presence or absence of the DNA band in a population of two or more discontinuous variants or genotypes. Molecular markers should be highly polymorphic in nature, have a codominant inheritance, neutral to environmental conditions or management practices, have frequent occurrence in the genome, show selective

neutral behavior, have easy access (availability), provide easy and fast assay, have high reproducibility, as well as facilitate easy exchange of data between laboratories. Molecular markers including AFLPs are extensively used in the 'green industry' because they have the potential to authenticate, assess genetic diversity, and aid in cultivar identification without being obscured by complex pedigree records, environmental conditions, or epistatic effects (Hongtrakul et al. 1997). AFLPs allow for the protection of a cultivar by producing a genetic profile that is unique to particular plant to the exclusion of all others (Bernet et al. 2003; Hodkinson et al. 2002; Krauss 1999; Loh et al. 1999; Lombard et al. 2000; Mitchell and Edwards 2001).

AFLP markers have been used for identifying genetic maps and diversity of accessions among lettuce samples (Jansen et al. 2006; Jeuken et al. 2001; Jeuken and Lindhout 2002, 2004; Witsenboer et al. 1997). Hale et al. (2006) and Holme et al. (2004) used AFLPs to differentiate 24 broccoli inbreds, while for *Spinacia oleracea* Martinez-Reyna et al. (2001), Correll et al. (2005) and Khattak et al. (2006) used molecular markers to genetically characterize their accessions. Tomato is one of the original plant for which AFLP markers have been developed, and there are 62 primers pairs recommended for its fingerprinting (Invitrogen. 2003). However, when IRD-800 labeled primers designed for *Eco*RI adaptors (E-primers) were used on six heirloom tomato varieties, only 39 primer pairs were found suitable for AFLP amplification from the tomato genome (Table 2.1). Incorporation of fluorescent dye molecules to primers has been reported to alter their annealing to templates (Aziz 2008). Also, with fluorescent dye IRD-800 labeled *Eco*RI, the 23 primer pairs providing the most vigorous AFLP amplifications for the three dark green vegetables were as following: E-AAC/M-CAT, E-AAC/M-CTC, E-AAG/M-CTC, E-AAG/M-CTT, E-ACA/M-CAG, E-ACA/M-CTC, E-ACA/M-CTT, E-ACC/M-CAT, E-ACC/M-CTG, E-ACT/M-CAT, E-ACT/M-CTG, E-ACT/M-CTT, E-AGC/M-CAC, E-AGC/M-CAG, E-AGC/M-CTA, E-AGC/M-CTC, E-AGC/M-CTG, E-AGC/M-CTT, E-AGG/M-CAA, E-AGG/M-CAC, E-AGG/M-CTC, E-AGG/M-CTG, and E-AGG/M-CTT. Under these conditions, the primers giving the most amplification success were E-AGC, E-AGG, M-CTC, and M-CTT; whereas, the primers with mid-range success were identified as E-AAC, E-ACA, M-CAG, M-CAT, and M-CTG (Table 2.2).

Jeuken et al. (2001), Jansen et al. (2006), Castenmiller et al. (1999), and Correll et al. (2005) also used same AFLP primer pairs reported in this study for broccoli, spinach, and lettuce marker-based analyses. For example, the common primer pairs in this study as well as used by Jansen et al. (2006) and Jeuken et al. (2001) for determination of genetic diversity between lettuce accessions through polymorphic AFLP markers are following: E-ACA/M-CAC, E-ACA/M-CAG, E-ACA/M-CTA, E-ACA/M-CTC, and E-ACA/M-CTT. AFLP profiles revealed ample polymorphism to distinguish each accession of the four vegetable types analyzed in this study through several hundred DNA fragments that were generated to compare their genetic similarities. Two hundred and sixty-three AFLP markers of each vegetable were selected for phenetic analyses, and clustering pictorial images of genetic distances among accessions were created using the estimation methods of Link et al. (1995) and Nei and Li (1979) via TreeCon (Scanalytics Inc., Fairfax, VA) software (Figs. 2.1, 2.2,

Table 2.1 Success rates of AFLP primers (designed for *Eco*RI and *Mse*I adaptors) and primer pairs during amplification of markers from the six varieties of heirloom tomatoes (*Lycopersicon esculentum*)

AFLP Primers (success rate[a])	M-CAA (6)	M-CAC (4)	M-CAG (7)	M-CAT (4)	M-CTA (8)	M-CTC (6)	M-CTG (0)	M-CTT (4)
E-AAC (1)	*[b]	*	**	**	***		*	
E-AAG (6)	***	***	***	**	***	***	*	***
E-ACA (4)	***	*	***		***	*	*	***
E-ACC (6)	***	***	***	***	***	***	*	**
E-ACG (7)	***	***	***	***	***	***	*	***
E-ACT (7)	***	***	***	***	***	***	*	***
E-AGC (3)	*	*	***		***	***	*	*
E-AGG (5)	***	*	***	***	***	***	*	*

[a]Success rate of the individual primer is the indicative of the number of pairing primers that it was found operational in amplifying AFLP markers

[b]Primer pairs are given empirical values (no amplification, *, ** and ***) based on the intensity and number of AFLP markers amplified through these. Thus, 39 primer pairs are identified that were most successful (***) in producing AFLPs from tomato samples under given amplification conditions

2.3, and 2.4). Similar dendrograms were also produced when transformed distance (Link et al. 1995, Nei and Li 1979) or neighbor joining method (Nei and Li 1979) were used to infer the tree topology (Scanalytics Inc., Fairfax, VA). All four of the trees (Figs. 2.1, 2.2, 2.3, and 2.4) were developed using the clustering and complete linkage methods (Link et al. 1995) provided in the algorithm and Tree-Con software (Scanalytic Inc., Fairfax, VA). Two phenotypically similar varieties of *Brassica oleracea*, i.e., premium crop' and 'green sprouting' clustered together (Fig. 2.1), while they also had very similar leaf patterns and texture. Their close relationship showed a bootstrap value of 99%, which means that out of one hundred comparisons of these two varieties were linked 99 times. The other two broccoli varieties 'Packman' and 'broccoflower' were distantly linked to this cluster, respectively.

When the three varieties of *Lactuca sativa* were compared via AFLP profiles, 'Bibb forming' and 'Deer tongue' were found linked most closely together with bootstrap value of 99% while 'Oak Leaf' remained distantly related to this pair (Fig. 2.2). The two varieties of *Spinacia oleracea* evaluated did not show close rela-

Table 2.2 Average success rates of AFLP primers (designed for *Eco*RI and *Mse*I adaptors) during amplification of markers from the nine varieties of three dark green vegetable (*Brassica oleracea*, *Lactuca virosa*, and *Spinacia oleracea*). Statistical analyses were performed using Microsoft Excel (Microsoft 2016) spreadsheets

AFLP primers	Average success rate*	Standard deviation from the mean
E_1 (E-AAC)	0.646^{ab}	−0.082
E_2 (E-AAG)	0.542^{a}	−1.132
E_3 (E-ACA)	0.688^{ab}	0.213
E_4 (E-ACC)	0.604^{a}	0.821
E_5 (E-ACG)	0.583^{a}	0.775
E_6 (E-ACT)	0.625^{a}	0.620
E_7 (E-AGC)	0.854^{b}	0.113
E_8 (E-AGG)	0.813^{b}	0.363
M_1 (M-CAA)	0.563^{a}	−0.156
M_2 (M-CAC)	0.563^{a}	−0.174
M_3 (M-CAG)	0.688^{ab}	0.802
M_4 (M-CAT)	0.688^{ab}	0.304
M_5 (M-CTA)	0.604^{a}	0.601
M_6 (M-CTC)	0.813^{b}	0.408
M_7 (M-CTG)	0.708^{ab}	0.550
M_8 (M-CTT)	0.792^{b}	0.848

*Success rate of the individual primer is based on the intensity and number of AFLP markers amplified through these. Means within a column having a different letter are significantly different at $P = 0.05$

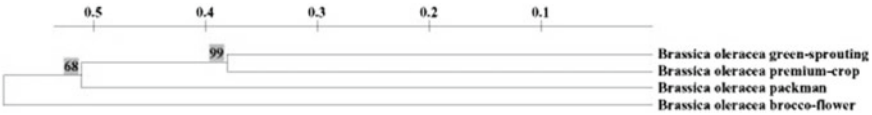

Fig. 2.1 Genetic distances among four broccoli (*Brassica oleracea*) varieties based on amplified fragment length polymorphic markers and as depicted by a Tree-Con Dendrogram software (Scanalytics Inc., Fairfax, VA, USA)

Fig. 2.2 Genetic distances among three lettuce (*Lactuca virosa*) varieties based on amplified fragment length polymorphic markers and as depicted by Tree-Con Dendrogram software (Scanalytics Inc., Fairfax, VA, USA)

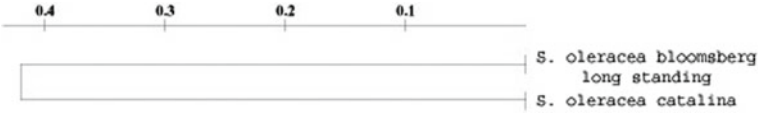

Fig. 2.3 Genetic distances among two spinach (*Spinacia oleracea*) varieties based on amplified fragment length polymorphic markers and as depicted by a Tree-Con Dendrogram software (Scanalytics Inc., Fairfax, VA, USA)

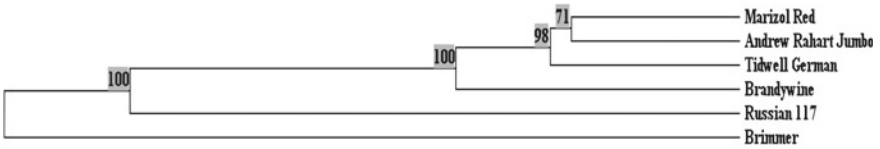

Fig. 2.4 Genetic distances among six heirloom tomatoes (*Lycopersicon esculentum*) varieties based on amplified fragment length polymorphic markers and as depicted by a Tree-Con Dendrogram software (Scanalytics Inc., Fairfax, VA, USA)

tionship with each other according to the tree topology (Fig. 2.3). Spinach DNA samples, however, showed high success with the most number of primer pairs for AFLP analyses depicted in Table 2.2. According to the dendrograms produced after AFLP analyses of heirloom tomatoes; 'Marizol Red' variety was found genetically more similar to 'Andrew Rahart Jumbo,' while 'Tidwell German,' 'Brandywine,' 'Russian 117,' and 'Brimmer' remained associated but, respectively, distant from this pair (Fig. 2.4). In many instances, similarities between phenotypically similar plants and their AFLP profiles were observed. AFLP profiles associated with each accession can also be used to identify plant lots and develop related genetic markers. The AFLP-based polymorphisms revealed by the DNA analyses in this study can be used to develop markers for tracking genetic inheritance within progeny populations. Thus, these results add to the body of knowledge compiled to help breeders, farm operators, and researchers in improving the commercial value of the *Brassica oleracea*, *Lactuca virosa*, *Spinacia oleracea*, and *Lycopersicon esculentum* as well as other crop plants. AFLP procedures were optimized for the use of fluorescent-labeled primers in analyses of these vitamin-E content relevant as well as heirloom varieties. Molecular methods used in this report would be easily adaptable for determining genetic linkage and marker-assisted breeding studies of these as well as other plants (Nybom et al. 2014). Using protocols presented here, plant breeders and horticulturists would be able to reduce the time and inputs spent in selecting parents and judging the presence of desirable characteristics in plant progenies.

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Chapter 3
Molecular and Quantitative Genetics of Stone Pine (*Pinus pinea*)

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Abstract The Mediterranean stone pine is currently on its way to domestication. Its genuine Mediterranean pine nuts are among the most expensive nuts in the world because they are mainly wild-collected from pine forests and woodlands. Despite the wide current distribution of stone pine over the whole Mediterranean biome, old-growth forests are scarce, often associated locally with dynamics on lose sands, coastal dunes or former estuary marshes. The species has been found to be genetically depauperate, putatively due to a population bottleneck in a local refugium during the Last Glacial Maximum confirmed in southern Iberia, and a possibly anthropic range expansion during Holocene. Only recently, cone harvesting and processing mechanisation have allowed for profitable pine nut production from orchard plantations. In Spain and Portugal, first elite clones have been registered for their use as grafted orchard crop.

Keywords Mediterranean stone pine · Pine nuts · Genetic depletion · Domestication

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3.1 Botany, Origin and Relevance of the Species

Edible nuts from gymnosperms are usually harvested from wild forests rather than constituting an agronomic crop. Rather uniquely, the Mediterranean stone pine, *Pinus pinea* L., is currently on its way to domestication for the production of pine nuts, one of the most expensive nuts in the world, known since Antiquity as gourmet ingredient of traditional Mediterranean dishes (Thirgood 1981; Agrimi and Ciancio 1993; Prada et al. 1997; Gil 1999).

The genus *Pinus* comprises over 100 species, including some major forest trees of the northern hemisphere providing relevant ecosystem services, notably timber. Among about twenty pine species with large, nut-like edible seed kernels, Mediterranean stone pine is known as the first one to have nourished humans: its pine nuts were already consumed by Neanderthals in the Middle Palaeolithic, before the Last Glacial Maximum in Europe (Weyrich et al. 2017). The small edible, ivory-white kernels are obtained by shelling pine nuts after extraction from cones. The seed coat is thick and woody, pine nuts yielding only 25% kernel in weight, i.e. in average 0.15 g out of 0.6 g per unit, respectively. Mean cone weight is 250–350 g when harvested in winter (relative humidity 50%), yielding 15–20% seeds and only 3.5–4% kernels in weight (Fig. 3.1). Cone picking has traditionally been done by tree climbers using long, hooked poles, but during the last decades, the use of specific mechanical tree shakers has been generalised where tractors can access tree stems (Mutke et al. 2012).

Mediterranean stone pine is an evergreen conifer tree that can reach 25–35 m height, exceeding 1 m stem diameter in monumental specimens, though in its natural, often resource-limited forest habitats, even dominant trees are more likely to culminate at 12–20 m height and 40–50 cm diameters at the end of the silvicultural rotation (80–120 years). Open-grown stone pines present a characteristic polyarchic (candelabra-like) crown, due to the low apical dominance at each branching point. The resulting crown shape is quite singular for a conifer, first spherical, later char-

Fig. 3.1 Mediterranean stone pine cone, green harvested and sundried opened, nuts in shell and kernels

Fig. 3.2 Young stone pines (left) and open-grown tree, overtopping oak woodlands (right)

acteristically umbrella-like when lower branches are dead or pruned (Fig. 3.2). In absence of pruning, stem form is poor due to thick branches. And given that volume growth is also slow, the timber is not appreciated. Most of the needle mass of the canopy is situated in an outward layer at the upper crown surface formed by many codominants, upward-growing shoots, which bear also the female cones (Lanner 1989; Fady et al. 2004; Mutke et al. 2005c, 2012).

Among the seven species of the taxonomic group of Mediterranean pines (subgenus and section *Pinus*, subsection *Pinaster*), stone pine is the only one with seeds larger than 15 mm, reaching up to 20 mm, and ripening only two and a half year after pollination, a year later than in most other pine species (Klaus 1989; Montero et al. 2004). Geographically, stone pine is native Mediterranean, sparsely spread from the Portuguese Atlantic coast to the shores of the Black Sea and the slopes of Mount Lebanon, from sea level up to 1000 m, occasionally to 1600 m (Quézel and Médail 2003). Within the last hundred years, its forest area has more than doubled to 0.75 million ha as results of forestation, for soil protection, ecosystem restoration and sustainable production of both timber and pine nuts. Additionally, during the last 30 years, private landowners have invested more than 20 million euros in new stone pine plantations aimed for cone production, motivated by high prices paid for pine nuts. Portugal and Turkey sum more than 0.25 million hectares of new plantations, often on abandoned farmland that yield higher cone crops than forest land (Calado 2012; Mutke et al. 2012; Kilci et al. 2014).

The actual geographic origin of Mediterranean stone pine has long been discussed. During the Antiquity, the species was already present in most Mediterranean countries. Despite its ubiquity as an old cultural element of Mediterranean landscape, the ecological or phytosociological role of stone pine is only secondary in mixed Mediterranean woodlands. Rather than forming its own, closed canopy layer, it grows as scattered trees or small groves overtopping the dominant lower oak or bush layer (Fig. 3.2), quite similarly to *Pinus sabiniana* spreading over blue oak woodlands and chaparrals in California (Lanner 1999). Pure or dominant stone pine forests are locally limited, often associated with poor sites such as coastal dunes, loose or

shifting sands, on sandpits or in former marshes or estuaries, or shallow soils over sandstone, gneiss or granite bedrocks, sometimes on limestone. In absence of forest management, on the long run, they will often be replaced by succession of mixed stands (Blanco et al. 1997; Prada et al. 1997; Gil 1999; Soto et al. 2010; Ganatsas 2007; Mutke 2013).

In some parts of its range (e.g. the southern Iberian Peninsula), Mediterranean stone pine was an integral element of the open woodland and dry steppe habitat dynamics that prevailed before the Last Glacial Maximum (LGM, 27–18 kyr BP). The oldest archaeologic evidences for human pine nut hoarding and consumption date from 49 kyr BP (Oakley 1958, cit. in Gil 1999), putatively even 150 kyr BP (Cortés-Sánchez et al. 2011), corresponding to Mousterian Neanderthals dwelling in caves and rock shelters along the Costa del Sol (Málaga, Spain). Pine nuts are ideal staple food, easy-to-store because the hard shell conserves dry-stored nuts for years. Kernels are highly nutritive, with 50% fats and 35% proteins, as much as raw soybeans. The northern shore of the Alboran Sea is actually considered to have been the refugium of the last ever Neanderthal population surviving until 28 kyr BP. Also in later periods, i.e. Upper Palaeolithic (Gravettian, 29–24 kyr BP), Epipalaeolithic (Solutrean, 23–19 kyr BP; Magdalenian 17–12 kyr BP) and early Neolithic (7500 yr BP), the continued presence of charred pine cone and seed fragments in caves and shelters such as Gorham's Cave (Gibraltar), Bajondillo and Nerja (Málaga, Spain) has confirmed that *P. pinea* was widely gathered by man along this littoral fringe (Badal 1998, 2001; Jordá Pardo et al. 2003; Finlayson et al. 2006, 2008; Cortés-Sánchez et al. 2008; Carrión et al. 2008; Stringer et al. 2008).

High-resolution palaeoclimate scenarios coincide to pinpoint the same coastal area as the most likely, if not the only, glacial refugium for Mediterranean stone pine on the Iberian Peninsula during the LGM (Benito Garzón et al. 2007). Later, during the Neolithic, Copper, Bronze and Iron Ages, the presence of stone pine also continued in this coastal region, where Phoenicians would establish the settlements of *Malaka* (Málaga, Spain) and *Calpe* (Gibraltar). By that time, the bioclimatic niche suitable for stone pine (Benito Garzón e al. 2007) would have already been expanded to all main areas in the Iberian Peninsula with presence of spontaneous stone pine forests today (Blanco et al. 1997; Prada et al. 1997). Nevertheless, the active role of men spreading useful tree species should not be neglected (Aranbarri et al. 2015; Levis et al. 2017). Protohistoric presence of stone pine has been confirmed by archaeologic evidences in the sandy estuaries of rivers like Tagus and Sado near the old Phoenician colonies *Keition* and *Bevipo* (Alcácer do Sal, Portugal), or Odiel, Tinto, Guadalete and Guadalquivir in the Gulf of Cadiz, territories of the ancient local *Tartessian* culture. When the Phoenician founded *Onoba* (Huelva) and *Gadir* (Cádiz, Spain), Mediterranean stone pine constituted already the dominant forest type in the sandy coastal plains. In eighth century BCE, pine nuts were being object of trade when Mycenaean and Phoenician goods started to make their way into Iberia (Gil 1999; Martínez et al. 2003; Martínez and Montero 2004). But even in the early third millennium BCE, long-distance trade had existed already in the area, confirmed for archaeological sites from megalithic SW Iberia by items such as amber, ostrich eggs, and even ivory not only from African, but also from Asian elephants (Fig. 3.3;

Fig. 3.3 Ivory vessel from Copper Age, carved in relief of four-sided pyramids, which might have been inspired in a pine cone [*Photograph* M. A. Blanco de la Rubia. Reproduction: courtesy of Research Group ATLAS (HUM-649), University of Sevilla]

García-Sanjuan et al. 2013). In the same epoch, Maritime Bell-Beaker ceramics were spreading from Western Iberia all over Europe, following Atlantic costs and river valleys (Cunliffe 2010).

The presence of stone pine has been confirmed also in other, later Iberian archaeological sites (Fig. 3.4), such as the Catalonian littoral near the Hellenic *Emporion* (Ampurias, Spain) or in the central Duero basin inhabited during Iron Age by the Celtiberian *Vaccaei*, where a region of several hundred square kilometres of inland sand deposits between Valladolid and Segovia has preserved its natural cover of extended pine forests with *Pinus pinaster, P. pinea*, and relicts of *P. silvestris* and *P.nigra*, called *Tierra de Pinares* ("Land of pinewoods") (Uzquiano 1995; Gil 1999; Franco-Múgica et al. 2005; García-Amorena et al. 2007; García-Antón et al. 2011; Morales-Molino et al. 2011).

It remains unclear, however, if any stone pine population survived glaciations in other refugia than southernmost Iberia. Elsewhere in the Mediterranean basin, anthracological evidence for its presence during the LGM and before the Holocene is absent, except one report by one author for two sites in southern France dated in 22 and 18 kyr BCE, though in a dubious floristic context with *Pinus sylvestris* and *Sorbus domestica* (Bazile-Robert 1981). In the late Neolithic (2500 BCE) and Bronze Age (2500–1200 BCE), stone pine might have been already present in the Crau, an open Mediterranean steppe south-east of Arles (Provence, France) shaped by agro-pastoral practices since the Neolithic. However, the corresponding charcoal remains, smaller than 2 mm, did not allow distinguishing between *Pinus pinea* and *P. halepensis*, the latter being definitively native (Henry et al. 2010).

From second millennium BCE onward, and especially during Classic Antiquity, stone pine items have been present in archaeological sites all over its present geographic range, including the Levant. In Phoenician and Roman times, stone pine

Fig. 3.4 Archaeological presence of *Pinus pinea* before the Roman Empire: 1 NW Portugal (Bronze Age to Roman, Figueiral 1993 cit. in Rubiales et al. 2011); 2 Ponta da Passadeira (Neolithic, Carrión Marco 2005 cit. in Rubiales et al. 2011); 3 Vila Nova de S. Pedro (Neolithic, Alfonso do Poço 1954 cit. in Rubiales et al. 2011); 4 Zambujal (Eneolithic, Hopf 1981 cit. in Rubiales et al. 2011); 5 Vale Pincel (Neolithic, Carrión Marco 2005 cit. in Rubiales et al. 2011); 6 Pontes de Marchil (Bronze Age, Pinto da Silva 1988 cit. in Rubiales et al. 2011); 7 Gorham's and Vanguard Caves (Middle/Upper Palaeolithic, Metcalf 1958 cit. in Rubiales et al. 2011; Finlayson 1999 cit. in Rubiales et al. 2011; Gale and Carruthers 2000 cit. in Rubiales et al. 2011); 8 La Falguera (Mesolithic-Neolithic-Bronze, Carrión Marco 2002 cit. in Rubiales et al. 2011); 9 Cova de l'Or (Neolithic, Vernet et al. 1987 cit. in Rubiales et al. 2011); 10 La Fonteta Ràquia (third century BCE, Jardón et al. 2009 cit. in Rubiales et al. 2011); 11 Lloma del Betxi (Neolithic, Grau 1991 cit. in Rubiales et al. 2011); 12 Fuente Álamo (Bronze Age, Schoch and Schweingruber 1982 cit. in Rubiales et al. 2011) 13 Cueva de Nerja (Upper Palaeolithic to Neolithic, Badal 1998); 14 El Cigarralejo (fifth to fourth century BCE, Cuadrado 1987 cit. in Rubiales et al. 2011; Rivera-Núñez and Obón 2005 cit. in Rubiales et al. 2011); 15 El Amarrejo (fourth to second century BCE, Broncano 1989 cit. in Rubiales et al. 2011); 16 Cabezo del tio Pio (fourth century BCE, Rivera-Núñez and Obón 1990 cit. in Rubiales et al. 2011); 17 Casa del Monte (fourth century BCE; Badal inéd.); 18 Castillo de Doña Blanca (Tartessian Bronze age, Chamorro 1994 cit. in Rubiales et al. 2011, Ruiz et al. 1995 cit. in Rubiales et al. 2011); 19 Hoyo de la Mina (Neolithic, Uzquiano 2002 cit. in Rubiales et al. 2011); 20 Huelva and Punta Umbía (seventh century BCE, Sánchez Hernando 2005 cit. in Rubiales et al. 2011); 21 Duero Basin (Iron Age, Uzquiano 1995; Rubiales et al. 2011; Hernandez et al. 2011 cit. in Rubiales et al. 2011); 22 Maures massif, Durance valley and Crau (Late Würm, Bazile-Robert 1981; fourth century BCE, Henry et al. 2010); 23 Pompeii (Roman, Robinson 2002 cit. in Rubiales et al. 2011); 24 Veli Brijun (Roman, Sostaric et al. 2001 cit. in Rubiales et al. 2011). Reproduction: courtesy of J. M. Rubiales, Technical University of Madrid; all references in Rubiales et al. (2011)

was a well-known tree, esteemed and often planted as ornamental (Feinbrun 1959; Thirgood 1981; Martínez and Montero 2004). Although, e.g. in Palestine, its introduction seems to have occurred as ornamental only in Hellenistic times (Kislev 1988), stone pine cones have still been found as burial gift in the 12th Egyptian dynasty already (nineteenth century BCE; Schweinfurth 1884). Cones and pine nuts were traded throughout the whole Imperium, from Syria up to Germania and Britannia, as gourmet food, and for religious uses associated with the cult of the mother goddess Cybele. During the modern era, it was widely used in forestation for soil protection, especially on dunes along Mediterranean coasts and in other similar climate zones in the world. During the troubled religious persecution times of the seventeenth century, stone pine is said to have been planted as a welcoming sign by Protestants in west-

ern France (Feinbrun 1959; Agrimi and Ciancio 1993; Prada et al. 1997; Gil 1999; Scarascia-Mugnozza et al. 2000; Fady et al. 2004; Konstantinidis and Tsiourlis 2011; Yılmaz et al. 2013; Loewe et al. 2012).

Definitively, the timeframe for palaeological references for stone pine, except in the southern coastal Iberia, falls plainly within the Holocene, when long-distance seafaring, trade and transfer of plant species have been documented at east since the spread of Neolithic Cardium Pottery Culture (Fugazzolla Delpino and Mineo 1999; Manen et al. 2007; Linstädter et al. 2012, 2016; Zilhão 2014). In consequence, in absence of further evidence, local pre-historic autochthony of the species is not easy to establish. Moreover, for many historic stone pine forests considered emblematic today, the current stands have clearly been established by man, in some cases only in the late nineteenth or even in the twentieth century, for instance along the Ligurian and Tyrrhenian littoral or on the slopes of Mount Somma/Vesuvius in Italy, the Strophylia forest west of Patras in Greece, or the Matn and Jezzine areas on the slopes of Mount Lebanon. The single-cohort structure of many of these pine forests still witnesses their origin from planting or sowing, similar to the twentieth-century protective forestations, e.g. in the Maghreb especially in northern Tunisia (George 1934; Ganatsas 2007; Ganatsas and Thanasis 2010; Mutke 2013).

Approximate current stone pine area is 490,000 ha in Spain (Alía et al. 2009), 195,000 ha in Turkey (Can 2016), 175,000 ha in Portugal (Eira et al. 2010), 46,000 ha in Italy (iStat 2011), 35,000 ha in Tunisia (Ammari et al. 2011), 13,500 in France, 12,700 ha in Lebanon (Stephan 2010; Hamade 2016), and minor areas in Morocco, Greece, Syria, Israel, Croatia or Albania (Mutke 2013). It is noteworthy that due to the presence of stone pine in mixed forests, range area estimates will differ depending on whether only stone-pine-dominated stands are computed, or any forest patch with presence of the species is included; for instance, the latter sum nearly 760,000 ha in Spain (Spanish National Forest Inventory, Alía et al. 2009).

The main objective of many stone pine forests is protection rather than production, and their cones are not always collected. This explains why in spite of huge differences in their total stone pine areas, respective annual commercial crops of the main producing countries, Spain, Portugal, Turkey and Lebanon, are quite similar in magnitude, yielding each around 4000–5000 t of pine nut in shell, Italy producing only about 1000 t. Moreover, the actual annual production of each country varies among years from one-half to five times the average yield, owing to masting synchronising regional crop fluctuations mainly by weather cues (Mutke et al. 2005a; Calama et al. 2016). In the whole Mediterranean region, about 0.96 million ha of stone pine (including recent plantations) yield 16,000–20,000 metric tons of pine nuts annually, i.e. 4000–5000 tons of shelled kernels (FAO 2010; INC 2012). During the twenty-first century, the increasing frequency of drought events in the Mediterranean has been reducing per-hectare yields (Mutke et al. 2005a; Calama et al. 2008, 2011, 2016). During the last few years, pine nut production has decreased drastically due to the invasion of an exotic seed pest, *Leptoglossus occidentalis*. Predation from this seed bug can make seeds or even whole cones to abort (Bracalini et al. 2013; Lesieur et al. 2014; Farinha et al. 2017).

Despite these hazards, the global pine nut production is expected to increase in the near future, once the aforementioned new plantations in Portugal and Turkey reach their full potential. With more than 250,000 ha planted since 1990, stone pine has multiplied nearly fourfold its historic area in both countries, about 50,000 ha in Portugal (DGF 1985) and 40,000 ha in Turkey (Acar 1995). Already today, they have relegated Spain from its former role as main cone producer, though part of Portuguese cones are still processed by Spanish and Italian firms (Calado 2012).

Despite the long cultural history of Mediterranean stone pine and its economic relevance, the first step towards domestication as agronomic nut crop has only been taken during the last decades. This transition has been triggered by the decrease of cone production in natural forests where cones have been traditionally harvested. Causes include climate change, pests and priority shifts in forest ecosystem management (e.g. urban forestry).

Mediterranean pine nuts are today among the most expensive nuts in the world, highly esteemed as gourmet and health food, rich in proteins and unsaturated fatty acids, dietary minerals (phosphorus, iron, zinc, magnesium), vitamin B1 (thiamine), B2 (riboflavin) and E (tocopherols), phytosterols and polyphenols (Nasri et al. 2005, 2007, 2009; Evaristo et al. 2010; Salas-Salvadó et al. 2011; Ruiz-Aceituno et al. 2012). Their high price is an opportunity as alternative crop on rain-fed farmland in Mediterranean climates. Stone pine performs well on poor soils even with reduced cultural practices, and it resists well climate adversities such as droughts and extreme or late frosts. Mechanic harvesting and modern automatised cone processing facilities have strongly lowered labour costs (Mutke et al. 2000, 2007a, 2012; Loewe and Delard 2012).

No cultivars or cultivated varieties have been defined for stone pine yet, but recently, several elite clones and a clone mixture, selected for outstanding cone production, have been registered in Spain and Portugal for their grafted use in agroforestry systems or orchards, as discussed in the third section of the chapter (Guadaño and Mutke 2016).

3.2 Molecular Diversity

Taxonomically, many authors regarded *P. pinea* as an enigmatic and isolated species (Mirov 1967; Klaus 1989). This species belongs to the so-called Mediterranean pines, which represent a greatly heterogeneous assembly, ranging from shore and island to mountain pines (Fady 2012). They diversified ca. 10 Myr ago and display distinct biogeographic and demographic histories. Recently, evolution patterns in the Mediterranean pines have been explored by means of a common set of low-copy loci (Grivet et al. 2013). The phylogenetic position of *P. pinea* was the most difficult to assess, but nevertheless the species was placed in the same cluster as *P. canariensis* and *P. roxburghii*, but not far from *P. pinaster*.

Stone pine is characterised by low genetic variation as discussed for different genetic markers in Fallour et al. (1997), Vendramin et al. (2008) and Pinzauti et al.

(2012) and by a high degree of phenotypic plasticity (Mutke et al. 2005b, 2010, 2013; Chambel et al. 2007; Carrasquinho and Gonçalves 2013a, b). The forest geneticists have long been confused about this genetically depauperate but widespread species. The absence of genetic variation is regarded negatively, and thus, it often remains unreported (Amos and Balmford 2001). Such condition makes it difficult to use the common analytical methods for population genetic studies (Soltis et al. 1992). But most important, the existence of species lacking of genetic variation questions the common belief that genetic diversity is essential for conservation (Lehman 1998). The absence of genetic variation has been associated with prolonged bottlenecks (O'Brien et al. 1985), and forest trees such as stone pine represent an excellent chance to test such hypotheses (Petit and Hampe 2006).

The genetic diversity of *P. pinea* was first investigated by Fallour et al. (1997) using isozymes, which are genetic markers exhibiting Mendelian inheritance, codominant expression and absence of pleiotropic and epistatic interactions. Seed tissues from ten populations of *P. pinea* from the northern Mediterranean were assayed with a total of 37 enzyme systems, and only one locus resulted to be polymorphic (with just two alleles). This study put in evidence that the values of genetic diversity in *P. pinea* were an order of magnitude lower than the mean value observed in other species of the genus *Pinus*, and much lower than Mediterranean pines with well-known low diversity such as *P. halepensis* (Fady 2005).

A research, representing to date the most complete survey of molecular diversity in this species, used paternally inherited chloroplast microsatellites to further analyse the genetic variation and possible phylogenetic origin of *P. pinea* (Vendramin et al. 2008). Chloroplast microsatellites have proven useful to study the geographic distribution of plant genetic diversity (Powell et al. 1995; Vendramin et al. 1999, 2000). Samples were collected from 34 populations distributed across the full range of *P. pinea* (Fig. 3.5).

All populations showed the same haplotype with the exception of all three Lebanese and two Spanish populations, where two and one additional low-frequency haplotypes were found, respectively (Fig. 3.5). Hence, this study extends the previous findings of Fallour et al. (1997) to the chloroplast genome. In this species, the chloroplast DNA variation is extremely low: total haplotype diversity is only 0.019 (0.37 in other conifers) and standardised allelic richness per microsatellite locus was 1.08 (2.58 in other conifers). Sequencing of the cpDNA fragments demonstrated that the absence of polymorphism was not due to loss or interruption of microsatellite repeat motifs. Furthermore, analyses and standardisation showed that the almost absent genetic variation of *P. pinea* was neither due to ascertainment bias caused by shorter alleles (i.e. a lower number of repeats microsatellite stretches) nor due to different sample sizes respect to studies in other conifers. Respect to allozymes, chloroplast microsatellites have higher mutation rates (Provan et al. 1999) and are generally highly variable in conifers (e.g. Powell et al. 1995; Vendramin et al. 1996; Petit et al. 2005). Therefore, the very low chloroplast diversity found in *P. pinea* is particularly remarkable. Comparing the level of diversity of *P. pinea* with other plant species, the possible effect of the different attributes (plant size, woody/herbaceous habit, geographic range size, native or introduced status, existence of asexual repro-

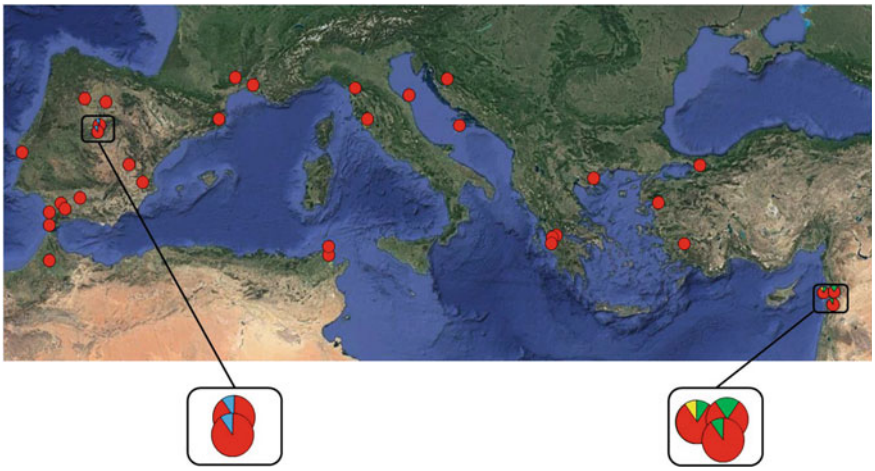

Fig. 3.5 Chloroplast DNA variation analysed in *P. pinea* populations using 13 microsatellites. Only four haplotypes were identified: H1: red (present in all populations), H2: green (private to Lebanon), H3: blue (private to the central Iberian Peninsula), H4: yellow (private to Lebanon) (Vendramin et al. 2008)

duction—through apomixis or vegetative growth—and mating system) was tested and taken into consideration. Genetically depauperate plants with characteristics similar to those of *P. pinea* are really remarkable, and the only other conifer with a similar low level of chloroplast microsatellite variation is *P. torreyana*, a Californian narrow endemic (Provan et al. 1999). New nuclear molecular markers (microsatellites and gene sequences) showed a comparable pattern of low genetic variation in stone pine (unpublished data), for example the level of nucleotide diversity at gene level (π) was one order of magnitude lower and heterozygosity (H_E) at microsatellite level significantly lower than in other conifer species. On the other hand, significant genetic differentiation (F_{ST}) was found among westernmost and easternmost stone pine origins, with central Spanish and Lebanese populations showing differentiated nuclear polymorphisms too.

The extreme situation of low polymorphism at molecular markers in this species is probably the result of a combination of factors that have acted in the same direction. The three rare cpDNA haplotypes (H2, H3 and H4 in Fig. 3.5) from Lebanon and central Spain, all differing by a single mutation from the widespread haplotype (H1), have as their goal the post-bottleneck mutations in areas of expansion and not the independent survival of haplotypes that existed prior to the bottleneck (Slatkin and Hudson 1991). This suggests that, at some point(s) during the evolutionary history of this species, one or more drastic range-wide decline events occurred, followed by the survival of a single (i.e. geographically circumscribed) population. The prolonged bottleneck experienced by *P. Pinea* could have been the consequence of the natural climatic and sea level fluctuations during the Quaternary. Furthermore, the unusual mating system of the species might have accelerated the loss of genetic

diversity. Forest trees normally are subjected to strong inbreeding depression, which causes the elimination of all inbred progeny prior to maturity (Petit and Hampe 2006; Scofield and Schultz 2006). Interestingly, along with *P. resinosa*, another pine species with low genetic diversity (Fowler 1964; Mosseler et al. 1991, 1992; Echt et al. 1998), *P. pinea* is one of the very few tree species that are not subjected to inbreeding depression: in this species, height growth is not reduced even by two successive generations of self-pollination (Ammannati 1988). Finally, unlike most of pine species, which are anemophilous, *P. pinea* seeds are dispersed only by means of mutualistic animals, such as birds, often scatter-hoarders that do disperse lots of several seeds from the same tree, resulting in a clustered kin dispersion. The scarcity of seed dispersers during some critical periods of the species history may have prejudiced its ability to colonise new territories, thus making stone pine more susceptible to range contractions and affecting its genetic diversity.

However, the most unusual finding is not only the bottleneck and the loss of diversity, but rather the fact that *P. pinea* has maintained a low level of diversity while spreading across a diverse and fragmented region. This spread probably took place during the Holocene, or even earlier, suggesting that stone pine has not been able to regain genetic diversity several hundred or maybe even thousands of generations after its decline. The existence of such genetically depauperate yet widespread species seems to be supported by the reduced evolutionary rates. It is obvious that, after the expansion, the low evolutionary rate maintains a low level of diversity, but at the same time it is not responsible for the success of the expansion itself. A number of not mutually exclusive explanations can be suggested. First, humans have played an important role by spreading the species for at least 3000 years. Second, during the bottleneck phase there might have been a loss of specific parasites and diseases (Amos and Balmford 2001): today *P. pinea* has comparatively few parasites and diseases (Fady et al. 2004). Third, variation at phenotypic traits, and not on marker diversity, may determine the successful adaptation to new environmental conditions. The small but not negligible amount of heritable variation found at adaptive traits (Mutke et al. 2005b, 2010) could have helped in colonising new environments, although the presence of a reduced genetic variation.

After a bottleneck, epigenetic variation can accumulate quickly (Rapp and Wendel 2005), and it might be responsible for the variation in functional traits of *P. pinea* trees grown in drought stress conditions (Sánchez-Gómez et al. 2011). Epigenetic mechanisms have been proposed to contribute to adaptation in plants (Richards et al. 2010; Bräutigam et al. 2013). Recently, Sáez-Laguna et al. (2014) analysed DNA cytosine methylation in *P. pinea* genome to identify potential epigenetic variability explaining the significant functional variation found in this species. The authors analysed the DNA of 20 vegetatively propagated individuals from five Spanish natural populations by using Amplified Fragment Length Polymorphism (AFLP) and Methylation Sensitive Amplified Polymorphism (MSAP) techniques. No polymorphic marker was identified by means of the AFLP technique, thus confirming the absence of genetic variation displayed by this species. The MSAP technique, however, discovered that approximately 65% of the analysed cytosines at CCGG motifs were methylated. This value is at least 10% higher than estimates indicated annual

and other perennial plants (Marfil et al. 2009; Herrera and Bazaga 2010; Li et al. 2011) and is in agreement with hypermethylation of conifer genomes (Nystedt et al. 2013), suggested to underlie genome evolution in conifers. The widespread methylation found in *P. pinea* genome might be closely associated with the repetitive nature of conifer genomes (Nystedt et al. 2013). Furthermore, this study reported a high level of cytosine methylation variability between the analysed trees that reflect the variation in 42% of the total MSAP markers analysed. Several studies suggest that cytosine methylation variability may be related to the phenotypic plasticity in adaptive traits (Zhang et al. 2013; Herrera and Bazaga 2010). Therefore, in *P. pinea* epigenetic variation could act as a source of variability alternative to genetic diversity, bringing about relevant evolutionary consequences for adaptation (Vendramin et al. 2008; Mutke et al. 2010).

3.3 Phenotypic Diversity, Selection and Breeding

Similarly to the reduced molecular diversity discussed in the previous section, studies of phenotypic variation in quantitative traits, evaluated in common garden tests, have detected only very moderate differences between populations of different geographic origins (provenances) in such adaptive traits as survival, total height and diameter, or phenology. Most of the analysed common gardens were established during the early 1990s as a reciprocal international provenance test. Accessions were exchanged from the full geographic range of stone pine, following an initiative of INRA within the framework of the FAO Committee *Silva Mediterranea*, which involved France, Portugal, Spain, Italy, Turkey, Tunisia and Morocco, and including further accessions from Greece and Lebanon (Fig. 3.6; Martín and Prada 1995; Carneiro et al. 2006; Court-Picon et al. 2004; Gordo et al. 2007; Mutke et al. 2008, 2010, 2013; Khaldi et al. 2009; Sbay et al. 2011; Acar et al. 2013; Carrasquinho and Gonçalves 2013a, b; Loewe et al. 2012; Hermida et al. 2016).

For French trial sites, Court-Picon et al. (2004) reported differences in germination capacity among provenances, attributed to differences in seed size and weight

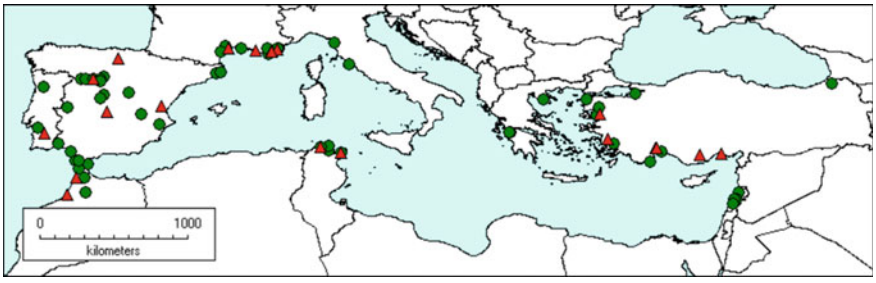

Fig. 3.6 Mediterranean stone pine provenances tested in FAO provenance trials (circles—provenances; triangles—test sites Mutke et al. 2013)

that also influenced positively the initial juvenile height growth. Greater vigour was related to earlier bud burst, though those precocious provenances also increased their risk of damages by late frosts. Accessions from limestone origin developed well on siliceous substrate, suggesting no adaptive differentiation regarding soil chemistry. But the variations in soil/micro-ecological conditions were reported to greatly influence the variation in size of the saplings, and the authors suggested that this strong individual variability due to the environment may have masked the differences of growth expected among provenances in their analysis (Court-Picon et al. 2004). Whether the small amount of variability detected in adaptive traits between provenances was only due to seed size, maternal effects, phenotypic plasticity, or also to genetic diversity, remained to be answered by further analyses and monitoring (Fady and Vauthier 2011).

Similarly, Carneiro et al. (2006) and Carrasquinho and Gonçalves (2013a) reported no significant differences between provenances regarding stem diameter at age 13 and strong provenance × site interactions for two Portuguese test sites. The provenance effect in height growth was significant, with among-provenance mean heights varying from 1.3 to 1.5 m at age 6 at one site and from 2.2 to 2.6 m at age 11 at the latter. Strong spatial autocorrelations were found within both sites, and this factor had to be taken into account in the models (Carrasquinho and Gonçalves 2013b).

Khaldi et al. (2009) reported a lack of significant differences in survival and growth among provenances (mean heights 2.0–2.2 m at age 10) for two Tunisian plots, but they did not take into account spatial effects. In contrast, Sbay et al. (2011) found significant differences in height growth up to age 12–13 at two Moroccan test sites, the best provenance exceeding by 9–10% the mean height (5.8 at age 12 in one site; 6.4 at age 13 in the other site). Two Turkish plots analysed by Acar et al. (2013) revealed also significant differences in height, diameter and conelet number at age 10. The cited studies did not find differences in survival, a trait more closely related to fitness, among provenances.

Analysing the Spanish test sites, Mutke et al. (2010) found strong phenotypic plasticity in response to microsite conditions, prevailing over small, though significant provenances differentiation. The same trend was found when comparing the four Spanish and three French test sites of the international provenance trial, after adjusting mean tree height values to micro-environment factors by iterative spatial analyses (Mutke et al. 2013). Height growth differed significantly between adjusted provenance means, ranging ±5% the average height at the two French sites, but as much as ±31% at the worst site in Spain, where mean height was only 1.1 m at age 14, due to the abundance of still juvenile trees without onset of adult whorled stem growth. In average, height growth was lower in trees from warmer (coastal) origins than colder (northern, inland) ones, similar to findings in a recent nursery trial with seedling from Spanish inland or coastal provenances (Pardos et al. 2013). Differences among provenances amounted to 3–12% of the observed phenotypic variance in height among trees at each site, clearly much less than the common, stable reaction norm associated to the microsite that explained 73–78% of the variation observed at each site, or between sites (Fig. 3.7; Mutke et al. 2013).

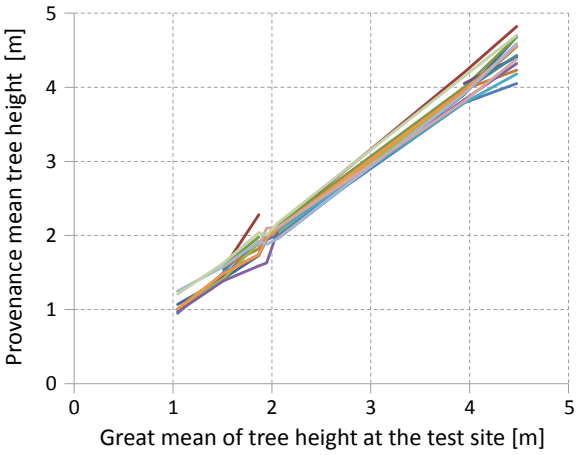

Fig. 3.7 Adjusted provenance mean heights plotted against the great mean at each site (15 provenances at 5–7 sites, age 9–16 yr; Mutke et al. 2013)

Another trial in Spain compared accessions from nine populations growing on contrasted soil conditions at seven test sites with an analogous range of geological variation, comprising river terraces with groundwater layers accessible for roots, deep sand deposits, shallow limestone leptosols, and even chalky gypsum marls. The adaptive differentiation in survival, vegetative phase change, height or diameter growth were absolutely non-significant among provenances, contributing only 0.02% of the total observed variation in tree height at 8 years, while variation between sites, as well as microsites within, explained as much as 84% of phenotypic variation (Gordo et al. 2007).

The reason for this overwhelming impact of microsite variation on stone pine performance is a strong phenotypic plasticity that allows high survival rates even in unfavourable environments, though with strong ontogenic delays as pay-off, such as the described delayed growth due to edaphic restrictions in one of the Spanish test sites. Stone pine is ecologically adapted to drought-prone environments and loose, well-aired soils, such as sand, sandy loam or gravel, where its adult root system can explore more than thirty metres horizontally and several metres in depth in search for water (Montero et al. 2004). In fact, growth can be restrained by too compact soils during the first phase of seedling establishment (less than 40% sand, and more than 40% silt or more than 30% clay), especially if occasional flooding does occur. In these conditions, the plant development can be arrested for many years in a persistent juvenile phase, resulting in a dwarf-juniper-like shrub habit. This juvenile "standby" stage in *Pinus pinea*, also observed in *P. canariensis*, can be seen as an evolutionary advantage to survive under harsh environments, as opposed to other pine species which do not demonstrate this ontogenic plasticity (Climent et al. 2011; Mutke et al. 2012).

Contrasting to the lack of genetic diversity of stone pine at provenance level both at molecular markers and at fitness related phenotypic traits, significant clonal variation has been detected for morphological and functional traits (Carneiro 2005; Mutke

et al. 2005b; Carrasquinho et al. 2010). Research on clonal selection and grafting techniques in stone pine has been under way for a few decades in Italy, Portugal and Spain (Magini 1965; Baudín 1967; Magini and Giannini 1971; Balguerías 1971; Gil and Abellanas 1989; Catalán 1990, 1998; Abellanas et al. 1997; Prada et al. 1997; Mutke et al. 2000, 2005b, 2007b; Gordo 2004; Castaño et al. 2004). When evaluating the cone productivity of different clones in several comparative grafted field tests, a similar common dependence on microsite was observed for tree growth and cone productivity, given that individual cone production correlates strongly with the crown size of the tree (r of 0.6–0.9) (Mutke et al. 2007b). The degree of (clonal) genetic determination for cone or nut production was estimated in 7–18% of overall phenotypic variation, based on the performance of 462 candidate clones in several grafted comparative trials in Spain, but it increased to as much as 24–57% once estimates were adjusted for variation in tree size. Corresponding estimations for genetic gain would be in the range from +12 to +39% of mean cone yield in each test site by selecting the top 10% (most productive clones), though genotype × site interactions were found to be significant, marking certain agroclimatic gradient from more genuine Mediterranean to colder, more continental climate zones. The genetic gain might be higher if referred to an overall average for the species, because the original selection of the clones compared in the grafted trials included only plus trees for cone production (Mutke et al. 2005b, 2007b).

Within the European Union, superior clones of forest trees can be registered and catalogued officially as *basic materials*, in the categories "*qualified*", i.e. phenotypically selected at the individual level, or "*tested*" when their superiority has been already demonstrated by comparative testing or estimated from genetic evaluation of their components. Their register is mandatory for admission as donors of commercial graft scions (forest reproductive material) under the frame of European regulation on the marketing of forest reproductive material (Council Directive 1999/105/EC).

For this register, clones must meet a list of requirements, among others the mandatory request to be identifiable by distinctive characters which have been approved and registered with the official body, similarly to markers or descriptors for plant varieties. In the case of stone pine, due to its great phenotypic homogeneity, no morphological or phenological descriptors could be singled out for clones: no clear differences in branching, crown form, needle shape, or phenological calendar do allow for identifying each one of them. Nuclear microsatellite variation (Pinzauti et al. 2012) made the identification of 18 Spanish elite clones possible, while other 31 outstanding clones could not be legally registered as basic materials because their nSSR genotype was not unique compared among 86 sampled clones. In 2015, 15 of these 18 clones were registered in the Spanish National Catalogue of Basic Materials for *Pinus pinea* (Table 3.1). Five clones, with consistent superiority for cone production at two or three different trial sites, were admitted as basic materials in the category "Tested"; while the other ten clones, having been evaluated only in one test site, were registered temporally in the category "Qualified", until their further characterisation in other, more recent trials (Guadaño and Mutke 2016).

In Portugal, a clonal mixture has been registered as "Qualified" Basic Material for stone pine, comprising 64 individual clones whose ortets had been phenotypically

Table 3.1 Stone pine elite clones registered as basic materials in Spain and expected genetic gain estimated from grafted trials

Unit of approval	Genetic gain (+% cone yield)
Category "Tested" (B.O.E., December 12, 2015)	
CL-C-23/Portillo-11	+25–27%
CL-C-23/Portillo-12	+12–29%
CL-C-23/La Vega	+12–17%
CL-C-23/Íscar	+11–20%
CL-C-23/Valdegalindo	+15–18%
Category "Qualified" (B.O.E., May 13, 2015)	
CL-Q-23/Hoyo de Pinares	+19%
CL-Q-23/Almorox	+19%
CL-Q-23/San Martín de Valdeiglesias	+24%
CL-Q-23/El Provencio	+31%
CL-Q-23/Pozoamargo	+21%
CL-Q-23/Casas de Haro	+23%
CL-Q-23/El Picazo	+22%
CL-Q-23/Santa Coloma de Farners	+9%
CL-Q-23/Llagostera	+11%
CL-Q-23/Dosrius	+9%

B.O.E.—Boletín Oficial del Estado, http://www.boe.es

selected as plus trees for outstanding cone production among 300 candidate trees sampled in the stone pine forests of southern Portugal. In 2004, two mother plant orchard with 50 ramets of each of these clones have been planted in two sites, Coruche (PNMQ01) and Alcácer do Sal (PNMQ02), for producing graft scions, commercialised since 2009 by the Private Forest Owners Associations APFC and ANSUB for in-field grafting of new plantations in the Alentejo region (Carneiro 2005; Carneiro et al. 2007; Carrasquinho et al. 2010; Guadaño and Mutke 2016).

For landowners who cannot count on skilled hands for in-field grafting, the release of the Spanish and Portuguese elite clones is a welcome solution that allows the development of orchard plantations from container-raised trees grafted in nursery. However, cross-border trade of grafted pines is currently hindered, at least partially, by a quarantine restriction for Portuguese forest plant materials due to the high biotic risk of spreading the pine wilt nematode, *Bursaphelenchus xylophilus*, one of the most dangerous threats for European coniferous forests, but that is asymptomatic in *Pinus pinea* (Nunes da Silva et al. 2015; Zas et al. 2015).

Definitively, though current agronomic knowledge about stone pine as orchard nut crop is still limited, and most plantations continue to be managed as extensive forestry or agroforestry systems with trees grown from seeds without selected

pedigree, Mediterranean stone pine nuts have lately taken their first steps from a wild-harvested forest commodity to a high-value agronomic crop.

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Part II
Genetic Diversity

Chapter 4
Genetic Diversity in Vegetable and Fruit Crops

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Abstract In order to overcome the challenges posed by the prevailing biotic and abiotic factors, plants have evolved various characteristics that confer survival and ensure adaptation to certain unfavorable conditions, consequently, leading to diversity. Increasing knowledge in the era of genome sequencing of organisms makes it evident that all forms of diversity have their origin rooted in the genes. In this context, genetic diversity analysis provides a powerful tool that enhances a comprehensive understanding of genetic variation and improves conservation strategies. Plant diversity refers to the variety of plants that exist on the Earth. Understanding genetic diversities among fruits and vegetable plants are of significant importance because of not only their agricultural importance but also their economic values. It is important to keep regular assessments of the conservation status of these plant species, in order to prioritize those in need of conservation action. In order to access genetic diversity easily by researchers and breeders, most crops are conserved in genebanks, seed, and/or field, typically as part of individual national germplasm systems. The improvement of fruits and vegetable plants considerably depends on

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the extent of genetic variability existing within the species as well as their crop wild relatives. The genetic variation that exists among plant populations is a basic requirement for efficient improvement of such populations. It also serves as an evidence to prove whether such a population can withstand unpredictable changes in the environment. Molecular studies provide insight into relationships and diversity among plant breeds. The extent and nature of genetic diversity of fruits and vegetable plants from all around the world has been investigated by typing DNA markers in a set of individuals belonging to several breeds. The availability of PCR-based molecular markers and next-generation sequencing allows for the detailed analyses and evaluation of genetic diversity in plants and to detect genes that influence certain desirable, important traits. In this chapter, we provide a comprehensive survey on the genetic diversity among some important families of fruits and vegetable plants.

Keywords Accessions · Biodiversity · Plant breeding · Germplasm · Geographic region · Population · Variability

4.1 Introduction

Biodiversity emphasizes the differences among living organisms, while genetic diversity refers to the inherited differences within and between populations of living organisms, and groups of related plants. This pool of inheritable differences within a mating population is the foundation for selection and improvement among plant populations. Along these lines, protection of the genetic diversity in plants is crucial for the satisfactory existence of man at present and in years to come (Rao and Hodgkin 2002). The importance of plant genetic diversity is now recognized as a significantly important area since exploding population with urbanization and decreasing cultivable lands are the critical factors contributing to food insecurity in the developing world. Agricultural scientists realized that genetic diversity can be captured and stored in the form of plant genetic resources such as gene bank, DNA library, and so forth, in biorepositories, which preserve genetic material for long periods (Govindaraj et al. 2015).

The genetic material of crops that are planted and grown as well as those that grow in the wild are essential to man as a source of sustenance, shelter, fuel, commercial, and cultural products. Breeders who raise plants to produce new and improved types need genetic variation (Kishor et al. 2017). Several techniques are accessible for examination of inherited differences within germplasm varieties, breeding lines, and other members of a population among which interbreeding exists (Mohammadi and Prasanna 2003).

The success of these strategies rely on family history, structural information, data with regard to crop production and soil management, biochemical processes, and DNA-based information (Mohammadi and Prasanna 2003). For a considerable length of time, research done has been directed toward identifying the hereditary differences among vegetable and fruit species in light of their agronomic and morphological

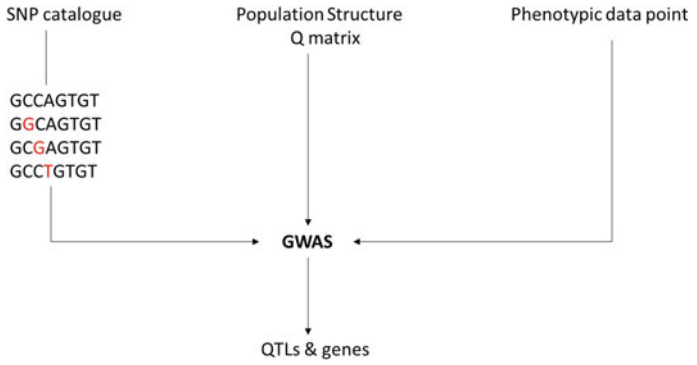

Fig. 4.1 Genome-wide association studies (GWAS) rely on three frameworks: (i) Single-nucleotide polymorphisms (SNP) catalogue obtained from diversity assessment; (ii) population structure and allele frequency spectrum (Q matrix); and (iii) phenotypic data points for each individual within the population

characteristics (Nunes et al. 2017). Genome-Wide Association Studies (GWAS) is the most recognized powerful tool to investigate genotype–phenotype association (Fig. 4.1). They have been useful in many crops like rice (*Oryza sativa* L.) (Huang et al. 2010), maize (*Zea mays* L.) (Kump et al. 2011) or barley (*Hordeum vulgare* L.) (Pasam et al. 2012).

However, certain limitations remain: (i) rare alleles are undetectable; (ii) alleles with minimal effects on the phenotype are not easily identified; and (iii) the most significant SNPs are not always the true predictive factors for any given trait (D'Agostino and Tripodi 2017). It is very likely that GWAS would be replaced by genomic selection (GS) strategies in the future (Bhat et al. 2016). Genomic selection (GS) is a type of selection that involves the use of markers and selection which is dependent on the genomic estimated breeding values (GEBV) of dominant members of the population as dictated by a statistical representation (Pérez and de los Campos 2014; Nakaya and Isobe 2012). In any case, the pattern of hereditary variability in plants is intricate and can be impacted by several elements such as: the crop production system, plant growth, seed distribution technique, geographical spread, and the plants grouping based on shared characteristics (Hamrick and Godt 1989). The degree and distribution of hereditary variation in a plant species depends on how the plant was bred, how much it has evolved over time, natural and topographical components, challenges in former times, and on many occasions by a variety of human factors (Rao and Hodgkin 2002). As such, molecular marker methods is one of the key techniques being utilized today as they are uninfluenced by climatic elements (Nunes et al. 2017).

There have been major breakthroughs in the utilization of molecular markers for the identification of genetic material in cultivated crops (Wünsch and Hormaza 2002). The molecular marker procedure involving the use of inter-simple sequence repeat (ISSR) markers has been exceptionally instrumental in researching the population structure of biological samples in different kinds of plant species (Nunes et al. 2017).

These types of markers are used largely because of their speed, productivity, ability to be reproduced, and their trademark capacity to distinguish incredible numbers of polymorphisms in a specific population (Reddy et al. 2002; Kuras et al. 2004). More importantly, molecular markers are major instruments for assessing the hereditary variations among living tissues or seeds from which new plants can be grown and among the genetic makeup of plants during the breeding process (Silva et al. 2017a), leading to marker-assisted selection (MAS). Other molecular markers have been successfully used in diversity studies in many species: Simple sequence repeats (SSRs: *Musa* spp. (Amorim et al. 2012; Irish et al. 2014; Kitavi et al. 2016), cassava (Ndung'u et al. 2014; Beovides et al. 2015) or wheat (Zeb et al. 2009; Li et al. 2013); restriction fragment length polymorphisms (RFLP: maize (Melchinger et al. 1990) and *Puntius* spp. (Balaraj and Basheer 2012); or single-nucleotide polymorphism (SNPs: barley (Sato et al. 2011), cassava (Kawuki et al. 2009), or common bean (Galeano et al. 2012).

In order to enhance and maintain the resources of our food crops and our tree assets, a major comprehension of the biology and inherited characteristics of major plant species is essential (Verde et al. 2013). Research has demonstrated that crossing different genotypes and choosing enhanced hybrids are vital to the generation of new plant varieties (Ortiz and Vuylsteke 1996). As indicated by Silva et al. (2017a), crossing affected the degree of genetic variation by lessening the gene distance and expanding the difference among a few genotypes. Learning about germplasm diversity and hereditary relationships among breeding materials can be an important guide in crop improvement techniques (Mohammadi and Prasanna 2003).

Among the most important crops around the world, vegetables and fruits play a significant role, especially as food source on local and regional development, contributing to not just food security, but also to nutrition security. That is not new; there are many evidences about their contribution, particularly indigenous fruits and vegetables, to a culturally acceptable, cost-effective, sustainable, and nutritious diet. Indigenous fruits and vegetables are defined as locally produced (usually within traditional systems), socially and culturally accepted as local foods, and eaten by previous generations or introduced for a very long time. While not a distinct category, these foods tend to be neglected and underutilized by research organizations, food processers, marketers, and consumers. Consistent evidences show that the consumption of diverse fruits and vegetables also has a significant positive association with health outcomes. The global dietary transition and associated negative nutrition and health outcomes are likely to be mitigated by increasing the consumption of more fruits and vegetables including indigenous varieties (Cogill 2015). For that reason, the study on the genetic diversity and conservation of fruits and vegetables for appropriate usage is an unavoidable activity for humanity.

In order to promote the conservation of genetic resources and ensure the discovery of novel genes, it becomes imperative to review past and most recent findings and identify the research gap. This is important in order to open doors for possible discoveries that can be made from the application of various tools in elucidating the genetic diversity among such plant species. A better understanding of genetic diversity and its distribution is essential for its conservation and use (Rao and Hodgkin

2002). This will aid in determining what to conserve as well as where to conserve while improving our understanding of the taxonomy, origin, and evolution of plant species of interest (Rao and Hodgkin 2002). Here, we provide a comprehensive review of the genetic diversity in some families of vegetable plants and fruits, and identify the research gap.

4.2 Genetic Diversity in Vegetables

4.2.1 Asteraceae

Asteraceae (*Compositae*) is the largest family of flowering plants, around 1,620 genera and more than 23,600 species. The family is distributed all over the continents except Antarctica and lettuce (*Lactuca sativa* L.) is an important leafy vegetable from this family, which is used for salad. The main center of origin of lettuce is the Mediterranean area, which has been cultivated since 2000 BC (Hancock 2004).

The main production center of lettuce in the world is Asia, North America, central Europe, Spain, and Italy. Mousavi et al. (2013) studied fifteen different accessions of Iranian lettuce cultivars and varieties by measuring the number and size of chromosomes. The study showed that they have nine chromosomes ($n = 9$) in all accessions, but had morphological dissimilarity and heteromorphisms between the chromosomes. The genetic diversity in *Asteraceae* family members remains for the most part elusive. Thus, further research is necessary to fully provide a comprehensive understanding of genetic diversity in this family.

4.2.2 Brassicaceae

Brassicaceae (Cruciferae) is one of the largest plant families, which comprises approximately 3000 described species and 350–380 genera. The family includes some of the world's most economically important crops, especially members of the genera *Brassica* L. (cauliflower, kale, cabbage, brussels sprouts, kohlrabi, and broccoli) (Al-Shehbaz et al. 2006). The 'cole' vegetables are grown and eaten throughout the world. These were domesticated from one ancestral species, the wild cabbage *Brassica oleracea* L. These cultivated species contain a large variety of leaf and root vegetables, oilseed, and condiment crops.

Cole crops are grown worldwide, except for some tropical areas. Among these, cabbage is most widely produced, followed by cauliflower and broccoli. Cabbage is more important in northern and eastern European countries, while cauliflower is more important in southern Europe, USA, and Mexico. Wild forms of *B. oleracea* were found in the Atlantic Coasts of Europe, Northern France, and England; related wild species are endemic to the Mediterranean basin. Sauer (1993) explained that

B. oleracea have been domesticated throughout Western Europe with the Greeks and Romans cultivating kales and non-heading cabbages since the first century AD. Guo et al. (2014) conducted a study to investigate the genetic structure and center of origin of *Brassica rapa* L., based on 51 SSRs and 715 alleles at polymorphic loci in 173 *B. rapa* accessions with a worldwide geographic distribution. The wild types belonged to the Old-World group (Europe, West Asia, and North Africa) and this group revealed the highest number and richness of private alleles. A second group with prominent levels of genetic diversity, representing East Asia was considered the effect of a secondary center of diversification, while the third group, with low levels of genetic diversity, included migrants into East, South, and Central Asia. Roots crops belonging to this family include turnips and radishes.

4.2.3 Convolvulaceae

Sweetpotato [*Ipomoea batatas* (L.) Lam] is perhaps the most important species belonging to the *Convolvulaceae* family and it is the seventh most important food crop. The origin of sweetpotato is in South Mexico and Central America. Access to genetic diversity is essential for any breeding program and to maintain in vitro or ex-vitro germplasm collections to conserve it (Fig. 4.2).

There exist wide germplasm collections around the word and the biggest is at International Potato Center in Peru; with more than 5500 cultivated *I. batatas* accessions from 57 countries conserved in 2000 (Zhang et al. 2000). Other countries like USA or Cuba (670 accessions) have maintained important germplasm collections for decades.

Fig. 4.2 Germplasm collections of *Ipomoea batatas* (L.) Lam help to conserve the genetic diversity for the future. *Source* INIVIT

The study of genetic diversity of sweetpotato is necessary due to the increased demand for food and for the conservation of genetic resources (Rodriguez-Bonilla et al. 2014). A study was carried out to assess the genetic diversity of sweetpotato utilizing 137 landraces from different locations in Puerto Rico (PR) using 23 SSR markers. The result showed presence of prominent levels of genetic diversity of sweetpotato across PR which can be related to the genetic makeup of sweetpotato, human intervention, and out-crossing nature of the plant. The study emphasized the importance of conserving this genetic diversity for future utilization (Rodriguez-Bonilla et al. 2014). Zhang et al. (2000) conducted a study to assess the genetic diversity of sweetpotato in tropical America utilizing 69 sweetpotato cultivars from different regions of Latin America with amplified fragment length polymorphism (AFLP) markers. Highest genetic diversity was found in Central America, whereas Peru–Ecuador exhibited low genetic diversity.

In many developing countries, sweetpotato serves as a secondary staple food. The creation of orange-fleshed sweetpotato, biofortified with β-Carotene, has been instrumental in alleviating vitamin A deficiency, especially in children in several countries (Van Jaarsveld et al. 2005).

4.2.4 Cucurbitaceae

Cucumis sativus L. (common name Cucumber) is a member of the *Cucurbitaceae* family found in the subtropical areas (Fig. 4.3). Qi et al. (2013) used a genomic variation map to provide insights into the genetic basis of cucumber domestication and diversity (that consists of approximately 3.6 million variants), by deep re-sequencing of 115 cucumber lines sampled from 3342 accessions worldwide. They were therefore able to identify 112 putative domestication sweeps; one of the regions which contained a gene involved in the loss of bitterness in fruits, an essential domestication trait of cucumber. Comparative analysis has suggested that fruit crops underwent narrower bottlenecks during domestication than grain crops (Qi et al. 2013).

Momordica charantia L. is commonly found in tropical and subtropical environments. It is commonly called bitter gourd or bitter melon, Chinese cundeamor or balsamina. 38 genotypes of the bitter gourd as well as few commercially available cultivars collected from different agro-ecological zones in India were evaluated in a diversity study both at morphological and molecular levels using random amplified polymorphic DNA (RAPD) markers and agronomic traits (Dey et al. 2006). They demonstrated from their research study that diversity based on yield-related traits and molecular analysis did not correspond with ecological distribution and that the cluster pattern based on yield-related traits and molecular variation differed in the bitter gourd cultivars.

Bottle gourd [*Lagenaria siceraria* (Molina) Standl.] is suited for tropical to subtropical climatic conditions. In 36 bottle gourd landraces of South African origin, Mashilo et al. (2017) accessed genetic diversity using 10 fruit qualitative traits and 11 polymorphic simple sequence repeat (SSR) markers. The bottle gourd landraces

Fig. 4.3 Cucumber (*Cucumis sativus* L.) from the *Cucurbitaceae* family is a vegetable, which is used for salad. *Source* Courtesy of J. A. Cruz-Alfonso

displayed a high level of genetic diversity based on the phenotypic fruit qualitative characteristics and thus fruit qualitative traits were identified as beneficial parameters for genetic analysis in bottle gourd.

Sechium edule (Jacq.) Sw.[1] or Chayote is found in tropical and subtropical environments. Genetic diversity among 26 accessions of chayote across 12 Indian states was identified using 18 morphological fruit traits and directed amplification of minisatellite DNA (DAMD) markers (Jain et al. 2017). The DAMD primers were used to determine the diversity of the chayote at the DNA level, producing 102 bands, of which 97, which were polymorphic, displayed between 66.66 and 100% polymorphism per primer. Furthermore, distinct cluster patterns were identified using the UPGMA method revealing a high diversity among the collected accessions (Jain et al. 2017).

Pumpkin (*Cucurbita pepo* L.) is grown in tropical and subtropical environments. The phytochemical and genetic diversity of three naked seed and eleven true pumpkin seed varieties was evaluated by Soltani et al. (2017) to discover traits that can be used in the selection of parent progeny during breeding. The fruit and seed sizes were found suitable for enhancement using breeding techniques in a bid to improve seed quality. The best variety tested by them, determined based on fruit and seed attributes such as unsaturated and saturated fatty acids, was *C. moschata* "Chaloos." The squash is commonly found in the Mediterranean regions but *C. maxima* Duchesne is a basic element in traditional subsistence agriculture in South America, where an enormous diversity of landraces exists (Lira-Saade 1995). The Centre for Conservation and Breeding of Agricultural Diversity at the Polytechnic University of Valencia

(Spain) has a collection of *C. maxima* with more than 300 accessions, which have been acquired since the 1980s (Ferriol et al. 2004). Molecular analysis using AFLP markers, which analyze neutral genetic diversity, and SRAP (sequence-related amplified polymorphism) markers, which preferentially amplify gene regions, showed a genetic diversity in squash concordant with the morphological variability in a study by Ferriol et al. (2004). The SRAP results agree with the more primitive traits showed by the South American landraces. With the use of these markers, the varieties were grouped by geographical descent: Spain, Central and South America implying the presence of two autonomous domestications in the two American regions, or potential introgressions from associated species. Besides, the accessions from the Canary Islands grouped separately from those from the Spanish peninsula. This divergence could be due to an earlier adaptation of *C. moschata* Duchesne ex Poir. to the tropical island environment, in addition to introduction of a different germplasm from America.

Zucchini (*Cucurbita pepo* L.) is native to the Mediterranean environments. Ferriol et al. (2003) evaluated the genetic diversity in a group of *C. pepo* using AFLP and SRAP PCR-based systems. Results from the cluster analysis showed the separation of cultivars into two subspecies (ssp. *ovifera* and ssp. *pepo*) with the information given by the SRAP markers more in tune with morphological variability and to the evolutionary history of the morphological types than the AFLP markers. Furthermore, in the ssp. *ovifera,* cultivars of varying morphological types were classified based on the fruit color with a possible indication of different types of development and also the extent of breeding in the accessions used in the experiment.

4.2.5 Malvaceae

Okra (*Abelmoschus esculentus* L.) is a member of the *Malvaceae* family. It is ideally cultivated in tropical regions. Ariyo (1993) observed the genetic variation between 30 varieties of West African Okra and discovered there was great diversity among the Okra varieties. Fruit characteristics and pigmentation were found to be major contributors to genetic variance. In a recent study, Saryam et al. (2017) also investigated the variability existing among fifty-five different varieties of Okra in New Delhi and discovered that leaf length, node at first flower appearance, yield of fruit, 100 seed weight, and number of locules were major contributors to genetic divergence with number of locules being the greatest contributor. Different genotypes with similar traits were revealed in the diverse clusters observed, depicting a potential for utilization in further hybridization. Nine clusters were observed to be monogenotypic while three clusters were polygenotypic including cluster I, the largest cluster with forty genotypes, and cluster III with seven genotypes. Forty-two genotypes were found susceptible to yellow vein mosaic virus; on the other hand, eleven varieties showed disease tolerance (Sarayam et al. 2017).

4.2.6 *Solanaceae*

Solanaceae is a plant family with around 2300 species, nearly one-half of which belong to the genus *Solanum*. Potato (*Solanum tuberosum* L.), tomato (*Lycopersicon esculentum* L.), and eggplant (*Solanum melongena* L.) are the three most important cultivated crops in the *Solanaceae* family (FAO 2007).

Eggplant (*S. melongena* L.) is a widely grown vegetable in Asia, Europe, Americas, and Africa. It is believed to have originated in Asia in the Indo-Burmese region (Isshiki et al. 1994), but other DNA studies mention that eggplant originated from Africa (Weese and Bohs 2010). Eggplant was derived from the subtropical species *S. incanum* L., native from North Africa and the Middle East and most of its wild relatives were found in Africa. The greatest diversity of eggplant has been found in India, known as Brinjal, where it was domesticated long ago. The World Vegetable Center holds a large public germplasm collection of eggplant, which includes the three cultivated species and more than 30 wild relatives, with more than 3200 accessions collected from 90 countries. Over the last 15 years, more than 10,000 seed samples from the Center's eggplant collection have been shared with public and private sector entities, including other genebanks (Taher et al. 2015).

Xiu and Knapp (2008) used ancient Chinese literature to explore the domestication process that has occurred with the eggplant (*S. melongena*). They found that the earliest documentation of eggplant was in Chinese literature from 59 BC. The domestication of the eggplant was determined by factors: fruit quality, size, shape, and taste. These aspects were changed from small to large in fruit size, taste changed from not palatable to sweetish, and then a wider variety of fruit shapes were cultivated. Lester and Hasan (1991) divided *S. melongena* into a series of morphological types or gene pools, identified as A (putative wild progenitors) to G (advanced cultivars), and suggested eastward movement of cultivated forms, with subsequent movement westward complicating patterns of character change.

Muñoz-Falcon et al. (2009) observed that the genetic diversity of modern cultivars of black eggplants was reduced, and the incorporation of black fruit materials from different origins could increase the genetic base of this cultivar type and can contribute to better exploitation of the heterosis resulting from the crosses of genetically distant materials. In another study, 28 accessions of eggplant from five species were analyzed by Singh et al. (2006) using RAPD markers and 144 polymorphic amplified products were identified from 14 decamer primers indicating a high level of genetic distinction in eggplant species. A tree diagram (dendrogram) used to show the distance or dissimilarity between clusters developed with UPGMA technique demonstrated that *S. incanum* was closest to *S. melongena* followed by *S. nigrum* L. and only one accession of *S. nigrum* and *S. surattense* Burm. f. showing grouping with one another.

Another most popular cultivated member of the *Solanaceae* family is the common potato (*Solanum tuberosum*) (Fig. 4.4), an important food crop after rice, wheat, and maize (Akkale et al. 2010). Potato was first domesticated and eaten by man in South America particularly in the region of the Andes about 8000 years ago. Cultivated potato and its wild relatives belong to the genus *Solanum*, the largest

Fig. 4.4 Common potato (*Solanum tuberosum* L.) from the Solanaceae family is one of the most important food crops after rice, wheat, and maize. *Source* INIVIT

genus with 1500–2000 species (PBI *Solanum* Project 2014). Juzepczuk and Bukasov (1929) proposed that potatoes were originally introduced into Europe from the Chiloe region in Chile. In contrast, Hawkes (1994) suggested that common potato was widely cultivated in the Andean highlands of Bolivia, Peru, and northern Argentina. Molecular analysis has revealed that the Andean potato predominated in the 1700s, and later the Chilean potato was introduced into Europe and became predominant long before the late blight epidemics (Ames and Spooner 2008; Ríos et al. 2007).

Primitive forms of cultivated potato and their wild relatives provide a rich, unique, and diverse source of genetic variation, which could be a source of various traits for potato breeding. The morphological traits are diverse in plant height, leaf and leaflet shape, flower color, stolon length, and size, color, and shape of tubers (Hanneman 1989). The world's largest potato collection at the International Potato Center (CIP) in Peru includes more than 5000 accessions of cultivated and wild potatoes (Centro Internacional de la Papa 2004); however, the world's potatoes have a narrow genetic basis due to its development from a few genotypes originated in South America. After the late blight (*Phytophthora infestans* (Mont.) de Bary) epidemics of the 1940s in Europe, that genetic basis became still narrower, due to the lack of resistance of available cultivars (Salaman 1954).

The *Lycopersicon esculentum* Mill., from the *Solanaceae* family, and its wild relatives exhibit a genetic variability that produce heritable differences in fruit chemistry that could be harnessed in identifying genes that regulate biosynthesis and accumulation of materials in the plant (Lee et al. 2012). The tomato is simple berry-type fruit that grows best in tropical environments. In their experiment utilizing combined

transcriptome, genetic diversity and metabolite profiles to determine the impact of the tomato fruit's genetic diversity on carotenoids, they discovered that the ethylene response factor SIERF6 played an essential role in the ripening process and on the carotenoid accumulation. While investigating the relationship between carotenoid levels and gene expression profiles, the existence of 953 carotenoid-related genes were explained (Lee et al. 2012).

Capsicum annuum L. (commonly called pepper plant) is a tropical and simple berry-type fruit belonging to the *Solanaceae* family. Aguilar-Meléndez et al. (2009) observed 80 accessions of Mexican cultivated *C. annuum* (22 domesticated, 58 semi-wild) and investigated their different nucleotide sequences in three single—or low—copy nuclear loci, *Dhn, G3pdh*, and *Waxy*. In all three loci, about 10% reduced diversity of domesticates relative to semi-wild plants and geographic structure was found (Aguilar-Meléndez et al. 2009). Ibiza et al. (2012) also characterized 260 *Capsicum* accessions from species in the Andean region. Intraspecific differences were identified in some species commonly used as fresh vegetables and spices (*C. chinense* Jacq., *C. baccatum* (Willd.) Eshb. and *C. pubescens* Ruiz & Pav.) and the principal cluster analysis showed a clear geographic division in relation to the country for the *Capsicum* species.

4.2.7 Umbelliferae

Carrot (*Daucus carota* L.) belongs to *Umbelliferae* family, which is an important root crop vegetable grown worldwide. It is the main source of antioxidants in the form of vitamin A carotenoids. This family contains about 250 genera and over 2500 species. Asia is considered origin of carrot cultivation, and before the tenth century, primitive yellow–purple rooted carrot was cultivated in the Afghanistan region, which is different in leaf morphology, root color, and shape (Simon et al. 2008). Large genetic variation is observed in cultivated carrot.

Baranski et al. (2012) studied the genetic diversity based on polymorphisms at 30 SSR loci in a collection of 88 cultivars of carrot between eastern (Asian) and western (European) genetic pools. Using a Bayesian approach, two clusters of 17 and 61 accessions were distinguished, which comprised the Asian and western type accessions, respectively. The result showed that genetic diversity of the Asian gene pool was higher than that of the western gene pool. Furthermore, the results of SSR analysis were supported by morphological characterization. In addition, genetic diversity of Iranian carrot accessions was evaluated using morphological traits, including 12 qualitative and 18 quantitative traits (Mehrabi et al. 2014). Results showed that Esfehan accession (1638.9 g/m^2) and Kerman (1650.9 g/m^2) accounted for the highest average yield than the other accessions. Similarly, in qualitative traits, root color in Shoshtar accession was purple and Esfehan accession had red root and yellow root compared to other accessions.

4.3 Genetic Diversity in Fruits

4.3.1 Anacardiaceae

Mangifera indica L. (the mango fruit) is a simple drupe member of the *Anacardiaceae* family that is mainly found growing in tropical areas. RAPD markers were used to screen fifty mango cultivars and a dendrogram based on Jaccard's coefficient of similarity implied a moderate degree of genetic diversity among the mango cultivars used in the experiment (Kumar et al. 2001). Another analysis using Pearson's coefficient of similarity revealed a high degree of genetic diversity. For both analyses, the "Mulgoa" was very distinct and to conclude, results from this study illustrated clearly that cultivars from the southern part of India showed the maximum diversity and RAPD markers were potentially useful in identifying mango germplasm for breeding uses (Kumar et al. 2001).

Spondias mombin L. or Yellow Mombin, is a, simple, drupe-type fruit that is tropical in nature and a member of the *Anacardiaceae* family. Silva et al. (2017b) measured the genetic diversity within and between *S. mombin* populations, native to the northern Mato Grosso State of Brazil, with ISSR markers. Molecular variances showed 77.38% of the total genetic diversity was seen within populations and 22.62% between populations. Populations located in Alta Floresta and Nova Bandeirantes are hereditarily the most alike and the nearest "structure" revealed genetic diversity among the genotypes of each population. Therefore, because there are hereditary differences between the two populations, and none of the individuals are genetically identical, the two populations can be a wellspring of genotypes for germplasm banks and for commercial fruit production in the future (Silva et al. 2017b).

4.3.2 Annonaceae

The Cherimoya (*Annona squamosa* L.) is to a wide variety of climatic conditions—tropical, subtropical, mild temperate, or Mediterranean environment and the fruit consists of an aggregate collection of berries. Van Zonneveld et al. (2012) in their experiment explored the possibilities of incorporating molecular marker data into geographic information systems (GIS) to allow visualization and better understanding of spatial patterns of genetic diversity as a key input to optimize conservation and use of plant genetic resources, using cherimoya as a case study evaluated on the basis of microsatellite molecular markers (SSRs). The results of their study found high levels of allelic richness and heterozygosity in cherimoya's putative center of origin, southern Ecuador and northern Peru, whereas levels of diversity in southern Peru and, especially, in Bolivia were significantly lower. The utilization of GIS on an extensive microsatellite dataset permitted a more definite prioritization of areas for in situ conservation and focused on accumulation over the Andean area spread of cherimoya than past studies could do (Van Zonneveld et al. 2012).

4.3.3 Arecaceae

The coconut palm (*Cocos nucifera* L.) is a tropical, simple drupe-type fruit that is a member of the family *Arecaceae*. Coconut is one of the important palms grown both as a homestead and plantation crop in countries and most island territories of tropical regions (Fig. 4.5). Coconut palms are found abundantly in coastal regions of most tropical islands and they have a versatile role in providing food, nutrition, fibers, beverage, medicine, shelter, and wide range of handicrafts (Rajesh et al. 2015). Southeast Asia is probably the main center of domestication of the species because the greatest morphological variability occurs in that region; *C. nucifera* is the only reported species under the genus *Cocos* (Persley 1992).

SSR and AFLP markers were used by Teulat et al. (2000) to estimate the genetic diversity between 31 cultivars from 14 coconut populations across a wide geographic range. The SSR results showed 2–16 alleles for every locus and a total of 339 alleles in all 14 populations. Two of the four dwarf populations tested known to carry out self-fertilizing type of reproduction were discovered to be homozygous at all 37 loci. Genetic diversity was greater in populations from South Pacific and Southeast Asia. East African populations were more different than those from West Africa while populations from Tonga and Fiji had more distinct alleles than those from the South Pacific.

Ribeiro et al. (2013) explored the genetic diversity in ten populations of Brazilian tall coconut using 13 microsatellite markers. A high level of genetic diversity was

Fig. 4.5 Coconut palm (*C. nucifera*) is a tropical fruit with many local uses around the world (*Source* Sarada Krishnan)

detected and twelve exclusive alleles were found distributed in six populations; four of them are considered as common localized alleles, one as a rare sporadic allele, and eight as rare localized alleles. These results provide an important tool to assist in collection activities, for greater efficiency in conservation of germplasm and give support to the coconut breeding program in Brazil.

Rajesh et al. (2015) used a simple and novel marker system, start codon targeted polymorphism (SCoT), for its evaluation as a potential marker system in coconut. SCoT markers were utilized for assessment of genetic diversity in 23 coconut accessions (10 talls and 13 dwarfs), representing different geographical regions. The extent of genetic diversity observed based on SCoT analysis of coconut accessions was comparable to earlier findings using other marker systems. Tall and dwarf coconut accessions were clearly demarcated, and in general, coconut accessions from the same geographical region clustered together. The results indicate the potential of SCoT markers to be utilized as molecular markers to detect DNA polymorphism in coconut accessions.

The oil palm (*Elaeis guineensis* Jacq.) is native to tropical environments. It is a simple drupe-type fruit that belongs to the *Arecaceae* family. *E. guineensis* is an allogamous arborescent monocot, cultivated extensively for the production of palm oil and palm kernel oil; it is the leading source of edible oil. Maizura et al. (2006) characterized 359 accessions of oil palm from 11 African countries using the RFLP method and the standard "Deli dura" variety as the control. The results revealed that all groups were more genetically diverse than the standard variety that lost 36 alleles when compared with the natural populations, thereby indicating a loss in diversity. They found that the genetic material of the oil palm from Nigeria showed the highest mean number of alleles per locus and percentage of polymorphic loci suggesting Nigeria should be the center of diversity of wild oil palm.

Bakoumé et al. (2014) assessed the extent of genetic diversity among 494 oil palms from 49 populations (representing ten African countries, three breeding materials, and one semi-wild material) using 16 SSR markers. In this study, the genetic diversity was high with a total of 209 alleles detected accounting for an average of 13.1 alleles per locus and a mean expected heterozygosity of 0.644. They concluded that the influence of human and environmental factors might have played a major role in grouping the African natural oil palm into three different groups as well as in the formation of a transition zone (formed by Ghana and Côte d'Ivoire). The high genetic diversity identified within the Malaysian Palm Oil Board's African oil palm germplasm collections provides ample scope for further genetic improvement. Pacheco et al. (2014) in Colombia made a similar study with microsatellites; they found high genetic diversity in 311 oil palm samples from the Republic of Cameroon, but the analysis did not show any defined population structure.

Palmae Phoenix dactylifera L. or date palm (family *Palmae*) is a simple drupe found in tropical, subtropical, and Mediterranean regions. Elshibli and Korpelainen (2008) studied the genetic variability in date palm germplasm derived from Sudan (37 female and 23 male accessions) and eight female accessions from Morocco. A high level of variability was observed among the Sudanese, Moroccan ,and male cultivars. The results of this study therefore demonstrated that the genetic groups

of the Sudanese cultivars and/or male cultivars did not follow a clear geographic pattern.

4.3.4 Bromeliaceae

Ananas comosus Merr. or the pineapple is the third most important tropical fruit in tropical and subtropical countries (Duval et al. 2001). It is a member of the *Bromeliaceae* family producing multiple sorosis type fruit. Duval et al. (2001) studied the molecular variability in a group of 301 accessions of pineapple RFLP markers. The result of their study showed that within *Ananas*, there was continuous variability majorly at the intraspecific level mostly in wild species. The cultivated species *A. comosus* was relatively homogenous despite its wide morphological variation and *A. bracteatus* (Lindl.) Schult. and Schult.f. which was grown as a fence and for fruit seemed to have less variability. According to that study, *A. lucidus* Miller cultivated by the Amerindians for fiber showed a great amount of polymorphism.

4.3.5 Cactaceae

The dragon fruit [*Hylocereus undatus* (Haw.) Britton and Rose], a member of the *Cactaceae* family, is a simple berry-type fruit mainly found in tropical habitats. However, the dragon fruit today still faces some challenges such as the determination of promising materials and lack of commercial cultivars in the country of Brazil, for example. Therefore, along these lines, in an effort to solve this problem, fertilization crosses were carried out manually by Silva et al. (2017c), between the *H. undatus* with *H. polyrhizus* (Weber) Britton and Rose and *H. setaceus* (Salm-Dyck ex DC.) Ralf Bauer. respectively.

To measure the genetic diversity of the progenies for future use in breeding programs, morphological tools were used to establish if there was genetic variability in interspecific dragon fruit hybrids (Silva et al. 2017c). By using the UPGMA clustering analysis method, based on cladodes characteristics (for 51 individuals of 45 progenies and six parental), six characters were assessed: length and diameter of stem, distance between areoles, arch height, and number and size of spines/areole. The findings from that study revealed great variability among dragon fruit hybrids, and eight of them have shown promise for use in breeding programs.

4.3.6 Caricaceae

The pawpaw or papaya (*Carica papaya* L.) is a simple berry-type fruit found in tropical to subtropical and temperate regions. It is a short-lived tree belongs to the

family *Caricaceae* (Fig. 4.6). The papaya variety "Maradol" in its yellow and red variants, obtained by the Cuban breeder and progressive farmer Damián Adolfo Rodríguez Rivera, has been recognized as among the best in the world for its excellent quality (Beovides 2017). Wild papaya (*C. papaya*) is a tropical nomadic pioneer tree that occurs naturally in lowland tropical and subtropical forests of Mesoamerica. The cultivated varieties of *C. papaya* represent the third most cultivated tropical crop worldwide (FAO 2012). Wild *C. papaya* is a key element of early successional tropical and subtropical forests in Mexico, and represents the genetic reservoir for the evolutionary potential of the species. Crosses between wild and domesticated papaya threaten the maintenance of the natural genetic pool of the species. Indeed, this genetic diversity forms the capital for current and future improvements to crop plants (Chavez-Pesqueira et al. 2014).

Kim et al. (2002) studied 63 accessions of *C. papaya* and six accessions of related species from different geographical origin. The study revealed that the limited genetic variation (about 12%) detected among this diverse group of materials did not correspond to the wide range of morphological characteristics observed in the field. The amount of genetic diversity found with AFLP markers is sufficient to distinguish between breeding lines for varietal protection. In addition, their results also indicated that self-pollinated hermaphrodite cultivars varied as much as the open pollinated dioecious cultivars.

Recently, Chavez-Pesqueira and Nuñez-Farfán (2016) used six nuclear and two chloroplast (cp) DNA markers to assess the genetic diversity and phylogeographical structure of 19 wild populations of *C. papaya* in its natural distribution in Northern

Fig. 4.6 *Carica papaya* L. is a tropical and subtropical fruit which biotechnology can help to conserve its genetic diversity and to improve the quality of cultivated varieties. *Source* INIVIT

Mesoamerica. They found high genetic diversity (Ho ¼ 0681 for nuclear markers, and h ¼ 0701 for cpDNA markers) and gene flow between populations of *C. papaya*. Their results suggest that the life history of *C. papaya* promoted its long dispersal and rapid colonization of lowland rain forests, thus maintaining genetic diversity throughout its range. However, recent human disturbance, mainly the fragmentation of tropical habitats in Northern Mesoamerica, appears to represent a threat to its dispersion and therefore to its genetic diversity and structure.

4.3.7 Clusiaceae

A member of the *Clusiaceae* family, the tropical and exotic Mangosteen (*Garcinia mangostana* L.) fruit, is a simple fleshy berry kind of fruit with seeds. The genetic relationship between 37 accessions of the mangosteen fruit and 11 accessions from other *Garcinia* species was evaluated using randomly amplified DNA fingerprinting (RAF) method by Ramage et al. (2004). From the 37 accessions evaluated, nine distinct genotypes were observed and clustered into three specific groups using corresponding analysis. The overall results showed that the three mangosteen groups were 63–70% diverse from the other species of *Garcinia* studied.

Santosh and Arakera (2017) evaluated the genetic differences in eight *Garcinia cambogia* Gaertn (a former scientific name) accessions, utilizing polymerase chain reaction-based random amplified DNA (PCR-RAPD) markers. An aggregate number of 227 bands were acquired from nine primers from a total of 20 that indicated polymorphism; out of the 227 bands, 225 showed 99.11% polymorphism. Each of the eight varieties were grouped into two main clusters and on analysis, it was observed that the yellow and red varieties could not be viewed as two diverse accessions and that their utilization for therapeutic or commercial purposes was based solely on the preference of the consumers.

4.3.8 Cucurbitaceae

Melon fruits (*Cucumis melo* L.) belonging to the family *Cucurbitaceae* are native to tropical and subtropical environments. Melons are simple berry (pepo)-type fruits that have a sweet aromatic flavor, great diversity and size, different flesh and rind colors, structural forms and dimensions and they can be broken down into seven different types based on these variations in species (Nuñez-Palenius et al. 2008). It is interesting to note that although there have been over 100 transgenic melon field trials in the USA since 1996, there are not any transgenic melon varieties in the market possibly due to technical or performance factors, intellectual property rights concerns, or a lack of public acceptance.

Sensoy et al. (2007) determined the genetic diversity of 56 varieties of melons of Turkish origin by comparing their phenotypic and molecular traits with those of

23 melons of local and foreign origin. They used sixty-one phenotypic characters and 109 polymorphic RAPD markers obtained from 33 primers in their analysis and discovered that related genotypes from particular regions were grouped in similar clusters. It was determined from the study results that the non-sweet melon was different from the sweet melon and the Turkish melon genotypes were more diverse than those of the sweet foreign varieties observed but similar to the reference accessions used. Lastly, the Turkish sweet melon genotypes belonging to the group's *inodorus* and *cantalupensis* were discovered to be very different and could have been crossed with the non-sweet types.

Monk fruit (*Siraitia grosvenorii* (Swingle) C. Jeffrey ex A. M. Lu and Zhi Y. Zhang) from the *Cucurbitaceae* family is a simple drupe-type fruit found in tropical and subtropical climatic environments. In a study to estimate the genetic differences among wild plant populations and varieties of Luohanguo in China using RAPD and AFLP markers, a high amount of diversity was observed at the species level due to differences in population (Tang et al. 2007). Although distinct variability was identified between Luohanguo varieties and the wild populations, genetic variances in Luohanguo varieties were lower than in the wild accessions.

Watermelons [*Citrullus lanatus* (Thunb.) Matsum. and Nakai] are a member of the *Cucurbitaceae* family too. They are a simple fleshy berry (pepo)-type fruit commonly found in temperate and subtropical environment. Campbell and Tishkoff (2008) studied 42 varieties of the genus *Citullus* and five watermelon cultivars using RAPD markers. They found that there was genetic diversity in all essential groups of *Citrullus* in terms of resistance to diseases such as anthracnose, watermelon mosaic virus, and downy or powdery mildew. Their research findings showed that molecular markers could be beneficial in measuring genetic variation and grouping *Citrullus* plants based on phylogenetic differences before evaluating them for pest or disease resistance.

4.3.9 Ebenaceae

Persimmon fruit (*Diospyros kaki* L.f.) is an exotic fruit that is part of the *Ebenaceae* family. It is a simple berry found mainly in temperate climatic regions. Del Mar Naval et al. (2010) measured the diversity of 71 persimmon cultivars from two European groups including cultivars from Japan, Italy, and Spain with 19 polymorphic microsatellite markers. 206 alleles were produced with an average of 10.8 alleles per locus. On measuring the cultivars' molecular variance, genetic variability between and within groups derived was noted to be significant, 85.2% for country of origin and 73.3% for the astringent type.

4.3.10 Ericaceae

A member of the *Ericaceae* family, *Vaccinium vitis-idaea* L. (popularly called Lingonberry), is a simple berry-type fruit native to cold and boreal environmental growing conditions. Wild clones and cultivars of Lingonberry were studied for their genetic variance using ISSR markers and four ISSR primers produced 113 polymorphic bands in 34 clones and eight cultivars (Debnath and Sion 2009). Cluster analysis using the unweighted pair-group method with averages (UPGMA) separated the 41 genotypes into three main clusters while identifying the one remaining clone as an outlier, thus, demonstrating a sufficient degree of polymorphism to separate Lingonberry cultivars. Geographical distribution on the basis of country of collection explained 12% of the total variation as revealed by the analysis of molecular variance (AMOVA).

4.3.11 Fabaceae

Tamarindus indica L. (common named as tamarind) is a simple leguminous, tropical fruit belonging to the *Fabaceae* family. Kumar et al. (2015) investigated the genetic diversity of nine tamarind genotypes using random amplified polymorphic DNA (RAPD) markers. Authors used ten primers to assess the genetic diversity in four flowering cultivars and five non-flowering of tamarind trees. Genotypes, which were morphological closely related, were found to be unrelated at the molecular level. A sizeable amount of intrapopulation diversity is recorded in the present study, which can be utilized in hybridization programmes to efficiently introgress the desirable trait of interest.

4.3.12 Fagaceae

The sweet chestnut (*Castanea sativa* Mill.), a simple nut type fruit is a widely spread and important multipurpose tree species in the Mediterranean area, which has played an important role in human history (Poljak et al. 2017). Natural events, such as glaciations, and human influence played significant roles in the distribution and genetic makeup of the sweet chestnut. Poljak et al. (2017) assessed populations from Central Europe and the western part of Balkan Peninsula by using ten polymorphic nuclear satellite markers. Their study showed three genetically variable and geographically separate well-defined groups of chestnut populations, of which two of the groups that were not entirely separate were seen in the northern region while one was observed in the southern part. From their study, they concluded that the genetic structure of sweet chestnut populations in Central Europe and in the Balkan Peninsula was because of natural colonization events causing significant and lengthy impact. They also noted

that although research has shown that gene flow between cultivated/grafted trees and wild chestnut trees can influence genetic structure, cultivated to wild introgression in the sweet chestnut depends on the proximity of the chestnut orchards and their naturally occurring populations.

4.3.13 Juglandaceae

Walnut (*Juglans regia* L.), a member of the *Juglandaceae* family, is a simple drupaceous nut-type fruit usually found growing in temperate and tropical environmental conditions. 215 wild accessions of walnut native to the Eastern Italian Alps forests were sampled, using SSRs markers and expressed sequence tag (EST)-derived SSR with 20 microsatellite loci selected from the literature (Vischi et al. 2017). The Walnut varieties native to the geographical location of this research study showed a moderate level of genetic diversity with the recognition of 80 alleles.

AMOVA revealed that most of the molecular diversity detected during that study was between individuals (nearly 98% of the explained variation). The model-based clustering algorithms revealed two clusters: the first one encompassed most of the samples and showed a great genetic admixture throughout the five sampling areas defined on the base of orographic characteristics of the region. The second cluster represented a small island with three samples traced back to an introduction from Russia at the beginning of the 20th century.

4.3.14 Lauraceae

Avocado (*Persea americana* Mill.) from the *Lauraceae* family is a simple, berry type of fruit. It is an exotic fruit found in tropical to subtropical regions. Studies have been done on the evaluation of genetic divergence by grouping agronomic traits phenotypically as such attributes are not difficult to measure and are not greatly influenced by the environment (Oliveira et al. 2017) while at times grouping may be done on biochemical or other characteristics. Peraza-Magallanes et al. (2017) analyzed the genetic differences between Avocado accessions in Mexico using microsatellite and SNP analysis of genes used in the biosynthesis of tocopherol. The analyzed cultivars displayed a moderately polymorphic set of genotypes, and a variation between the local Mexican Avocado accessions and the widely commercially grown Hass cultivar were identified. Results from this study provided a new insight into choosing and growing Avocados adapted to hot climatic conditions too.

4.3.15 *Lythraceae*

Pomegranate (*Punica granatum* L.), family *Lythraceae* is a simple berry (balausta) type fruit that can be found in tropical to subtropical and Mediterranean environments. The diversity of soft seeded pomegranate accessions from different parts of Iran were studied using their fruit morphology characteristics and DNA markers (Sarkhosh et al. 2009). Of the random primers studied, 14 of them demonstrated great polymorphism and amplification of specimen and an aggregate of 43 RAPD markers was generated. Estimated genetic similarity based on Jaccard's coefficient was between 0.13 and 1.0 when the RAPD data were used and no noteworthy correlation was found while categorizing on the basis of fruit traits in comparison to genetic similarity on the basis of RAPD data.

Using fluorescent AFLP markers, Yuan et al. (2007) also measured the population genetic diversity of 85 pomegranate cultivars from six geographical regions in China and found the existence of a lot of genetic diversity. Variance analysis showed that there were significant differences between populations in genetic diversity and that at the genetic diversity was higher at the species level than at the population level. Additionally, the UPGMA cluster analysis showed most accessions from the same populations were clustered together and there was partial gene exchange.

4.3.16 *Moraceae*

Morus alba L. (common name: Mulberry), a member of the family *Moraceae*, is a multiple sorosis type fruit found in temperate and Mediterranean environments. The Mulberry is an economically viable and biologically essential, woody plant in many parts of the world. It has served people for more than several years, and it is broadly utilized today still in the pharmaceutical and food industry as well as in on-farm cultivation (Wang et al. 2017).

The genetic variability among mulberry genotypes from seven countries was measured by Wang et al. (2017). From the 42 genotypes, 175 bands were shown, of which 169 were 96.57% polymorphic. Cluster analysis and PCA indicated a low level of diversity in the mulberry, and the 42 genotypes when grouped together showed some hybrid combinations. Likewise, there was an association between mulberry diseases and their genotypes, showing a possible utilization of ISSR researching the ability of mulberry to withstand diseases. According this study, using modern techniques, deeper understanding in classification and conservation resources of mulberry will present a higher-efficiency hybrid among populations.

Mulberry is a multipurpose plant, which originated in China, due to its excellent nutritional qualities, especially its high content of protein and energy. In addition, it is used in several regions of the world for livestock feeding. This species is included in the group "foliages of high quality," with a digestibility between 70 and 90% and similar protein contents to the leguminous ones (20–24%). Therefore, mulberry has

a great potential on animal feed with other products and local by-products (Leyva and López 2006; Martín et al. 2007). Mulberry is also an important crop cultivated widely as food for silkworm larvae in commercial silk production.

4.3.17 Musaceae

Bananas and plantains (*Musa* spp.) are very important food crops. *Musa acuminata* Colla and *M. balbisiana* Colla represent the two principal genomic groups from the *Musaceae* family. Banana is a simple berry fruit type that is cultivated in tropical and subtropical regions. In order to decrease the loss of genetic information in the *Musa* spp. and to broaden the utilization of genotypes in breeding programs, there is a need to pay close attention to recognizing, gathering, and protecting genotypes that are members of the *Musa* spp. (Venkatachalam et al. 2008).

For years, it was not only challenging and hard but also thought to be impossible in practice to breed bananas because of issues such as triploidy, clones with low fertility, vegetative propagation due to pest, disease and climate change problems, time taken to generate a cultivar with superior traits and limitations in the progression of fast breeding (Vuylsteke 2001; Nyine et al. 2017). Genotypes of different clusters were observed to be very similar genetically due to the presence of a lot of closely related alleles and therefore cultivars in the same sub-cluster are likely to be of same origin (Creste et al. 2003; Silva et al. 2017a). In order to cater for the growing needs for food to care for the increasing world population and to address the challenges prevalent in banana production today, it is sustainable to breed high yielding and resistant cultivars (Simmonds 1986; Rowe 1990).

Breeding for resistant cultivars and at the same time keeping the sensory and color attributes of the fruit are not easy for banana breeders and thus it requires a committed effort and cautious determination of cross-combinations. Understanding how such attributes differ among varieties and how economically essential attributes are similar is an important initial step in the improvement and determination of appropriate genomic choice models for banana (Nyine et al. 2017). By employing new breeding tools for genomic selection such as microsatellites (SSR) markers, several researchers have been able to effectively measure existing genetic variability among banana cultivars (Rout et al. 2009; Mattos et al. 2010; Ying et al. 2011; Librelon et al. 2013; Kiran et al. 2015).

In their investigation of variation among banana cultivars after genotyping by utilizing SSR markers, Nyine et al. (2017) found that the population being studied was hereditarily different, mirroring an intricate family tree, generally affected by the male progenitor. In addition, they discovered that the reaction of the banana genotypes to agricultural cultivation practices fluctuates greatly as regards to traits. Likewise, for examining genetic diversity, similar to SSR markers, RAPD and ISSR markers can be utilized.

Silva et al. (2017a, b, c) assessed the genetic divergence in 21 banana cultivars using ISSR markers and discovered the tested primers effectively determined enough

polymorphism needed to measure genetic variation in banana genotypes. According to Silva et al. (2017a), existing variations could be explained by existing somaclonal variations between different banana cultivars and the great diversity in diploid varieties may be linked to their hybrid origin.

Using AFLP technique, Wong et al. (2001) measured the distinction between three subspecies of the wild banana *M. acuminata* in Malaysia and found eight primer combinations that showed molecular markers specific for each taxon. They however found subsp. *truncata* which is endemic to the Malaysian peninsula to be genetically separate from the other two subspecies *malaccensis and microcarpa* tested.

Issues such as ploidy should be analyzed from time to time in breeding programs to separate ploidy levels so that different standards may be utilized to choose hybrids for breeding purposes from those qualified for variety release. In spite of a large portion of the enhanced crossbreeds being triploids, their fertility levels ought to be tested such that further upgrades can be done on them to accomplish gene pyramiding while at the same time limiting inbreeding. Dedicated attempts are needed to comprehend the structural organization of the banana genome through cytological techniques (Nyine et al. 2017).

The plantain (*Musa* spp.) is a simple berry commonly found in tropical and subtropical environments. Ude et al. (2003) evaluated the genetic diversity in an African plantain core collection from 25 plantains from different parts of West and Central Africa, using AFLP and RAPD markers. They found the AFLP technique to be highly discriminatory in determining how genetically diverse the plantains were as they produced markers with greater polymorphic information content (PIC) and their clusters produced also indicated similar relationships between similar inflorescence types than that of the RAPD markers and clusters. In addition, they also discovered that a small group of Cameroonian cultivars were separate from the bulk of the other plantains suggesting that Cameroon might have cultivars that have beneficial genes.

4.3.18 Myrtaceae

A member of the *Myrtaceae* family, Guava (*Psidium guajava* L.) is a simple berry type fruit that is tropical and subtropical in nature. RAPD markers were used to estimate molecular diversity of 41 genotypes of guava consisting of five *Psidium* species, 23 varieties, 12 selections, and a hybrid (Prakash et al. 2002). Based on Ward's method of cluster analysis, all the individuals on the dendrogram were grouped into two major clusters according to their geographical locations and species and the species were interspersed between the varieties. Overall, the study showed that the genetic base of Indian guava can be rated as low to moderate diversity and also indicated that various triploid seedless cultivars of guava are not genetically identical and have independent origins. All the species used in the study could be subspecies of *P. guajava*.

Myrciaria dubia (Kunth) McVaugh, the tart fruit tree, also commonly known as camu camu (family *Myrtaceae*) is a simple berry-type fruit found mainly in tropi-

cal and subtropical areas. Research has been done evaluating the genetic diversity in the tart fruit tree populations and other crops belonging to the same family using ISSR markers. In an experiment to determine the genetic differences between ten tart fruit tree populations in Roraima, Brazil, Nunes et al. (2017) discovered 108 alleles (indicating a large amount of variability) by employing the use of 14 polymorphic ISSR markers. Of the fourteen primers used, UBC827, GCV and UBC810 provided the greatest number of alleles. Previously, Nascimento et al. (2014) also observed high levels of genotypic differences based on morpho-agronomic attributes. Nunes et al. (2017) attributed the large genetic variability noticed due to the non-existence of breeding programs at the time of the study and also to the low level of species domestication. The authors showed high genetic variability among progenies of *M. dubia* and recommended a direct selection method to assist the process of domestication and improvement of this species. In addition, Santana et al. (2016) and Brandão et al. (2011) using the mentioned markers to study genetic differences in *M. tenella* (DC.) O.Berg and *Myrcia splendens* (Sw.) DC. found 71 and 70 polymorphic markers, respectively. The polymorphic loci amount generally measures how efficient the primers are in accessing the genetic variability within a population (Luz et al. 2015).

4.3.19 Oleaceae

A member of the *Oleaceae* family, the olive fruit (*Olea europaea* L.) is a simple drupe-type fruit grown in subtropical and Mediterranean regional environments. Belaj et al. (2002) used the RAPD method to measure the genetic variability in 103 olive cultivars from the world germplasm bank in Spain and AMOVA was used to differentiate the phenotypic differences among regions and among countries. It was observed that the greater part of the genetic variance noted was as a result of the diversity that existed among the plant varieties within a country. The results confirmed the majorly allogamous nature of the *Olea europaea* species.

4.3.20 Passifloraceae

Passiflora edulis Sims (family *Passifloraceae*) is a simple berry-type fruit with the family consisting of 17 genera and approximately 525 species. Brazil is the largest center of genetic diversity of the genus *Passiflora*. Commonly referred to as passion fruit, it is popularly grown in tropical, subtropical, and temperate climatic regions. In 2008, Negreiros and others conducted studies on the genetic diversity of yellow passion fruits and related half-sibling related family lines and discovered that some fruits produced desirable traits beneficial to the fresh market consumers and some varieties had superior traits that could be integrated into breeding programs. Castro et al. (2012) assessed 28 morphological attributes to determine the variation between

passion fruit varieties and found that 22 of those attributes contributed greatly to the existing genetic differences amongst the passion fruit genotypes tested.

In a study in Brazil, Oliveira et al. (2017) assessed the genetic divergence among fifteen varieties of passion fruit by determining the relative contribution of fourteen of their phenotypic agronomic attributes. Their results showed that phenotypic grouping and analysis based on the quantitative attributes of flowers and fruits contributes the diversity in the *Passiflora* spp. Sousa et al. (2015) investigated interspecific genetic variation in 25 wild species of *Passiflora* preserved in the active *Passiflora* germplasm bank of the Universidade Estadual de Santa Cruz (Brazil). Of 31 primers tested, 20 identified polymorphic loci with a total of 331 bands, suggesting high polymorphism in the sample.

4.3.21 Rhamnaceae

Jujube (*Ziziphus jujube* Mill.) belongs to the *Rhamnaceae* family producing a simple drupe-type fruit that can be grown in tropical, subtropical, and Mediterranean regions. Genetic diversity in Indian Jujube cultivars using SCoT, ISSR, and ribosomal DNA (rDNA) markers was assessed and a high level of polymorphism was identified in all primers used (Singh et al. 2017). A higher amount of variability and a higher gene flow was observed over multiple loci among seven populations of *Z. mauritiana* Lam. thus showing a greater rate of exchange of genes or alleles from one population to the next. Majority of the genetic diversity was also seen to exist within than among populations and cultivars from the same place of origin, separated genetically into clusters in each marker system. thereby indicating wide genetic diversity and distribution exists across agro-climatic zones and that the marker systems were robust.

4.3.22 Rosaceae

The family *Rosaceae* includes species grown for their fruits (for example, peaches, apples, and strawberries), lumber (black cherry), and ornamental value (roses). *Rosaceae* consists of a wide variety of fruit types (such as follicles, drupes, and pome) and growth habits (ranging from bushy tree to herbaceous types). Those fruit tree species that are drupes (such as almonds, apricots, and peaches) are essential crops produced by farmers around the world (FAOSTAT 2015, http://faostat.fao.org/), providing vitamins, minerals, fiber, and antioxidant compounds for healthy diets (Verde et al. 2013).

Prunus dulcis (Mill.) D. A. Webb commonly referred to as almond is a simple drupe type of fruit that grows well in temperate and desert climatic conditions. Sorkheh et al. (2007) measured the genetic distances among 45 cultivated and related wild-type species of almonds from Iran, Europe, and America and their relationship with agronomic traits including flowering, kernel and fruit properties, maturity times,

and self-incompatibility. Nineteen primer combinations were assayed using AFLP fingerprints and molecular characterization. Cluster analysis in view of AFLP information plainly isolated the genotypes and wild species as per their source and family, while cluster analysis on the basis of agronomic information grouped by morphological traits.

Ideally, suited to temperate habitats, apples (*Malus pumila* Miller) are a simple fleshy-type fruit with seeds classified as a pome fruit. Richards et al. (2009), in a study performed using microsatellite variation at seven loci, discovered that the eight populations of the wild apple cultivar collected from different locations in Kazakhstan exhibited variation among individual half-sibling families and geographical cultivation sites. The wild apple cultivar populations from the southwestern regions were more diverse than those from the Northern sites, and however, variation among half siblings was three times greater than variation among sites. Ma et al. (2017) on the other hand, demonstrated in their study, genetic variations in 20 cultivated and 10 wild-type apples through specific locus amplified fragment sequencing. Patterns of linkage disequilibrium (LD) blocks were observed to be different in both cultivated and wild apples, and for cultivated apples, LD patterns were found to be in the quantitative trait loci areas controlling fruit quality. The findings by Ma et al. (2017) thus indicated the possibility that fruit quality had possibly undergone selection during domestication of apple. Findings would help in genetic breeding in the future and in the analyzing of complex traits in apple.

The apricot (*Prunus armeniaca* L.*)* is a dimple drupe type of fruit indigenous to Mediterranean and temperate regions. The genetic diversity of 47 cultivars of apricot from various geographical regions was studied by Hagen et al. (2002) using AFLP markers. A UPGMA dendrogram showed genetic diversity decreased in varieties from the former USSR to Southern Europe. This is consistent with ancient historical information that talks about the spread of apricot from its birthplace in Asia (Hagen et al. 2002).

The North American cultivars on the other hand were intermediate revealing a different genetic base than the European and/or Mediterranean cultivars (Hagen et al. 2002). In a separate study by Romero et al. (2003), the genetic variability between different apricot geographical groups using SSR markers was investigated and a total of 34 alleles were identified with a mean value of 3.1 alleles/locus and observed heterozygosity of 32% on average. The UPGMA cluster analysis of their study, based on Nei's genetic distance, also grouped genotypes according to their geographic origins and pedigrees.

Sweet cherry (*Prunus avium* L.) is a simple drupe fruit-type native to temperate climatic regions. Ganopoulos et al. (2011) accessed the genetic diversity in 19 traditional sweet cherry varieties cultivated in Greece and characterized them for 17 morpho-physiological traits using 15 SSR loci and 10 ISSR markers. 77.33% of the total variability was explained by the PCA of the nine qualitative and eight quantitative morphological parameters observed. Furthermore, findings from their study based on stepwise multiple regression analysis (MRA) showed that SSR alleles found were associated with harvest time and fruit polar diameter. In addition, it was discovered that three ISSR markers were correlated with fruit harvest and soluble

solids while four ISSR markers had a correlation with the skin color of the fruits (Ganopoulos et al. 2011).

The loquat fruit *(Eriobotrya japonica* (Thunb) Lindl) is a simple fleshy berry-type pome that can be grown in tropical to subtropical and temperate regions. Genetic ties between 40 loquat accessions that originated from different countries were evaluated using microsatellites (Soriano et al. 2005). A total of 39 alleles were detected in the population studied, with a mean value of 2.4 alleles per locus. The expected and observed heterozygosities were 46 and 51% on average, respectively, leading to a negative value of the Wright's fixation index (–0.20) indicating a smaller degree of genetic diversity in the set of loquat accessions analyzed than in other members of the *Rosaceae* family. UPGMA cluster analysis, based on Nei's genetic distance, generally grouped genotypes according to their geographic origins and pedigrees. In this study, six accessions showing identical marker patterns were Spanish local varieties thought to have been derived from "Algerie" by a mutational process very common in loquat species.

Native to temperate climatic regions, peaches [*Prunus persica* (L.) Batsch] are simple drupe fruit types. Xu et al. (2006) evaluated the genetic diversity and relationships in 17 Japanese peach cultivars and six traditional varieties using AFLP markers and pedigree tracing. They discovered that although genetic relationships derived by AFLP tallied with genetic information shown by pedigree information, there were some contradictions. In all peach varieties, differences were identified by a minimum of 10 polymorphic bands and results suggested the commercial Japanese peach cultivars were majorly derived from traditional cultivars from China.

Pear (*Pyrus communis* L.) is a simple berry (pome) fruit types that grow well in the temperate environment. Populations of *Pyrus* spp. all over the world are threatened with more than 85% pear genotypes going extinct in the nineteenth century alone; losses of some varieties resistant to abiotic and biotic stresses can be attributed to insect pest and pathogen problems (Sindelar 2002; Fowler and Mooney 1990). The germplasm of the wild pear has a large amount of genetic divergence brought about by hybridization, mutation, and natural selection of seed, and therefore it is crucial to study genetic differences in wild relatives as not all will have genes that can withstand biotic and abiotic stresses (Kishor et al. 2017).

A study was done on nine varieties of wild pear (*Pyrus pashia* L.) to assess its genetic diversity in morphology and variances were observed in morphology, seed, and fruit attributes between various cultivars (Kishor et al. 2017). Most of the cultivars had erect and spreading tops were considerably large in size and were not affected much by pests and diseases suggesting that variability observed for these characteristics tested was possibly due to the environmental conditions in the region, the cultivars themselves, or a combination of both factors. Kishor et al. (2017) also explained that the differences in seed traits may either be due to changes in agro-ecological conditions, bad mutations, hybridization, or sexual propagation.

Raspberry (*Rubus idaeus* L.) is an aggregate of drupe fruit type found mainly in warm temperate areas. Published studies by Dale et al. (1993) revealed the genetic diversity of 137 raspberry varieties gathered from all over the world since 1960. They discovered that varieties where separated into groups genetically on the basis

of geographical origin. Varieties that were developed from the USSR formed a separate cluster while all other varieties were grouped into either derivation from North American or European origin.

Fragaria vesca L. popularly known as strawberry is an aggregate assessor fruit type, classified as a pseudocarp. The strawberry can be found cultivated in tropical to cold temperate and desert climatic regions. In an experiment to evaluate the genetic diversity of strawberry according to phenotypical data, using PCA, Chhetri et al. (2017) grouped nineteen genotypes into five clusters. The results of the study depicted that strawberry genotypes had a high level of genetic divergence that could be beneficial for breeding programs. The essential traits for the classification of the strawberry varieties were fruit traits and plant structure, and these need to be employed in further characterization and analysis of the breeding materials and genetic resource of strawberries.

4.3.23 Rutaceae

Citrus species are basic hesperidium-type fruit members of the family *Rutaceae* that can be grown in tropical, subtropical, and in the Mediterranean areas. Several species have been discovered that ought to be taken into consideration when testing new genotypes in the search for wanted traits for the complete and proficient use of genetic resources in citrus development (Herrero et al. 1996). They studied a large distribution of 198 cultivars and accessions of 54 species from the *Citrus* genus and 13 of their relatives. They reported a cluster of the citrus species in two major groups that is the orange–mandarin and the lime–lemon–citron–pummelo groups. Additionally, in a study by Coletta Filho et al. (1998), the genetic similarity assessed within 35 mandarin varieties using RAPD markers was found to be high, suggesting, therefore, that cultivated mandarins have a narrow genetic base. In this manner, they determined that the mandarin group, *C. reticulata* Blanco, was one species consisting of a few hereditarily unique individuals and numerous hybrids instead of an incredible number of species as detailed by some taxonomic research studies.

Pummelo (*Citrus maxima* Merr.) is a simple hesperidium-type fruit most commonly found in tropical and subtropical regions. An investigation carried out by Yu et al. (2017) gives information into genetic variation, encourages future genome-wide association research, and provides an advancement of the pummelo breeding program. Yu et al. (2017) measured the genetic variation between 274 varieties of pummelo in China using nuclear simple sequence repeat (nSSR) markers and discovered genetic differentiation F_{ST} as 0.077 and heterozygosity calculated as 0.325. The entire germplasm by structure further splits the pummelo accessions into three subpopulations from southeast, southwest, and central China.

One of the biggest challenges facing the citrus industry is the citrus greening disease caused by the bacterium *Candidatus Liberibacter asiaticus* and spread by the insect Asian citrus psyllid. Millions of acres of citrus crop have been devastated

in the USA and other countries by this disease (USDA APHIS). Developing cultivars resistant to this disease will be a big priority in the near future.

4.3.24 Solanaceae

Lulo (*Solanum quitoense* Lam*)* is a tropical and subtropical simple berry (pepo)-type fruit that belongs to the *Solanaceae* family. Coronado et al. (2017) characterized the genetic diversity of Lulo fruit in Colombia using random amplified microsatellite markers (RAM). They performed this by collecting 21 Lulo plant materials from three districts in Colombia and calculated both the genetic diversity and similarity indices. Analysis using the Nei-Li coefficient resulted in formation of three groups at the 0.60 likeness level, with a free conveyance of materials, without an association with geographical location nor the existence or non-existence of thorns. In conclusion, the RAM markers identified genetic diversity in the Boyaca-Colombia study area.

4.3.25 Vitaceae

Grape vine (*Vitis vinifera* L.) is a temperate Mediterranean simple berry-type fruit of the *Vitaceae* family. Emanuelli et al. (2013) observed a high level of genetic variation among interspecific hybrid cultivars and rootstocks of the domesticated grapevine sub-species *sativa* and its wild relative sub-species *sylvestris*, despite many putative duplicates and extensive clonal relationships. In the whole germplasm collection assessed, the average genetic diversity as measured by the expected heterozygosity was higher for the SSR loci than for the SNP's. The analysis of the genetic structure in the grape germplasm collection revealed several levels of stratification. According to this study by Emanuelli et al. (2013), the major difference was between accessions of *V. vinifera* and non-*vinifera*, followed by the distinction between wild and domesticated grapevine. Intraspecific subgroups were detected within cultivated grapevine representing different eco-geographic groups. The comparison of phenological core collections and genetic core collections showed that the latter retained more genetic diversity, while maintaining a similar phenotypic variability.

4.4 Future Perspectives

Measuring and confirming both the intra- and interspecific variation among plant accessions is of most extreme significance, since it enables genetic resources to be all the more productively utilized by breeders. To build an effective breeding program, among other criteria, it is critical to characterize varietal accessions in such a way as to get the essential data about the genotypes that would be utilized in crosses. A major

step in the grouping and assessment processes is the addition of more information to the existing knowledge of descriptors (either agronomic, morphological, genomic or molecular).

It is presumed that genomic selection (GS) can enhance the proficiency of cross-breeding programs particularly for crops with a lengthy selection and reproductive cycle such as banana. Developing ISSR molecular markers or similar in societies where agriculture is of great importance would provide more data on genetic variation, which is very essential when such hereditary assets are utilized as a part of breeding and germplasm protection programs. Molecular markers showed that plants that are more genetically diverse can be utilized as parent material in breeding programs to produce hybrids. As such, genetic diversity knowledge is crucial for efficiency in breeding programs and for identifying hybrid mixes with greater heterosis and heterozygosity.

There exists a high level of genetic variability among both accessions held in genebanks and wild populations, especially on fruit tree and vegetable crop species, that can be exploited in future breeding programs and conservation activities. The data so far accumulated in molecular fingerprinting of fruit and vegetable species utilizing DNA markers should continue to be studied with a major objective of acquiring a shared view that will permit a simpler and quicker genetic identification that is reproducible among research centers.

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Chapter 5
The Genetic Diversity of Popular African Leafy Vegetables in Western Kenya

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Abstract African leafy vegetables are widely consumed in western Kenya. The most popular being *Solanum* spp. (African nightshade), C*leome gynandra* (spider plant), *Vigna unguiculata* (cowpea leaves), jute mallow, *Amaranthus* spp. (amaranth), *Crotalaria* spp. (slender leaf) and *Cucurbita* spp. (pumpkin leaves). Other vegetables that are consumed but to a lesser extent are the *Basella alba* (vine spinach) and *Brassica carinata* (Ethiopian kale). The wide genetic diversity of African leafy vegetable species contributes to their wide distribution and ability to perform over a wide range of agro-ecological zones in western Kenya. African leafy vegetable domestication is a recent occurrence with some still being harvested in the wild. The genetic diversity of African leafy vegetables therefore comprises of the wild landraces and the recently developed improved varieties. The differences are mostly brought about in taste and morphology. Yield aspects (leaves and seed) of these vegetables are contributed to by the genetic diversity. Morphological characteristic differences in some species like amaranth are distinct and easily observable, but in other species like spider plant differences are not that obvious. These differences bring about variations in popularity, depending on age groups, locations and utilization. An example is African nightshade, where the landraces known for bitter tastes are preferred by the older people and the improved varieties that are mild in taste are popular with the young generation who like the mild ones. The urban women prefer the improved varieties which have wider leaves and are easy to prepare. The landraces are threatened with extinction because of population increase in western Kenya. People have encroached into the wild such as the Kakamega Forest and open fields, thereby reducing the biodiversity of ALVs. The diversity is also threatened by change in climate, people's culture and eating habits due to the influence from the west. Efforts being made to conserve the genetic biodiversity of ALVs are in-situ by local farmers and ex-situ by institutions such the Kenya Agricultural & Livestock and Research Organization gene bank.

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5.1 Introduction

Genetic diversity is defined as the variety of alleles and genotypes found in a population, and this is seen in the differences in morphology, physiology and habits between individual and populations (Toro and Caballero 2004). Genetic diversity is brought about by the variation in the quantity of genetic information found within and among individuals of a species (Biodiversity 2014). Currently, the two major methods known to determine genetic diversity in a species are morphological characterization and molecular markers. It is recommended to use both molecular and morphological methods to analyse genetic diversity because they complement each other. Although the use of molecular markers is efficient, it is expensive, while the morphological method has many limitations mainly phenotypic plasticity (Ojiewo et al. 2013). Other disadvantages of morphological analysis are that fewer characters are displayed (Rao and Hodgkin 2002) and are affected by environment interactions (Jacoby et al. 2003). For example, the size and maturity period of a species may give different results depending on moisture and nutrient availability. However, morphological characterization is the main method used to determine genetic diversity and plays a crucial role in the analysis and evaluation of germplasm. The descriptions given to indicate diversity in the ALV species in this chapter will be based on the morphological characteristics.

Several terms are used to describe African vegetables. These include traditional African vegetables (TAV), indigenous African vegetable (IAV), indigenous leafy vegetable (ILV) and African leafy vegetable (ALV). These terms may mean different things to different people. According to the United Nations Food and Agriculture Organization (FAO), ALVs are all categories of plants whose leaves are acceptable and used as vegetables by communities through custom, habit and tradition (Ambrose-Oji 2009). Before the introduction of exotic vegetables like cucurbits, spinach and carrots, ALVs would be found in the wild or were semi-domesticated species that are part of traditional diets for many parts of Kenya (Ambrose-Oji 2009). This is the case in western Kenya as well where even currently ALVs are still found wild in forest areas and grow as weeds in abandoned areas. Some are semi-domesticated in the backyard of homesteads and sugar cane plantations where they are less demanding of management and inputs. Efforts to maintain ALVs as part of our traditional diet have resulted in increased cultivation in kitchen gardens or inter-cropping with staples or among themselves. They grow quickly and get harvested within a short time making them important for food security.

African leafy vegetables (ALVs) are important for their medicinal value (Orech et al. 2007; Ndinya 2005), high micronutrient contents (Kwenin et al 2011; Kamga et al. 2013), source of food security (Gogo et al. 2016) and income in the continent, but are still among the underutilized species. The origin of these vegetables is Africa, but some originate from elsewhere because they have been consumed for many

years in the continent they are now considered traditional in Africa. The diversity of indigenous and naturalized species in Africa is estimated at 40,000 (Maundu et al. 2009). About 8–10% of these are utilized as food locally with 1000 species being used as vegetables in Africa but only about 400 that are well defined and found within 53 botanical families (PROTA 2004). Out of the many species of African vegetables species, only 8 per cent are recent introductions that are regarded as standard global vegetables (PROTA 2009).

Only to a small extent are indigenous vegetables still collected from the wild and volunteer plants in crop fields harvested, because domestication and cultivation have been on a steady rise (Oniang'o et al. 2006). Larger sizes of land are now allocated to amaranth (*Amaranthus* spp.), spider plant (*Gynandropsis gynandra*), African nightshade (*Solanum* spp.), vegetable cowpea (*Vigna unguiculata*) and jute mallow (*Corchorus olitorius*) than some 10 years before. These vegetables are considered as the most important vegetable species across communities and borders (PROTA 2004), but they are also of great importance locally among the western Kenya communities. A plant's popularity and potential as a vegetable are often known to specific geographic communities and may not be appreciated as part local food culture in other communities or regions, and this poses its own challenges in conducting research and promoting specific vegetables nationally.

The government and donors may consider these vegetables as secondary and not worth for research because they are utilized by a minority and are restricted to marginal conditions (Mnzava 1997). Such species are of critical importance during food shortages because they grow in marginal conditions. Majority of ALVs therefore have remained neglected. Some other species, however, are widely used across the continent (Chweya and Eyzaguirre 1999), and therefore any results of their improvement are expected to reach a wider population and therefore create tangible impact. The neglect in research of ALVs provides a greater scope for quick advances as shown in a report by Bioversity International (Khire 2013). The report states that between 1996 and 2007 Bioversity International worked with partners in Kenya on various aspects of ALVs. Their aim was to revive the interest of researchers, growers and consumers in ALVs. Results of this work were that 89 neglected species were identified, 23 types of cowpea were conserved in-situ and agronomic protocols were produced at Maseno University in western Kenya.

The western Kenya region has a rich biodiversity of ALVs, initially from landraces but added to that are the recent introductions from outside and breeding of improved varieties. Consumption of ALVs in western Kenya has remained high because diets mainly comprise of ALVs which are sold at the local markets and even big stores. Except for the coastal part of Kenya, the western region uses more ALVs species than any other region in Kenya and has the most common cultivated species such as *Corchorus* spp., *Crotalaria ochroleuca*, *G. gynandra* and *V. unguiculata*, (Pasquini et al. 2009). Earlier on these vegetable species were only grown in pure stands in kitchen gardens, on farmlands, built-up areas, hedges and wastelands (Orech et al. 2007), but with sensitization of communities on importance of ALVs and with increased research, farmers are now setting aside bigger land solely for ALVs production.

5.2 Western Kenya

Western Kenya comprises of four major tribes: the Luo, Luhya, Kisii and Kalenjin. They have been associated with consumption of ALV for many years. Vegetables, traditionally used to be collected from Kakamega rain forest, the wild surrounding bushes or weedy forms in cultivated fields in the rural areas. Increase in population in western Kenya has led to division of land in small sizes, and collection of wild ALVs from the bushes is becoming a thing of the past. Efforts of domestication began, and eventually most of the African indigenous vegetables consumed in Nairobi were majorly being sourced from farms in western and transported at night using busses. The amount of ALV traded from western Kenya was estimated to be 31 tons per month (Mwangi and Kimathi 2006). Vegetables bought directly at farm gate level were estimated to be worth USD 6000, and by 2006 this had increased to a value of USD 142,860 (Mwangi and Kimathi 2006). The great potential of ALVs in western Kenya has not only drawn business interests but also created awareness in the need of conducting studies to develop and introduce improved new varieties which have greater yield potential. The result of such studies has been an increase in the diversity of ALVs in the region.

5.3 Characterization of ALVs Found in Western Kenya

Genetic diversity studies are directed towards crop improvement programmes, germplasm characterization and conservation efforts. Characterization is the description of plant germplasm. Morphological characterization allows for thorough sampling and analysis of a plant, but it is time-consuming, requires a lot of labour, is subjected to phenotypic changes and does not provide information on particular genes and their number and neither do they account for variations observed in plants (Jacoby et al. 2003). Examples of studies on morphological characterization of ALVs, especially on African nightshade, have been done by the authors such as Ojiewo et al. (2013), Wesonga et al. (2016) and Kuruma et al. (2016) among others.

When compared to morphological characterization, molecular markers not only provide a large number of characters for analysis but also provide a detailed characterization of genetic diversity regardless of environmental influences. The challenge is the high cost of molecular analyses which makes it difficult to sample a large number of individuals, and the inability to include herbarium materials (Jacoby et al. 2003). Research on genetic diversity of these ALVs using molecular markers has been increasing over time. However, more ALVs diversity studies using molecular markers still need to be conducted because so far, the focus has been on few vegetable species like Solanum, Amaranthus, Cleome and Vigna, which when compared, the ALV genetic diversity studies using molecular markers were Vigna 58%, Solanum 24%, Amaranthus 15% and *C. gynandra* 3% (Omondi et al. 2016).

To determine the genetic diversity of different varieties or landraces of a crop species, characteristics that differentiate them have to be identified. Traditional methods used to determine genetic diversity in western Kenya are morphological and agronomic aspects that include the physical appearance of different parts of the plant, yield, maturity period, taste and cooking qualities. Scientifically, detailed information about genetic differences among groups of accessions usually referred to as descriptor is helpful for management and utilization of germplasm collections (Loumerem and Alercia 2016). Descriptor lists assist curators, breeders, scientists and those managing crop genetic resource collections (Bhattacharjee 2017). Although descriptors of most of the exotic crops are internationally available through the International Union for the Protection of New Varieties of plants (UPOV), this is lacking in ALVs because these species are not globally recognized except for a few species like pumpkin. Nevertheless, progress has been made and through the morphological characterization and molecular marker studies, traits that can be used to differentiate varieties have been identified regionally within the African continent. Observed are slight differences in the format of presentations, morphological traits emphasized and total number of traits included. Descriptor lists for varieties of the major ALV species that can be used in breeding and quality seed production are therefore now available. The biodiversity and World Vegetable Centre (WVC) in Arusha have played an important role in facilitating and developing improved varieties of ALV species as well as the descriptors.

5.3.1 African Nightshade

The African nightshade is considered a complex of at least eight leaf species that include *Solanum americanum* Mill, *Solanum eldorettii*, *Solanum retroflexum* Dunal, *Solanum scabrum* Mill and *Solanum villosum* Mill (Edmonds and Chwewa 1997). After joined research in the region and exchange of germplasm, the species are now commonly used as leafy greens in much of sub-Saharan Africa. Some of these provide for large-leafed types that have now become popular in western Kenya for their mild taste and ease of preparation. The nightshades are among top priority ALVs identified for improvement and promotion through research. A major constraint facing this objective is the inadequate knowledge on the taxonomy and nomenclature on African nightshades, making it difficult for breeders to make good selection for those with high nutritional value and good agronomic traits for genetic enhancement (Mwai et al. 2007). This confusion is especially seen in S. *eldorettii* where in the literature it is often used to refer to two species: *S. florulentum* and *S. florulentum* species that are very closely related (Mwai and Schippers 2004). The *Solanum* species in the black nightshade complex group can be taxonomically confused by intermediate forms and hybridization between the edible African nightshade species such as *S. americanum, S. scrabrum and S. vilosum* with *S. nigrum* which is poisonous (Wikipedia 2018). To separate the edible and the poisonous Solanum species, it is often recommended that the former be referred to as African nightshade and the latter the black nightshade.

To improve conservation and utilization of available diversity in African nightshade, several studies are still being conducted to resolve the taxonomic confusion.

The *S. scrabrum* is the largest species, growing to 1.5 m, with large purple–black fruits and very leafy (Fig. 5.1). There is considerable variation in this species, in that some forms have large leaves while others have small serrated leaves (Figs. 5.1 and 5.2); some types have non serrated leaves (Fig. 5.3), others have thick stems to support large plants types (Fig. 5.4); some types produce large quantities of fruit but with relatively less dense foliage (Fig. 5.5). The *S. scrabrum* species an introduction from elsewhere has become popular in western Kenya such that currently it is the most cultivated African nightshade species because of the high leaf yields. Leaves can be as large as those of common kales, and because it is not very bitter the youth consume it easily. *Solanum villosum* Mill has thin stems and edible orange-coloured small berries (Fig. 5.6) that are loved by children, a trait that is peculiar to it and easily distinguishes it from the rest of the nightshades. This species is easily found in the wild from where it is usually picked. Communities consuming these vegetables do not have specific names for the different landraces. But improved varieties are now being given specific local names as this is a requirement during official release of a variety. Morphological traits that easily show genetic diversity in African nightshade are:

- Plant habit
- Leaf shape
- Leaf margin
- Leaf size
- Flower colour
- Colour of anther
- Colour of mature berries

Fig. 5.1 Large-leafed A. nightshade with few berries

Fig. 5.2 Serrated small-leafed A. nightshade

Fig. 5.3 A. Nightshade with non serrated leaves

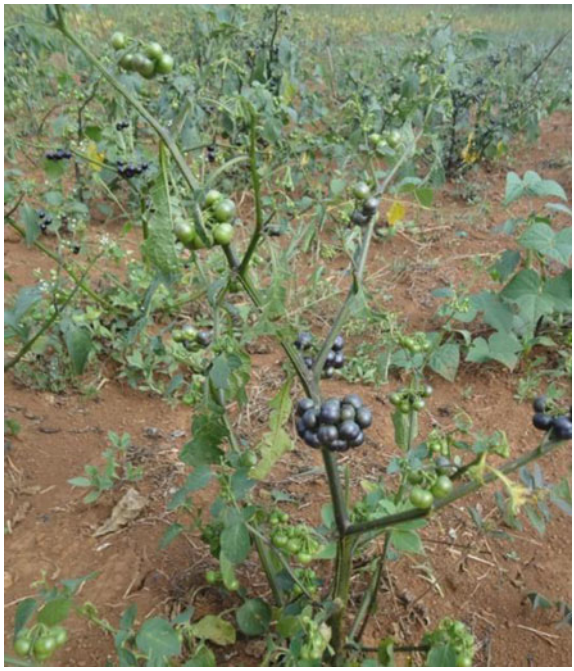

Fig. 5.4 Thick-stemmed A, nightshade

Fig. 5.5 Large-leafed A. nightshade with many berries

- Plant height
- Berry size
- Plant branching habit

Fig. 5.6 Thin stemmed, A. nightshade

- Colour of stems.

5.3.2 Amaranth

Family: Amaranthaceae. Amaranths are important species in Kenya. The more important species include *Amaranthus cruentus* (Fig. 5.7), occasionally with large leaf forms; *A. lividus* (Fig. 5.9), commonly used in western Kenya; and *A. hypochondriacus* (Fig. 5.8), which, together with *A. cruentus*, constitute most of the local seed of amaranth (Maundu et al. 2009). These species are wild as well as cultivated. Other species include the wild spiny amaranth (*Amaranthus spinosus*) (Fig. 5.10), which has spines on its stem. *Amaranthus* is a relatively difficult group to master because of similarity between species (Maundu et al., 2009). Figure 5.12 shows different types of Amaranthus including improved varieties currently found in western Kenya. The landraces (Fig. 5.11) are short, have small leaves and mature early even within 2 months unlike the improved varieties which grow tall, have large leaves or a lot of foliage if leaves are small and can stay up to 4 months. The utilization

Fig. 5.7 *A. cruentus*

Fig. 5.8 *A. hypochondriacus*

of grain amaranth is a more recent phenomenon encouraged by the government for flour composites, animal and baby feeds. Grain Amaranth produce fewer leaves and are not popular for vegetables. Morphological traits that show genetic diversity in Amaranth are:

- Plant habit
- Leaf shape
- Leaf margin
- Leaf size
- Plant height

Fig. 5.9 *A. lividus*

Fig. 5.10 *A. spinosus* (wild type)

- Berry size
- Plant branching habit
- Colour of inflorescence
- Inflorescence growth habit
- Inflorescence type
- Seed colour.

Fig. 5.11 *A. retroflexus* (local landrace)

Fig. 5.12 Different types of improved amaranth varieties

5.3.3 Cowpea

Vigna unguiculata (L.) Walp. Family: Fabaceae.

Cowpea is used as a pulse, a source of leafy vegetable and fodder as well. It is a very important African crop and mainly grown in warm sub-humid to semi-arid areas where it is able to survive with little rain. Within each region, the importance attached to either the seed or leaves varies. Some communities grow it for pulse and not the leaves; others grow it for its leaf only and still others for both. In western Kenya where the crop is very popular, the Luhya community grows it more for the leaf than seed while the Luo community grows it for both leaves and pulse. Most conspicuous morphological characters include the colour of flowers; colour, size and shape of leaves, seeds (Figs. 5.13) and pods; and the habit of the plant either determinate (Fig. 5.14), indeterminate or climbing (Fig. 5.15).

The centre of origin of cowpea appears to be West Africa, although the wild type originated from Southern Africa (Timko et al. 2007). According to Kuruma et al. (2016), there is a low level of similarity among the cowpea germplasm tested using morphological traits analysed in Kenya, making it necessary therefore to broaden the genetic base of the Kenyan cowpea germplasm. In research morphological traits, e.g. growth habit, raceme position, days to flower, pod length, yield and 100 seed weight are keys in selection for the best cultivars with good attributes to improve other breeding lines (Kuruma et al. 2016). However, among the local communities the characteristics of the pulse are what is used to discriminate the different types. The women selling grain as seed in local markets are able from experience to guide a buyer on how the crop will behave in the field. Often, different types of seeds representing different genotypes are sold when all are mixed together and it is up to the buyer to do selection if need be. This is a common method by which farmers

Fig. 5.13 Cowpea seeds of different shapes and sizes

Fig. 5.14 Determinate type of cowpea

Fig. 5.15 Indeterminate type of cowpea

traditionally conserve majority of local germplasm. Morphological traits of cowpea that show genetic diversity are:

- Growth habit
- Stem/branch rooting
- Growth pattern
- Twinning tendency
- Plant pigment
- Stem pigment
- Stem width
- Terminal leaf shape
- Leaf texture
- Flower colour
- Pod length
- Number of locules per pod
- Seed shape
- Seed colour
- Eye colour
- Seed testa texture.

5.3.4 Ethiopian Mustard (Brassica Carinata A. Braun)

Family: Brassicaceae. Synonyms: Ethiopian kale/mustard. *Brassica carinata* is a kale, often characterized by purplish stem and thin soft leaves. The plant is traditionally grown in western Kenya where it is known as *kanzira* or *kandhira*. It also has a wild type that is commonly found growing as weeds in fields. The wild has small leaves (Fig. 5.16), while the improved varieties have larger leaves (Fig. 5.17). The morphology traits for genetic diversity characterization include:

- Leaf shape
- Leaf margins

Fig. 5.16 Wild type of Ethiopian mustard in western Kenya

Fig. 5.17 An improved variety of Ethiopian mustard

- Leaf size
- Leaf colour
- Plant height
- Primary branching
- Stem colour
- Flower colour
- Seed colour.

*5.3.5 Indian Spinach (*Basella alba *L.)*

Family: Basellaceae. Synonyms: Ceylon spinach, Indian spinach. *Basella alba* is a creeping plant with heart-shaped leaves that is widely used as a vegetable and hardly dries up even in dry seasons. In western Kenya, it is commonly planted on hedges or staked on banana trees (Fig. 5.18) where leaves are easily accessible, but sometimes is picked from the wild. It is used in western Kenya where it is called *nderema* but is not that popular and will not be found in the local markets in large quantities. The species is found in two forms, a green form (*alba*), which is the more widely distributed, and a red type (*rubra*), which is more commonly used as an ornamental. In western Kenya, only the green type is grown (Fig. 5.18). Studies by Reddy et al. (2014) on selected landraces of the green form resulted in identification of nine morphological traits that indicate genetic diversity. These included:

- Growth habit
- Stem colour

Fig. 5.18 Indian spinach found in western Kenya

- Stem shape
- Leaf colour
- Leaf shape
- Leaf margin
- Leaf margin colour
- Leaf apex
- Petiole colour
- Flower colour.

5.3.6 Jute Mallow

Corchorus olitorius (L.). Family: Tiliaceae. Jute, or Jew's mallow, is specific to communities originating from western Kenya. It is an erect annual that is spontaneous, grows easily and is now commonly planted and sold. It does best in low, warm to hot humid areas with well-drained soils (Maundu et al. 2009). The Corchorus vegetable is very popular with communities in western Kenya where in some parts it can be prepared alone, but mostly because of the mucilaginous trait, it is mixed with cowpea leaves or Crotalaria to make the dish slimy. Genetic improvement of the cultivars of jute (*Corchorus olitorius* L.) is needed to widen the genetic base of new cultivars and the diversity required to achieve this, which according to Basu et al. (2004) exists. Qualitative traits such as leaf morphology show some having entire margins (Fig. 5.19) and others very serrated leaf margins (Fig. 5.20); branching habit, pod distribution and stem colour (Figs. 5.21 and 5.22) are taxonomically the most informative characters. The highest morphological variability from studies occurred

Fig. 5.19 Plant with entire leaf margin

Fig. 5.20 Plant with serrated leaf margins

Fig. 5.21 Narrow leaved Jutemallow

Fig. 5.22 Broad leaved jutemallow

within African accessions, indicating that this species originally evolved in Africa (Benor et al. 2012). The important morphological traits for genetic characterization are:

- Stem colour
- Stem hair
- Branching habit
- Leaf vein colour
- Leaf petiole colour
- Leaf petiole length
- Leaf margin
- Petal colour
- Pod shape
- Pod shattering
- Pod distribution
- Testa texture.

5.3.7 Pumpkin

Pumpkin (*Cucurbita* spp.) Family: Cucurbitaceae. Pumpkin is a species that was introduced but its leaves are now among the most widely used as vegetables in western Kenya. The three species of pumpkins are *C. pepo* (L.), *C. maxima* Duchesne and *C. moschata* Duchesne. All these three species of pumpkins are widely grown in western Kenya; but the C. pepo and C. Maxima are more preferred for leaves than fruit. Pumpkins are important vegetables in western Kenya for leaves (Fig. 5.23) and fruits (Figs. 5.24 and 5.25), both of which are heavily utilized and are often sold in local and supermarkets. Leaves are sold mainly in their fresh form. The various types of pumpkins are differentiated from the morphological traits such as:

- Plant growth habit
- Fruit shape
- Fruit colour
- Fruit texture skin
- Fruit size
- Fruit flesh colour.

5.3.8 Slender Leaf

Slender leaf (*Crotalaria* spp.); Family: Fabaceae. Other names include rattle pod, rattle box, sunhemp or slenderleaf (Abukutsa-Onyango 2007). The two African species used as a vegetable are *Crotalaria ochroleuca* and *Crotalaria brevidens.* The former

Fig. 5.23 Pumpkin leaves used as vegetables

Fig. 5.24 Pumpkins selling at a roadside in western Kenya

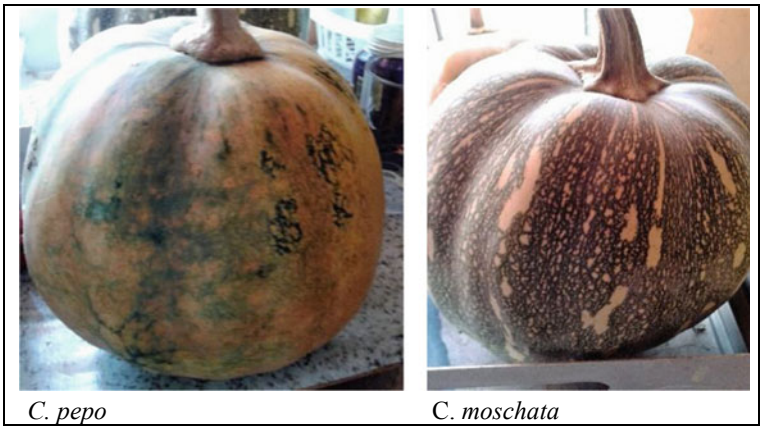

Fig. 5.25 Two types of pumpkin fruits found in western Kenya

has a mild taste, whereas the latter has a bitter taste. *Crotalaria ochroleuca* (Fig. 5.26) has bright green leaves and grows taller than *C. brevidens* (Buleti et al. 2015). The flowers are pale yellow or creamish in colour, and the seeds are normally light yellow with pods that are wider in diameter and big. *Crotalaria brevidens* has bluish green leaves and has bright yellow flowers, and the seed colour is light brown in colour with pods being small and narrow in shape (Schippers 2002). Locally, the main dis-

Fig. 5.26 *Crotalaria ochroleuca*

tinguishing features of the two species are the taste and pod size. Both species are commonly cultivated and consumed in Kenya. A market survey conducted in three markets in western Kenya revealed that slenderleaf was among the top ten priority African indigenous vegetables in the region (Abukutsa-Onyango 2007). The bitter taste of slenderleaf could be due to the presence of alkaloids and phenolic compounds (Schippers 2002). The two species are very similar in appearance, but morphology traits that can be used to differentiate them include:

- Leaf colour
- Leaf length
- Leaf width
- Plant height
- Pod length
- Seed colour.

5.3.9 Spider Plant

Cleome gynandra (L.). Family: Capparaceae. Synonym: *Gynandropsis gynandra* (L.) Briq.

Cleome gynandra is also known as spider plant, spider flower and cat's whiskers (Fig. 5.27). It is herbaceous vegetable that is important in western Kenya. This vegetable is usually cultivated in kitchen gardens and grows easily on cattle sheds or places with a lot of animal or poultry manure. The spider plant is particularly important to Luo and Luhyia communities who live in western Kenya. The most noticeable difference is the colour of the stem and fruit, which may vary from green to purple.

Fig. 5.27 Spider plant vegetables

Differences in the colour of flowers also vary from white, pink and purple. Research on genetic diversity determined by molecular markers of four spider plant morphotypes: green petiole/green stem, green stem/purple petiole, purple stem/green petiole and purple stem/purple petiole showed that four were well differentiated and that genetic variation exists within the species (K'Opondo et al. 2009). The high level of genetic variation of traits from different spider plant accessions studied is confirmed by Wasonga et al. (2015) and is an indication of the potential there is in genetic improvement. The information on its phenotypic diversity should aid on selection of germplasm for not only breeding but conservation as well (K'Opondo 2011). In communities where spider plant is popular, neither farmers nor consumers of spider plant are particular about these morphological differences because to them, the accessions of these vegetables have similar tastes and maturity period. The distinct difference is usually only seen in the colour of flowers and plant stems. Principal components for characterizing spider plant accessions are stem colour, stem hairiness, petiole colour, petiole hairiness, leaf hairiness, leaf shape and number of leaflets per leaf. Other useful traits of spider plant descriptors include growth habit, flower colour, leaf colour, leaf pubescence and leaf blade tip shape.

5.4 Conservation of Genetic Diversity in ALVs

The genetic variation within a species, in the wild or domesticated is important for the survival and therefore it is important to conserve genetic diversity. Through conservation, genetic diversity is maintained and this ensures availability of a wide variation of genetic resources within species for utilization whenever the need be. Genetic variability provides varieties that can be adopted in different environments, tolerate or resist diseases and provide food security. A great loss of genetic resources has been realized and is being felt in many places. The loss of plant genetic diversity within a species is estimated at 25–30% (Westmoreland 1999). In Mexico, only 20% of the maize varieties reported in 1930 now exist. Ten thousand wheat varieties were present in China in 1949; by 1970, only 1000 varieties remained (UN FAO 1996). In the Philippines, several thousand rice cultivars existed before the introduction of high-yield varieties. Currently, a few hundred upland traditional cultivars are left in the fields (Salazar 1992). The rapid erosion of genetic diversity is becoming a great concern.

A great deal has been accomplished in the last decade to protect the plant genetic wealth. However, much still remains to be done in improving the conservation strategies and enriching the collections, which involves a wide range of diversity comprising wild relatives, and landraces, weedy forms, unimproved and modern cultivars. In genetic resource conservation, the aim should be to maintain as large as possible, within each species, a genetic diversity with value that can be exploited when necessary (Khanna and Singh 2016). Therefore, Rao and Hodgkin (2002) caution that conservation efforts be they in-situ or ex-situ should be done only with enough information on the genetic diversity that is being conserved.

The main centre for Plant Genetic Resources (PGR) activities under the Consultative Group of International Agricultural Research (CGIAR) is Bioversity. The purpose of the CGIAR support to PGR is to ensure that the diversity of germplasm is safely maintained and made freely available for use in research and crop improvement for the benefit of all people. The CGIAR seeks to achieve this goal both directly through the institutions it supports and indirectly through strengthening national capabilities. The PGR activities supported by the CGIAR include exploration and collection, characterization, multiplication, evaluation, conservation, data management, information service, the supply of germplasm to plant breeder's, research workers and training where appropriate (Arora et al. 1991). The ALVs have benefited from this support, and genetic diversity research activities specifically on the priority wild relatives and the domesticated types have enhanced understanding of the origin, natural selection and changes that have occurred.

Other players in the conservation of ALVs are donors (USAID, GIZ) that have been providing funds for research in the various aspects of ALVs that have eventually generated interest in ALVs that have now captured the attention of many and resulted in calls for the conservation of these underexploited crops.

5.4.1 Genetic Erosion in ALVs

African leafy vegetables have a high species and habitat diversity, but they are threatened by genetic erosion because of factors such as over-exploitation in the recent years, neglect, habitat fragmentation and competition from exotic vegetables (Orech et al. 2007). Other threats are from changes in climate, urbanization and increased human populations. The ALVs have also been neglected as the demand is local and not global.

5.4.1.1 Over-exploitation (Overgrazing and Excessive Harvesting)

Communities found in close proximity to Kakamega forest graze animals and harvest vegetables in the forest without giving any thought to conservation. This is where most of the wild landraces can still be sourced from. With the disappearance of these wild types, the result is reduction in the diversity which may be essential for breeding in future. The status of ALVs as weeds or wild species made them perpetually abundant with an inexhaustible seed pool. The tendency had been therefore not to conserve them but to eradicate them by weeding and foraging (Mnzava 1997) without any thoughts about their value and the need to conserve them. Yet with increase in human population and reduction of arable land, the availability of ALVs spontaneously growing as weeds has become less.

5.4.1.2 Competition from Exotics

The introduction and emphasis of global vegetables in Kenya had some negative impact on the cultivation and use of African indigenous vegetables. The national agricultural policies advocated the use of exotic species at the expense of traditional ones (Abukutsa-Onyango 2011). This created change in eating habits and adoption of Western type of diets (Frison et al. 2005). The global vegetables were appealing to the young because of the taste and the rich because of status. Although ALVs are nutritious, unfortunately nutritious plant materials are usually rejected unless they appeal to the consumer, whether or not they have nutritional value. Therefore, some of the poorest vegetables nutritionally became also the most popular. However, this trend is changing with the desire to embrace culture more and better education on importance of traditional species.

5.4.1.3 Habitat Fragmentation

Amount and distribution of genetic diversity in a place often change when there is interference due to habitat loss and fragmentation. Degradation of vegetation follows, and the stability of plants, their diversity and survival cannot be guaranteed. The highly threatened genetic resources of ALV are the wild races which have not been domesticated. As open land and forest reduce because of population increase and repeated cultivation on small pieces of land, less of the ALV become available. No deliberate efforts are being made to maintain wild species because this would be expensive.

5.4.1.4 Utilization of Limited Genetic Resource

Mankind depends on only a few varieties of existing species. Genetic erosion occurs to those varieties that are not consumed often and eventually leading to loss of their knowledge. The loss of plant genetic resources is mainly blamed on the modern agriculture. In modern agriculture, the objective is commercial rather than subsistence. High yields and uniformity become essential because a modern farmer requires to plant a variety that they will harvest in great quantity and at once then sell immediately. Whereas the traditional agriculture was based on diversity that would meet farmer's various objectives such as long period of harvest, taste, tolerance to diseases, this has been replaced with modern hybrid varieties (Westmoreland 1999) which even though few and low in genetic base are high yielding and uniform. The introduction of improved varieties of ALVs has become popular in the communities, and the use of landraces for most vegetable species may be on the decline. The continued use of these modern varieties and neglect of the wild and landraces could lead to the loss of traditional varieties that have potential value such as drought tolerance and resilience to climate change.

5.4.1.5 Promotion of Industrial Crops

The major cash crop in western Kenya is sugar cane. Sugar cane is produced under a monoculture system and has a three-year cropping cycle, and the growth habit is such that it becomes impossible to inter-crop in the second year of growth. This change in land use causes a decline in the land area for other crops such as indigenous vegetables. During the peak periods of sugar cane production in western Kenya, production of ALVs reduced and so did the diverse vegetable species and indigenous knowledge that contributed to their production and utilization (Netondo et al. 2010). This has caused genetic erosion of varieties of ALVs that were cultivated and consumed by people in this area for many years, playing a significant role in food security among local households (Wemali 2010). In areas of sugar production, large factories are built and alongside a degree of urbanization sets in and contributes to genetic erosion of ALVs.

5.4.1.6 Climate Change

Climate change will have an impact on the ability of wild species to survive because it will cause changes in species distribution, population sizes and selection pressures. About 22–27% of the wild relatives of the species that are valuable to agriculture may be in danger of extinction (Jarvis et al. 2016). Considering that ALVs are still collected in the wild state, Africa then becomes one of the most vulnerable continents to climate change effects such as loss of diversity (Neaves et al. 2015). In the recent past, rainfall patterns in western Kenya have become unpredictable. Climate change is a reality, and a great challenge this will be to ALVs wild species and landraces that are much less adaptable to these new threats if no deliberate efforts are put in place to maintain them. There will exist individuals able to survive flooding, drought or attacks by emerging new insect and disease pests. These extreme situations seem to be on the increase, and the conserved ALV wild relatives will become vital resources for the adaptation of climate change. It is estimated that areas under semi-arid and arid types of climate in Africa will increase by 5–8% (Jarvis et al. 2016) which will be a challenge to the continuous cropping of some improved ALV varieties that have not been developed for hardship environments. According to recent studies, communities not able to mitigate against the challenges of climate change are the most vulnerable to genetic diversity losses (CTA 2010).

5.4.2 Conservation Methods

The current emphasis is to understand the distribution and extent of genetic diversity available to communities in useful plant species, so that the genetic diversity can be conserved and used properly (Rao and Hodgkins 2002). Conservation efforts are associated with particular constraints depending on the method of conservation,

but the benefits of a variety can enhance its use and therefore efforts towards its conservation. Apart from their food value, traditional vegetables are useful for other purposes, such as medicine, cash income and cultural observance, factors which contribute to the conservation of these species. However, much still remains to be done in improving the conservation strategies and upgrading the collections of ALVs, which has a wide range of diversity, comprising wild types, landraces, edible weeds, traditional varieties and improved cultivars. The taxonomy of many of the species is also not known. Globally, an important step by Bioversity International is the support of the crop wild relative (CWR) project and is supporting countries to manage and conserve CWR by generating lists of descriptors that can provide information which these counties can collect and utilize when needed (Thormann et al. 2013). The main conservation methods for plant genetic resources are in-situ and ex-situ.

5.4.2.1 In-Situ

In-situ conservation is the maintenance of populations of species in their natural surroundings. In the case of cultivated species, conservation is done in the places where they have developed their distinctive characteristics. *In-situ* conservation enables plants to change and adapt to stress brought about by unfavourable environmental conditions such as drought (Worede 1992). Common approaches for *in-situ* conservation are genetic reserve conservation and on-farm conservation (FAO 2016).

Increased attention has been given to conserving the diversity of our agriculture through existing seed. The farmer is crucial for conservation of plant genetic resources for food and agriculture (PGRFA). Success can be achieved if conservation of plant genetic diversity within the farming environment is not hindered and the traditional cultural systems are not done away with but promoted (Westmoreland 1999). The recent approach of farmer seed banks set within communities does not only save seed for when it is required but conserve farmer germplasm. The deliberate in-situ conservation initiatives for ALV crop plants and their wild relatives are still minimal. However, efforts by Bioversity through the support of CGIAR resulted in 23 types of cowpea to be conserved in-situ (Khire 2013). As development of improved varieties continues, the number of ALV varieties that can be cultivated will increase. It will be a challenge to prevent the not so popular and less common varieties from disappearing (Cernasky 2015).

Important issues in management of conserved germplasm have led to Rao and Hodgkins (2002), recommending that there should be development of various types of monitoring procedures for in-situ conserved germplasm. The basis of this recommendation is that Nevo and colleagues established ways in which diversity varies according to different environmental factors including variation in soil type and available moisture. They have also shown ways in which patterns of variation can change over time and alter both the numbers and the types of alleles present.

5.4.2.2 Ex-Situ

Ex-situ conservation is the maintenance of parts of biological diversity outside their natural habitats (Maxted et al. 1997). Ex-situ collections are stored in seed gene banks, field gene banks and in-vitro. The main storage infrastructures for ex-situ conservation techniques are gene banks. The seed gene bank collection of the WVC holds 2659 indigenous vegetable accessions of 48 species, the largest in Africa to date (Ojiewo et al. 2013). This gene bank acts as the primary source of breeding material for the development of new varieties by WVC and its partners in the public and private sectors. It is an essential resource for the farmer participatory variety selection process that the WVC and its partners have adopted (Ojiewo et al. 2013). In Kenya, the national gene bank found in the outskirts of the capital city Nairobi preserves some genetic resources of ALV (Kemei et al. 1995). Seeds stored in the local gene banks can be easily reintroduced into farmer's fields when needed. FAO plays a lead role in strengthening the conservation of PGRFA through policy assistance and technical support and by raising awareness. Collaboration of international, regional and national partners, in multiple projects, strengthens capacities; addresses technical and policy aspects; and prepares gene bank standards and technical guidelines for crop-specific conservation techniques (Arora et al. 1991).

5.4.2.3 Integrated Conservation Approaches

In the integrated conservation system, farmers and researchers work together to restore landraces that were lost and to set up community gene banks where farmers can source a diverse range of crops and varieties. Scientists have recognized that ex-situ conservation and in-situ conservation are complementary, each having particular advantages and disadvantages but equally important (Arora and Paroda 1991). There are now conservation systems that have elements of both in-situ and ex-situ to improve conservation. It is therefore necessary to have a balanced application of both in-situ and ex-situ approaches to conservation. Within the latter category, a further balance needs to be struck between seed, field gene bank, in-vitro, pollen and, perhaps in future, DNA storage (Withers 1991). In these cases, in-vitro conservation may never replace conventional technologies entirely but will complement them within a strategy that balances the traditional approaches of in-situ, seed and field gene bank conservation with the best of the new *in-vitro* conservation, pollen storage and even DNA storage which should be used where they apply (Chandel and Pandey 1988). There has been in recent years significant advancement in the application of modern preservation of in-vitro (tissue culture) techniques and cryopreservation technology in the genetic conservation of germplasm. These techniques can be experimented on ALVs as well.

5.5 Conclusion

There is a need to increase the production and genetic diversity to meet the high demand in the area. Lack of genetic material of underutilized crops remains a major problem, and yet availability of genetic biodiversity is important if African vegetable improvement is to be done. The green revolution is dependent on availability of genetic diversity for crop improvement (CGIAR, 1994). Equally important is the safeguarding of the genetic diversity and ensuring its full use. Germplasm collecting efforts of the ALV species that are partially domesticated and those that are still in the wild need to be stepped up to increase the genetic diversity for conservation and breeding. As environmental degradation continues to take place, the wild landraces will continue to diminish. The government and partners should lead in research, development and conservation efforts of these vegetables because though regarded highly only at community level they have economic and nutritional importance and should be popularized nationally. African leafy vegetables can provide a variety of nutrient-rich food to an increasing population. More documentation on the ALV and the availability of this information is necessary if strides are to be made.

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Chapter 6
Genetic Diversity in Nutritious Leafy Green Vegetable—Chaya (*Cnidoscolus aconitifolius*)

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Abstract Chaya (*Cnidoscolus aconitifolius* ssp. *aconitifolius* Breckon) is a fast-growing, semi-perennial, and semi-woody Mesoamerican euphorbiaceous. It is used as a leafy green vegetable and prevailingly cropped in tropical savanna climate. However, cropping of chaya is possible in both dryer and more humid climates. Although the crop has its origin in the Maya region of Southeast Mexico, Guatemala, and Belize, chaya is popular throughout Mesoamerica. Due to its high nutritional value, cooked chaya leaves are an essential ingredient of the diet of Maya communities, especially in Southeast Mexico. Chaya is also used as an ornamental plant, for forage, and in traditional Maya medicine, where it is used to cure a wide range of diseases such as diabetes, kidney problems, arteriosclerosis, gallstones, and high cholesterol. Chaya can be called a semi-domesticated plant: Apart from wild chaya, there are four chaya varieties, whose grade of domestication varies from cropped almost wild phenotypes to entirely domesticated: 'Chayamansa,' 'Redonda,' 'Estrella,' and 'Picuda.'

Keywords *Euphorbiaceae* · Spinach tree · Agroforestry · Maya cuisine · Traditional Mesoamerican agriculture · Family home gardens · Edible tropical plants

6.1 Introduction

Chaya (*Cnidoscolus aconitifolius* ssp. *aconitifolius* Breckon), also known as spinach tree, is an attractive shrub that slightly resembles a hibiscus or cassava plant. With a protein content of 57 g kg^{-1} fresh leaves (with outstandingly well-balanced amino

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acids), chaya is considered one of the most nutritious leafy green vegetables grown on land. Furthermore, the plant is rich in vitamin A, vitamin C, calcium, potassium, iron, and antioxidants.

Chaya production systems are noteworthy in that they require few external inputs as well as simple crop management. The shrub, which is usually reproduced by clones, can be grown on different soils. Given that no significant pests or diseases have been reported for the crop, and due to its modest crop management and high nutritional value, chaya has undoubtedly the potential to alleviate nutritional deficits in hot regions of developing countries all around the globe. Thanks to its high protein content, the crop can additionally serve as an attractive enrichment of vegetarian diets; no wonder that chaya is considered one of the most promising edible horticultural crops worldwide. Furthermore, a more widespread use of chaya as medicinal plant is highly conceivable—and its potential use as forage plant is still underexploited. With all this potential, chaya can be undoubtedly labeled one of the new potential 'super crops.'

6.2 Taxonomy

The chaya is a leafy and shrubby *Euphorbiacea* (Quezada Tristán et al. 2006). *Euphorbiaceae* comprehends over 8000 species structured in five subfamilies: *Phyllanthoideae*, *Oldfieldioideae*, *Acalyphoideae*, *Euphorbioideae*, and *Crotonoideae.* The latter includes *Cnidoscolus* spp., classified in the tribe *Manihoteae* (Webster 1994). All *Euphorbiaceae* are dicotyledons with unisexual and often small flowers. In Mexico, there are 43 genera and 782 species (as well as 32 intraspecific taxa) of *Euphorbiaceae*, which can be found from sea level to 3000 m altitude. The most diversified genera are *Euphorbia* spp. (241 spp., 31% of the species of *Euphorbiaceae* known of Mexico) and *Croton* spp. (124 spp., 16%). Fifty-seven percent of the Mexican species are endemic, which represents 9% of *Euphorbiaceae* worldwide. *Euphorbia pulcherrima* Willd. ex Klotzsch, the poinsettia, is probably the best known *Euphorbiaceae*. Of the introduced species, *Manihot esculenta* Crantz, cassava or manioc, and *Ricinus communis* L., the castor bean, are most frequently cropped in Mexico (Steinmann 2002).

Cnidoscolus spp. itself includes 67 species. The genus is endemic to the Americas and mainly distributed in Mexico and Brazil, but is also found in the Caribbean and in remaining South America (Fernández Casas 2008). In Mexico, 24 species of Cnidoscolus have been identified, 19 of them are endemic. Formerly, many species of *Cnidoscolus* spp. were grouped with the genera *Manihot* and *Jatropha* (Steinmann 2002). Due to their ardent trichomes, petiolar or foliar glands, and a characteristic single white floral envelope, *Cnidoscolus* spp. was considered an independent genus by McVaugh (1944). This classification was upheld by later anatomic and pollen morphological studies (Miller and Webster 1962).

C. aconitifolius is one of the most widespread species of this genus. The word *Cnidoscolus* is derived from two ancient Greek words that mean nettle and thorn,

while *aconitifolius* means leaves like aconitum (Fernández Casas 2007). Being commonly cultivated, it is assumed the species is not under threat (Ocampo and Balick 2010). *C. souzae* McVaugh is the morphologically closest species to cropped chaya. Chaya is correctly denominated as *C. aconitifolius* ssp. *aconitifolius* Breckon. Yet, there is serious taxonomic confusion regarding the plant: In the literature, the synonym *C. chayamansa* McVaugh is found frequently, even in recent publications, where it refers to the domesticated varieties of chaya (Ross-Ibarra 2003). Contrariwise, *C. aconitifolius* refers to wild phenotypes as well as to four cultivated varieties of chaya, including one called 'Chayamansa.' *C. multilobus* (Pax) I. M. Johnston, a different plant, is occasionally also called chaya (Fernández Casas 2008). There are acknowledged researchers, such as Breckon (1975), that do not consider chaya a domesticated plant. Still, for pioneers on chaya such as Jeffrey Ross-Ibarra (2003), chaya meets all the requirements of a domesticated crop.

The name chaya comes from the Yucatec Maya word *chay*, which refers to both cultivated and wild *C. aconitifolius.* Additionally, chaya has common names from diverse linguistic backgrounds for chaya (Fernández Casas 2007). Many of these names are Spanish and refer to the urticating hairs of the plant, e.g., *picar* (to sting). Other names denote the use of chaya as a leafy green vegetable, hence the English term 'tree spinach' (Ross-Ibarra and Molina-Cruz 2002; Table 6.1).

Table 6.1 Common names for chaya (Fernández Casas 2007; González Arce 2008; Mariaca-Méndez and de Tabasco 2012; Ocampo and Balick 2010; Ross-Ibarra 2003)

Region or country	Common names
Yucatan Peninsula	Chaya, s picar, chaya amarilla, chaya brava, chaya del monte, chaya pica, jom chaay, k'an chaay, keken-chay, ts'its'ik-chay, x'etel, xtsaj, chaay, chaay kool, k'eek en chaay, xe'tel, saj, tsaaj
Chiapas	Kulis ek, salik la, sla ek
Oaxaca	Mala mujer
Veracruz-Llave	Chaya
Remaining Mexico and Belize	Chaya, picar, mala mujer, tza, tsats, x'chay, ya'ax chay
Guatemala	Chaya, chay, chatate, chayo, copapayo
Honduras, Cuba	Chaya
Nicaragua	Quelite
Costa Rica	Chicazquil, chicsaquil
Cuba	Chaya
Virgin Islands	Tree spinach
USA	Chaya, tree spinach, spinach tree
Philippines	Chaya

6.3 Origin and Actual Distribution

Chaya is both wild and domesticated. Wild chaya has extensive distribution, growing from southern Texas south along the Gulf Coast, throughout Southeastern Mexico and the entire Central America down to Colombia, with a disjunct population in the Mexican state of Guerrero on the Pacific coast (Colunga-García Marín and Zizumbo-Villarreal 2004; Ross-Ibarra and Molina-Cruz 2002).

There is agreement that wild chaya is of Mesoamerican American origin (Quezada Tristán et al. 2006) and that it was domesticated in a region that includes Guatemala, Belize, South, and Southeast Mexico (Ross-Ibarra and Molina-Cruz 2002). Yet, there are contrary theories as to where chaya was first domesticated. Like many acknowledged researchers, Ross-Ibarra (2003) favors the Yucatan Peninsula and the Mexican state of Chiapas as the origin of domesticated chaya. Ross-Ibarra sustains this theory through ethnobotanical arguments: These are the areas with the most extensive and diversified medicinal and nutritional uses of chaya by the local population.

However, Breckon (1975) argues that at the very least the variety 'Chayamansa' comes from the Department of Petén in Guatemala, which is also the only region where all four varieties of chaya ('Chayamansa,' 'Estrella,' 'Picuda,' and 'Redonda'; the latter is almost exclusively found in Guatemala), are being cropped. Since there was continuous information and plant material exchange between Mayas in the lowlands of Petén and on the neighboring Yucatan Peninsula (Ford 2008), it is certainly plausible that 'Chayamansa' later moved to Yucatan. There it could have become quickly widespread due to the poor soil fertility and low soil depth on the peninsula, which favors the cropping of chaya over more demanding traditional Mesoamerican crops. What is for sure in this debate is that domesticated chaya has been cultivated since pre-Hispanic times in all regions inhabited by Maya population. Although cropped Chaya is still most common in Mesoamerica (Fernández Casas 2007), it can now be found all over the world, often in milder, more humid, and even under significantly dryer conditions than in Mesoamerica. There is evidence of chaya being cropped from 0 to 1000 m above sea level—which does not necessarily imply its altitude limit.

Cropped chaya is mostly distributed throughout the Yucatan Peninsula, which apart from the Mexican states of Campeche, Quintana Roo, and Yucatán, and also includes the Petén in Guatemala and the northern part of Belize (De Clerck and Negreros-Castillo 2000; Fedick et al. 2008; Flores-Delgadillo et al. 2011; Mariaca-Méndez and de Tabasco 2012; Ocampo and Balick 2010; Ross-Ibarra and Molina-Cruz 2002; Standley and Steyermark 1958). As for the rest of Mexico, there is evidence that chaya is currently cropped in Aguascalientes, Chiapas, Coahuila, Colima, Jalisco, Guerrero, Guanajuato, Hidalgo, Morelos, Oaxaca, Puebla, Querétaro, San Luis Potosí, Tabasco, Tamaulipas, and Veracruz-Llave. In Chiapas and Veracruz-Llave, chaya has been known for centuries; in most of the other states, chaya was introduced recently (Colunga-García Marín and Zizumbo-Villarreal 2004; Fernández Casas 2007; Quezada Tristán et al. 2006; Ross-Ibarra 2003).

Cropping of chaya is evidenced all over Central America. It has a strong presence in Belize (Berkelaar 2006; Fernández Casas 2007; Ross-Ibarra 2003), Guatemala, and Costa Rica, where it was brought to from Mexico in the 1980s (Ocampo and Balick 2010; Ross-Ibarra and Molina-Cruz 2002). Chaya is also cropped in Honduras, El Salvador, Nicaragua, and Panama (Fernández Casas 2007; Ross-Ibarra 2003). The shrub grows in South America (Poot-López et al. 2012) and has been documented in Bolivia, Brazil (Berkelaar 2006), and Colombia (Ross-Ibarra and Molina-Cruz 2002).

After Mesoamerica, the Caribbean is the region with the second highest distribution of chaya. Use and production of the crop are particularly popular in Cuba (Fernández Casas 2007; Ross-Ibarra and Molina-Cruz 2002; Stephens 1994). Additionally, the shrub can be found on the Virgin Islands (Fernández Casas 2007), in Puerto Rico (Newton 1984), the Bahamas, and the Dominican Republic (Berkelaar 2006). Chaya cuttings from Cuba were also introduced to southern Florida, where it is often found as a wild shrub, but rarely used as food (Stephens 1994). In suburban areas of the Southwestern United States, especially in south Texas, however, chaya has been introduced recently by its Hispanic population for its use as a vegetable and as medicinal plant (Kuti and Torres 1996).

Not only did chaya spread from the Caribbean to the USA, but also to Africa: In the second half of the twentieth century, it was introduced to Ghana from Puerto Rico. Subsequently, its cultivation spread into neighboring Nigeria (Newton 1984). Then, it became popular in most tropical Africa, especially in Eastern Africa. There, it can be found in Kenya, Zambia, and Tanzania (Berkelaar 2006). Shortly after being brought to Africa, chaya production also started in Asia, where it was first evidenced in Brunei (Peregrine 1983). The Philippines (Fernández Casas 2007) and Indonesia are further Asian countries for which chaya consumption and cropping are documented. Recently, chaya was introduced to the Marshall Islands, the Federated States of Micronesia, Fiji, and Vanuatu. Hawaii is the part of Oceania, where chaya has the longest history: In the mid-twentieth century, it was established by the US government as a new perennial vegetable. Despite little presence in Hawaiian cuisine, the shrub can still be observed growing wild (Berkelaar 2006).

6.4 Ethnobotany

Since the limestone bedrock of the Yucatan Peninsula is covered by a thin layer of soil, the region is often characterized as a challenging environment for agriculture. Yet the Maya inhabitants, both ancient and modern, have managed to successfully cultivate this landscape through a variety of innovative techniques and microscale adaptations (Fedick et al. 2008). In the Peninsula, the most relevant cropping system—which is found throughout Mesoamerica—is undoubtedly the *milpa* (Hernández 1995). In the *milpa*, at least two varieties of maize (*Zea mays* L.) are associated with diverse legumes, squash (*Cucurbita moschata* Duchesne) and a varying number of other crops (Toledo 2003). Before and long after the conquest, the *milpa* system has sus-

tained a large indigenous population on the Yucatan Peninsula in a relatively secure food situation.

The Yucatec Maya also integrated other production systems in their agroecological portfolio, such as the family home gardens (Fig. 6.1). These gardens are highly agrobiodiverse, providing the peasant family with esculents as well as medicinal and ornamental benefits. They also provide material for construction and secondary functions, such as generating shadow and delivering an optimum environment for small-scale backyard animal breeding. Home gardens are based on native and introduced shrub and tree species, among them wild and domesticated chaya (Mariaca-Méndez and de Tabasco 2012; Colunga-García Marín and Zizumbo-Villarreal 2004). The soil of most home gardens does not show major signs of physical and chemical degradation despite prolonged and intensive land use, a rare situation for agricultural soil in the tropics. The healthy state of these soils is contributed to the cultivating skills of Maya women, who are usually in charge of home gardens and the fact that plantings are situated within natural cavities that are common in the karstic Yucatec bedrock. These cavities are filled with both natural and household organic residues, providing an efficient substrate for cropping perennial plants. Home gardens are usually located on family-owned parcels or *solars* (Fedick et al. 2008). In contrast to *milpas* which are often walking or biking distance from the homes, *solars* are close to or in the family homes plots. Chaya has been an integral part of these gardens for generations.

Such a long-lasting relationship between Maya people and chaya is accompanied by cultural practices for the cultivation and care of the chaya shrub. A widespread belief in Mesoamerica, for example, is that one must ask a chaya plant for permission before harvesting to avoid being stung by its spines. Similarly, chaya is said to

Fig. 6.1 Chaya is commonly used as a hedge plant in traditional Yucatec Maya home gardens

require a special way of cutting to avoid harmful twitches and greetings such as 'Good morning Mrs. Chaya! Will you give me leaf?' are common. It is also believed that the plant, and thus its spines, wakes up with the arrival of the sun, and that to harvest leaves safely, they should be cut in the early morning or late evening (Mariaca-Méndez and de Tabasco 2012; Ross-Ibarra and Molina-Cruz 2002).

6.5 Morphology

Chaya is an evergreen or drought-deciduous, fast-growing, semi-perennial, and semi-woody tropical shrub (Berkelaar 2006; Ross-Ibarra and Molina-Cruz 2002; Standley and Steyermark 1958). Some authors, such as Ocampo and Balick (2010), report that chaya reaches a height maximum of three meters, but most have observed that it reaches a height between three and five meters (Breckon 1975; Ross-Ibarra and Molina-Cruz 2002; Standley and Steyermark 1958). This discrepancy is probably due to the observation of higher wild and cropped, respectively pruned, lower plants. Its width is approximately two meters (Berkelaar 2006).

Chaya leaves look like those of okra. They are dark green and commonly surfaced with some hairs. Each leaf is borne on a slender 10–30 cm long and succulent petiole. Young leaves are often lacking lobes, while mature chaya leaves are shallowly or deeply, palmately lobed with three to seven alternately arranged lobes per leaf. The blades of mature leaves are about 30 cm long and broad, and the leaves exhibit entire to slightly dentate margins and abundant latex. Where the stem connects to the leaf, the veins are fleshy and cuplike (Ocampo and Balick 2010; Ross-Ibarra and Molina-Cruz 2002; Standley and Steyermark 1958; Stephens 1994).

Like many *Euphorbiaceae*, *Cnidoscolus* spp. is characterized by irritating latex and highly stinging trichomes (Fig. 6.2) that contain toxic compounds like linamarin and glycoside flavonoids. For chaya, they are an effective defense mechanism, preventing leaf damage by herbivorous insects; also, human contact results in a very strong skin irritation (Rates 2001; Scheman and Conde 2001; Torres-González and García-Guzmán 2014; Tuberville et al. 1996). These stinging hairs are predominantly found on the young chaya stem (Stephens 1994). Noteworthy, more irritating leaf latex means less presence of urticant pubescences on stems, leaves, flowers, fruits, and vice versa (Miranda Velásquez et al. 2016; Parra-Tabla et al. 2004; Torres-González and García-Guzmán 2014).

There is a considerable inter- and intravarietal morphological diversity of chaya leaves. In a study of Quezada Tristán et al. (2006), chaya plants from 13 Mexican states were observed for differences on their leaves. The results showed that 91.9% of the monitored plants had three-lobed leaves, 7.9% five-lobed, and 0.2% four-lobed leaves. Furthermore, 80% of the leaves had sagittate shape, while 10% were decurrent and 10% showed chordate shape. Nineteen percent of observed leaves manifested pubescence and 96% glands.

Chaya is a self-compatible, monoecious species with separate male and female flowers located at the end of long flower stems. Each flower exhibits defunct reproduc-

Fig. 6.2 Urticant pubescences on stems and leaves of wild chaya

tive organs of its opposite sex (Parra-Tabla et al. 2004; Ross-Ibarra and Molina-Cruz 2002; Stephens 1994). Its flowers are white and tubular. They are usually arranged on cyme-branched and three-forked inflorescences, in which the pistillate flowers are located on the basal fork and the staminate flowers are expanded distally from the base of the lobes (Kuti and Torres 1996; Parra-Tabla et al. 2004). Both kinds of flowers are small and less than 10 mm long. The white male flowers are much more abundant (Stephens 1994). Chaya flowers bloom frequently (Ocampo and Balick 2010). They are considerably fragrant, making them attractive to pollinators, predominantly butterflies and several bee species, including *Apis mellifera* L. (CC Grow 2015; Parra-Tabla et al. 2004). The fruits of *C. aconitifolius* are schizocarpic, with rounded pods, approximately 2.5 cm wide, have three seeds, and are dehiscent by explosion (Ocampo and Balick 2010; Standley and Steyermark 1958).

Chaya has fibrous roots, which are a consequence of their asexual reproduction using stem cuttings (Aguilar et al. 2011). The wood of young stems is soft, easily broken, and susceptible to rot. When cut, the stem exudes a white latex (Stephens 1994).

6.6 Composition

Since chaya contains high amounts of crude protein, minerals, and vitamins, the plant can be considered among one of the most complete vegetables (Table 6.2).

The chaya leaf contains high levels of all amino acids, with the exception of arginine and glutamine (Kuti and Kuti 1999). Its well-balanced protein content over

Table 6.2 Content of water, nutritional composition, and nutritional value of 1 kg fresh leaves of chaya (*C. aconitifolius*), spinach (*Spinacia oleracea* L.), chard (*Beta vulgaris* L. subsp. *vulgaris* DC), lettuce (*Lactuca sativa* L.), and curly kale (*Brassica oleracea* convar. *acephala* Alef. var *sabellica* L.) (Aguilar et al. 2000; Kuti and Torres 1996; Vogel 1996)

Crop	Water	Protein	Fat	Crude fiber	Carbohydrates	Ash	Nutritional value
	%	g	g	g	g	g	kJ
Chaya	85.3	57	4	19	42	22	1140
Spinach	91.6	25	2	18	5	18	640
Chard	92.2	21	3	10	7	17	590
Lettuce	95	13	2	15	11	6	480
Kale	86.3	43	9	42	25	11	1550

Table 6.3 Mineral, metal, and vitamin content of 1 kg fresh leaves of chaya, spinach, chard, lettuce, and kale (Kuti and Torres 1996; Vogel 1996)

Crop	Ca	K	P	Fe	Provitamin A	Vitamin B	Vitamin C
	mg	mg	mg	mg	mg	mg	mg
Chaya	1994	2172	390	114	8	8	1650
Spinach	1250	6350	550	40	4	3	500
Chard	1050	3750	400	27	35	3	400
Lettuce	350	2250	350	11	8	2	130
Kale	2100	4900	850	19	41	3	1050

performs popular leafy greens such as spinach, chard, lettuce, and kale (Aguilar et al. 2000; Kuti and Torres 1996; Santacruz et al. 2013; Sarmiento-Franco et al. 2003a, b). In regard to mineral and metal content, chaya is also an outstanding vegetable: Compared to spinach, chaya offers almost three times more iron and twice the amount of calcium. The same applies for its contents of vitamin B and C, where chaya is superior to most leafy greens (Table 6.3).

6.7 Biodiversity

Compared to other crops, the domestication of chaya had a relatively short-term process of approximately 500 years (Ross-Ibarra and Molina-Cruz 2002). The principal selection criteria were leaf size and form, the reduction of trichomes, and a decrease of reproductive growth. Given the climatic and edaphic diversity of the regions where chaya is historically cropped, an adaptation to different environments certainly also played an important role in its diversification process (Ross-Ibarra 2003). Apart from wild phenotypes, four cropped varieties of chaya have been identified: 'Estrella,' 'Picuda,' 'Redonda,' and 'Chayamansa.' Since wild and cropped chaya is one and

the same plant, morphological and physiological differences between them are fluent. The four recognized varieties differ from wild chaya in their leaf morphology and leaf size (Stephens 1994). Chaya varieties also differ in the presence and density of stinging hairs and their cyanide content (Abdala-Roberts and Parra-Tabla 2005). A further distinction criterion is fertility (Table 6.4).

Wild chaya (Fig. 6.3), which is called 'Tzin-tzin chay' or 'X'etel' on the Yucatan Peninsula, has relatively heterogenic leaves and high fertility. It is rarely eaten (Ross-Ibarra 2003; Stephens 1994). This circumstance can undoubtedly be ascribed to its frequent, white, tall, stiff, and highly irritating stinging hairs; they are found on all leaf veins and margins. Compared to the domesticated varieties, wild chaya is characterized by notably big leaves. They can have five to nine lobes, of which the central one stands out for being the longest and widest. The apex of all lobes is acuminated. The petiole of wild chaya leaves is remarkably long and entirely covered by trichomes. The trunk of wild chaya is slightly lignified and has a round form. It does not break as easily as the stems of domesticated chayas and is entirely covered by trichomes. Wild chaya blooms all year and produces abundant fruits. In traditional Maya medicine, it is used to cure chronic wounds.

In contrast, 'Chayamansa' (Fig. 6.4), often simply referred to as 'chaya,' 'sweet chaya,' or 'plegada,' is clearly the most domesticated variety. The usual overlapping of the central three lobes cannot be found in wild chaya. Furthermore, leaves are not covered by stinging hairs (Ross-Ibarra and Molina-Cruz 2002). The petioles are short and smooth. Normally, 'Chayamansa' achieves a maximum height of 170 cm. The

Table 6.4 Morphological differences of four identified chaya varieties (Ross-Ibarra 2003; Ross-Ibarra and Molina-Cruz 2002; Stephens 1994)

Variety	Leaf morphology	Number of lobes	Stinging hairs	Fertility
'Chayamansa'	Three obovate central lobes overlap two adjacent ones	5	Degenerated hairs along the petiole and the bottom margin of the lamina	Low and never viable seed production; commonly empty anther sacks
'Redonda'	Juvenile leaves: often entire; mature leaves: entire to slightly dentate margins	3 (mostly)	Very scarce and atrophied	Mature plants flower and actively produce pollen
'Picuda'	Lobes are strongly dentate to pinnatifid	5–9	Trichomes are scarce but usually filled; they appear empty and flaccid	Produces abundant quantities of seeds, pollen, and mature fruit
'Estrella'	Spreading, non-overlapping dentate lobes	5	Similar to 'Picuda'	Scarce flowers; low fertility; no mature fruits

Fig. 6.3 Leaves of wild chaya show five to nine lobes and large trichomes

Fig. 6.4 'Chayamansa' is the most popular variety in the Yucatan Peninsula

stems are scarcely lignified, plain, succulent, and quadrangular. They break easily. ‘Chayamansa’ flowers intensely with up to fifty flowers per plant. Its anthers produce at best a few, usually deformed, pollen grains. On the Yucatan Peninsula, this variety is appreciated for its sweet taste and the softness of its leaves.

‘Redonda’ (Fig. 6.5), occasionally called ‘Mansa,’ ‘In Sul,’ or ‘Pig Chaya,’ represents the least known and unrecognized chaya variety. In Maya home gardens, ‘Redonda’ reaches heights between two to three meters. It has small leaves with a smooth lamina and (normally three) relatively broad, rounded lobes. ‘Redonda’ has a low density of atrophied trichomes on its leaves. Leaf petioles are long and plain. The variety is characterized by scarcely lignified, plain, and quadrangular stems, which tend to break easily. Tests showed that less than 1% of this pollen is viable and mature fruit and seed are extremely rare (Ross-Ibarra and Molina-Cruz 2002; Stephens 1994). Different from other varieties, ‘Redonda’ is prepared by frying instead of boiling. Its resin is occasionally used to cure insect bites.

Also, ‘Picuda’ (Fig. 6.6) rarely exhibits trichomes. On the Yucatan Peninsula, ‘Picuda’ is frequently labeled ‘Tzin-tzin chay,’ which refers to the resemblance of its leaf morphology with wild chaya. The plant usually has five to seven lobes, occasionally up to nine, which are strongly dentate to pinnatifid and the apex is acuminate or caudate. Scarce trichomes are observed on the veins and are considerably denser on

Fig. 6.5 Juvenile leaves of ‘Redonda’ are rarely lobed

Fig. 6.6 'Picuda' is a highly fertile chaya variety

the long leaf petioles. The stems of 'Picuda' are thin and round and are not covered by trichomes. 'Picuda' is considered a highly fertile variety of chaya. Nonetheless, samples in Maya home gardens have demonstrated that there are also completely unfertile individuals. Noteworthily, it is the only variety whose roots are used in traditional medicine, particularly to cure kidney infections.

'Estrella' (Fig. 6.7), also known as 'X'etel' on the Yucatan Peninsula, shows leaf similarities with wild chaya. It shares this characteristic with 'Picuda,' but differs from it in having less fine lobes (Ross-Ibarra 2003; Ross-Ibarra and Molina-Cruz 2002). The leaf lamina of 'Estrella' is smooth. The leaves usually have five lobes

Fig. 6.7 'Estrella' is characterized by spreading, non-overlapping dentate lobes

whose apex is acuminate and the leaf margins are scarcely toothed. Trichomes are dense and frequent on the large petioles of 'Estrella' leaves, yet sparsely located on the margins and central leaf vein. The lignified stems are covered by short, brown trichomes. The variety never produces mature fruit. Only young leaves are eaten; their taste is considered less sweet compared to 'Chayamansa.'

'Estrella' and 'Picuda' are usually not considered entirely domesticated and rather represent cropped wild plants versus varieties. Both varieties exhibit few morphological differences compared to wild chaya and can be reproduced sexually. Consequently, 'Estrella' also shows the highest genetic diversity of all varieties; it is followed by 'Picuda,' which is still more diverse than wild chaya. In comparison, 'Redonda' and 'Chayamansa' meet all of the requirements for a domesticated crop; they have lost their ability of sexual reproduction, which makes human intervention indispensable, and they demonstrate low genetic diversity and have morphological characteristics (especially on their leaves; Fig. 6.8) which are rarely found in wild chaya (Ocampo and Balick 2010; Ross-Ibarra 2003). The clonal structure is another meaningful indicator for the current state of domestication of a crop. Ross-Ibarra (2003) identified in his benchmark publication on the origin of chaya, that throughout Mexico and Guatemala, only one clone for 'Chayamansa,' and three clones of 'Redonda' existed, while 'Estrella' and 'Picuda' possessed significantly more clones.

On the Yucatan Peninsula, 'Chayamansa' is the most common variety, while 'Estrella' and 'Redonda' are rare, and 'Picuda' is almost unknown. On the contrary, in Central and South America (except Guatemala, where there is only ornamental use), 'Picuda' is the most popular variety, whereas 'Chayamansa' and 'Estrella' are less frequent. Finally, 'Redonda' is popular in Guatemala and the Mexican state of Chiapas (Ross-Ibarra 2003; Ross-Ibarra and Molina-Cruz 2002).

6.8 Uses

6.8.1 Nutritional Use and Dishes

Chaya is a highly nutritious food, whose leaves taste somewhat like spinach. For consumption, the leaves and shoots are cut into manageable pieces and can be fried, boiled, frozen, or canned for later use (CC Grow 2015; Loarca-Piña et al. 2010; Ross-Ibarra and Molina-Cruz 2002). An obstacle regarding the usability of chaya as food or fodder is the presence of cyanogenic glycosides in fresh leaves (García-Rodríguez et al. 2014; Miranda Velásquez et al. 2016; Stephens 1994). González-Laredo et al. (2003) demonstrated a hydrogen cyanide concentration of 23.7–42.5 mg kg^{-1} dry leaves. Fortunately, boiling eliminates this anti-nutritional compound (Castro-Juárez et al. 2014; Molina-Cruz et al. 1997). Up to 15 min is recommended for boiling (Molina-Cruz et al. 1997; Quezada Tristán et al. 2006; Stephens 1994). Although drying the chaya leaves also reduces their cyanogenic glycosides content,

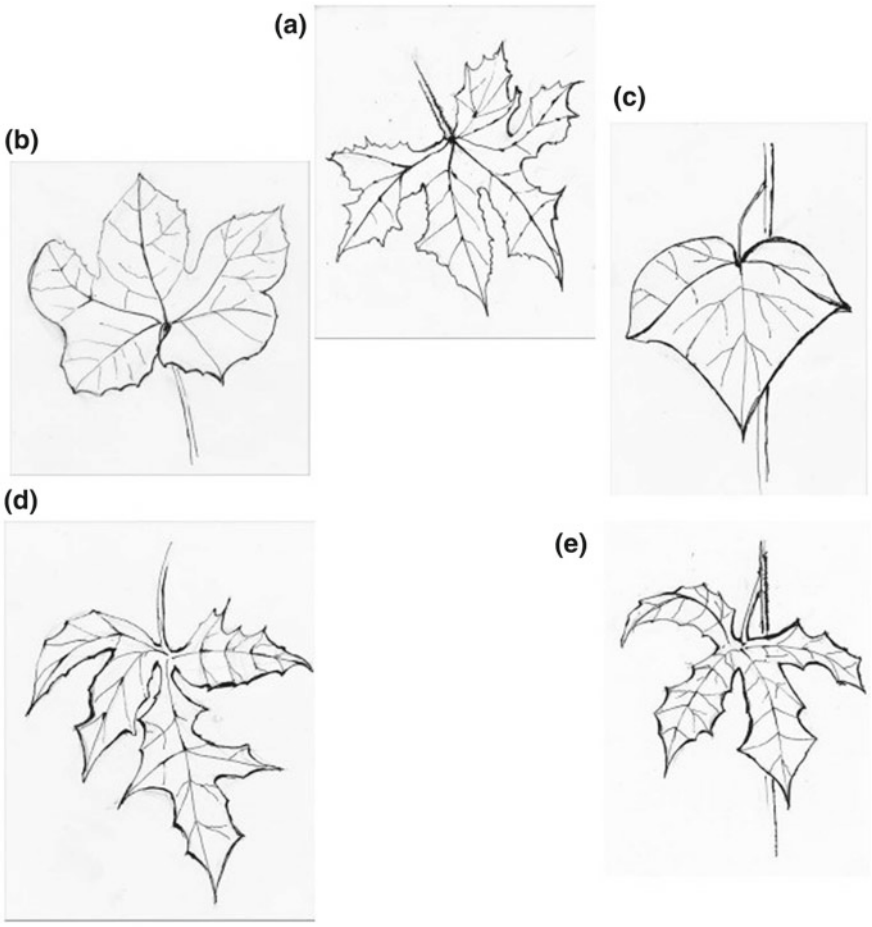

Fig. 6.8 Leaf morphology of wild chaya (**a**), variety 'Chayamansa' (**b**), juvenile leaves of variety 'Redonda' (**c**), variety 'Picuda' (**d**), and variety 'Estrella' (**e**). Drawing: Sylvia Ebel

complete elimination still requires subsequent cooking (Berkelaar 2006; González-Laredo et al. 2003).

Removing cyanides in fresh chaya through drying or cooking affects the nutritional content of the chaya leaves. Drying chaya leaves increases the ash, fiber, and protein content more than four times and the content of fats even more, but also significantly decreases the vitamin C content (Durán Mendoza et al. 2012; Molina-Cruz et al. 1999). Boiling the chaya leaves is more common. It causes a relative increase of carbohydrates, fat, calcium, phosphorous, and iron and decreases crude fiber, protein as well as potassium (Kuti and Torres 1996; Table 6.5). Vitamin C is transferred from the leaves to the water when boiling, while vitamin B is conserved through cooking (Molina-Cruz et al. 1997).

Table 6.5 Nutrient content of 1 kg fresh and cooked chaya leaves (Kuti and Torres 1996)

	Protein	Fat	Crude fiber	Carbohydrates	Ca	K	P	Fe
	g	g	g	g	g	g	g	g
Fresh leaves	57	4	19	42	1.99	2.17	0.39	0.11
Cooked leaves	41	5	14	77	2.15	1.4	0.45	0.2

Since pre-Columbian times chaya leaves and young shoots have been consumed on the Yucatan Peninsula (Martin et al. 1977), the region where most information about chaya as food is available. Two sixteenth-century Spanish chronicles mention a wide use of the plant on the Yucatan Peninsula at the time of the European conquest. Its continuity is evidenced up to the twenty-first century. Even now, chaya is consumed weekly in rural communities of the Peninsula, where it is sold in almost all markets and supermarkets (Ross-Ibarra 2003; Ross-Ibarra and Molina-Cruz 2002). Chaya is eaten all year long, although it is most abundant during the rainy season.

For the Yucatec Maya population, chaya is the essential vegetable protein source after legumes (Salazar et al. 2016). In times of food scarcity, both domesticated and wild chaya even serve as a replacement for meat, which is why chaya is occasionally associated as food for the poor (Fernández Casas 2007; Ross-Ibarra and Molina-Cruz 2002). Nevertheless, chaya continues to be an essential ingredient of plentiful regional dishes. Usually, the larger mature leaves are used as wraps and the young leaves and apical shoots are eaten (Loarca-Piña et al. 2010).

Modern usage of chaya on the Yucatan Peninsula is highly diverse, and at least 70 recipes are documented. Often leaves are simply eaten after boiling them in water with salt (Ross-Ibarra and Molina-Cruz 2002) or served with oil (Stephens 1994). Various traditional Maya dishes combine chaya with crops obtained from the *milpa*, such as maize, bean, squash, and chili pepper, and other products from the home garden. In a study done in a representative Maya community, 5% of regularly consumed dishes included chaya and 3% showed chaya as primary ingredient (Salazar et al. 2016).

Different variations of the following traditional recipes exist throughout the Yucatan Peninsula: A well-known chaya dish is *Dzotobichay*, also called *Brazo de la India*, a *tamal* filled with diced chaya leaves, covered with a sauce made of tomato or other vegetables, wrapped in banana or chaya leaves. *Tamal* is a dish, where nixtamalized[1] maize dough and lard are mixed, covered by a maize husk or diverse leaves and later steamed. Usually, they are filled with vegetables or meat. Also popular is *Pibxcatic*, stuffed peppers served over chaya leaves, which is often combined with *Cochinita Pibil*, slow-roasted shoat, intensively marinated with sour orange and annatto and wrapped in banana leaves. A traditional dish known as *Chay je'* in which previously boiled chaya leaves are mixed with eggs, onions, and occasionally tomatoes, is gaining popularity in the larger cities of the Yucatan Peninsula. *Chakbilchay* is a soup made of chaya and lime; *Caldillo je'* is chaya with eggs and

[1] Nixtamalization refers to soaking and cooking maize kernels in water with a lye of lime, chalk, or wood ash.

pasta, and *Chayiwaaj* is a *tamal* that only contains chaya and lard (Ross-Ibarra and Molina-Cruz 2002; Salazar et al. 2016).

Since chaya is so versatile and can be added to soups, pasta, sauces, salads, eggs, and breads (Ross-Ibarra 2003), it is being integrated into many common Yucatan and Mexican dishes. Some examples are *Chaya K'abax* (cooked beans with chaya), *Tzajbi chay* (fried chaya), *Jorochitos de Chaya* (maize dumplings with chaya), *Enchiladas de chaya* (fried chaya, wrapped in tortilla and covered by a sauce made of tomato or tomatillo), or *Escabeche de Chaya* (pickled chaya).

As for drinks, chaya infusions are consumed regularly in the Maya region of the Yucatan Peninsula—but with a predominantly medicinal purpose (Andrade-Cetto et al. 2006). In restaurants with non-indigenous clients, shakes made of blended fresh chaya leaves, fruits, and sugar are offered regularly.

6.8.2 Medicinal Use

Chaya is a versatile medicinal plant. A total of 15 constitutional effects are reported for the plant:

- Antidiabetic and hypoglycemic (Loarca-Piña et al. 2010; Palos Suárez et al. 2006; Valenzuela Soto et al. 2015);
- Anti-inflammatory;
- Antimicrobial (Obichi et al. 2015);
- Antimutagenic (González-Laredo et al. 2003);
- Antioxidant (González-Laredo et al. 2003; Gutiérrez Zavala et al. 2007; Kuti and Konuru 2003; Loarca-Piña et al. 2010);
- Bone and muscle-building (León de Gutiérrez and Díaz Bolio 1971);
- Cardioprotective and circulation restorative (Diaz-Bolio 1974);
- Cholesterol lowering (Diaz-Bolio 1974; Mariaca-Méndez and de Tabasco 2012);
- Diuretic (Andrade-Cetto et al. 2006; Diaz-Bolio 1974);
- Hepatic (Fernández Casas 2008);
- Lactation stimulant (Fernández Casas 2008);
- Laxative (Diaz-Bolio 1974)
- Memory restorative and brain stimulant (Donkoh et al. 1999);
- Nail strengthening (Diaz-Bolio 1974);
- Vision improving (Diaz-Bolio 1974; Donkoh et al. 1999).

On the Yucatan Peninsula, chaya is widely sold in local markets as a medicinal plant, although occasionally other plants are traded under the name chaya. The respective knowledge is passed from generation to generation. Although most of its medicinal properties have not been clinically tested (Ross-Ibarra and Molina-Cruz 2002), chaya is used to cure a large variety of ailments:

- Alcoholism (Donkoh et al. 1999);
- Anemia (Fernández Casas 2008; León de Gutiérrez and Díaz Bolio 1971);

- Atherosclerosis (Diaz-Bolio 1974);
- Constipation (León de Gutiérrez and Díaz Bolio 1971);
- Cough (León de Gutiérrez and Díaz Bolio 1971);
- Diabetes (Castro-Juárez et al. 2014; Kuti and Kuti 1999; Kuti and Torres 1996; León de Gutiérrez and Díaz Bolio 1971; Loarca-Piña et al. 2010; Valenzuela Soto et al. 2015);
- Elevated uric acid (León de Gutiérrez and Díaz Bolio 1971);
- Eye irritations (León de Gutiérrez and Díaz Bolio 1971);
- Fatigue (Ross-Ibarra and Molina-Cruz 2002);
- Gallstones (Diaz-Bolio 1974);
- Gastrointestinal disorders;
- Gout (Donkoh et al. 1999);
- Headache (Fernández Casas 2008);
- Hemorrhoids (León de Gutiérrez and Díaz Bolio 1971);
- Infections of teeth, tongue, and gum (León de Gutiérrez and Díaz Bolio 1971);
- Inflammation of veins (León de Gutiérrez and Díaz Bolio 1971);
- Insomnia (Donkoh et al. 1999);
- Menstruation pain and fatigue (León de Gutiérrez and Díaz Bolio 1971);
- Muscle disorders (Ross-Ibarra and Molina-Cruz 2002);
- Overweight (León de Gutiérrez and Díaz Bolio 1971).

Chaya is also increasingly popular in Western herbal and naturopathic medicine, for many reasons, including inefficiencies in conventional medicine or abusive or incorrect use of synthetic drugs resulting in severe side effects. Additionally, a large percentage of the world's population does not have access to conventional medicine and plants like chaya are an accessible and effective source for treating illnesses (Rates 2001). However, caution should be used with chaya. Although the plant is not lethal at a single dose, chaya does contain cyanide. Thus, the current research on chaya places emphasizes on the importance of the dose for receiving benefits from this plant (García-Rodríguez et al. 2014).

In traditional Mesoamerican medicine, chaya is most applied as a remedy for the treatment of diabetes mellitus (Castro-Juárez et al. 2014; García-Rodríguez et al. 2014), particularly in the treatment of noninsulin-dependent (type II) diabetes (Kuti and Kuti 1999; Loarca-Pina et al. 2010). This application is also popular among Hispanics in the USA (Kuti and Torres 1996). Chaya decreases the level of blood glucose, which is most likely related to the level of glycosides found in the plant. In medical animal experiments, chaya consumption was shown to reduce blood glucose levels of diabetic rats (Valenzuela Soto et al. 2015) and rabbits (Kuti and Torres 1996). Such studies, however, are not considered to be entirely conclusive. For example, Palos Suárez et al. (2006) reported that feeding chaya to diabetic rats did not affect their blood levels of glucose, but did decrease their triglyceride levels. It is also noteworthy that chaya may only be hypoglycemic for diabetic individuals: blood sugar levels increased for non-diabetic rabbits fed with chaya tea (Kuti and Torres 1996).

The cardioprotective activity of chaya is associated with its content of antioxidant glycosides (García-Rodríguez et al. 2014; Kuti and Konuru 2003). Yet, there is an academic dispute regarding the intensity of chaya's antioxidant potential: Gutiérrez Zavala et al. (2007) detected high antioxidant properties, while González-Laredo et al. (2003) and Loarca-Piña et al. (2010) attest chaya which has poor antioxidant capacity.

Chaya's content of glycosides has been clearly proven. As for phenolic glycosides, they involve protocatechuic acid (Loarca-Piña et al. 2010), related to the antimutagenic (González-Laredo et al. 2003) and anti-inflammatory effect of chaya. Most studies suggest that chaya is poor in phenols (García-Rodríguez et al. 2014; Santacruz et al. 2013). In contrast, a considerable part of the medicinal properties attributed to chaya is related to its high content of flavonoids: Chaya leaves contain the antioxidants catechin and kaempferol, as well as the main anti-inflammatory agents, rutin and quercetin (González-Laredo et al. 2003; Loarca-Piña et al. 2010; Molina-Cruz et al. 1997). Chaya also shows presence of saponins (Santacruz et al. 2013) and glycosides that protect against hypertension and have antibiotic and anti-inflammatory properties (Obichi et al. 2015). Cholesterol-lowering sterols as well as diuretic (but also moderately toxic) coumarins were identified in chaya leaves (García-Rodríguez et al. 2014). Chaya also presents low contents of alkaloids and tannins, particularly antioxidant, anti-inflammatory, and potentially antidiabetic gallic acid (Loarca-Piña et al. 2010; Miranda Velásquez et al. 2016; Molina-Cruz et al. 1997; Santacruz et al. 2013). In addition to traditionally used aqueous extraction, the active compounds of chaya have been detected using extractions with ethanol, methanol, and hexane (Table 6.6).

Chaya is exceptionally high in calcium and iron (Berkelaar 2006). Calcium supports bone and dental health and is alleged to mitigate weight; iron supports the

Table 6.6 Chemically identified pharmacologically functional groups and active substances of chaya and respective extraction methods (García-Rodríguez et al. 2014; González-Laredo et al. 2003; Kuti and Kuti 1999; Kuti and Torres 1996; Loarca-Piña et al. 2010; Miranda Velásquez et al. 2016; Molina-Cruz et al. 1997; Obichi et al. 2015; Santacruz et al. 2013; Sarmiento-Franco et al. 2003a, b; Valenzuela Soto et al. 2015)

Functional group	Active substances	Water	Ethanol	Methanol	Hexane
Flavonoid glycosides	Amentoflavone, astragalin, catechin, dihydromyricetin, kaempferol, quercetin, rutin		X	X	
Phenolic glycosides	Protocatechuic acid		X	X	
Saponin glycosides			X		
Steroidal glycosides					X
Alkaloids		X	X		
Tannins	Gallic acid	X	X		

X Extraction with the respective substance evidenced

hemoglobin synthesis (Lukaski 2004). Chaya also contains copper, manganese, zinc, and magnesium, essential cofactors for proper enzyme activity relevant to cardiovascular homeostasis (García-Rodríguez et al. 2014; Kuti and Kuti 1999). Furthermore, the plant contains proteolytic enzymes (Iturbe-Chiñas and Lopez-Munguia 1986), which have anti-inflammatory properties and support the immune system.

Finally, numerous health benefits of chaya are related to its vitamin content. Chaya is notably high in provitamin A (Sarmiento-Franco et al. 2003a, b) and vitamin B (Obichi et al. 2015) and shows an exceptionally high vitamin C content: Only 25 g cooked chaya leaves can provide the daily dosage recommended for adults of antioxidant vitamin C. Provitamin A, which is conserved through cooking, is linked to improvement of vision, has anti-inflammatory and antimutagenic properties, and possibly also has a positive effect on human skin (Molina-Cruz et al. 1997). Vitamin B is associated with lowering cholesterol and has hypoglycemic and cardioprotective effects (Lukaski 2004).

For medicinal purposes, chaya leaves are usually used orally (Castro-Juárez et al. 2014; Ross-Ibarra and Molina-Cruz 2002). The most common preparations are aqueous extracts, such as teas and infusions (Mellen 1974; Valenzuela Soto et al. 2015). The latter are characterized by higher herbal concentrations and by a longer steeping time than for making tea. Fresh chaya leaves are cooked in water (Valenzuela Soto et al. 2015) or occasionally infusions prepared with lukewarm water are also drunken (Castro-Juárez et al. 2014). A rather unusual technique is the use of dried chaya leaves for preparing aqueous extracts (Loarca-Piña et al. 2010). Apart from the medicinal use as tea or infusion, cooked chaya leaves can be eaten (Mellen 1974) and drunk for medicinal purposes (Ross-Ibarra and Molina-Cruz 2002; Valenzuela Soto et al. 2015).

Different parts of the plant are also used to treat body ailments directly. The sap of the plant is applied directly for treating skin disorders; chaya stems or leaves are rubbed on sore or injured muscles and joints; and the painful stinging caused by chaya's trichomes is alleged to cure muscle disorders, rheumatism and arthritis (Ross-Ibarra and Molina-Cruz 2002).

6.8.3 Use as Forage

Free range pigs and chickens are common in Maya home gardens, where they are fed with in situ forages such as the chaya (Acosta et al. 1998). Due to its high content of proteins, chaya can be used as fodder in diverse silvopastoral or agroforestal systems (Santacruz et al. 2013) but also for intense animal breeding, predominantly chicken production (Aguilar et al. 2000). Chaya even serves as a functional forage for fishes, particularly tilapia (Poot-López et al. 2012). The use as forage is undoubtedly one of the most promising areas regarding innovative applications of chaya. Further research in this regard should consider diverse processing techniques to enhance digestibility and resource-efficient use of chaya.

6.9 Cropping

Chaya is commonly cropped in soil but has also been grown in black polyethylene bags with a substrate mixture of sand and compost (Valenzuela Soto et al. 2015). Dry and hot conditions are ideal for transplanting chaya (Sarmiento-Franco et al. 2003a, b; Stephens 1994), and it performs best under full sun to partial shade. The plant grows slowly under full shade but tolerates it. Chaya demands well drained, even sandy soils (Kuti and Torres 1996; Ross-Ibarra and Molina-Cruz 2002). Good soil drainage is a requirement for a healthy development of chaya (Berkelaar 2006). Since soils of the Yucatan Peninsula are tendentially alkaline, chaya might have problems with highly acid conditions.

Despite chayas' multifunctional use, there is a lack of precise data regarding the cropping of chaya, which has changed little from pre-hispanic times (Cuanalo de la Cerda and Guerra Mukul 2008). This indicates the opportunity for further research in cropping and the potential to increase chaya yield and quality.

Chaya is frequently found in the biodiverse and agroforest like Maya home gardens, rather than grown in agricultural fields. There, it usually forms part of hedges (Ross-Ibarra and Molina-Cruz 2002), often with a fencing purpose. In these hedges, both wild and cropped chaya are found and often not directly associated with other plants (Mariaca-Méndez and de Tabasco 2012). In an experiment documented by Aguilar et al. (2011), chaya was successfully polycropped with Spanish cedar (*Cedrela odorata* L.) and Persian lime (*Citrus* × *latifolia* Tanaka ex Q. Jiménez). Principally, chaya can be associated with numerous other plants and even tolerates the invasion of weeds (Berkelaar 2006). Thus, polycropped plants need to be selected carefully: *Hibiscus sabdariffa* L., for example, does not result in an improvement of its annual yield (Ebel et al. 2016).

Cropped chaya is reproduced almost exclusively by cuttings (Mariaca-Méndez and de Tabasco 2012). Only the variety 'Picuda' is occasionally reproduced by seed (Ross-Ibarra and Molina-Cruz 2002). Thick and woody stem cuttings are normally selected for transplanting (Stephens 1994). It is recommended to let the cuttings dry for up to one month before using them because they require intense watering after transplanting and tend to rot easily (Ross-Ibarra and Molina-Cruz 2002). As for their preferable length, dimensions of 10–60 cm are reported (Aguilar et al. 2011; Sarmiento-Franco et al. 2003a, b). In the Maya region of Quintana Roo, cuttings of 40 cm (without leaves) are most common. They are planted upright in excavations of about 25 cm depth and only partially refilled with earth or substrates.

Since chaya is rarely produced commercially, little information is available regarding optimum planting densities. Circular arrangements (Aguilar et al. 2011) as well as linear ones (Ebel et al. 2016) are reported. As for the distance between plants in a linear arrangement, CC Grow (2015) recommends 20 to 30 cm, while Ebel et al. (2016) worked successfully with 50 cm and with 2 m between rows. Aguilar et al. (2011, 2012) report that in polycropping systems, a planting density of up to 3770 chaya plants ha^{-1} delivers the highest survival of cuttings and most cuttings with satisfactorily long roots; maximum 2650 plants ha^{-1} result in the highest develop-

ment of callus, while most roots per cutting are obtained with 3000 plants ha^{-1} and the best vegetative growth with up to 2890 plants ha^{-1}. In monocropping models, densities of 5000 plants ha^{-1} generate a positive vegetative development of chaya. Thus, 10,000 plants ha^{-1} deliver the highest yield. Polycropping favors yield in the dry season, although monocropping clearly produces the highest annual output (Ebel et al. 2016).

As cuttings, the initial aerial growth of the vegetative parts is fast, but the root growth is slow (Aguilar et al. 2011). Therefore, the leaves are not harvested until the second year (Sarmiento-Franco et al. 2003a, b). Leaf biomass is also directly related to leaf length (Parra-Tabla et al. 2004). Pruning chaya in its first year results in rapid vegetative growth (Stephens 1994), decreases generative growth but also stimulates its trichome density (Abdala-Roberts and Parra-Tabla 2005). It is recommended to break the stems rather than to cut them, as this seems to decrease the incidence of infection. Pruning also prevents branches from breaking easily by the wind (Ross-Ibarra and Molina-Cruz 2002).

Chaya is tolerant of heavy rain as well as moderate drought (Peregrine 1983). According to Stephens (1994), chaya requires 650–1500 mm of annual precipitation. Yet successful production in non-irrigated desert conditions (Ross-Ibarra and Molina-Cruz 2002) suggests that chaya can withstand dryer conditions. Drought does not affect plant quality since protein, ash, iron, and zinc content is not affected by changing soil moisture levels (Cifuentes and Bressnniz 2010). During the dry season on the Yucatan Peninsula, Maya peasants tend to irrigate chaya weekly with two liters per plant.

Apart from common synthetic fertilizers, chaya can be manured with organic inputs, such as compost and liquid manure (Berkelaar 2006). In regard to nutrients, the plant requires 370 kg ha^{-1} of nitrogen (Cifuentes and Molina 2000). Potassium plays a smaller role (Cifuentes and Bressnniz 2010), while the most limiting nutrient in the poor Yucatec soils is plant-available phosphorus (Fedick et al. 2008). There are also varietal differences regarding nutrient response: 'Estrella' converts up to 400 kg ha^{-1} a^{-1} nitrogen and 225 kg ha^{-1} a^{-1} phosphate in abundant biomass, while 'Chayamansa' does not respond that strongly to the application of N and P. For both varieties, the application of N has a positive effect of their protein content but reduces ash, Fe, and Zn in the leaves. The application P and K does not impact neither of these parameters (Cifuentes and Bressnniz 2010).

Chaya has very few problems with pests and diseases (Kuti and Torres 1996; Peregrine 1983). Although chaya is exposed to diverse herbivores on the Yucatan Peninsula (Arango et al. 2000), the plant shows a fast and flexible response to leaf damage (Parra-Tabla et al. 2004). The few herbivores mentioned in this context include the caterpillars of the white-striped long tail (*Chioides catillus* ssp. *albofasciatus* Hewitson) and the yellow angled-sulfur (*Anteos maerula* Fabricius) as well as grasshoppers (Parra-Tabla et al. 2004). In Quintana Roo, the red spider mite (*Tetranychus urticae* C. L. Koch) and unspecified species of leafcutter ants are occasionally observed. Outside of the Yucatan Peninsula, the tomato hornworm (*Manduca quinquemaculata* Haworth) is reported as a potential pest (CC Grow 2015).

Since there is a relatively low risk of entomopathogenic fungi in tropical ecosystems (Gilbert 2002), few fungal diseases are associated with chaya. The same applies for bacteria. However, according to Berkelaar (2006), if an older chaya is cut back too close to the ground the entire plant can be exposed to fungal or bacterial diseases. Virus represents a more serious threat to chaya, especially the cassava common mosaic virus. Fortunately, the effect of this pathogen on chaya is minimal (Elliott and Zettler 1987). It is presumably transmitted mechanically through infected knives during stem cutting (Lozano et al. 1981).

For harvesting, it is recommended to cut leaves on their petioles and to select soft and fresh material, starting on the bottom of the shrub (CC Grow 2015). The use of gloves is highly recommended to protect the hands from chaya's trichomes and spines (Stephens 1994). Harvesting can be started in the first ten weeks of the second year of establishment of chaya (Kuti and Torres 1996). It should then be repeated every two to three months (Ross-Ibarra and Molina-Cruz 2002), never exceeding the half of total foliage biomass (Sarmiento-Franco et al. 2003a, b). Berkelaar (2006) reports that under tropical winter, chaya shows an almost dormant appearance and does not produce harvestable material.

In the Yucatec home gardens, highest level yields are reported for between five and ten years, although yields have been reported for after ten years. An output of approximately 5.5 t ha^{-1} dry or 38 t ha^{-1} fresh leaves can be expected (Cifuentes and Molina 2000). Dense planting may improve this potential considerably (Ebel et al. 2016). Comparing the most frequent varieties, 'Estrella' produces more biomass than 'Chayamansa' and correspondingly higher yields, especially if the input of nitrogen and phosphorous is abundant (Cifuentes and Bressnniz 2010). Research on storing chaya is poor, although it is known that chaya can be stored for at least one week in a refrigerator (CC Grow 2015). Internationally, chaya is occasionally sold in pickled form (Kuti and Torres 1996).

6.10 Chaya and Maya Identity: What Does Chaya Taste Like?

The plant called chaya is an important ingredient in many different recipes of all kinds of Yucatecan cuisine. It is regarded as part of the culinary heritage of the Maya indigenous people and as the paradigm of the 'typical' food of the Yucatan Peninsula. However, the term 'typical food' is pejorative. Rather, the gastronomically correct meaning is that it seasons Maya food as an exquisite gourmet dish.

The concept 'typical food' is the result of centuries of colonialism and neocolonialism (Palacios 2014). It is derived from the premise of binary oppositions such as Maya versus *dzul* (roughly understood as 'white male') or Indian food versus rational people's food.

Explanations, interpretations, and analyses based on binary oppositions place indigenous people and non-indigenous people in different, exclusive, antagonistic,

and irreconcilable social structures. This is not to say that 'both' social groups live in perfect harmony with each other. In fact, conflict has been always present between them (Castillo Cocom 2005).

Bartra (2007) notes that the naturalist and explorer Alexander von Humboldt wrote that corn was a staple food of the Mexican people and animals. He adds:

> Corn is what Indians eat, what peasants eat, what the *peladaje* [low social class with little education] eats. And Creoles and their heirs, who despise the Indians, and also despise the grain that feeds them. Corn has been overshadowed by racist considerations. The racial contempt for indigenous peoples has been a constant of the Mexican right wing, both creole, and later the Frenchified and nowadays Americanized. (Bartra 2007)

However, Bartra has a somewhat romantic view of the Maya. They are not a homogeneous and compact group, neither a big fraternity governed by feelings of equality, fairness, and respect. They are just like any human being: not good or bad, just humans. Sometimes, the Indians also insult one another using the same words that some white people use to insult them: Indio! [Bow Bender!].

Chaya as food for Indians is an ethnic marker among Maya indigenous peoples themselves. In Xocenpich, Yucatán, Nardo, a Maya Indian, was watching Juan, another Maya, eat a toasted Chaya taco with its ubiquitous *chile maax* (*Capsicum annuum* L.) *tamulado* (roasted and wrung out *maax* chili pepper) and to which Juan added lemon juice, and Nardo asked his wife:

– What does chaya taste like?
– I have never tasted it!

His question and statement confer him to the status of people of reason.

Chaya is used by Maya indigenous people and by people of reason to express not only the socioeconomic and cultural group to which they belong, but social agents also associate the plant with humor through nicknames (affective, derogatory, racist, and euphemistic). Amaro Gamboa (1999) in its compendium of regionalisms *Vocabulario de el Uayeísmo en la Cultura de Yucatán* includes the word chaya as part of the Spanish language spoken in Yucatán.

Chaya f. Poor woman 'ill-mannered' and poor, badly dressed, low social class; word of the terminology of racial prejudice in Yucatan. Dim. *chayita* doubly pejorative. Nickname: so-and-so *chaya*. Plant Bot. *Jatropha Urens*, L. *Euforbiáceas* The equivalent for men is common to both terms: the *chaya* so-and-so or chayote, which also means freeloader; a person who hangs around and does not work (Amaro Gamboa 1999).

Chayote (V. *Chaya*) 'the *Chaya* Pepe Gómez'… (applied) to the individual man (or women) of the lower class… its synonym chayote it has also has other meanings: in: scrounger, freeloader, and sponger, parasite… (Amaro Gamboa 1999).

Chaya is much more than a simple ethnic marker or something that boosts our immune system or the paradigm of the food of Maya indigenous people. Chaya is a mirror of the consensual and not so consensual resonances that construct new reflexivities but also explore spaces of noise and racism, residues of colonization.

6.11 Outlook

The versatility and accessibility of chaya make it a plant that has incredible potential to alleviate hunger and nutritional challenges in many areas of the world and provide health and healing benefits for benign and life-threatening ailments and diseases, and nutritious forage for livestock. However, research is lacking in all of these areas to develop the plants full potential.

Since chaya grows well on the thin and low fertile soil of the Yucatan Peninsula, the shrub could be cropped in other areas with similar conditions (Ross-Ibarra and Molina-Cruz 2002). In this regard, its potential to provide food security and additional income for Latin American farmers is not exploited to its full potential. Furthermore, although the crop has achieved certain distribution in some tropical countries, chaya can be cultivated in hot (tropical but also dryer) regions of Africa, Asia, and Oceania. Chaya, with its high protein and mineral content, has serious potential to alleviate nutritional deficits around the world. Chaya also has the potential to make a significant nutritional contribution to the diet in the industrialized world, particularly because of its high and diverse protein content; chaya may be the new 'superfood' for vegans.

The processing of chaya is still an almost undiscovered area. It may be relevant for its use as food, forage, and as medicinal plant. In regard to medicinal purposes, only its antidiabetic properties have been demonstrated in scientific research. Chaya's cardioprotective and cholesterol-lowering potential, well known in traditional medicine, deserve deeper examination. Understanding the constitutional effects of chaya on the human body would also allow the development of efficient therapies. Similar to chaya as food, its use as medicine and forage can deliver substantial benefits for regions with difficult cropping conditions (as long as they are hot enough).

There are other potential applications of chaya. For instance, chaya's high content of nitrogen, calcium, potassium, phosphorous, and iron makes it an ideal resource to produce fermented bio-fertilizers for foliar application. Additionally, due to its glycoside content, thinking of chaya as organic pesticide has potential.

In short, although chaya is already sold on the international market, the development of chaya as a new horticultural crop would transcend its current ethnic popularity and create a worldwide market for the plant and its products (Kuti and Torres 1996; Ocampo and Balick 2010), whether as a leafy green vegetable, as a therapeutic herbal tea, as new chicken fodder, or as something totally different.

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Chapter 7
Genetic Diversity in Taro (*Colocasia esculenta*)

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Abstract Taro [*Colocasia esculenta* (L.) Schott] is an ancient, tropical root crop that is morphologically diverse with over 10,000 landraces. It is the fifth most produced root crop in the world and is mainly grown in tropical Africa, China, New Guinea, and many Pacific islands. Taro typically is grown for its starchy corm (i.e., underground stem), although leaves and flowers also are eaten as vegetables. There is controversy over its geographic center of origin, but this is likely to be in the Indo-Malayan area. Evidence indicates that it was domesticated, possibly independently, across an area that ranges from northeast India to Yunnan province in China to New Guinea. Within Micronesia and Polynesia, where taro is a staple crop, the genetic base is very narrow. Genetic diversity within the taro germplasm is significantly greater in Asia and New Guinea. The exploitation of this diversity could lead to the development of cultivars with greater disease resistance, and improved yields and corm quality. Taro is a neglected crop in terms of recent advances in molecular biology, with only a limited number of studies utilizing next-generation transcriptome and genome sequencing. At present, a high-quality reference genome is lacking; however, recent genotyping-by-sequencing (GBS) approaches promise to improve our understanding of taro genetics.

Keywords *Colocasia esculenta* · Tropical root crop · Genetic diversity · Genetic markers

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7.1 Introduction

Taro [*Colocasia esculenta* (L.) Schott] belongs to the family Araceae (i.e., Arum family), a large, ancient, monocot plant family with mainly tropical distribution across the world. This family is characterized by its morphological diversity, the presence of many forms of calcium oxalate crystals, and flowers with a spadix of small, bisexual, or unisexual flowers, covered by a spathe (Henriquez et al. 2014; Matthews 1995).

The number of species in the genus *Colocasia* is disputed, but ranges from 5 to 10, with approximately 60 synonyms (Catalogue of Life 2017; The Plant List 2017; Matthews 1991, 1995). Three species are only known from single specimens in herbarium collections: *C. gracilis* from Sumatra, *C. manii* from upper Assam, and *C. virosa* from Bengal. Of the more common species, *C. affinis* and *C. fallax* occur in Northeast India and Southeast Asia, while *C. gigantea* is found wild in Indonesia and cultivated throughout Southeast Asia. Taro (*Colocasia esculenta*) is the most widely cultivated species with over 10,000 landraces worldwide (Ivancic and Lebot 2000).

Taro, as a species, is characterized by rare and erratic flowering (Ivancic et al. 2004a; Ivancic and Lebot 2000). The female, pistillate flowers are located at the base of the spadix, and male, staminate flowers are located near the top. Typically, cross-pollination is required, because the female flowers become receptive before the pollen is shed. Following successful pollination, the highest number of seeds recorded per fruit cluster was over 22,000 seeds (Ivancic and Lebot 2000).

Taro is one of the oldest cultivated crops, with evidence of its use by at least 6950–6440 cal yr B.P. in New Guinea (Denham et al. 2003). It is the fifth most produced root crop in the world, with global production of 10.1 million metric tons in 2014 (FAOSTAT 2014). Taro is consumed primarily for its starchy corm or underground stem. In Hawaii, the corm is cooked and mashed into a paste (i.e., poi) that could be served fresh or fermented after several days. Taro leaves serve as a vegetable, providing good sources of dietary fiber and vitamin C (Ferguson et al. 1992). Interestingly, in Yunnan province in China, one morphotype of taro produces abundant flowers that are eaten as a high-value vegetable (Jianchu et al. 2001).

In Hawaii, poi has been reported to be easily digested, with potential uses as a probiotic due to high levels of *Lactococcus lactis* bacteria found during fermentation (Brown and Valiere 2004). Its ease of digestibility may be due to the small size of its starch granules (1–7 μm) compared to those of arrowroot (*Zamia floridana*), canna (*Canna edulis*), or potato (Langworthy and Deuel 1922). Also, poi has been reported to be hypoallergenic due to its low protein content, and it has been fed to infants with failure to thrive or those with allergies to cow's milk (Brown and Valiere 2004). In China, taro was used to treat some gastrointestinal disorders in traditional Chinese medicine (Yu et al. 2015).

Taro is the root crop that has the greatest diversity of flavonols when compared to sweet potatoes, cassava, five species of yams, and giant taro, with 20 compounds identified (Champagne et al. 2011). Phenolic compounds (including flavonols) have

been reported to protect against a variety of human diseases, such as cancers, cardiovascular disease, diabetes, and Alzheimer's (Soto-Vaca et al. 2012).

Taro also contains anti-nutritive compounds, such as oxalates, trypsin inhibitors, uracil, and lectins. Oxalates could lead to hypocalcemia and kidney stones in humans, while trypsin inhibitors could cause growth depression, pancreatic hypertrophy, and hyperplasia (Guchhait et al. 2008). Uracil and glycol–protein lectin have been identified as compounds that could explain the acridity (i.e., irritation) of taro mucilage (Yu et al. 2015).

In the wet tropics and subtropics, taro is a vital component of many subsistence farming communities. In 2014, Nigeria was the world's largest taro producer (3.27 million metric tons), followed by China, Cameroon, Ghana, and Papua New Guinea (PNG) (FAOSTAT 2014). In the Pacific Islands, it is one of the most important staple food crops, and it is also widely cultivated throughout the Caribbean and South America (Kreike et al. 2004; Plucknett 1970; Plucknett et al. 1970).

Taro is typically grown in subsistence farming societies, so production issues that affect supply are serious food security concerns. In Vanua Lava (in Vanuatu) where it is grown as the staple crop, taro is consumed at 0.43 kg of dry matter per person per day (Caillon et al. 2006). Regional taro collections have been made through Taro Genetic Resources: Conservation and Utilization (TaroGen) and Taro Network for Southeast Asia and Oceania (TANSAO) (Singh et al. 2010). However, despite its emerging importance as a crop in many areas of the world, and its cultural significance in Pacific Island societies, no International Agricultural Research Center (i.e., CGIAR) has a mandate to conserve and carry out research on taro. Similarly, in the USA, there is no USDA Germplasm Repository with responsibility for conserving and distributing taro germplasm.

7.2 Morphological Diversity of Taro

As a species, taro is highly polymorphic (Purseglove 1972), with phenotypic descriptors related to size of corms and abundance of cormels. Jianchu et al. (2001) studied taro diversity in the Yunnan Province of China and determined that there were five uses based on morphotypes of taro categorized by farmers: (1) inflorescence, that produces abundant flowers eaten as a vegetable; (2) single corm, of up to 2 kg fresh weight with few cormels; (3) multicormel, having many cormels with better quality and yield than the corm; (4) multicorm, that has similar sized corms and cormels; and (5) petiole, where that structure is eaten as a vegetable but corms are poorly developed and long stolons are produced. In addition, there is the stolon or wild-type morphotype (*Colocasia esculenta* var. *aquatilis*) that has a poorly developed corm, no cormels and many, long stolons that are eaten as a pickled vegetable.

Two variants of taro are widely cultivated: (1) *C. esculenta* var. *esculenta* and (2) *C. esculenta* var. *antiquorium* (Deo et al. 2009). *Colocasia esculenta* var. *esculenta*, called the 'dasheen' type of taro, has a large, cylindrical corm with only a few cormels and is similar to the single-corm morphotype described by Jianchu et al. (2001).

Colocasia esculenta var. *antiquorium* called the 'eddoe' type has a small, globular corm with relatively large cormels and is similar to the multicormel morphotype described earlier (Jianchu et al. 2001). Most taros cultivated in Asia and the Pacific are the dasheen type. Analysis of 32 accessions of these two variants from India using randomly amplified polymorphic DNA (RAPD) was not able to distinguish between these two phenotypes based on these genetic markers (Lakhanpaul et al. 2003).

Cheema et al. (2007) analyzed 24 accessions of taro grown in India for 14 characters: number of leaves per plant, plant height, petiole length, days to maturity, number of corms per plant, corm weight, corm length, corm girth, number of cormels per plant, total yield per plant, dry matter, protein, starch, and oxalate content. Highly significant differences were found among accessions for all 14 characters. Singh et al. (2008) evaluated 859 accessions from PNG using 10 quantitative characters: number of cormels, weight of cormels, corm length, corm breadth, corm weight, leaf length, leaf width, plant height, number of stolons, and number of suckers. In addition, they measured 20 qualitative traits, including color of corm flesh and fiber, color of various parts of the leaf blade and petiole, and Taro Leaf Blight (TLB) resistance. They found high variability among taro accessions for these phenotypic traits.

Typically, taro is grown from vegetative propagules and not from seed. Species-specific insect pollinators of taro are endemic to New Guinea and Indonesia, with one insect species found also in northern Queensland (Hunt et al. 2013; Matthews 1995; Carson and Okada 1980). In countries without these insect pollinators, there is a little natural hybridization which results in the development of morphotypes that are quite distinct, even though they share the same genotype (Kuruvilla and Singh 1981).

7.3 Genetic Diversity of Taro

Cytological studies show that taro has diploid forms ($2x = 2n = 28$) as well as triploid forms ($3x = 3n = 42$) (Kokubugata and Konishi 1999; Coates et al. 1988; Yen and Wheeler 1968). Analysis of triploid clones indicates autotriploidy, which occurs when unreduced gametes are produced by a diploid parent during meiosis (Ochiai et al. 2001; Matthews 1995; Coates et al. 1988). Triploids are inherently sterile, because of their uneven number of chromosome sets. However, while they are characterized by increased hardiness at high altitudes or latitudes and are found in cooler climates, they must have developed in tropical areas where sexual reproduction of diploids was possible. Triploids are common in Asia (including China, India, Indonesia, Japan, Thailand, and Vietnam), Africa, and South America (e.g., Costa Rica) (Chaïr et al. 2016, 2016; Lebot et al. 2004; Matsuda and Nawata 2002; Ochiai et al. 2001; see Table 7.1). Diploids are common throughout Asia, Oceania, and South America. Interestingly, in Polynesia, only diploid forms are found (Kreike et al. 2004; Coates et al. 1988).

Several types of genetic markers have been applied to study the genetic diversity of taro (Table 7.1). In general, these markers fall into two categories: (1) band-based,

Table 7.1 Summary of taro studies that have incorporated genetic markers

Authors	Marker type	Purpose	Collections sampled	Sample details
Caillon et al. (2006)	AFLP/ethnobotanical data	Morphological and genetic variation	Vanuatu	118 accessions (collected for Vanuatu Agricultural Research and Technical Centre); 74 accessions genotyped
Chaïr et al. (2016)	SSR	Origin, diversification, and dispersal/genetic diversity/ploidy	19 countries in Asia, the Pacific, Africa, and America	357 cultivars
Cheema et al. (2007)	Heritability	Phenotypic and genotypic coefficients of variation	North India	24 clones
Coates et al. (1988)	Cytotype	Chromosome variation	Australia, New Zealand, PNG, the Philippines, Thailand, Japan, Nepal, Huahine in the Society Islands, and Easter Island	~112 cultivars
Das et al. (2015)	Chromosomal variation/RAPD	Chromosomal variation/phylogenetics/drought resistance	India	1 sample from each of 10 populations
Deo et al. (2009)	Genetic transformation	Disease and pests of taro	Not applicable (n/a)	n/a
Doungous et al. (2015)	Inter-retrotransposon amplified polymorphism (IRAP) fingerprints	Intraspecific variability	Cameroon (Africa)	7 accessions of *Colocasia esculenta* var *antiquorum* Schott compared to 20 accessions of *Xanthosoma sagittifolium*

(continued)

Table 7.1 (continued)

Authors	Marker type	Purpose	Collections sampled	Sample details
Henriquez et al. (2014)	Illumina sequencing/plastid and mitochondrial	Phylogenetics/family Araceae	No data	32 genera of Araceae; 5 other samples including *Colocasia esculenta*
Hunt et al. (2013)	SSR	Field evidence for natural breeding	Northern Queensland Australia; PNG	Australia Hopevale site, 45 wild; PNG 2 wild, 1 feral, 1 cultivar
Irwin et al. (1998)	RAPD	Phylogeographics/genetic diversity	Thailand, Hawaii, Indonesia, Micronesia, Western Samoa, PNG	44 taro accessions, 2 *Xanthosoma* spp. and 1 *C. gigantes*
Ivancic et al. (2004b)	Agro-morphological characters/crosses	Genetic control of traits	Vanuatu national taro germplasm collection	453 accessions
Ivancic and Lebot (2000)	Origin, domestication, and spread	Review	n/a	n/a
Ivancic and Lebot (1999)	Isozyme/morphological traits/reproduction systems	Evaluate endemism	SW Pacific, New Caledonian Wild Taro	3 morphotypes, up to 160 samples for morphological characterization
Kokubugata and Konishi (1999)	Ploidy	Chromosomal variation	India, Thailand, Costa Rica, Japan, Pakistan	7 cultivars
Kreike et al. (2004)	AFLP/ploidy	Genetic diversity; association between botanical variety and ploidy level	Southeast Asia and Oceania: Vietnam, Thailand,, Indonesia, Malaysia, the Philippines, PNG, Vanuatu	170 accessions from TANSAO
Lakhanpaul et al. (2003)	RAPD	Phylogeographics/intraspecific variability	India	32 taro accessions belonging to 28 morphotypes

(continued)

Table 7.1 (continued)

Authors	Marker type	Purpose	Collections sampled	Sample details
Lebot et al. (2004)	AFLP/isozymes/ploidy/morpho-agronomic descriptors	National germplasm collections as part of TANSAO	Indonesia, Malaysia, Thailand, Vietnam, the Philippines, PNG, and Vanuatu	2298 accessions
Lebot and Aradhya (1991)	Isozyme (compared to morphological/ploidy other studies)	Genetic variation/diversity	Asia and Oceania	1417 cultivars and wild forms
Liu et al. (2015)	Illumina sequencing/transcriptome	Whole transcriptome profiling; gene annotation	China	Leaf tissue
Mace et al. (2006)	SSR	Identify duplicates/assess allelic diversity within national collection	Oceania: PNG, Solomon Islands, Vanuatu, New Caledonia, Fiji, Palau, Niue, Tonga, Cook Islands, and Samoa	515 accessions
Mace and Godwin (2002)	SSR	Geographical structure/identify duplicates in collections	Vanuatu, Hawaii, Samoa, Fiji, Niue, Tonga, Cook Isle, New Caledonia, Palau, PNG, Japan, China, Vietnam	17 accessions: 13 *C. esculenta* var. esculenta (dasheen type); from Fiji, 1 *C. esculenta* var. *antiquorum* (eddoe type), and one *Xanthosoma* species
Matsuda and Nawata (2002)	RFLP at ribosomal DNA/isozymes	Geographical structure/routes of human transfer	China (mostly Yunnan province), Japan, Taiwan, and Vietnam	227 accessions

(continued)

Table 7.1 (continued)

Authors	Marker type	Purpose	Collections sampled	Sample details
Matthews et al. (1992)	Restriction digest with rRNA ITS/mtDNA genes/ploidy	Genetic diversity and routes of dispersal	Japan compared to Australia	Taro collection at National Research Institute of Vegetables, Ornamental Plants and Tea: 80 Japanese accessions and 1 Queensland, Australia sample
Ochiai et al. (2001)	RAPD/isozyme	Geographical differentiation/phylogenetic relationships	China	121 accessions
Okpul et al. (2004)	Agro-morphological characters	Formation of TANSAO regional core collection	PNG	276 accessions
Parvin et al. (2008)	Karyotype	Karyotype	Bangladesh	7 cultivars
Paul et al. (2014)	Morphological characters/genotypic trait correlations	Genotypic–phenotypic correlations	Bangladesh	457 samples from 13 districts
Quero-Garcia et al. (2006)	AFLP/SSR	Quantitative trail loci for trait mapping	Vanuatu	2 crosses; 223 progeny
Quero-Garcia et al. (2004)	AFLP/agro-morphological descriptors	Vanuatu taro national germplasm collection	Vanuatu	452 accessions
Sardos et al. (2012)	SSR	Eco-geographical survey of landraces in Vanuatu/genetic diversity/relatedness	Vanuatu, Indonesia, Philippines, Thailand	344 landraces
Sharma et al. (2009)	Subtractive hybridization/expressed sequence tags	Differentially expressed genes/host–pathogen interactions	India	63 cultivars and 5 wild wetland taros

(continued)

Table 7.1 (continued)

Authors	Marker type	Purpose	Collections sampled	Sample details
Sharma et al. (2008a)	AFLP	Geographical differentiation/phylogenetics/disease resistance	India	14 accessions
Sharma et al. (2008b)	RAPD/isozyme	Geographical differentiation/phylogenetics/disease resistance	India	14 accessions
Shintaku et al. (2016)	Illumina GBS to identify SNPs	Linkage map/disease resistance	Hawaii	2 parents and 96 progeny
Singh et al. (2012)	RAPD/ISSR/agro-morphological characters	Genetic diversity/phylogenetics/association with agro-morphological characters	Andaman islands, India	21 accessions
Singh et al. (2008)	SSR/agro-morphological traits	Core collection development	15 provinces of PNG	859 accessions for creation of a core collection of 81 accessions; 151 genotypes characterized
Sreekumari and Mathew (1991)	Karyomorphology	Karyomorphology	India	5 morphotypes
Trujillo et al. (2002)	Disease resistance	Cultivate cultivars resistant to taro leaf blight	Palau, Polynesia	Developed three new TLB-resistant cultivars
Xu et al. (2001)	Morphotypes/cytotypes	Ethnobotany/folk taxonomy	China	53 samples
You et al. (2015)	SSR	Marker development; 5278 SSRs developed, identified 62 polymorphic SSRs	China	Two lines of taro, TLB resistant and susceptible

including isozymes (enzymes), RAPDs, amplified fragment length polymorphisms (AFLPs) and simple sequence repeats (SSRs, microsatellites), and (2) sequence-based, including single-nucleotide polymorphisms (SNPs), expressed sequence tags, and transcriptome profiling with next-generation sequencing (RNA-seq). The disadvantage of RAPD and AFLP markers is that the bands are 'anonymous,' with genomic locations unknown, so they provide no corresponding gene-specific data. The RAPD technique was somewhat supplanted by AFLPs, due to the latter marker's ability to generate more reproducible fingerprints. The SSR markers are better than RAPDs and AFLPs in terms of simplicity, amplification reliability, and co-dominance. In taro, SSRs are bi-allelic or tri-allelic depending on ploidy: due to the ploidy issue, partial heterozygosity makes it impossible to score genotypes exactly (Chaïr et al. 2016). The scoring of SSRs is generally considered more reliable and consistent than for RAPDs and AFLPs, and SSR scoring can be standardized across laboratories (Seeb et al. 2007). The advantages of sequence-based SNP markers in comparison to SSR markers will be discussed further in Sect. 7.5.

Diversity in morphological traits is not a good indicator of genetic diversity (Singh et al. 2008; Lebot et al. 2004; Okpul et al. 2004). In a study of 814 taro accessions from PNG, taro with contrasting morphological traits would cluster together in a dendrogram produced from 7 SSR markers, whereas accessions with the same morphological traits could be widely separated (Singh et al. 2008). In spite of morphological variability identified from 2298 accessions collected in Indonesia, Malaysia, Thailand, Vietnam, the Philippines, Papua New Guinea and Vanuatu, the genetic base of these accessions was narrow, based on isozymes and AFLP fingerprinting (Lebot et al. 2004).

Taro has a large genome, which is estimated to be 4.08 Gbp (C-value mean, Kew Royal Botanic Gardens 2016). This large size coupled with high heterozygosity due to its outcrossing nature has thus far prevented the creation of a reference genome of the species. However, a reference transcriptome was developed from messenger RNA isolated from leaf tissue and sequenced using next-generation sequencing strategies (Liu et al. 2015; Table 7.1), providing a first look at gene family characterizations within the species. Genomic efforts are further complicated due to the limited amount of information available on genetic maps. There is a single linkage map of the taro genome based on 169 AFLP markers and 8 SSR markers available (Quero-Garcia et al. 2006; Table 7.1); however, this map does not provide chromosome-level resolution, with the study identifying 18 linkage groups rather than the expected 14 (diploid taro having $2n = 2x = 28$ chromosomes).

Advances in high-throughput sequencing have resulted in the identification of 5278 SSR markers, of which 62% were identified as polymorphic based on a test set of 100 primers (You et al. 2015). Prior to this study, only 52 SSR taro-specific markers had been characterized (Lu et al. 2011; Hu et al. 2009; Mace and Godwin 2002; Table 7.1). Less attention has been focused on the development of SNP markers; however, Shintaku et al. (2016) published the first report on the use of SNPs in a population of taro that segregated for resistance to TLB. Unfortunately, they were not able to identify SNPs associated with resistance to TLB. More recently, Helmkampf et al. (2018) developed >1700 SNP markers for taro. In the future, the increasing

number of genetic and genomic tools associated with taro will provide breeders with many new types of resources.

While genetic resources are limited to recent times, taro is well studied with respect to its domestication history (Chaïr et al. 2016; Coates et al. 1988; Yen and Wheeler 1968). Human dispersal of taro across the globe has been studied using chromosome number, morphology, and genetics, as well as through references in ancient texts. Taro has a long history of use, having been consumed for ~9000 years (Rao et al. 2010). It is mentioned in texts as early as 2000 years ago (Whitney et al. 1939).

The center of origin for taro is uncertain (Chaïr et al. 2016). The fact that three species (now possibly extinct) of *Colocasia* were found in Sumatra (Indonesia), upper Assam (India), and Bengal (Bangladesh and India) is an indication that the Indo-Malayan region is the geographic center of origin for *C. esculenta* (Matthews 1991, 1995). However, an argument for a secondary center of origin in New Guinea for *Colocasia* is the co-evolution of several *Colocasiomyia* spp. (Diptera, Drosophilidae) as specific pollinators for species in the genus *Colocasia* (Sultana et al. 2006). Fruit flies *Colocasiomyia stamenicola* and *C. pistilicola* (Carson and Okada 1980) are endemic to New Guinea, and their entire life cycle depends on inflorescences of *Colocasia*. However, it is possible that these fruit flies evolved earlier elsewhere, but became extinct in those locations later. Another possible center of origin for taro is Hainan Island China, based on the co-evolution of *Phytophthora colocasiae*, the oomycete pathogen that causes taro leaf blight. *Phytophthora colocasiae* is host-specific to taro and Zhang et al. (1994) suggested that its center of origin was Hainan Island, based on the presence of three different mating types. Shrestha et al. (2014) confirmed the presence of three mating types in Hainan, but questioned whether this was sufficient evidence to show that the center of origin of *P. colocasiae* was on Hainan Island.

The center of origin for taro is expected to harbor the greatest genetic diversity (Fig. 7.1). Accordingly, genetic diversity is the focal subject of numerous studies that have utilized an array of molecular biological methods (Table 7.1). Irwin et al. (1998) evaluated 44 taro, two tanier (*Xanthosoma* spp.), and one C. *gigantea* accession using RAPD markers, and found the highest genetic diversity in taro accessions from Indonesia. Kreike et al. (2004) used AFLP markers to evaluate gene diversity in 255 taro accessions from Indonesia, Malaysia, New Guinea, the Philippines, Thailand, Vanuatu, and Vietnam and found that Thailand had the greatest gene diversity. Within the Pacific Island region, seven SSR markers indicated that the geographic areas with the greatest sources of genetic diversity were New Guinea and the Solomon Islands (Mace et al. 2006). Chaïr et al. (2016) used 11 SSR markers to compare taro accessions from 19 countries in Asia, the Pacific, Africa, and America, and found the greatest genetic diversity in Asian accessions (mainly from India).

It is possible that wild taro was widespread over a geographic area ranging from northeast India to Southeast Asia to Melanesia (including New Guinea), and that domestication happened at several independent locations (Chaïr et al. 2016; Matthews 1991, 1995). Domestication of taro is hypothesized to have occurred in two separate geographic locations based on archaeobotanical evidence (Denham

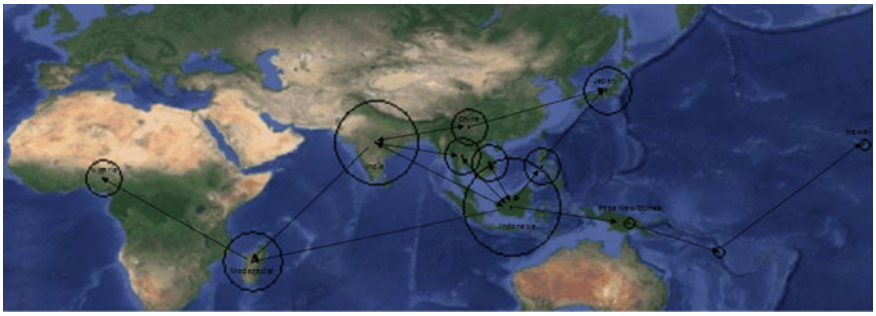

Fig. 7.1 Potential movement of taro from the putative center of origin of the species to areas of production and diversity today. The literature is not consistent as to the center of origin and center of domestication (Coates et al. 1988; Matthews 1991, 1995), but concurs that centers of diversity are found in Indonesia and India. Here, size of the circle represents the reported genetic diversity (Lebot and Aradhya 1991; Lebot et al. 2004; Chaïr et al. 2016) within the region and arrows indicate potential movement patterns (Ivancic and Lebot 2000; Xu et al. 2001; Matsuda and Nawata 2002)

et al. 2003), cytological studies (Coates et al. 1988), and SSR markers (Sardos et al. 2012): (1) India–Southeast Asia and (2) New Guinea. Two distinct, separate gene pools (one in Asia and one in the Pacific) have been confirmed by isozyme analysis (Lebot and Aradhya 1991), AFLP markers (Kreike et al. 2004; Lebot et al. 2004; Quero-Garcia et al. 2004), and SSR markers (Chaïr et al. 2016). Ochiai et al. (2001) used isozyme analysis and RAPD analyses to support their contention that Yunnan province in China was another center of taro diversification and dispersal into temperate Far East Asia, particularly for triploid taros.

Identifying the center of origin for taro and subsequent domestication patterns has important ramifications for understanding human migrations. The distribution of some taro cultivars is understood, with Western African cultivars believed to have come from India (Ivancic and Lebot 2000). When taro started moving across the Pacific, only a few domesticated genotypes were carried by Austronesians as they spread from PNG to Polynesian and Micronesian islands, with Hawaii being settled in A.D. 1190–1290 (Wilmshurst et al. 2010).

7.4 Conventional Breeding of Taro

Modern taro breeding programs started during the 1970s after the discovery that treatment of plants with gibberellic acid (GA, 300–1000 mg GA L^{-1}) induced flowering and allowed synchrony of flowering of parents, making hand-pollination possible (Ivancic and Lebot 2000; Wilson 1979). Hand-pollination is required because specialized insect pollinators are either rare or non-existent outside of the Solomon Islands, Australia, and New Guinea (Hunt et al. 2013; Sultana et al. 2006; Plucknett 1970).

Sustainability of existing taro landraces or cultivars is limited by low yields (see Sect. 7.4.1), diseases (see Sect. 7.4.2), or quality issues (see Sect. 7.4.3). Taro breeders seek to improve plant architecture (e.g. optimal number of suckers, absence of stolons, optimal number of leaves, vertical petioles), corm yield, and quality traits (e.g., high dry matter content, low levels of irritant substances) (Quero-Garcia et al. 2009). In addition, in Hawaii two other desirable qualities are purple corm color and 'stickiness' of the mashed corm to produce poi.

Plant vigor is often associated with heterozygosity (Quero-Garcia et al. 2009; Lebot et al. 2005). Breeding of taro with parents that come from diverse genetic pools could result in improved vigor and yield of progeny. However, breeding of taro is problematic, because inbred lines are not possible due to predominant self-incompatibility and severe inbreeding depression (Quero-Garcia et al. 2009; Ivancic and Lebot 2000). The use of wild-type parents in conventional breeding requires seven generations of modified backcrosses to introgress the desired gene into traditional landraces/cultivars, while removing such undesirable traits as irregular corm shapes, high numbers of stolons, and high levels of acridity (Okpul 2002 as cited in Quero-Garcia et al. 2009). Breeders also must take care to avoid the introduction of viruses when trying to broaden the genetic base in breeding programs (Sukal et al., 2015). For taro, the chances of breeding and selecting a high-yielding clone with excellent eating qualities are generally very low and become much lower when additional traits are sought (e.g., disease resistance). In PNG, a recurrent selection program for taro only produced eight clones from over 100,000 progeny (less than 0.008%) that had high yield, good eating quality, and resistance to TLB (Okpul 1997 as reported by Lebot et al. 2005).

The success of breeding programs depends on the availability of diverse genetic resources (Okpul et al. 2004). However, conservation of taro collections is difficult with many national collections having been made and lost over the years, due to natural disasters and loss of funding (Lebot et al. 2005). A method of selecting a limited core collection has been proposed to represent a useful diversity of taro landraces/cultivars, based on diverse geographic origins, wide genetic distances, quality, agronomic performances, and functional sexuality (Lebot et al. 2005).

Taro breeding has been initiated in many countries within the South Pacific including Samoa, PNG, and Vanuatu under two main programs, TaroGen and TANSAO (Singh et al. 2010). The main thrust of these breeding programs was developing through sexual hybridization, new cultivars that would maintain taste and yield, while incorporating disease resistance. Modern taro breeding at the University of Hawaii started in the late 1980s. This program focused on bringing in novel diversity from different geographic regions to increase yield and disease resistance (Cho et al. 2007).

7.4.1 Phenotypic Trait: Yield

Taro has the lowest average yield (5.83 t ha^{-1}) of the major root crops (Quero-Garcia et al. 2006). In Oceania and elsewhere, there are only two options for meeting the food needs of a rapidly growing human population: (1) increase agricultural production or (2) increase food imports (Lebot 2013). Increasing agricultural production of taro could involve improved cultivation methods, such as increased irrigation. In Vanuatu, taro productivity was reported to vary from 7.1 t ha^{-1} of dry matter when grown under non-permanent irrigation to 20.1 t ha^{-1} when inundated between river stones.

Morphological characters such as number of cormels per plant, protein, corm weight, and corm length were highly and positively correlated with total yield (Cheema et al. 2007). Quero-Garcia et al. (2006) studied F_1 progenies of 123 and 100 individuals obtained from crosses between local cultivars from Vanuatu and found the strongest correlation between corm length, corm width, and corm yield. They found several quantitative trait loci (QTLs) associated with corm yield and corm dimensions, as well as a dominant gene responsible for the yellow color of corm flesh.

7.4.2 Phenotypic Trait: Disease Resistance

There are several oomycete and fungal pathogens that infect taro, suppressing yield (Ivancic and Lebot 2000; Ooka 1994). The oomycete *Phytophthora colocasiae* causes TLB, resulting in water-soaked lesions on leaf blades that spread rapidly under warm, wet, humid conditions (Miyasaka et al. 2013). This pathogen can also cause a rot of the petiole and corm. *Pythium aphanidermatum*, as well as several other *Pythium* species, is another oomycete that causes corm rot. Fungal pathogens that cause leaf spots, decreasing functional photosynthetic tissue, are *Cladosporium colocasiae* and *Phyllosticta colocasiophila*. Other fungal pathogens that cause corm rots are *Sclerotium rolfsii* and *Ceratocystis fimbriatum*. Under specific conditions, these various oomycete and fungal pathogens could cause significant losses. However, the greatest losses in yield are caused by *P. colocasiae*.

Several viruses infect taro and reduce its yield (Sukal et al. 2015). The most widespread is dasheen mosaic virus, and it is believed to infect the majority of vegetative planting material in Hawaii (Ooka 1994). Taro vein chlorosis virus (TaVCV) has been recently established in the Hawaiian Islands, providing another challenge, but its effects on yield are still unknown (Long et al. 2016). Colocasia bobone disease virus (CBDV) and taro bacilliform virus (TaBV) occur in New Guinea and the Solomon Islands (Ivancic and Lebot 2000). Importantly, co-infection of taro plants with CBDV and TaBV results in the lethal alomae–bobone disease. An effort to breed taro plants with resistance to the alomae–bobone disease resulted in a few hybrids that recovered after initial infection, gaining some tolerance (Ivancic et al. 1993).

Some nematodes also cause taro diseases, including *Hirschmaniella miticausa*, *Pratylenchus* sp., *Helicotylenchus* sp., and *Meloidogyne* sp. (Ooka 1994). Mitimiti disease caused by *H. miticausa* could result in dramatic losses, but is limited to the Solomon Islands (Bridge et al. 1983). Ortiz et al. (2008) screened taro germplasm from Thailand, Vietnam, and Nepal, as well as 11 taro cultivars (derived from Hawaiian, Thai, Samoan, Guamanian, New Guinean, Palauan, and Indonesian parents) for resistance to root-knot nematode *Meloidogyne javanica*. They found one accession from Thailand, and one cultivar (#19) had consistently lower reproduction factors (Rf) and higher growth ranking, suggesting possible resistance.

In Hawaii and in many other areas of the world, the most important taro disease is TLB. *Phytophthora colocasiae* was present in Hawaii during the 1920s and probably contributed toward the extinction of more than 270 traditional Hawaiian cultivars (CTAHR 2009). Current research on the genetic diversity of *P. colocasiae* using SNPs and mating types confirmed that this pathogen was introduced into Hawaii (Shrestha et al. 2014). Evaluation of existing, traditional Hawaiian taro genotypes showed that only a few had moderate resistance to this disease and almost all were very susceptible (Miyasaka et al. 2012). In Hawaii, it was estimated that 25–50% of taro corms were lost due to oomycete and fungal diseases (Miyasaka et al. 2001; Trujillo 1967).

When TLB spread to the Samoan Islands during the 1990s, it resulted in 95% losses in traditional, TLB-susceptible taro genotypes (Fig. 7.2). The introduction of TLB-resistant taro cultivars helped to increase the production of taro in Samoa after 1998 (Trujillo and Menezes 1995). When TLB reached the Dominican Republic in 2004, 70–95% of commercial taro plantings were lost and dramatic losses in production of the TLB-susceptible, commercial taro genotype occurred (R. P. Duverge, personal communications).

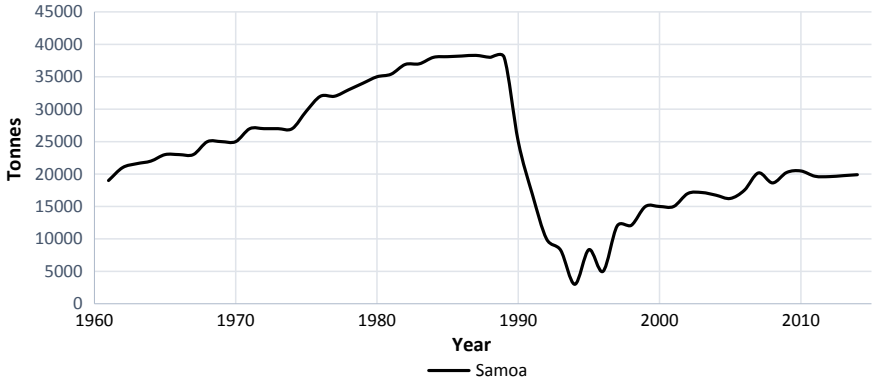

Fig. 7.2 Production of taro in Samoa between 1961 and 2014 (FAOSTAT, 1961 to 2014). The dramatic decrease in taro production during the early 1990s was due to the introduction of taro leaf blight (TLB), followed by some recovery of production due to the development of TLB-resistant taro cultivars

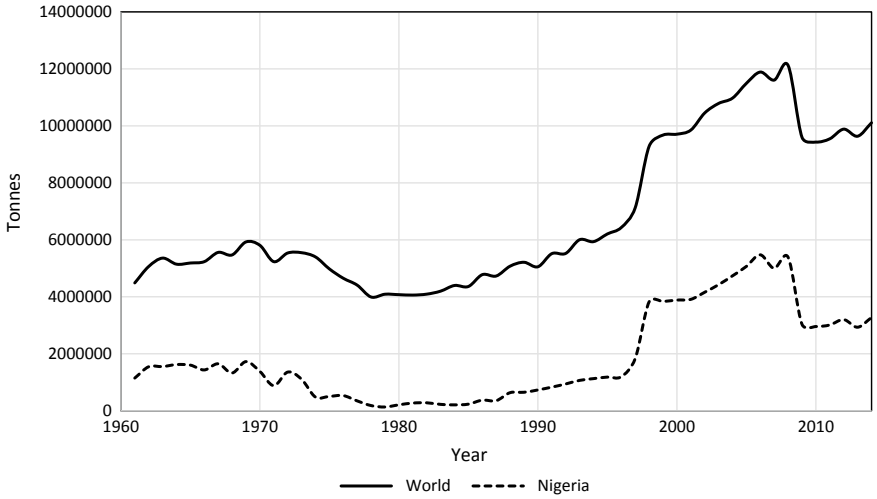

Fig. 7.3 Global taro production compared with Nigeria's production from 1961 to 2014 (FAOSTAT, 1961 to 2014). The downward deflection observed in 2009 resulted from the arrival of TLB in Western Africa

Nigeria has tripled its production of taro since 1996 and is now responsible for over a third of the world's taro output (Fig. 7.3). In 2009, many farmers there reported complete destruction of their crop due to TLB (Bandyopadhyay et al. 2011). Cameroon and Ghana also started to experience losses due to TLB around that time (Omane et al. 2012). The significance of Western Africa as source of taro production and the impact of TLB there on global taro production are illustrated in Fig. 7.3. Similar to Samoa, Western Africa is unlikely to regain former production levels without TLB-resistant cultivars.

Natural resistance to TLB has been found within the taro germplasm from Palau and Pohnpei (Miyasaka et al. 2012), and there have been efforts in Hawaii from the mid-1980s to increase yields and TLB resistance of traditional taro genotypes in Hawaii using conventional breeding (Miyasaka et al. 2013; Cho et al. 2007; Trujillo et al. 2002; Trujillo and Menezes 1995). After the efforts of three separate Hawaiian taro breeding programs that were conducted over the past 30 years, three new taro cultivars (BC99-6, BC99-7, and BC99-9) have been accepted by farmers (Miyasaka et al. 2013; Cho et al. 2007). However, there are still problems due to the breakdown of TLB resistance, loss of vigor, and/or susceptibility to other pests (R. Yamakawa, personal communications).

In Hawaii, current research efforts are focused toward transferring TLB resistance from TLB-resistant taro cultivars into traditional Hawaiian taro genotypes by hand-pollination. A cross between '230' × '255' is promising, based on a larger proportion (22%) of progeny with TLB resistance as measured by a detached leaf assay (Shintaku et al. 2016; Brooks 2008). The identification of molecular markers for disease resistance or other desirable traits may help to reduce the number of gen-

erations needed to produce taro cultivars with desirable traits, high yield, and disease resistance.

7.4.3 Phenotypic Trait: Quality

Lebot et al. (2004) characterized over 2000 accessions preserved in seven national germplasm collections in TANSAO for quality traits. They found that several corm quality characteristics were highly variable and likely to be genetically controlled. These quality traits were dry matter, minerals, and amounts of lipids, proteins, amylose, glucose, fructose, saccharose, and maltose. In addition, they found that good taste was correlated with high contents of dry matter, starch, and amylose. Dry matter ranged from 1.5 to 55.9%, while starch content ranged from 36.6 to 55.9%.

Ferreres et al. (2012) measured 41 phenolic compounds in leaves of two taro cultivars from the Azores and found quantitative differences among individual compounds. In particular, 'red' taro was richer in hydroxycinnamic acid derivatives than 'giant white' taro, and these compounds could be important for improved nutritional value.

Taro corms are rich in carotenoids and flavonoids that could have healthful properties that protect against cancers, cardiovascular diseases, and cell dysfunction. Lebot and Legendre (2015) used high-performance thin-layer chromatography (HPTLC) to screen corms of more than 1800 taro hybrids for flavonoids (e.g., anthocyanins, flavonols, and flavanols). Variation in contents of screened compounds was found, and the characteristics were heritable. Similarly, Guchhait et al. (2008) found significant differences among genotypes for dry matter content, mineral concentrations (potassium, calcium, magnesium, and phosphorus), anti-nutrient compounds (trypsin inhibitor, soluble oxalate, calcium oxalate, and total oxalate), and antioxidant enzymes (peroxidase, polyphenol oxidase, and catalase) in cormels from 31 taro cultivars from West Bengal, India. Based on these results, it is evident that breeding of taro for improved nutritional quality is possible.

7.5 Our Current Research Efforts on SNPs as Genetic Markers

Next-generation sequencing methods (GBS and RNA-seq) have advantages of providing thousands if not millions of markers that could be used for phylogenomics, trait mapping, and genome-wide association study, and to understand tissue-specific response to pathogens. Similar to microsatellite markers, SNP markers have bi-allelic or possibly tri-allelic states (triploid only); tri-allelic genotypes are otherwise predicted to be rare because of the low likelihood of a point mutation occurring three times at the same chromosomal location. Unlike SSRs, SNP markers do not require

standardization across laboratories, making them ideal for joint research efforts conducted across multiple laboratories. For trait mapping, many loci are necessary to cover genome intervals that capture linkage groups. While SSRs and SNPs are both options, recent advances in sequencing permit scoring great numbers of SNPs (thousands to millions) at much lower costs and greater ease (Schielzeth and Husby 2014).

Taking advantage of a taro breeding program established at the University of Hawaii (Cho et al. 2007), progress has been made recently to acquire a genome-wide set of genetic markers representing a range of taro genotypes from Hawaii, the South Pacific, and mainland Asia (primarily from China). The objectives of these efforts are to study the genetic basis of phenotypic traits relevant to taro breeding (e.g., TLB resistance), obtain a linkage map of the taro genome, and shed light on the phylogeography and cultivation history of taro in the Pacific.

Approximately 60 samples representing the majority of extant genotypes from Hawaii, several genotypes from Palau, as well as several genotypes each of South Pacific and mainland Asian origin (introduced to Hawaii post-European contact) were SNP typed using reduced representation methods based on restriction enzyme digest (GBS) and Illumina next-generation sequencing. After pooling and assembling the resulting libraries to serve as a reference, >1700 SNPs, were identified after quality filtering (Helmkampf et al. 2018).

Principal component analysis of this dataset (Fig. 7.4) revealed several distinct groups among the represented Hawaiian, South Pacific, and mainland Asian genotypes. The largest differences were found between a large but tightly clustered Hawaiian group containing many genotypes selected for their purple corm color (e.g., 'Lehua') and all remaining cultivars. Hawaiian cultivars characterized by striped petioles ('Manini') and large, undulating leaves ('Lauloa') also clustered separately. Phylogenetic analysis provided further resolution within the remaining cultivars: alongside the above-mentioned groups 'Lehua,' 'Manini' and 'Lauloa,' 'Ula'ula' (Hawaiian, red petioles), 'Mana' (Hawaiian, branched petioles), 'Kāī'(Hawaiian), Palauan, and Asian cultivars were also recovered as monophyletic groups with high to moderate support.

While the phylogenetic relationships between groups could not be resolved reliably, these results demonstrate that the traditional Hawaiian classification scheme based on morphological traits (elaborated upon by Whitney et al. 1939) is largely congruent with phylogenetic kinship. The fact that traits used to define groups (e.g., corm color, petiole color, leaf shape) are consistently and usually only found within monophyletic groups further suggests that these traits are under strong genetic control and were carefully maintained during centuries of selection. Hybridization between established Hawaiian groups consequently seems to have occurred rarely.

Interestingly, five cultivars introduced to Hawaii from the South Pacific after European contact were found contained within groups consisting of old Hawaiian genotypes (e.g., 'Tahitian,' within Mana group), instead of being paraphyletic with respect to Hawaiian genotypes. This finding caused us to hypothesize that the split between the Hawaiian taro groups occurred before Hawaii (and possibly other East Polynesian islands) was colonized by the first Austronesian settlers. It is congruent with recent evidence that Austronesians colonized East Polynesia in one major pulse

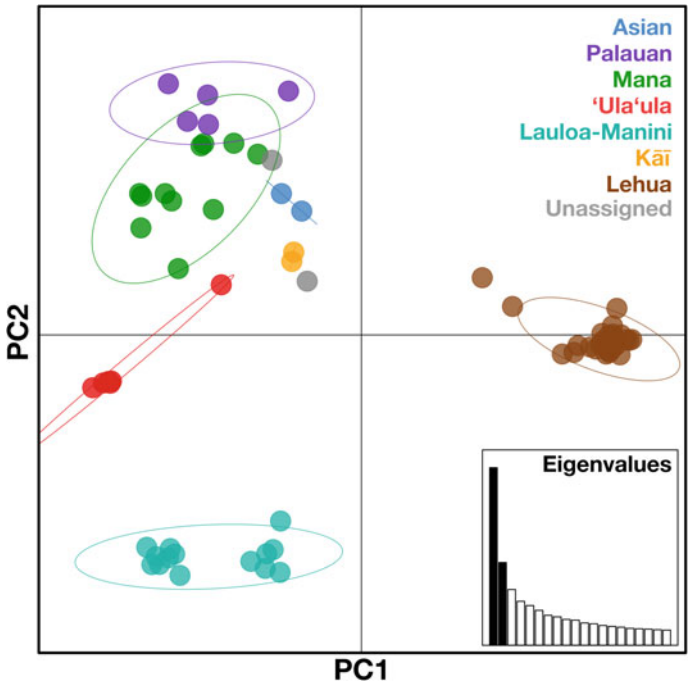

Fig. 7.4 Principal component analysis (PCA) of >1700 SNPs obtained from Hawaiian, Palauan, and Asian (e.g., China) taro landraces/cultivars (Helmkampf et al. 2018). Samples fall into several distinct clusters that are consistent with results from phylogenetic analyses (indicated by symbols or colors) and traditional, morphology-based nomenclature (Whitney et al. 1939). The first two principal components explain 16.3% (PC1) and 7.6% (PC2) of the variance. Reproduced with permission of Oxford University Press

between A.D. ~1190 and 1290 (Wilmshurst et al. 2010). In conclusion, the taro groups probably originated further back in the colonization history of the Pacific, and were brought to Hawaii as established groups where they further diversified by selection of desirable mutations or occasionally occurring cross-pollinations.

7.6 Summary

Taro is one of the oldest cultivated crops. As a species, it has great morphological diversity, with over 10,000 landraces. Various genetic markers have been used to study the genetic diversity of taro, and although the center of origin is uncertain, there is general agreement that there are two separate gene pools: (1) India to Southeast Asia and (2) New Guinea. When breeding taro for improved yield, disease resistance, and quality, it is important to include taro genotypes with wide genetic diversity. Although it is the fifth most produced root crop in the world, taro has been neglected in terms

of genetic resources. While there are regional collections of taro cultivars, there is no international center (i.e., CGIAR) with a mandate to conserve and carry out research on taro. Taro has been neglected also in regard to recent advances in molecular biology, with only a limited number of studies utilizing next-generation sequencing to generate genetic markers for trait mapping and one next-generation transcriptome. At present, a high-quality reference genome is lacking; however, recent genotyping-by-sequencing (GBS) approaches promise to improve our understanding of taro genetics.

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Chapter 8
Genetic Diversity in Banana

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Abstract Banana and plantain are one of the globally important commercial crops supporting livelihood and food security of millions across the globe. In view of environmental degradation, climate change, loss of diversity of crop plants and thereafter the need for diversity-based farming for the sustainable food system, the importance of conservation and utilization of genetic diversity of *Musa* are globally signified. The edible banana fruit is available entirely from section Eumusa and rarely from section Australimusa of the genus *Musa* under family Musaceae and developed from two ancestor species *M. acuminata* and *M. balbisiana* in the South East Asian centre of origin. The diversity and distribution of species, subspecies and groups are important aspects in evolutionary and conservation studies. There are so far three major geographical regions of distributions of cultivated bananas, viz. Asia and the Pacific (29%), Africa (35%) and Latin America and the Caribbean (36%). A large proportion (70–85%) of the gene pool of the domesticated banana is available within Asia and the Pacific regions. The conservation of *Musa* germplasm is the priority objective of programmes and activities related to *Musa* (Banana and Plantain) diversity. The strategy for *Musa* conservation has been developed by the International Network for the Improvement of Banana and Plantain (INIBAP). The international banana germplasm collection is managed by the International Transit Centre (ITC) with INIBAP/Bioversity International, having the world's largest *Musa* germplasm collection and conservation with around 1500 accessions. Morphological and molecular characterization is useful in the classification of cultivars and newly discovered wild *Musa* species. There are two major utilizations of the collected and evaluated *Musa*

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germplasm, viz. use of accession in breeding and crop improvement programmes and secondly, boosting production through direct distribution of better accession to the small-scale banana growers. The International *Musa* Testing Programme (IMTP), coordinated by INIBAP with the NARS and breeding programmes, resulted in several promising banana hybrids. Direct use of *Musa* accessions by the farmers from the ITC also showed encouraging impact on small banana growers in Cuba, Northern Tanzania and Nicaragua for their food security and better income. There is a need for more intensification of *Musa* germplasm collection, conservation and utilization, so as to achieve the call of the 'Delhi Declaration on Agrobiodiversity Management 2016'.

Keywords Agrobiodiversity · Genetic diversity · *Musa* · Germplasm · Species · Wild · Banana · Plantain · Cultivar · Variety · Collection · Characterization · Utilization

8.1 Introduction

Banana and plantain are one of the globally important commercial crops, belonging to the genus *Musa* and supporting a significant contribution to the global food commodity sources (in terms of gross value of production) after rice, wheat and milk (Horry 2000). It is grown in more than 300 countries in the world (Singh 2008) with an annual production of 106.84 million tons from 5.03 million hectares of the plantation (Anon 2015). Livelihood and food security of millions across the globe are dependent on this crop. The sustainable banana production system is supposed to be one of the main pillars of the sustainable food system with minimal impact on the environment, for the much needed focus on addressing world hunger and malnutrition especially under the changing scenario of climate. But the real challenges are multidimensional and interconnected in respect of sustainability, as recognized by the Sustainable Development Goals, signed by 193 world leaders in 2015. In the past, it was needed to increase food production but could not reimburse due attention for the environment, water and biodiversity, and hence, contributed to considerable environmental degradation and loss of crop diversity. For a sustainable solution, agricultural diversity and diversity-based farming have been advocated as the major backbones of sustainable agricultural intensification and sustainable food system (Tutwiler et al. 2017). This line of sustainable food system signifies the importance of genetic diversity of *Musa* and its conservation and utilization. The genetic diversity is the sum of the gene pool in wild relatives, native landraces, local selections, elite cultivars and released and introduced varieties ranging from its centre of origin, diversification and commercial cultivation. The primary responsibility is the collection and conservation of the existing diversity in a systematic manner (Dinesh and Veena 2015), followed by its ultimate objectives of characterization, evaluation and utilization of the gene pool for a sustainable banana production system. Our effort was to compile

the constructive information selectively into a simplified and summarized article on genetic diversity in banana.

8.2 Family (Taxonomic) Background of Banana

The commercially available edible banana and plantain are the finest representative members of the plant family Musaceae and genus *Musa* that resulted from historical evolution and domestication processes. The order Zingiberales (previously called Scitaminae) accommodates the Musaceae family along with other seven related families. Altogether of these eight families have over 2000 species, of which there are about 50 species of the banana family, Musaceae. However, when genetic diversity of edible banana is concerned, interest is directed towards Musaceae family only, with special emphasis on genus *Musa* and its sections Eumusa and Australimusa. Under the family Musaceae, there are two genera, viz. *Ensete* and *Musa*. The genus *Musa* includes four sections—Callimusa, Rhodochlamys, Australimusa and Eumusa, of which edible fruit is available only from Eumusa and rarely from Australimusa (Table 8.1). The economic importance of the sections Callimusa and Rhodochlamys is limited to commercial exploitation of natural fibre and ornamental plant (Valmayor 2000).

Table 8.1 Genus and species of the banana family Musaceae[a]

Genus[b]	Section	Species	Distribution	Uses	Chromosome
Ensete	–	7–8	West Africa to Papua New Guinea	Vegetable, fibre	9
Musa	Callimusa	5–6	Indo-China, Thailand, Malaysia and Indonesia	Ornamental	10
	Rhodochlamys	5–6	India to Indo-China	Ornamental	11
	Australimusa	5–6	Queensland, Australia to the Philippines	Fibre, fruit	10
	Eumusa	9–10	India, South East Asia, Papua New Guinea, to South Pacific and Japan	Fruit, fibre, vegetable	11

[a] *Source* Valmayor (2000)
[b] Inclusion of the third genera, *Musella* was controversial, while Insertae sedis was a new section added to genus *Musa* (Uma et al. 2005)

8.3 Origin and Evolution

Earlier it was considered that banana is originated in India, owing to its antique evidence in Indian culture, epics and wide diversity and adoption of banana (Valmayor 2000). Later, based on a systematic study by Simmonds and Shepherd (1955) with expertise in genetics and cytotaxonomy, it was concluded that South East Asia is the centre of origin of the ancestors of edible banana. Those ancestors of the present day seedless edible banana were seedy, non-pulpy and non-edible (Uma et al. 2005). There were two most widespread ancestor species under the genus *Musa*, viz. *M. acuminata* and *M. balbisiana*, of which *M. acuminata* types had its primary centre of origin in the Malayan region while *M. balbisiana* was of Indian origin (Horry 2000; Chandel and Agarwal 2000). The natural distribution of the genus *Musa* stretches in the north from Nepal and southern mountainous China and in the south to the southern islands of Indonesia and New Guinea, with an outlier in the wet tropical rainforests of Queensland. The western limit is India, with an outlier on Pemba Island near the East African coast. To the east, wild *Musa* were recorded in Melanesia, with an outlier on Samoa. No wild *Musa* have been recorded on the African continent or in the Americas. These boundaries define the area of primary (natural) diversity of *Musa* (Langhe et al. 2009). Evolution of edible banana was initiated with the *M. acuminata* types. Due to the gradual development of parthenocarpic traits, early human settlers discovered the edible types of banana fruit, leading to the most striking human interventions in the form of selection and perpetuation of edible types of banana across geographical locations. As a result, the *M. acuminata* types come across the Indian subcontinent where it introgressed with *M. balbisiana*, and hence, the earliest bispecific types, i.e. the natural hybrids of banana were evolved. Differential combinations of these two wild progenitor species resulted in the development of different genomic constituents and groups, ranging from diploid to tetraploids. Considering 'A' genome contribution from *M. acuminata*c olla and 'B' genome contribution from *M. balbisiana* colla, the bispecific genomic groups of diploid edible bananas are AA, AB and BB, triploid bananas are AAA, AAB and ABB and the tetraploids bananas are AABB and ABBB (Uma et al. 2005). As the present day edible banana is the outcome of the initial natural hybrids and carrying genome contributions of two species (*M. acuminata* and *M. balbisiana*), hence giving a species name to edible banana is inappropriate. The name of edible banana is to be decided as per genomic nomenclature based on the proportionate genomic contribution of the progenitor species (Valmayor 2000), for example, 'Musa (AAB) Martaman' is the genomic nomenclature of the banana cultivar 'Martaman'. However, the recent molecular genetic studies suggested that the cultivated bananas might have been derived through intra- and inter-specific hybridizations of four wild Musa species, namely *M. acuminata* (A-genome), *M. balbisiana* (B-genome), *M. schizocarpa* (S-genome) and *M. textilis* (T-genome) with identification of two more genotypes (AS and AT) and the S- and T-genome cultivars being occurring in New Guinea (Li and Ge 2017). A few, mostly tetraploid edible banana varieties in the Melanesia-Philippines region show some characteristics of the species *Musa schizocarpa* Simmonds (Section Eumusa) and

Musa textilis Née (Section Australimusa) besides those of the major contributing species, *M. acuminata* and *M. balbisiana*. However, the contribution of *M. schizocarpa* and *M. textilis* to the generation of edible bananas is very minor and probably relatively recent (Langhe et al. 2009).

8.4 Diversity and Distribution of Different Sections, Species and Subspecies of the Genus *Musa*

8.4.1 Diversity and Distribution of Different Sections of the Genus Musa

There are four sections of the genus *Musa*, viz. Australimusa, Callimusa, Rhodochlamys and Eumusa, and they have major diversity and distribution in South East Asia, except Australimusa. Champion (1967) presented the geographical distribution of these sections and that had been accepted traditionally and widely. However, a need was felt for revision of these traditionally accepted boundaries of distribution of sections to develop more accurate ones and could reasonably justify the presence of Callimusa in China and Australimusa in Borneo (Fig. 8.1) (Pollefeys et al. 2004).

The Philippines, Queensland and Australia are the major regions of diversity, distribution and commercial utilization of the species of section Australimusa that comprises of 5–6 species. The commercial production of textile fibres in the Philippines, called 'Abacca' or 'Manila hemp' is done from *Musa textilis* which is a species under the section Australimusa. Another species under this section is *Musa fehi*, grown in the South Pacific (locally known as 'Fe'i banana') and Indonesia (locally known as 'Pisang Tongat Langit') for edible fruits that are borne on upward-erect bunch with its male axis pointed to the sky (in contrast to the species of Eumusa section which bear fruit on hanging bunches). The species (about 5–6) under the section Callimusa are mostly non-domesticated and wild, with major distribution in Indo-China and Indonesia, and utilization is limited to its ornamental values. The section Rhodochlamys comprises the species which are distributed in India, Indo-China, the Philippines, Thailand and Malaysia and mostly ornamental with small plant size and bright colour, erect to semi-erect inflorescences (Horry 2000; Valmayor 2000). Uma et al. (2006) redefined the distribution of the section Rhodochlamys in India, based on their explorations and earlier literature and reported the distribution of six species across 11 states of the country. There are at least ten species under the section Eumusa, and they are distributed in India, South East Asia, Papua New Guinea, to South Pacific and Japan (Table 8.2).

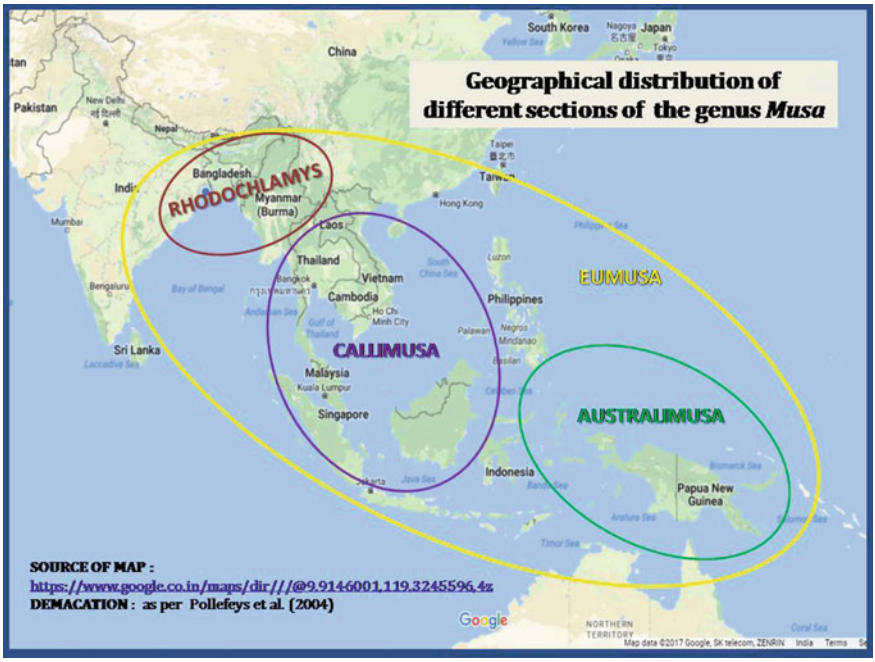

Fig. 8.1 Geographical distribution of different sections of the genus *Musa*. Figure construction is based on a map source (Google maps 2018) of https://www.google.co.in/maps/dir///@9.9146001.119.3245596.4z and information source of Pollefeys et al. (2004)

8.4.2 Diversity and Distribution of Different Species and Subspecies of the Genus Musa

Among the species of Eumusa, *M. acuminata* and *M. balbisiana* are two very wide spread species across the centre of origin and diversity. However, the distribution of *M. balbisiana* was supposed to be less extensive than *M. acuminata*. The southern India, northern Myanmar (Burma) and the Philippines were considered as the regions of the original population of this species, from where they were introduced to other locations (Cheesman 1948; Argent 1976). In the studies for clarification on the issue of origin of 'Butuhan' clone (an ancient hybrid of *M. balbisiana* and *M. textilis*), it was indicated that *M. balbisiana* was an introduced species also in the Philippines, and hence the origin of *M. balbisiana* was limited to India and Myanmar only (Carreel 1994; Horry 2000). Shepherd (1990) presented the diversity and geographical distribution of the subdivisions of *M. acuminata* (Table 8.3, Fig. 8.2). Based on molecular studies, some of the subspecies were similar to others (ssp. *banksi* vs. *microcarpa; zebrine* vs. *sumatrana*) and were tentatively summarized by Horry (2000) into six subspecies, distributed in six regions (Table 8.4). However, Perrier et al. (2009) differentiated *M. acuminata* seeded diploids into four basic clusters on the basis of molecular markers (RFLP and SSR), viz. (i) *M. acuminata* spp. *banksii*

Table 8.2 Diversity and distribution of species under different sections of the genus *Musa*

Section	Selected species	Distribution	Uses
Australimusa (Basic chromosome No. 10)	*M. textilis* *M.maclayi* *M. lolodensis* *M. peekelii* *M. fehi*	Queensland New Caledonia Philippines Australia	Fibre, fruit and vegetable
Callimusa (Basic chromosome No. 10)	*M. coccinea* *M. violascens* *M. gracilis*	Indo-China Indonesia	Ornamental
Eumusa (Basic chromosome No. 11)	*M. acuminate* *M. balbisiana* *M. schizocarpa* *M. itinerans* *M. flaviflora* *M. sikkimensis* *M. cheesmani* *M. nagensium* *M. halabanensis* *M. ochracea*	India	Fruit, vegetable, fibre and medicinal
Rhodochlamys (Basic chromosome No. 11)	*M. ornate* *M. velutina* *M. laterita* *M. sanguinea* *M. mannii* *M. aurantiaca* *M. rosea* *M. rubra*	India Indo-China Philippines Thailand Malaysia	Ornamental
Incertaesedis (Basic chromosome No. 7, 9)	*M. boman* *M. ingens* *M. lasiocarpa*	–	Medicinal

Source Uma et al. (2005)

cluster from New Guinea, (ii) *M. acuminata* spp. *malaccensis* cluster from Malayan Peninsula, (iii) *M. acuminata* spp. *burmanica, burmanicoides, siamea* from north-east India, Burma, southern China and Thailand and (iv) *M. acuminata* ssp. *zebrina* cluster from Java.

8.4.3 Diversity and Distribution of Different Groups and Subgroups of Edible Bananas

The diversity and distribution of different groups and subgroups of cultivated bananas and plantains have been the consequences of the long history of continuous evolutionary and domestication pathways through its wild, cultiwild and neutralized forms, towards its basic cultivars (clones) and derived cultivars. The genome assemblies

Table 8.3 Diversity and geographical distribution of the subdivisions of *M. acuminata*[a]

Subspecies of *M. acuminata*	Geographical distribution
*M. acuminata*ssp. *urmannica/burmannicoides*	Sri Lanka, Eastern India (including Assam), Myanmar (Burma), northwest Thailand
*M. acuminata*ssp. *siamea*	Thailand (central, northern, eastern-?), Indo-China
*M. acuminata*ssp. *malaccensis*	Thailand (southern), Malaysia (northern), Sumatra
*M. acuminata*ssp. *truncata*	Malaysia (upland areas), Thailand (southern-?)
*M. acuminata*ssp. *microcarpa*	Only at Sabah and Sarawak
*M. acuminata*ssp. *banksii*	Irian Jaya, Papua New Guinea and neighbouring islands, Australia (northern)
*M. acuminata*ssp. *errans*	The Philippines
Non-chracterized Indonesian forms *(zebrina, M. sumtrana?)*	Sumatra, Java, Kalimantan, Sulawesi
'Pemba' forms	Zanzibar (?)

[a]*Source* Shepherd (1990)

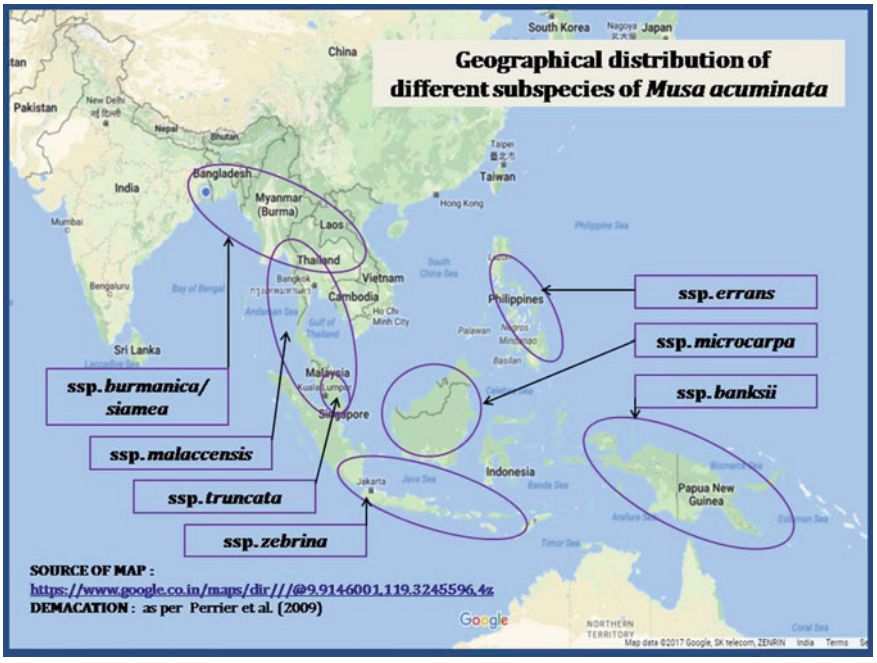

Fig. 8.2 Geographical distribution of different subspecies of *Musa acuminata*. Figure construction is based on a map source (Google maps 2018) of https://www.google.co.in/maps/dir///@9.9146001.119.3245596.4z and information source of Perrier et al. (2009)

Table 8.4 Revised diversity and geographical distribution of the subspecies of *M. acuminata*[a]

Subspecies of *M. acuminata*	Geographical zones of distribution
M. acuminata ssp. *urmannica/burmannicoides/siamea*	**Northern zone**—India, Myanmar, Thailand (central, northern)
M. acuminata ssp. *truncata*	**Malaysian mountains**
M. acuminata ssp. *malaccensis*	**Central-western zone**—Thailand (southern), Malaysia (northern), Sumatra
M. acuminata ssp. *microcarpa*	**Central-eastern zone**—Borneo
M. acuminata ssp. *banksii/errans*	**Eastern zone**—The Philippines, Papua New Guinea, Australia (northern)
M. acuminata ssp. *zebrine* (*M. acuminata?*)	**Southern zone—**Indonesian archipelago

[a]*Source* Horry (2000)

that constitute the entire spectrum of edible banana and plantains, such as AA, AAA, AAB or ABB, are designated as the 'Groups', while the total set of a basic cultivar and its derived clones form a 'Subgroup'. Development of subgroups appeared to occur in regions distant from the primary (natural) distribution of *Musa*. These distant regions are usually known as the centres of secondary and tertiary diversity. Derived cultivars can undergo somatic mutation, thus leading to new derived cultivars. Hence, continued diversifications at secondary and tertiary centres of diversity are supposed to result in distributions of the high density of specific banana cultivar groups at notable geographical areas, as given in Table 8.5 (Langhe et al. 2009). There are so far three major geographical regions of distributions of the cultivated bananas and plantains, with a somewhat one-third contribution by each to the total global production, viz. Asia and the Pacific (29%), Africa (35%) and Latin America and the Caribbean (36%). Each of these three geographical regions has a separate set of diversity with respect to cultivated bananas. Among the groups of cultivated

Table 8.5 Geographical distribution of the main banana cultivar groups[a]

	Group	Geographical distribution
1	The AA and AAA cultivars	The 'Indonesia-Philippines-Melanesian' region, with exceptional AA density in New Guinea and around
2	The Highland AAA bananas (East African Highland bananas, EA-AAA)	The Great Lakes region in East Africa
3	The AAB-Plantains	The rainforest zone in Africa
4	The AAB Maia Maoli-Popoulu-Iholena cultivars	Oceania
5	The AB and other AAB	South India
6	The Eastern ABB cultivars	Philippines and Vietnam
7	The Western ABB subgroup	Northeastt India and South India

[a]*Source* Langhe et al. (2009)

banana in South East Asia, the dessert and cooking banana belonging to AAB and ABB groups showed the highest diversities in that regions. In Africa, the highland banana (EA-AAA) of AAA group is predominantly cultivated in East Africa, while the AAB clones of Plantain subgroup are grown in the Central and West Africa. The cultivated bananas of the Latin America and the Caribbean belong to three major subgroups, viz. Cavendish subgroup of AAA, Plantain subgroup of AAB and Pome and Silk subgroups of AAB group. The banana varieties exported throughout the world are only from the Cavendish subgroup of the AAA group (Table 8.6) (Horry 2000; INIBAP-IBPGR 1990).

Table 8.6 Diversity and distribution of triploid subgroups of cultivated banana and plantains[a]

Subgroup	Distribution
AAA—Cavendish	It is a main export of banana and grown for local consumption in many countries. Humid tropical regions between 20°N and 20°S also extended to 20° and 30° latitude of both hemispheres
AAB—Plantain	Mainly found in hillsides of humid tropics to the lowland humid tropical forest of Americas, West Africa and South India. It can grow in very poor to fertile alluvial soil
AAB—Silk, Mysore and Pome	These types of bananas are slightly acidic in taste. They are very popular in the southern states of India. Other than India, they can be found in Brazil, few restricted areas of Mexico and Venezuela. Pome type can be found in Australia; whereas, Silk type is grown in the Caribbean and South East Asia
ABB—Bluggoe and Pisang Awak	These are hardy cooking-type banana but also used as a dual purpose (dessert type). Bluggoe and Pisang Awak can grow even well in marginal land and harsh environment, as well as higher altitudes in tropical regions. Bananas from this group used a staple food in South America, especially indigenous people of Savannahs and Amazon basin. Pisang Awak is popular in the backyard garden of Asia and grown on small to medium scale
AAB—Maia Maoli/Popoulu	Maia Maoli/Popoulu cultivars are mainly grown as a backyard banana in Pacific Island. They are very important cooking-type banana in this area. They are also found in the west coast of South America ('Maqueno' cv. In Ecuador)
AAA—Mutika/Lujugira	These types are also known as East African Highland banana, grown between higher altitudes of 1000–2000 m. Major food crop of Uganda, cultivated in 40% of total arable land

[a]*Source* Horry (2000)

8.5 Collection and Conservation of *Musa* Germplasm

The major proportion (70–85%) of the gene pool of domesticated dessert banana is constituted from the Asia and the Pacific regions. Whereas the gene pool of the cooking type banana and plantain predominate in the African continent. More than 60 cooking banana types are available in the East African Highlands, and more than 120 plantain types are available in the West and Central Africa. Introduction of banana to the African continent dated back to about 3000 years ago and its remarkable diversification resulted in a source of staple food for the people of many countries of this continent based on those cooking-type banana and plantains. Another edible banana group, known as Fe'I bananas, is confined to the Pacific. However, the threats posed by habitat destruction and the replacement or loss of traditional cultivars intensify the urgency for collection and conservation efforts. Besides, the crop improvement strategy needs a well collected and conserved gene pool (INIBAP 2006). Widespread diseases and pests of *Musa* such as banana bunchy top virus, weevil borer, Sigatoka leaf spot and Fusarium wilt are major problems for the conservation of *Musa* germplasm at ex situ field gene bank. Many uncommon and rare species are on the verge of extinction even in in situ conditions. Various species of *Musa* face challenges due to the destruction of their wild habitat by deforestation, shifting cultivation and wild animals. Therefore, there is utmost importance for conserving natural habitats of different rare and uncommon species (Menon 2016). According to the approach of the diversity-based production system, productivity and sustainability may be enhanced by integrating inter- and/or intracrop diversity within the production system and the losses from epidemic diseases can be mitigated by planting mixed genotypes in place of extensive monocrops of a single variety of banana. In view of the 'Global Conservation Strategy for *Musa* (Banana and Plantain)' has been developed by INIBAP to provide a framework for the efficient ex-situ conservation of the globally important collections of *Musa species* (INIBAP 2006).

The planning for conservation of *Musa* germplasm was laid out at an international level at a seminar in 1989 in Belgium under the aegis of INIBAP and IBPGR. Conservation of *Musa* genotypes in their natural habitat requires the protection of their centre of origin and secondary centre of diversification. It is very challenging to implement any recommendation due to the vast land area. For supporting regional research and development initiatives and conservation efforts, there are four regional banana research networks, viz. BARNESA (for Southern and Eastern Africa), *MUSA*CO (for West and Central Africa), BAPNET (for Asia and the Pacific) and *MUSA*LAC (for Latin America and the Caribbean) (Fig. 8.3). These networks are integrated with the national research organizations of respective banana-producing countries, coordinated by a regionally posted INIBAP scientist. Several countries having high *Musa* diversity have been supported by INIBAP for collection, conservation and characterization of *Musa* germplasm. The banana germplasm collections and conservations of major gene banks in Asia and the Pacific Region have been presented in Table 8.7 (Valmayor 2000).

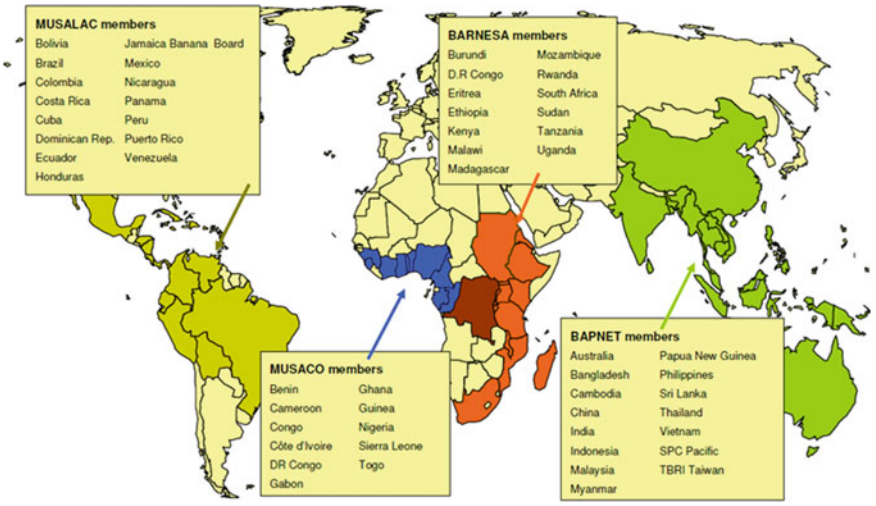

Fig. 8.3 Regional banana research networks—BARNESA, *MUSA*CO, BAPNET and *MUSA*LAC

8.5.1 Methods for Conservation of Musa *Germplasm*

The conservation of *Musa* germplasm is the priority objective of programmes and activities related to *Musa* diversity. The methods generally followed for the conservation of *Musa* germplasm are broadly categorized as in situ and ex situ conservation, depending on the site of conservation with respect to its natural habitat.

(1) In situ conservation: It refers to the conservation efforts of *Musa* germplasm on-site under natural habitat where those were originated and evolved. Conservation of traditional local cultivars in the farmer's field under the observation of farmer with continuous cultivation and improvement of cultivars may also be considered under this type of conservation.

(2) Ex situ conservation: The ex situ conservation refers to the conservation and maintenance of samples of living organisms outside their natural habitat. The plant parts used for ex situ conservation are whole plant, seed, pollen, vegetative propagule, tissue or cell culture, and accordingly the ex situ conservation techniques are also different. For example, short to medium period conservation of the whole plant can be done in field gene bank, while medium to long-term conservation is possible in cryopreservation using vegetative propagule or tissue culture materials.

   (a) Seed conservation: Long-term conservation of genetic diversity of the natural population of *Musa* species by seed can be possible through different technological intervention (Darjo and Bakry 1990). The feasibility of conserving the wider *Musa* wild diversity needs to be explored, and with available knowledge of seed biology, seed conservation needs to be researched

Table 8.7 Banana germplasm collections of major gene banks in Asia and Pacific Region[a]

Country	Germplasm collection	Institute
Australia	5 AA, 23 AAA, 7AAAA, 1 AB, 9 AAB, 4 ABB, 3 wild *Musa* species: *M. acuminata, M. balbisiana, M. zebrina*	Maroochy Horticultural Research Station, Department of Primary Industries, Nambour, Queensland 4560, Australia
	81 AA, 13 AAA, 24 AAB, 9 ABB, 1 ABBB, 3 BBB, 1 Fe'i banana, 5 AS, 3 AAT, 27 wild *Musa* species	South Johnstone Research Station, Department of Primary Industries, South Johnstone, Queensland 4859, Australia
Indonesia	9 AA, 9 AAA, 9 ABB, 2 BBB, 1 each *Musa acuminata* and *M. balbisiana*	Centre for Research and Development in Biotechnology, Cibinong, Java, Indonesia
	10 wild *Musa* species and 3 subspecies of *Musa acuminata*	Bogor Botanical Garden, Bogor, Java, Indonesia
Malaysia	16 AA, 16 AAA, 12 AAB, 6 ABB, 2 ABBB, 3 BBB, 6 wild *Musa* species and 6 *Musa acuminata* subspecies and 1 Ensete	Malaysian Agricultural Research and Development Institute, Serdang, Selangor, Malaysia
Philippines	18 AA, 25 AAA, 16 AAB, 9 ABB, 1 ABBB, 1 BB, 9 BBB, 9 wild *Musa* species, 1 Ensete	Bureau of Plant Industry, Department of Agriculture, Davao National Crop Research and Development Centre, Bago Oshiro, Davao City, Philippine
Thailand	9 AA, 8 AAA, 10 AAB, 10 ABB, 1 ABBB, 1 BBB, 3 wild species of *Musa* species, 4 *Musa acuminata* subspecies and 2 Ensete	Kesetsart University, Department of Horticulture, Pakchong Research Station, Nakhon Rachasim, Thailand
Vietnam	10 AA, 18 AAA, 12 AB, 9 AAB, 14 ABB, 9 wild *Musa* species, 1 Ensete	Phu Ho Fruit Research Centre, VinhPhu Province, Vietnam
Taiwan	15 AA, 86 AAA, 8 AAAA, 4 AB, 36 AAB, 20 ABB, 4 BBB, wild and ornamental bananas	Taiwan Banana Research Institute, Chiuju, Pingtung, Taiwan
Papua New Guinea	106 AA, 71 AAA, 60 AAB, 38 ABB, 1 AB, 6 AAAB/AABB, 1 ABBB, 2 AS, 3 AAS, 3 ABBS, 1 AAAT, 1 Fe'i, and 103 unclassified, 6 wild *Musa* species and 1 Ensete	Department of Agriculture and Livestock Laloki, Konedobu, Port Moresby, Papua New Guinea

(continued)

Table 8.7 (continued)

Country	Germplasm collection	Institute
India	8 AA, 48 AAA, 1 AAAA, 12 AB, 84 AAB, 78 ABB, 3 ABBB, 52 unidentified, 6 wild *Musa* species	Consolidated data from field gene banks at National Research Centre for Banana (Trichy, 540 accession), Indian Institute of Horticultural Research (Bangalore, 241), Tamil Nadu Agricultural University (Coimbatore, 125), Banana Research Station (Kannara, 121), Banana Research Station (Hajipur, 115) and others

[a]*Source* Valmayor (2000)

more widely. Seed and embryo cryopreservation have very good prospects but seed storage behaviour and seed germination after a long period of storage are very unpredictable; therefore, embryo rescue protocols are necessary with expertise for successful regeneration of new plantlets (MusaNet 2016). Although properly dried banana seeds can live for a few months to two years, but the germination requirement for different species varies from each other depending upon the germination temperature (Chin 1996). Seed conservation of banana is limited to wild species only, and this option cannot be used to local varieties and cultivars since female sterility is observed.

(b) Field collections and on-farm conservation: Small number of accession to several hundreds of accession is distributed as field collection throughout the whole inter-tropical zone, and this type of field conservation is very essential for assessing and characterization of existing *Musa* germplasm. Establishment of regional field collection in each geographic region made it possible to conserve the diversity on-site in each continent (INIBAP-IBPGR 1990), e.g. for Asia, the Davao in the Philippines (Department of Agriculture, Bureau of Plant Industry-BPI); for East Africa, the Gitega in Burundi (Institut de Recherches Agronomique et Zootechnique-IRAZ); for Central and West Africa, one in Nigeria (IITA) and for Latin America and the Caribbean, another at La Lima in Honduras (FHIA). But, maintaining this large on-site field germplasm centre is very challenging due to natural hazard, and disease–pest problems. A significant amount of *Musa* diversity continues to be maintained in farmers' fields. Many farmers are already practicing de facto on-farm conservation through the continued cultivation of landraces or traditional cultivars or landraces of bananas and plantains. Hence, the traditional local cultivars and local landraces with cultural significance and nutritional value are conserved.

(c) In vitro collections and cryopreservation: In 1989, setting up of an international in vitro collection centre at INIBAP transit centre (INIBAP TC) at Leuven, Belgium energized the collection of various banana germplasm in vitro and duplicating the material in other collection centre around

the different region. Cryopreservation or cryo-conservation is a process where plant parts are preserved by cooling to very low temperatures (typically at −80 °C using solid carbon dioxide or at −196 °C using liquid nitrogen). Satisfactory conservation in liquid nitrogen, followed by explants generation and plantlet formation was reported in banana (Villalobos and Abdelnour 1991; Agrawal et al. 2004). Improved protocol for cryopreservation of *Musa* species has been developed at ITC (KU, Leuven) which involves the use cryoprotectants, followed by slow freezing and plunging into liquid nitrogen. Recovery of banana cell suspensions after five years of storage in liquid nitrogen has been reported and those stored materials recorded with 95% regeneration (Panis et al. 2004, 2007). For conservation of the elite and rare banana germplasm, the ITC took up the programme of cryopreservation of *Musa* germplasm at the global level.

In 1984, the International Network for the Improvement of Banana and Plantain (INIBAP) was established at Leuven, Belgium. Collection of germplasm, its conservation and distribution of diseases-free germplasm were the major activities under *Musa* germplasm management programmes of INIBAP. As per the decision of the CGIAR, the INIBAP was brought under the governance and administration of the International Plant Genetic Resources Institute (IPGRI) in 1993 and henceforth, the international banana germplasm collection is managed by the IPGRI/INIBAP Transit Centre (ITC). For the benefit of the international community, the collections were placed under the auspices of FAO and are held in trust by INIBAP in 1994. With the contribution from 44 countries across the world, including the accessions of wild, cultivated and improved bananas, the collection of INIBAP consisted of approximately 1200 accessions (Van Den Houwe et al. 2003). The genetic diversity of genus *Musa* was largely covered in the collections located at INIBAP Transit Centre (ITC) in Catholic University in Leuven, Belgium, which was the largest assemblage of *Musa* collections and conservation with around 1500 accessions (Table 8.8) and represented by about 15% wild relatives, 75% landraces and 10% advanced cultivars of the genus. The accessions are maintained permanently by in vitro conservation techniques using proliferating shoot culture and slow growth conditions at low temperature (16 °C) and reduced light intensity (25 μmol/m^2/s). Standardized indexing processes against five viral diseases are followed for the incoming germplasm in collaboration with three indexing centres located at Australia, France and South Africa. Only the pathogen-free accessions are made freely available for international distribution (Van Den Houwe et al. 2003).

Major objectives of collection and conservation efforts by the ITC are:

(i) Long-term conservation of *Musa* genetic resources,
(ii) Maintaining a diversity in public domain,
(iii) Contributing to understand *Musa* diversity through characterization,
(iv) Services for safe movement of germplasm and related information,
(v) Developing and transferring ex situ conservation.

Table 8.8 *Musa* germplasm collection and conservation at ITC[a]

Type of source	Donor	Accessions	Genotypes
Major Field collections	FHIA, Honduras (1988)	97	Wild/cultivated forms
	IITA, Nigeria (1986–1987)	85	AAB-plantain
	IRAZ, Burundi (1987)	54	EA-Highland bananas
	CIRAD, Guadeloupe (1987–1990)	267	Wild/cultivated forms
	CARBAP, Cameroon (2010)	41	AAB-plantain
	NRCB, India (2010–2011)	57	AB, AAB and ABBs
Collecting missions	Papua New Guinea (1989–1990)	278	Diploid wild/cultivated
	Vietnam (1996)	43	Wild/cultivated forms
	Tanzania (2002–2005)	56	EA-highland bananas
	DR Congo (2005)	38	Semi-dwarf AAB-plantains
Breeding programmes	CARBAP, CIRAD, EMBRAPA, FHIA, IAEA, IITA, INIVIT, TBRI	126	Improved high-yielding and disease-resistant cultivars
Others	Other collections, botanical gardens, private persons	337	Wild/cultivated forms
Total		1479	

[a]*Source* Musanet (2016)

8.6 Characterization and Evaluation of *Musa* Germplasm

The collected *Musa* germplasm essentially need proper characterization and evaluation to ascertain its genetic relatedness, diversity, conservation strategy and utilization. The characterization is done on the basis of morphological characters, essentially supported by cytological, biochemical and molecular studies.

8.6.1 Morphological Characterization

The most widely used technique of morphological characterization for tentative genomic classification of *Musa* germplasm was given by Simmonds and Shepherd (1955), and it was on the basis of 15 morphological characters contributing to the development of score card for classification. Despite its classical nature and useful-

Table 8.9 Modified score card for assigning tentative genomic groups to collected *Musa* germplasm[a]

Genome	Score card		
	Simmonds and Shepherd (1955)	Silayoi and ChomChalow (1987)	Singh and Uma (1996)
AA/AAA	15–23	15–25	15–25
AAB	24–46	26–46	26–45
AB	49	–	46–49
ABB	59–63	59–63	59–65
ABBB	67	–	66–69
BB/BBB	–	70–75	70–75

[a]*Source* Singh and Uma (2000)

ness, this score card technique has had some lacunae like discontinuity and ambiguity with respect to score ranges. In efforts to overcome the lacunae, modified score cards have been the development by Silayoi and Chomchalow (1987) and Singh and Uma (1996) for assigning tentative genomic groups to collected *Musa* germplasm (Table 8.9). The modifications of score cards were useful for addressing the collected germplasm belonging to AB, ABBB and BB/BBB genomes for morphological characterization. However, a major limitation of score card technique had not been resolved and contributed to the difficulty in distinct differentiation of genomes like AAB and AB or ABB and ABBB, due to overlapping values of scoring. For African plantain cultivars (AAB group), Swennen (1990) developed a morphology-based key and discussed the limitations of morpho-taxonomy for clonal identification. He noted that the incidence of somaclonal variation during in vitro propagation or germplasm maintenance could further complicate the correct identification of clones and confound efforts to use morphological variation to estimate genetic diversity. The ability of (CMT) classical morpho-taxonomy to discriminate various clones weakens as the genetic base of the clones under examination narrows (Jarret and Gawel 1995). The 'Descriptor for banana' developed by IPGRI-INIBAP/CIRAD is a more comprehensive in nature and available since 1996 for use of *Musa* germplasm collectors. A minimum set of descriptors was agreed upon by the Taxonomy Advisory Group (TAG) to field verified accessions from ITC, with the aims to establish a standardized procedure for a routine morphological characterization of banana plants and providing instructions on how to document with photographs, etc. (Gueco et al. 2017).

Another major limitation of morphological character-based score card technique is the unreliability and authenticity of those plant morphological characters which are supposed to be readily influenced by biotic and abiotic factors, resulting in misleading score estimates. The difficulty arises due to the presence of several duplicates and synonyms in a large gene pool that of like *Musa* is also not easy for handling. Hence, apart from morphological characters, cytological, biochemical and molecular studies are also encouraged for fine-tuned characterization and authentic identity of *Musa* germplasm (Singh and Uma 2000).

It is required to using stable and reproducible characters for the classification of recent cultivars and newly discovered wild *Musa* species. From the view point of cytology, one of the basic and stable characteristics of a species is its nuclear genome size, which has been estimated on many edible bananas and their wild ancestors. A genome size of 600–650 Mbp was determined in *M. acuminata* and 550 Mbp in *M. balbisiana*, clearly discriminating both species (Doležel et al. 1994; Lysák et al. 1999). Another important characteristic of a species is karyotype, i.e. the number and morphology of chromosomes. The chromosome number had been shown to determine the sectional classification of individual species of the genus *Musa*. There were some difficulty and complication in cytogenetic studies for detailing and supporting the classification, because of the small size of chromosomes, similarity in the morphology of chromosomes and absence of landmark specific to chromosome. Determination of the ploidy level of collected *Musa* germplasm is very important and that can be done by 'flow cytometry' technique. The INIBAP Transit Centre (ITC) collaborated with the Institute of Experimental Botany, Olomouc and the Czech Republic for determination of ploidy of every ITC accession using flow cytometry (ProMusa 2011).

8.6.2 Molecular Characterization

The biochemical and molecular approaches have been successfully utilized for reliable characterization of *Musa* germplasm by overcoming or supplementing to overcome many known lacunae or limitations those encountered in sole dependence on morphological character-based characterization. There is always a possibility of variations in morphological characters or altered expression of the genetic makeup of the germplasm under the influence of climatic factors and management practices. Whereas, the DNA and its genetic makeup is stable and specific to the germplasm and provides dependable and distinguished characterizations. To identify the germplasm of the *Musa* cultivars, biotechnologists used different markers such as isozymes (MDH, EST, PRX, PGM, GOT, ME, ADH, GDH, SUDH, SDH, GUDH, etc.), random amplified polymorphic DNA (RAPD), restriction fragment length polymorphisms (RFLP), amplified fragment length polymorphisms (AFLP), simple sequence repeats (SSR), diversity arrays technology (DArT), etc. (Uma et al. 2005; Pillay et al. 2012). Microsatellite-based molecular characterization was adopted more widely for *Musa* germplasm and resulted in dependable genotyping and strengthening the knowledge of phylogenetic relationships (Čížková et al. 2015).

8.7 Utilization of *Musa* Germplasm

The Delhi Declaration on Agrobiodiversity Management, adopted at the first International Agrobiodiversity Congress, held in November 2016 in India, called for 'An agrobiodiversity index to help monitor conservation and use of agrobiodiversity'. The Prime Minister of India (the host country), Sri Narendra Modi addressed the Congress and pointed that we a treasure of valuable agrobiodiversity that need to be explored scientifically (Bioversity International 2017). It appeared to be a message of thrust area for widening the utilization of agrobiodiversity. It is equally applicable to the utilization of *Musa* germplasm, like other crops. The thrust for utilization of *Musa* germplasm is an integral part of the strategic framework of the INIBAP (a network of Bioversity International) in their 'Global Conservation Strategy for *Musa* (Banana and Plantain)', where the use of diversity and the services of collections had been targeted to promote worldwide by upgrading collections, serving users' needs and providing easy access to key information about the use of accessions. In this strategy, high priority was given to partnership of germplasm collections with multiplication, demonstration and dissemination facilities. Web-based portal has been initiated, and it provides a comprehensive one-stop reference system on *Musa* taxonomy, accession availability, characterization, evaluation and practical experiences in using diversity for improving livelihoods, along with other initiatives and programmes like *Musa* Germplasm Information System (MGIS) Database, International *Musa* Testing Programme (IMPT), Regional networks (LACNET, ASPNET), the HarvestPlus and Generation Challenge Programmes, etc. (INIBAP 2006; Horry 2000).

8.7.1 Utilization in Banana Breeding and Improvement Programmes

There are two major utilizations of the collected and evaluated *Musa* germplasm, viz. use of accession in breeding and crop improvement programmes and secondly, boosting production through distribution of better accession to the small-scale banana growers (Van Den Houwe et al. 2003). The important research centres involved in banana breeding programmes are—(i) Honduran Agricultural Research Foundation (FHIA: Fundaci´on Hondure˜na de Investigaci´ on Agrıcola) in Honduras, (ii) Brazilian Agricultural Research Centre (EMBRAPA-CNPMF: EmpresaBrasileira de PesquisaAgropecu´aria Centro) in Brazil, (iii) International Institute of Tropical Agriculture (IITA) in Nigeria, (iv) The African Centre for Research on Banana and Plantain (CARBAP) in Cameroon, (v) ICAR-National Research Centre for Banana (ICAR-NRCB) in India, (vi) Banana Research Station (BRS) of Kerala Agricultural University in India, (vii) Tamil Nadu Agricultural University (TNAU) in India and (viii) French Agricultural Research Centre for International Development (CIRAD) in France (Bakry et al. 2009). The National Agriculture Research System (NARS) of each banana growing country in the world has research activities related to *Musa*

germplasm collection, evaluation and utilization and regional strategy of cropping and breeding objectives (Table 8.10).

In conjunction with NARS, breeding programmes and researchers, INIBAP coordinates the International *Musa* Testing Programme (IMTP) (Van Den Houwe et al. 2003). The establishment of the IMTP phase I began in 1989 as a programme to evaluate germplasm from the FHIA breeding programme in Honduras for resistance to banana leaf spot diseases (BLSD) and released three varieties, of which two

Table 8.10 Breeding objectives for bananas[a]

Subgroup	Regions of production	Cropping strategy	Breeding objectives
Cavendish (AAA) Dessert type	Latin America, Caribbean, Philippines, India, West Africa, Mediterranean countries	Export banana intensive system, Local markets	Resistance to diseases, (Black Leaf Streak, Sigatoka, *Fusarium* wilt race 4), nematodes, weevils. Slow fruit ripening. Tolerance to drought and cold temperature
Silk and Pome (AAB) Dessert type	Brazil, India, Australia, South East Asia	Local and regional markets, Food crop system Extensive system. Intensification in progress	Resistance to diseases (Black Leaf Streak, Sigatoka, Fusarium wilt), nematodes, weevils. Fruit quality (fragility). Adaptation to cold temperature
Bananas of East Africa (AAA) Dessert and beer types	East Africa	Local market, Food crop system	Resistance to diseases (Black Leaf Streak, Sigatoka, Fusarium wilt), nematodes, weevils
Plantains (ABA) Cooking type	West Africa, India, Latin America	Local and regional markets. Food crop system. Intensification in progress	Resistance to diseases (Black Leaf Streak), nematodes, weevils. Productivity, Sucker production
Popoulou/Maia Maoli (ABB) Cooking type	Pacific	Local market, Food crop system	Resistance to diseases (Black Leaf Streak, Fusarium wilt)
Saba, Bluggoe (ABB) Cooking type	South East Asia, All marginal zones, Latin America, Caribbean	Local market, Food crop system. Processing industry	Resistance to Fusarium wilt, Moko disease and nematodes

[a]*Source* Bakry et al. (2009)

dessert banana varieties (viz. FHIA-01 and FHIA-02) and one cooking banana variety (FHIA-03). The second phase of the IMTP started in 1996 involving 37 testing sites for developing hybrids resistant or tolerant to BLSD, Sigatoka and *Fusarium* wilt, and the results suggested that FHIA-23 and SH-3436-9 were the most tolerant to BLSD and GCTCV-119 (an improved cultivar) had good yield under good management (Bakry et al. 2009).

Under phase III of IMTP, twenty-one varieties were introduced and were evaluated and tested against black and yellow Sigatoka, Fusarium wilt and nematodes. Information on pathogen populations, host-pathogen relationships and adaptability and productivity were obtained through the evaluation trials (Table 8.11) (Molina et al. 2005).

8.7.2 Direct Use of Genetic Resources by the Farmers

Regarding direct use of genetic resources by the farmers from the International Transit Centre (ITC) for impacting on small-scale banana growers and local consumption, the accessions (that do not contain virus particles) are freely available on request to bonafide users, provided that should be maintained in public domain (Van Den Houwe et al. 2003). The ITC provided 42 *Musa* accessions of different genomes and subgroups, which were characterized at Lapanday Foods Corporation, Davao City, Philippines, and published a catalogue including 25 ITC *Musa* accessions to assist in distinguishing highly heritable characters with supplemental information on the disease reaction of the accessions against Fusarium Wilt (Foc TR4) and Banana Bunchy Top Virus (Gueco et al. 2017). Similarly, 70 ITC accessions were introduced to India through the ICAR-NBPGR, New Delhi, of which 44 accessions are being evaluated at the ICAR-NRC for Banana, Trichy (ICAR-NRCB 2017). Selected accessions through evaluation and framework of IMTP are demonstrated at research stations and extension fields so that small-scale banana growers can observe the better characteristics (more productive and disease resistance) and taste fruit qualities of the new introductions. The superior variety selected by farmers is then multiplied and distributed for direct use by farmers for boosting production and consumption. These efforts recorded an encouraging impact on small banana growers in Cuba, Northern Tanzania and Nicaragua for their food security and better income. Feedbacks by the small banana growers also indicated the need for more intensification of these activities for *Musa* germplasm conservation and utilization (Van Den Houwe et al. 2003; Garming et al. 2010).

Table 8.11 List of hybrids evaluated in IMTP Phase III[a]

Hybrid	Type	Characteristic
FHIA-01	Dessert/cooking	Resistant to BS[b] and FW[c]
FHIA-02	Dessert/cooking	Resistant to BS
FHIA-03	Dessert/cooking	Resistant to BS and FW, drought tolerant
FHIA-17	Dessert/cooking	Tolerant to BS and resistant to FW race 1
FHIA-18	Dessert	Resistant to BS
FHIA-21	Plantain	Resistant to BS
FHIA-23	Dessert/cooking	Tolerant to BS and FW
FHIA-25	Cooking	Resistant to BS
SH-3640	Dessert/cooking	Resistant to BS
BITA-2	Cooking	Resistant to BS, Susceptible FW
BITA-3	Cooking	Resistant to BS
CRBP-39	Plantain	Resistant to BS
SH-3436-9	Dessert	Tolerant to BS
IRFA-911	Plantain	Resistant to BS
GCTCV-119	Dessert	Resistant to FW race 1
GCTCV-106	Dessert	Resistant to FW race 1
GCTCV-247	Dessert	Resistant to FW race 1
'Yangambi km 5'	Dessert/cooking	Reference clone (Sigatoka)
'PisangCeylan'	Dessert	Reference clone (Sigatoka)
'Gros Michel'	Dessert	Reference clone (Fusarium)
'Williams'	Dessert	Reference clone (Fusarium)
'Cultivar Rose'	Dessert	Reference clone (Fusarium)
'Cachaco'	Cooking/dessert	Reference clone (Fusarium)
'PisangJariBuaya'	Dessert	Reference clone

[a] *Source* Molina et al. (2005)
[b] *BS* black Sigatoka
[c] *FW* Fusarium wilt

8.8 Conclusion

Remarkable progress has been made in *Musa* germplasm collection, conservation and utilization with intensive efforts at regional levels and by INIBAP and Bioversity International at international level. Scientific clarification and technical feasibility in these processes have been increased by adopting advancement in cytology, molecular biology and cryopreservation. The diversity and distribution of species, subspecies, groups and subgroups of *Musa* across growing regions and hotspots have been studied by the banana workers. Present collection and conservation of *Musa* germplasm at ITC are the world's largest with around 1500 accessions conserved. Banana breeding and improvement programmes coordinated by INIBAP with the NARS have yielded several promising banana hybrids through IMTP. Evaluated *Musa* accessions being directly utilized by farmers have impacted positively on food security and better income of small banana growers in Cuba, Northern Tanzania and Nicaragua for their food security and better income. These mankind services of *Musa* diversity conservation and utilization are the vivid proposer of the need for more intensification of *Musa* germplasm collection, conservation and utilization, so as to achieve the call of the 'Delhi Declaration on Agrobiodiversity Management 2016'.

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Part III
Agrobiodiversity Utilization

Chapter 9
The Role of Agrobiodiversity in Sustainable Food Systems Design and Management

Ciaccia Corrado, Testani Elena, Roccuzzo Giancarlo and Canali Stefano

Abstract Biodiversity is intended as the whole of living organisms within an ecosystem, whereby the ecosystem functioning strictly depends on the complex interaction among its biotic and abiotic components. Ecosystem services, as the set of benefits provided by the ecosystems to humans, are related to biodiversity conservation and promotion. Similarly, the agrobiodiversity, as the whole of cropped/bred and associated wild biodiversity under agricultural management, can foster agroecological services provided by agroecosystems. Understanding the linkages between agrobiodiversity and services should drive on the agricultural management strategies. By this, the agroecosystems should be designed through biodiversification in space and time, and managed in order to promote those agrobiodiversity traits connected to ecological services (functional biodiversity). However, since successfully diversification implies changes at field production as well as in downstream, the entire food system—and not only the primary production phase—should be considered to proceed towards the ultimate goal of sustainability. In this context, the adoption of agroecology principles, intended as the ecology of the entire food system, can drive towards biodiversified agroecosystems, which are the sustainable from an environmental, economical and social perspective. In this chapter, the actual food system is described, underlining the negative externalities associated to modern agriculture. Agroecology is described as a possible approach to change production paradigm, from the cropped field to the landscape scale, achieving new models for food provisioning in a globalized context. In the meanwhile, the central role of agrobiodiversity in this redesign approach is clarified, focusing on its definition, measurement and management practices aimed to foster the ecological services provided.

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9.1 Introduction

The post-war and the Green Revolution led to a significant intensification of the agriculture in developed countries as a result of the improvement in mechanization, the large-scale use of inputs, and highly productive genotypes. This intensification was accompanied by the progressive specialization of farms and the parallel structuring of agroindustrial sectors with the aim to be more effective in quality control and supply logistics. This pathway has been accompanied by—sometimes extreme—simplification of agriculture production systems at different scales (i.e. cropping system, farm, territory and supply chain) in terms of structures, elements and processes (i.e. biodiversity and its exploitation for production purposes) (Meynard et al. 2013).

Simplification and specialization have been progressively recognized to be the origin of the growing negative external impacts of agriculture, mainly at environmental level, as pollution of local environments, greenhouse gas emissions and loss of biodiversity. These externalities are combined with the effects of the so-called climate change, which exacerbates the burden of uncertainty and variability of environmental conditions. Furthermore, the current unfair distribution of resources, food and individuals' access to them worldwide highlights how agriculture should achieve the challenge of food security without compromising environment, through the reduction of negative impacts, and possibly enforcing the positive contribution on the same environment (Smith 2013).

To face this challenge, it is clear the need to intensify agriculture in a different and sustainable way. Agricultural intensification is a complex concept that has given a series of nuances that are not always clarified when used (Wezel et al. 2015). Sustainable intensification of agriculture relates to the increase of food production while conserving resources, reducing negative environmental impacts and enhancing natural capital and the flow of environmental services (Pretty and Bharucha 2014). This approach does not necessarily imply significant changes in the current 'business as usual' agricultural system. The concept of ecological intensification, instead, arises partially overlapping sustainable intensification in the scientific literature, but it comes from the effort to integrate the understanding of the biological and ecological processes and functions in agroecosystem, to intensify the ecosystem service providing (FAO 2009; Tittonell 2014; Doré et al. 2011). In this perspective, ecological intensification actually constitutes an upgrading of the sustainable intensification concept from farm to landscape scale (Bommarco et al. 2013).

In this upscaling framework of definitions, we can further drive on the issue of sustainability by introducing the concept of agroecology and agroecological intensification. Agroecological intensification of agriculture inserts social and cultural perspectives in its practical implications, which are missing in sustainable and ecological intensification, just orientated to economic and environmental aspects (Wezel et al.

2015). Actually, agroecological intensification focuses on farmers and consumers as key players in agricultural systems.

Gliessman (2016) frames these aspects at the highest levels of the process needed to transform the food system, going beyond the farm to the whole food system and the societies in which they are embedded. He claims that '*food system transformation occurs within a cultural and economic context, and this transformation must promote the transition to more sustainable practices*'. Gliessman argues on agroecology as the way to '*build a new global food system, based on equity, participation, democracy, and justice, that is not only sustainable but helps restore and protects earth's life support systems upon which we all depend*'. A complete definition of agroecology is indeed the 'ecology of the entire food system' (Francis et al. 2003), from soil to the organization of human society, which integrates the ecological, social and economic dimensions. In this prospective, agroecology is contemporary considered as a science, towards action research and interdisciplinarity, following a participatory and holistic approach, as a social movement, supporting rural communities, food sovereignty and short food supply chain, and, clearly, as a practice (Wezel et al. 2011). Indeed, although the conceptual evolution of the term, agroecological principles rely on practical choices, recognizing the ecosystem functions within the agricultural environment as a driving force to obtain sustainable production systems.

9.2 Agroecology as a Practice

As a set of practices, agroecology heads towards the sustainable use of local and renewable resources, enhancing knowledge of local farmers focusing on their priorities, the conscious use of biodiversity as a tool to provide ecosystem services, resilience and environmental, economic and social benefits, from local to global scale (Altieri 2009). At cropping system or farm scale, three main strategies encompassing the soil conservation, the organic matter loop cycling and the diversification have been identified.

9.2.1 Soil Conservation

Conservation agriculture is based on three main principles: minimizing soil disturbance or eliminating the deep ploughing, permanent soil covering with cover crops during the non-cultivation period and diversifying crops in the rotation. The conservative soil management is indeed proven to enhance natural biological processes above and below the ground. On the other hand, there are many challenges faced with the use of conservation agriculture practices, including soil compaction, low nitrogen availability in the raining periods and sourcing of adapted equipment for controlling weeds (Peigné et al. 2016). Therefore, the introduction of conservative practices implies the redesigning of the agroecosystem. For instance, to enhance weed con-

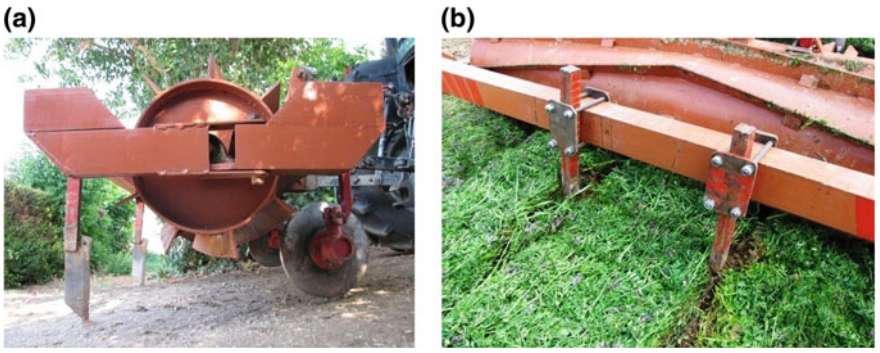


Fig. 9.1 Managing the agroecological service crops: the in-line roller crimper technique (ILRC) for reduced tillage transplanting of vegetable crops. The technique is a reduced tillage system relying with the concept of the in-line tillage and the use of the roller crimper: a sharp vertical disk and a coulter (or chisel) are in-line installed in sequence of the roller crimper. This machinery allows i) to flatten the ASCs and, simultaneously, ii) to obtain a 0.2–0.3 m deep and few centimeters wide transplanting furrow, without disturbing the mulch layer. The mulch covers the soil surface, controlling weeds, reducing water losses and protecting soil. Tillage zone is extremely reduced: soil disturbance and energy consumption are minimized. A = the In Line Roller Crimper machinery; B = coulters opening the transplanting furrow (Canali et al. 2013)

trol, considered as one of main limits to soil conservative management (Mäder and Berner 2012), it is necessary to rethink the cropping system as a whole, by modifying the choice of crops and crop rotations. As an example, the no-till or minimum tillage systems based on roller crimper use (Fig. 9.1) to terminate cover crops have been proven to have a great potential to control weeds and to provide additional relevant ecosystem services (i.e. soil temperature control, energy and water saving), particularly in organically managed vegetable cropping systems (Canali et al. 2013). The no till-roller crimper technology could be used as in alternative to green manuring and allows to terminate cover crops by flattening, and to obtain a natural mulch able to face weed development, simultaneously preparing the cash crop transplanting bed (Luna et al. 2012).

9.2.2 Organic Matter Loop Cycling

According to agroecological principles, system management should be based on an efficient recycling of plant nutrients. At farm level, the nutrient cycling depends on: (i) the virtuous use of plant and animal residues (e.g. composting process); (ii) the combination of production systems (i.e. mixed systems; agroforestry); (iii) the synchronization of nutrient availability with plant demands (Ciaccia et al. 2017a). Since the aim of agriculture is to provide food to humans, the yield removal from agricultural lands inevitably determines nutrient mining conditions on the short and long run. It implies the need to reintegrate nutrients from off-farm input, opening the

Fig. 9.2 Closing the organic matter loop cycle: municipal waste compost. Closing the organic matter loop cycle at territorial scale is promoted by composting of municipal organic wastes. Compost is then reutilized for farming in periurban agricultural areas. Photo: Municipal composting plan at Maccarese, Rome, Italy. A = waste material after collection using degradable bioplastic bags; B = compost at end of the process

discussion on which sources should be used. From an agroecological perspective, the choice should fall on renewable resources of natural origin, respecting a circular cycling of materials from and to the farm. By this, the organic matter loop cycle should be redesigned to redirect its flow from the field to the society and closing the loop back to the field (Fig. 9.2). Strategies aimed at achieving this result (e.g. municipal waste compost—MWC—production and use) are actually encompassed by the framework of the circular economy (EC 2015) and tailored at local level (e.g. MWC use in periurban agricultural areas).

9.2.3 Cropping System Diversification

Actually, agroecosystem redesign is often supported by system diversification by increasing the diversity of cultivars and crops in the rotation, or by valourizing natural biodiversity for biological control. In this perspective, agroecological practices should be intended as a 'design work', in which functional elements (directly or not directly connected to production, such as crops in rotation, cover crops, intercrops, animals and landscape elements) could be introduced, spatially and temporary, and properly managed, with the aim to intensify the system diversity, provided ecosystem services to sustain resilience and, as a consequence, production. As an example, several authors report the effectiveness of cover crops on agroecosystems biodiversity in space and time, influencing beneficial and pest arthropods presence, crop disease control, water and nutrient cycles and the weed community dynamics (Magagnoli et al. 2017; DuPont et al. 2009; Manici et al. 2004). They can be, therefore, defined as Agroecological Service providing Crops, ASC (Canali et al. 2015).

The key point is to identify the desirable kind of biodiversity, able to maintain and/or enhance ecological services, and thus determining the best practices (i.e. functional elements and their management) to build the desired biodiversity components. To do this, a deep comprehension of the complex interrelationships among the components of the agroecosystem is necessary to maximize services and avoid disservices.

9.3 The Role of Agrobiodiversity

9.3.1 What Is Agrobiodiversity?

The target crops, as far as the animal breeds, represent the interaction between the environmental genetic resources and the management practices and systems over millennia. These domestication processes, besides human selection, were also characterized by introgression from wild relatives, hybridization among varieties and races, and mutation phenomena that led to the definition of adapted cultivars and breeds to local abiotic and biotic environmental variation (Benckiser and Schnell 2006). The same processes determined a co-evolution with the wild flora and fauna, accompanying the target species in the agroecosystems (Marshall 2006). The agrobiodiversity, defined as the diversity of organism living in landscapes that are under agricultural management (Wood et al. 2015), includes not only the genetic resources (varieties, breeds, etc.) used for the production of fodder, fibre, fuel and pharmaceuticals, but also the associated diversity that supports production (e.g. soil micro-organisms, natural pest enemies, pollinators). With Green Revolution, changes in food production have led to a reduction in varieties and breeds choice for agricultural purposes. The industrial and intensive agriculture resulting from globalization contributed then to reduce the actual species and varieties of over than 90% (Love and Spaner 2007; Benckiser and Schnell 2006). Thus, during the middle of the last century, agriculture determines loss of biodiversity, including the associated wild component, leading to changes in species composition, and in addition, to environmental pollution due to increased inputs of different origins (Tilman et al. 2001). Beyond that, since agricultural ecosystems cover nearly 40% of terrestrial surface of the heart, modern agriculture contributes up to 30% of total global GHG emission and 70% of water consumption (De Clerck et al. 2016). Several studies showed the links between biodiversity and ecosystem functions, services and resilience (Landis 2017; Wood et al. 2015), highlighting that the loss of biodiversity due to mainstream agricultural practices threatens ecosystems functioning (Power 2010). On the other hand, appropriate management can reduce the 'disservices' provided by agriculture or even generate ecological services. The conservation and restoring of agricultural biodiversity (the agrobiodiversity) is then the key point in obtaining sustainable, produce-and service-providing agroecosystems (Love and Spaner 2007). Moreover, being the associated biodiversity governed by ecological processes that allow them to persist in agri-

cultural settings, but also supporting the same agroecosystems (Wood et al. 2015), recognizing and studying the processes help protect specific services and to manage chosen agrobiodiversity components in order to promote other services. These processes are mainly interference phenomena (plants, micro-organisms), detritivore and nutrient recycling (soil microflora, meso- and micro-fauna), immobilization (micro-organisms), assimilation of inorganic pools (plants), herbivory and predation (animals, including insects). Thus, an in-depth comprehension of mechanisms regulating the expression of beneficial ecological services in agroecosystems will be the key factor also to redesign farm systems.

9.3.2 Agrobiodiversity and Agroecological Services

Ecological service is the suite of benefits provided by functioning ecosystem to the human, including provisioning, regulating and cultural services (Cardinale et al. 2012). Agroecosystems as sources of just provisioning services, strongly dependent on ecosystem services provided by natural ecosystems, can be considered an outdated perception. Agrobiodiversity can affect ecological services directly (e.g. increased crop diversity increases human nutrition) and indirectly (e.g. increased crop diversity reduces the waste of water and runoff), as far as enhance the same production with supporting services. The components of agrobiodiversity supply critical services to the farming process through interaction among them and with the surrounding physical environment, thereby, understanding the linkages between agrobiodiversity and services (Fig. 9.3) can be crucial in managing agroecosystems (Wood et al. 2015; Kremen et al. 2012).

One of the most important agroecological services provided is the biological pest control. Indeed, the spontaneous flora and non-harvested crop (i.e. ASC; perennial vegetation) can be attractive for several beneficial organisms, representing the habitat for antagonistic microorganisms (Power 2010) as far as predator (Fig. 9.4), parasitoid and pollinator arthropods (Landis 2017; Burgio et al. 2015) or weed seed granivore species of birds, rodents and insects (Navntoft et al. 2009). Moreover, high plant biodiversity (wild and/or ASC introduction and/or intercropping) contributes to weed suppression by filling the ecological niches otherwise occupied by competitive weeds (Ciaccia et al. 2017b). This suite of bio-control services can reduce the need for pesticides, including herbicides (Fig. 9.3), contributing to reduce chemical pollution, which is one of the main disservices provided by agriculture. Since soil structure (i.e. soil porosity and aggregation) and nutrient availability are strictly related to organic matter amount and decomposition, soil fertility enhancement can be considered an important ecological service provided by micro-organisms (Altomare and Tringovska 2011) and macro-fauna, such as heartworms. Together with nitrogen fixation due to Rhizobium bacteria activity, decomposer richness potentially allows to reduce synthetic fertilizer use, if organic residues are regularly incorporated into the soil and/or the soil disturbance is reduced (e.g. no-till or minimum tillage). These few examples underpin the importance of agrobiodiversity recognition and evaluation, as

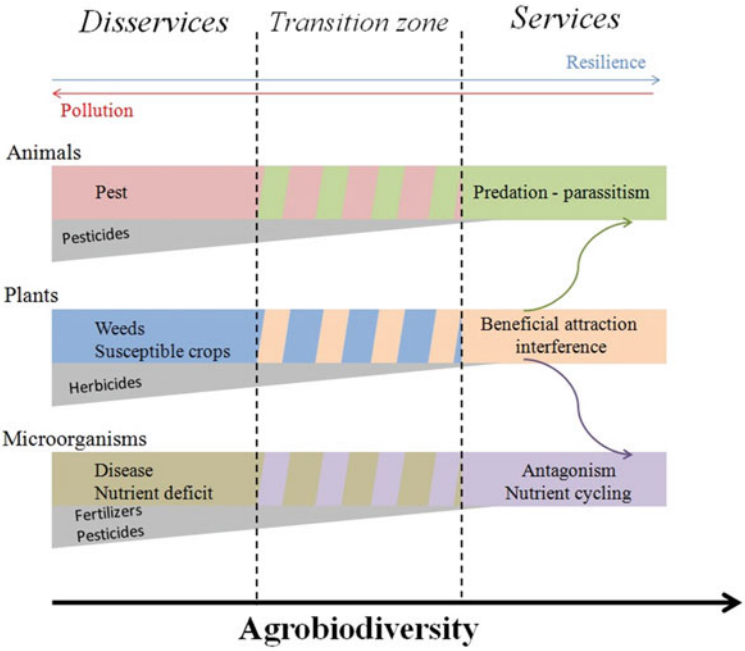


Fig. 9.3 Linkages between agrobiodiversity and ecological services. The increase of agrobiodiversity enhances the presence and the activity of beneficial arthropods, reduces the ecological niches otherwise available for weeds and promotes nutrient cycling, in turn contributing to input use reduction

Fig. 9.4 Aphid predation by *Syrphus vitripennis* (Meigen) on artichoke. *Syrphus vitripennis* contributes to aphids bio-control in agroecosystems (Burgio et al. 2015). Smart designing of agroecosystems fosters the presence of predator arthropods of pest insects. Non-harvested or spontaneous plants can increase the micro-habitats

guide for management strategies able to foster the agroecological service and reduce the disservices.

9.3.3 The Agrobiodiversity Evaluation

Investigations on agrobiodiversity are often restricted to species richness (*S*), which determine the number of species within an area by giving equal weight to each species. They give information about the so-called *System Diversity* of an agroecosystem (Benckiser and Schnell 2006). There is, however, huge interest of ecologists in the relative abundances of species, since no community consists of species of equal abundance. By this, several diversity indices and functions have been developed over time (Table 9.1). Among these, the species evenness (e)—individuals of each species in a community—Simpson (D) and Shannon-Wiener (H′)—heterogeneity of a community, combining richness and evenness—are the most used in agrobiodiversity assessments (Love and Spaner 2007; Peet 1974). Agrobiodiversity can be measured also in terms of genes variation within and among individuals at different levels: species, race, variety and population (Fu 2015). The *Genetic Diversity* assessment is an evaluable instrument to analyse and predict hybridization phenomena of a community, ensuring adaptability of the same community to changing environment and selection pressures (including climate change and anthropic activities), thus being used as an instrument for evaluating the agroecosystem resilience and plasticity. Moreover, the genetic diversity allows the evaluation of genetic erosion within an area/agroecosystems. Based on the broad definition of genetic diversity, the use of different diversity parameters, and the application of different molecular markers allows the assessment at different levels, included the impact from specific plant breeding programmes evaluation (Fu 2015).

High species and genetic richness may not result in a wide range of ecosystem services. It could be due to species redundancy in the agroecosystems (Naeem 1998). From an ecological point of view, redundancy is referred to the presence of different species contributing to the same ecological process or service: this overlap determines that relation between ecosystem functioning and biodiversity richness is not necessarily linear (Fig. 9.5). It means that in an agroecosystem, no apparent ecological distinctions among entities could be highlighted, so that, although they are reproductively distinct gene pools, and separate species, they constitute in effect a single functional ecological unit (Walker 1992): there are many more species than there are functions, thereby, redundancy is built into the system (Vandermeer et al. 1998). As a consequence, agrobiodiversity can be also evaluated and measured in terms of functional groups, defined as groups of species that share common biogeochemical attributes, such as pollination, nutrient cycles or trophic levels (Naeem 1998) identifying their functional identity (Costanzo and Bárberi 2014). It provides information about the so-called *Functional Biodiversity*, describing providing ecosystem functions by species richness (Marshall 2006). It assesses the richness of functional

Table 9.1 Indices and functions for agrobiodiversity biodiversity assessment

Name		Formula	Meaning	Main category	Reference
Species Richness	S or R	Count of species	*n° of species per defined area*	*Richness*	Magurran (1988)
Individual Richness	N	Count of individuals	*n° of individuals per defined area*	*Richness*	Magurran (1988)
Rarefaction	$E(S)^a$	$\sum\left\{1-\left[\binom{N-Ni}{n}/\binom{N}{n}\right]\right\}$	*Expected n° of species per defined area*	*Richness*	Hurlbert (1971)
Margalef's index	D_{Mg}	*(S-1)/lnN*	*Relationship between species R and N*	*Richness*	Clifford and Stepheson (1975)
Menhinck's index	D_{Mn}	$S/\sqrt{N}$	*Relationship between species R and N*	*Richness*	Whittaker (1977)
Simpson index	D	$\sum\left(\frac{Ni}{N}\right)^2$	*Probability that two randomly selected individuals in a sample belong to the same species*	*Heterogeneity*	Peet (1974)
Shannon-Wiener Shannon-Weaver	H'	$\sum\left(\frac{Ni}{N}\right)\ln\left(\frac{Ni}{N}\right)$	*Diversity of a community. The H'_{max} occurs when all species have the same n° of individuals*	*Heterogeneity*	Peet (1974)

(continued)

Table 9.1 (continued)

Name		Formula	Meaning	Main category	Reference
Evenness (Pielou)	J	$\frac{H'}{H'max} = \frac{H'}{\ln S}$	*Evenness of a community. Ranges between 0 and 1, where 1 is for communities with equal n°of individuals per species*	*Equitability*	Peet (1974)
Redundancy	-	$(D_{max}\text{-}D)/(D_{max}\text{-}D_{min})^b$	*Response behavior of the heterogeneity when the sample size and species number are fixed*	*Equitability*	Peet (1974)

[a] n = standardized sample size; Ni = number of individuals in the ith species

[b] D_{max}, D_{min} = maximum and minimum heterogeneity possible values for a given species number and sample size

Species diversity measures can be divided into three main categories. First are the species richness indices. These indices are essentially a measure of the number of species in a defined sampling area. Second are the indices related with heterogeneity of a community. Third are indices and functions related to the number of species and the distribution of individuals among those species (equitability)

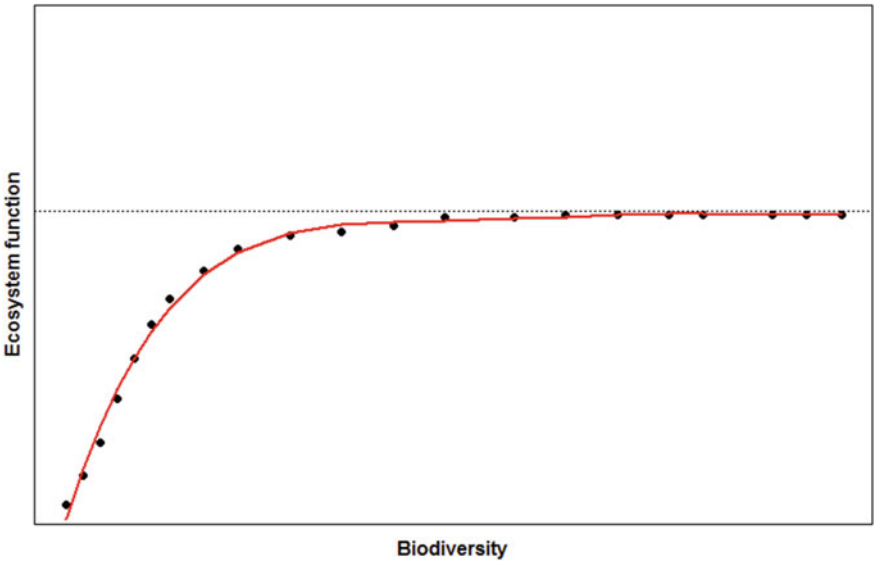


Fig. 9.5 Relation between biodiversity, richness and ecological function. The increase in biodiversity and ecosystem functions are not linearly related. Different species my contribute, in different ways to the same ecological services, both fostering the ecosystem functioning or overlapping other groups activity (redundancy) (from Naeem 1998; modified)

features and interrelations in an area by identifying food webs, keystone species and guilds (Benckiser and Schnell 2006).

9.3.4 Trade-Off Between Agrobiodiversity and Production

The functional diversity assessment drives into the need to: (i) identify the traits related with ecological services (and disservices) and (ii) adopt management strategies and agroecosystem design supporting functional groups with the identified traits (Landis 2017; Wood et al. 2015). It implies the need for a system redesign from intensive and specialized agriculture, by introducing ecological infrastructure, large rotations, multicropping, intercropping and promoting mixed farming systems (i.e. crop-livestock farming and agroforestry). As a consequence, the trade-off between provisioning services (production of agricultural goods) and the regulating services gained by biodiversity-based approach design should be accepted. Moreover, another lock-in is the requirement of actions moving to scales larger than individual farms, involving the need of common planning and coordination (Landis 2017; Frison 2016).

9.4 Managing the Agrobiodiversity: An Agroecological Perspective

9.4.1 Diversification in Space and Time

The uniformity of the input-intensive crop monocultures and industrial-scale feedlots of modern agriculture and their reliance on chemical inputs can be overtaken by different model of agriculture based on diversifying farms and farming landscapes, '*optimizing biodiversity and stimulating interactions between different species, as part of holistic strategies to build long-term fertility, healthy agro-ecosystems and secure livelihoods, i.e. diversified agroecological systems*' (Frison 2016). A farming system can be defined 'diversified' when it includes functional biodiversity at multiple spatial and/or temporal scales, through the use of 'agroecological' practices and strategies (Kremen et al. 2012). Then, diversified systems have to be heterogeneous not only in spatial terms, but variable also across time for both natural succession processes and human actions, such as management practices or landuse changes. Among practices, the crop rotations are one of the most important practice, largely used for the disease cycles interruption effectiveness (Lin 2011). The diversified agroecosystem can rely on diversification in crop and variety choice, introduction of ecological infrastructures, mixed systems and it may upscale from the field and farm scales to the landscape one (Fig. 9.6).

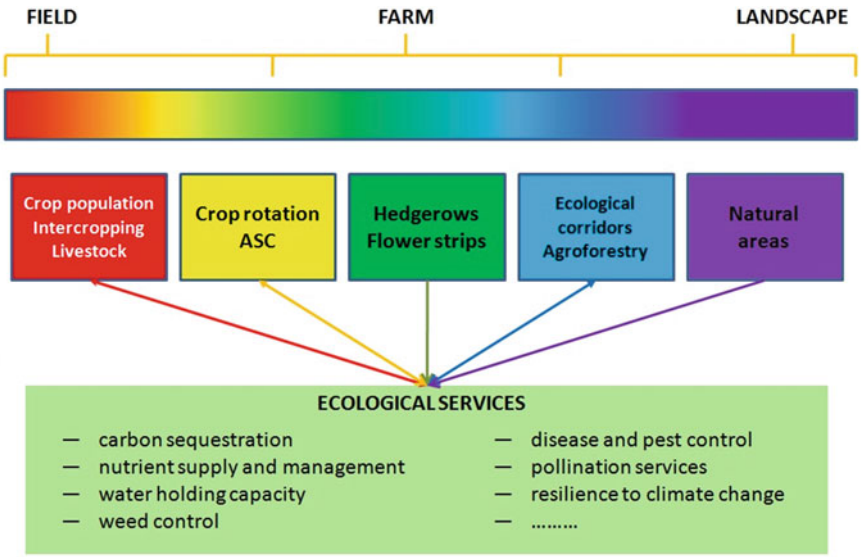


Fig. 9.6 Diversification strategies and ecological services. Moving from high resolution (field) to low resolution (landscape) scale, different strategies and techniques are mobilized and implemented to support and to foster ecological services (from Kremen et al. 2012—modified)

9.4.2 Diversification at Field Scale

The intensive use of same varieties for cropped species, also within rotation systems, may result in crop vulnerability phenomena due to soil disease, selection of competitive weeds or susceptibility to environment and climate changes (Lin 2011; Ceccarelli et al. 2010; Neve et al. 2009). The use of mixed varieties in monocropping can be addressed as a possible solution to this vulnerability, increasing the genetic diversity and enhancing the disease suppression and the production stability (Kremen et al. 2012; Lin 2011). On this basis, the adoption of evolutionary breeding populations may result in variety selections adapted to specific environments, hence increasing agrobiodiversity functionality over time (Campanelli et al. 2015; Ceccarelli 2015; Costanzo and Bárberi 2014). On the other hand, the use of intercropping (i.e. contemporary cultivation of different crop species) is to be considered an evaluable strategy to produce yield, suppressing belowground soil diseases and reducing the ecological niche available for weed establishment (Hiddink et al. 2010; Poggio 2005). Several studies address the effectiveness of intercropping of cereals and leguminous crops in ensuring yield, nitrogen efficiency and weed reduction (De Stefanis et al. 2017; Costanzo and Bárberi 2014). The utility of intercropping is fostered correctly spacing the different crops, such that they root systems could interact without compete for limiting resources (Kremen et al. 2012).

The introduction of agroecological service crops (ASC), being an important link among soil, crop, insect pest and weed management (Canali et al. 2015; Bárberi 2002), can be considered another evaluable diversification strategy at field scale. The agroecological services provided by ASC range in function of three main aspects: (i) ASC species choice, (ii) position within the rotation, (iii) management strategies. Different species differently contribute to ecological services, depending on the specific functional identity (set of homogeneous phenotypic traits related to the expression of a given agroecosystem service) and composition (complementarity effect of different traits) (Costanzo and Bárberi 2014). As a consequence, the agroecosystem services provided depend also by the species mixture introduced as ASC (Fig. 9.7). Moreover, the choice of the species and the deriving services strongly depend also by the position within the rotation, as break crop (between two cash crops) or living mulch (intercropped with cash crop). ASC break crops may contribute to soil protection (cover crops), nutrient management (catch crops and green manures) or to disease control (trap crops); living mulch to weed suppression and nutrient management (Figs. 9.8 and 9.9). Finally, the ASC management has a role in service provisioning. Termination of break crops may enhance nutrient cycling (i.e. green manure) or pest and weed control (i.e. flattening the ASC; Magagnoli et al. 2017; Canali et al. 2015) as far as the living mulch sowing time or spatial distribution respect to the main crop (i.e. strip or broad distribution) can foster services reducing risk of interference between cash crop and ASC (Ciaccia et al. 2017a). Allelopathy aspects related to ASCs represent another variable which could be moved by the agroecosystem 'designer'. The knowledge of the allelopathy potentiality of the ASCs against weeds, but also cash crops, is an added value to the process of 'rethought'

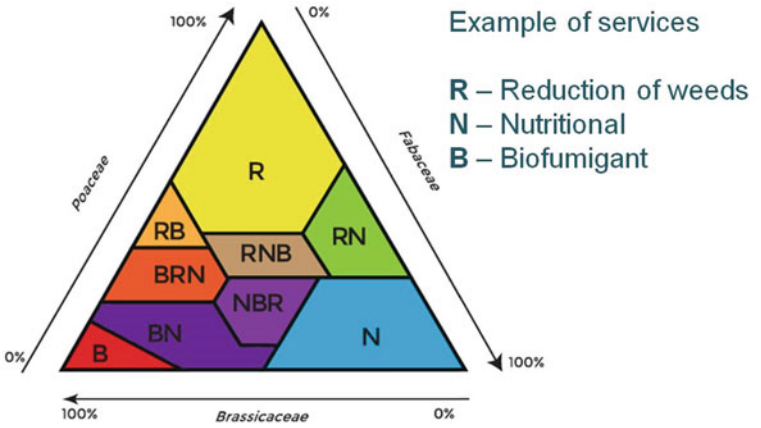


Fig. 9.7 Ecological services provided by ASC mixture of different botanical families. In a ASC mixture, in accordance to the relative ratio of one genotype in respect to the others a specific ecological service can be promoted. *Brassicaceae* are known for their biocide and nematicidal effects by releasing glucosinolate compounds (Lazzeri et al. 2009); *Poaceae* for their ability as catch crops, to prevent leaching during winter time and/or for their smoother effect against weeds (Barberi 2002; Ciaccia et al. 2015a, b); *Fabaceae* for their soil *N* building capacity. *Poligonaceae*, *Borraginaceae* and *Asteraceae* can act as trap plant for nematodes and other pests. The ability of ASC mixtures to provide the needed services is then dependent by farmer choice. It implies the farmer's knowledge of the system in which the ASC are introduced (from Ciaccia and Canali 2016—modified)

the whole system and it is capacity to maximize the benefits provided by the ASCs. Crops such as rye (*Secale cereale* L.), sorghum genus (*Sorghum* Moench) and vetch (*Vicia sativa* L.), are well known to exhibit allelopathic activity against weeds in field conditions, so they can be used to address and boost the ASC service on weed control (Ciaccia et al. 2015a, b; Teasdale et al. 2007; Barnes and Putnam 1987; Tesio and Ferrero 2010). In the newest and most comprehensive definition of allelopathy, in which not only plant–plant interactions are conceived, specific ASCs can also act as repellent against some pests or attractors of beneficial insects through chemical signalling modulation (Narwal 2010).

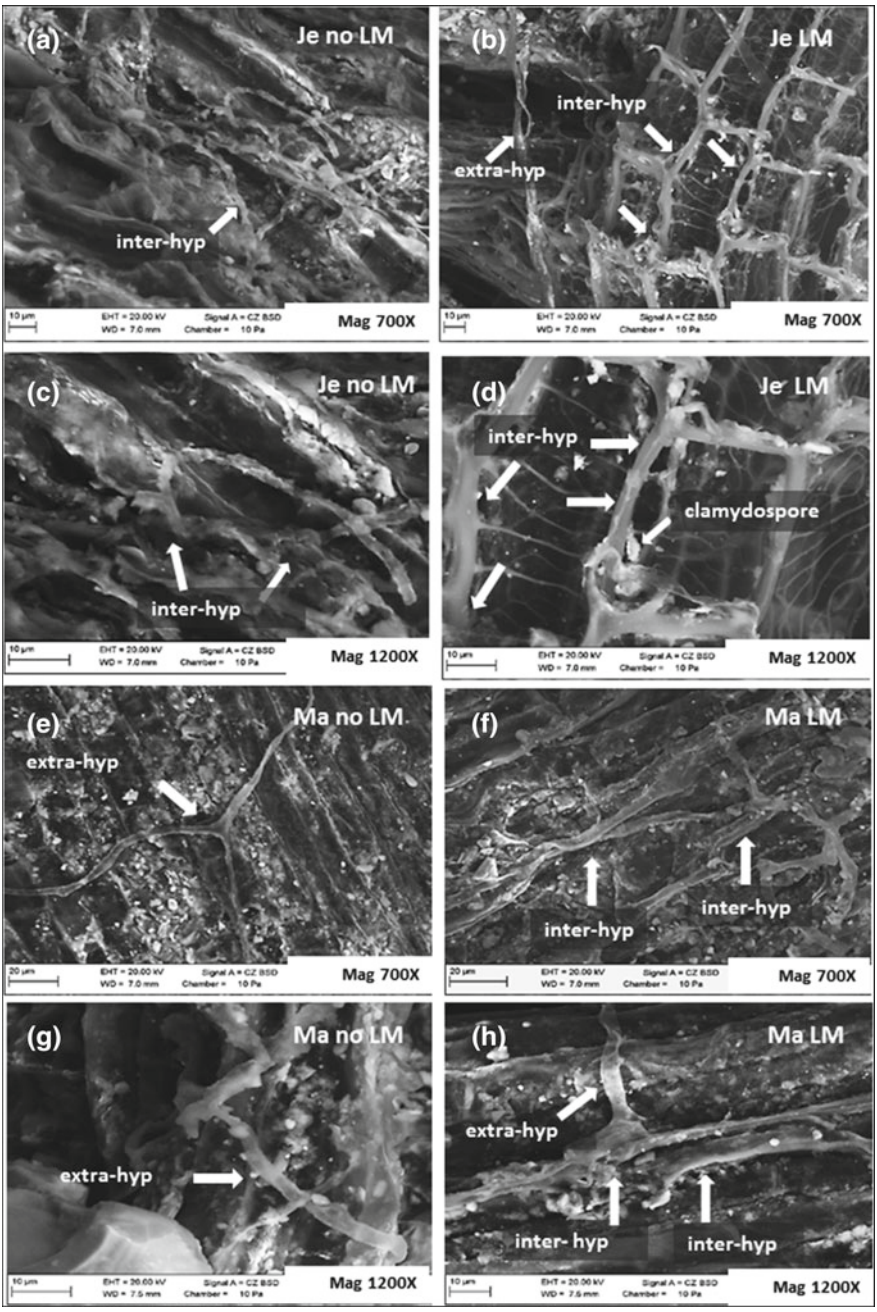
9.4.3 Diversification at Farm Scale

Diversification at farm scale encompasses both the temporal dimension (the rotation and the practice choices) and the spatial one. The inclusion within the farm of functional herbaceous strips and/or hedgerows can contribute to the overall agroecosystem biodiversity and functioning (Kremen et al. 2012), representing important ecological infrastructures. They can be of natural, residual, origin or new planted area and occupy field margins or go through the fields (Caporali 1991). These areas,

Fig. 9.8 Cauliflower/medic living mulch. Cauliflower (*Brassica oleracea* var. *botrytis*) and burr medic (*Medicago polymorpha* var. *anglona*) living mulch contributes to weed suppression occupyingthe ecological niches available to weeds in the sole crop systems. Competition between cauliflower and medic is managed by shifting the sowing time of the legume in respect to the transplanting of the vegetable. Suitable solutions allow to exploit complementarity resource use between species (Ciaccia et al. 2017b)

mainly through addition of floral resources, may enhance beneficial arthropods abundance and parasitism incrops (Burgio et al. 2015; Morandin et al. 2014). In particular, farm-scale hedgerow can provide pest control benefits up to 100 or 200 m into fields and partial crop pollination, thus multiple hedgerows around fields could enhance pest control and pollinator activity throughout entire farm, reducing the need for chemical pest control and contributing to preserve wild bees (Sardiňas and Kremen 2015; Morandin et al. 2014). Since species from soil seed bank or dispersing from neighbouring vegetation can strongly influence the herbaceous species present in the ecological infrastructures as far as represent a source of weeds or habitats for pests, it is necessary to provide for their management over time (Burgio et al. 2015). Uyttenbroeck et al. (2015) suggest the need to develop tailored flower strips to maximize the services delivered, settle in the strip and the adaptability of the species to the specific environment.

A system where trees are grown together with annual crops and/or animals results in enhancing complementary relations between components, increasing multiple use of the agroecosystem (Altieri and Rosset 1996). Agroforestry can be considered the higher stage of diversification at farm level, representing systems in which woody perennials (trees, shrubs, etc.) are grown in association with herbaceous plants (crops and pastures) and/or livestock, in a spatial arrangement, a rotation or both (Lundgren 1982). These systems allow the complete loop cycle of organic materials within the agroecosystem, overtaking risks linked to animal waste management as far as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◀**Fig. 9.9** Mycorrhization of roots of artichoke cultivated with living mulch or as sole crop. Effect of living mulch (LM) on mycorrhization of cortex cells in fine, third order lateral roots of Jesino (Je) and Mazzaferrata (Ma) artichoke cultivars. Images are referred to 700X (A-B-E-F) and 1200X (C-D-G-H) magnifications by Scanning Electron Microscope (SEM-VP mode). White arrows indicate AMF extra hyphae (extra-hyp) and AMF internal hyphae (inter-hyp). Mycorrhization intensity in Je in presence of LM was higher (M: 43%) than in Je with no LM (M: 15%). No difference was recorded between Ma with LM and Ma with no LM (Trinchera et al. 2016)

phosphorus mining condition typical of stockless systems (Ciaccia et al. 2017a) and enhance pest and disease control, reducing the need for agrochemical inputs (Smith 2010). The recycling of animal wastes (as manure and/or compost) stimulates microbial activity, enhancing the system functioning and reducing the need of off-farm fertilizers, indirectly contributing to reduce agriculture disservices, such as pollution and energy consumption (Canali et al. 2004). There are many examples of agroforestry systems, both in the tropics and in Europe, representing a feasible solution to face land degradation or desertion, providing financial, environmental and agronomic benefits (Leaky 2014; Pantera et al. 2018).

9.4.4 Diversification at Landscape Scale: Landscape Elements

Structural complication of an agroecosystem is decisive to obtain biodiversification, which can act in restoring equilibrium conditions after disturbance and optimize the use of internal resources limiting external inputs use. Species richness represents indeed the fundamental requirement for the self-maintenance of each ecosystem, since the mutual interaction among components plays a role in its stability. Ecological infrastructure is effective elements of heterogeneity, with a separate role then agricultural crops. Actually, ecological infrastructures, or areas of ecological compensation, such as hedgerows, vegetation strips, groves, plant corridors, field margins and ponds constitute the 'non-productive' vegetal components within the agroecosystem, but which are functional in the providing of ecosystem services (Burgio et al. 2006; Burgio 2007). In this perspective, the insertion of landscape elements in the agroecosystem should be opportunely designed and weighted on the basis of the obtainable positive interactions. Hedgerows, as example, could be designed with a landscape perspective, as a link between the components of the landscape itself, helping to reduce its fragmentation (one of the main causes of biodiversity loss) (Baudry 1984). At the same time, hedgerows can represent a refuge for beneficial insect fauna and a natural 'biofarm' of useful insects and mites (predators and/or parasitoids) that easily move on the surrounding crops, feeding phytophagous harmful for the cultivated species (Paoletti et al. 1997).

9.5 Beyond the Cropping System: Upscaling to Food System

Francis et al. (2003) argued that focusing only on the production sector in agriculture, even if upscaling from the farm to the landscape level, essentially ignore the large investment in energy and materials connected with food chains. Since the most of energy consumption relies after the field production processes, and then also the environmental impact of agriculture, the agroecology attention should focus on the entire food systems if it wants to act as a transformative approach (Levidow et al. 2014). These considerations drive into the need to redesign the entire food systems beyond the production sector of agriculture, towards the ultimate goal of sustainability, encompassing also the economic and social aspects (Frison 2016; Gliessman 2016).

The simplified and specialized systems derived by the Green Revolution and globalization have been recognized to be the origin of negative externalities of modern agriculture, of which environmental impact is just one component. In particular, unfair distribution of the added value produced within the supply chain actors and, accordingly, these intensive production systems have been acknowledged to be anymore not able to guarantee also the economic and social sustainability. Indeed, they are considered ineffective to respond to the present societal challenges which imply to move from a purely agricultural perspective towards an agrofood system aiming at: (i) producing sufficient healthful, safe and affordable food for the global increasing population, (ii) reducing pollution and greenhouse gas emissions derived from food production, processing, trading and consumption, (iii) developing food chains driven by renewable energy and recycled nutrients, (iv) adapting to climate change and mitigating greenhouse gas emissions, (v) protecting soils, water, air, biodiversity and landscapes, while (vi) taking into account current and emerging ethics, food habits, lifestyles and consumer needs (Geiger et al. 2010; Godfray et al. 2010; Rahmann et al. 2017).

In the last four decades, in order to respond to the growing request of the society for a lower impact of agricultural production processes, policies aimed at increasing the efficiency of industrial and conventional agricultural practices have been implemented in all developed areas of Europe and USA, to reduce use and consumption of costly, scarce or environmentally damaging inputs. These policies, included in the frame of the so-called integrated approach, promoted the use of techniques and practices that help farmers in maintaining or increasing production through improved seeds optimum planting density, more efficient pesticide and fertilizer application, and more precise water use. More recently, among the technologies developed and implemented in the contest, the digital and precision agriculture belong to the conceptual framework of increased input efficiency. Despite the application of these novel technologies led to the mitigation of negative environmental impacts of agriculture, seldom they help to break the heavy dependence of industrial agriculture from external inputs (Gliessman 2016).

Later on, the demand for safe food (without synthetic inputs, i.e. pesticides and fertilizers), the definition of a framework of environmental policies, addressed to environmental protection and to certification systems for agricultural products, determined the development of production based on the replacement of inputs with higher energy costs and environmental impact, with renewable ones, with higher environmental compatibility, and perceived as healthy by consumers. The development of the organic agricultural model, which took place in Europe since the 1990s, has to be encompassed in this context. However, the substitution of inputs does not necessarily aim at modifying the basic structure and functioning of agricultural production systems, which therefore can remain characterized by a high degree of intensity and specialization, without solving the intrinsic shortcomings of the industrial model. Furthermore, the implementation of this production model does not necessarily imply to get away from operational schemes of transformation and commercialization typical of industrial production and, therefore, does not ensure a different and fairer way of distributing the value produced along the supply chain.

Nevertheless, the agricultural models based on the substitution of inputs can represent a crucial point of transition from the conventional/industrial agricultural model to agricultural models based on the principles of agroecology as it has happened in Europe—and in some extent in the USA, as well—and resulting in the wide spreading of organic agriculture (Tittonell 2014).

Indeed, it is the recognition of the weaknesses of the input substitution model and the attempts to overcome them which lead to the definition and the development of the redesign approach as leading strategy for the sustainable transformation of food systems.

The redesign approach is essentially based on the application of agroecology principles, implemented at field and farm scale. Actually, agroecologial management bring to complex agroecosystems which, being diversified over time and space, are able to supply a wide range of products. Diversified agroecosystems, socially and economically sustainable, are functionally linked to appropriately structured supply networks, able to deliver food and services to the end users, guaranteeing participated governance and ensuring the conservation of the relationship between food, territories and cultures, together with an equitable and responsible distribution of product value (Meynard et al. 2013).

The transition resulting from the system redesign and the consequent horizontal and vertical diversification of production systems to a wider spatial scale can lead to further ecological, economic and social advantages. In fact, it may happen that the ecological functions needed to sustain the functioning of agroecosystems, thus permitting their self-support and auto-regulation, cannot operate at field and/or farm scale. In this scenario, agroecosystems design must take into consideration the scaling up to wider and more complex perspective, considering the territorial scale. Obviously, the transition from the farm to the territorial scale implies a radical change in the decision-making and governance processes, which turns from individual to collective and which must, therefore, provide effective mechanisms of participation (Gliessman 2016).

9.6 Agrobiodiversity, Agroecology and Organic Farming

Agrobiodiversity is recognized to have a key role in obtaining sustainable agricultural systems. In fact, diversification is promoted by all models of sustainable agriculture based on ecological principles and debated in recent years, including organic farming and agroecology (Migliorini and Wezel 2017; Bellon et al. 2011). Despite this, organic farming and agroecology have some points of divergence from conceptual and technical points of view (Fig. 9.10). At a conceptual level, the main difference is referred to the attention on agrobiodiversity: organic farming has an 'impact-oriented' approach, analysing the impact of practices on biodiversity; agroecology has a 'resource-oriented' approach, fostering the benefits deriving by agrobiodiversity management. From a technical perspective, organic farming has sharp restrictions and is mandatory for farmers to obtain a third-part certification complying the regulations (e.g. EC 2007, 2008) to access the market. This point could represent the weak link of the organic farming certification system, opening to the so-called conventionalized systems (based on an input substitution criteria), whereas there are few examples worldwide of policy funding of agroecological practices and systems, currently not market-driven (Wezel and Francis 2017; Darnhofer et al. 2010). Nevertheless, several findings from scientific research and practical applications suggest organic agriculture as a means through which meet the future challenges in food production (Muller et al. 2017). IFOAM, according to the four principles of organic agriculture, has conceived the following recent definition: '*Organic Agriculture is a production system that sustains the health of soils, ecosystems and people. It relies on ecological processes, biodiversity and cycles adapted to local conditions, rather*

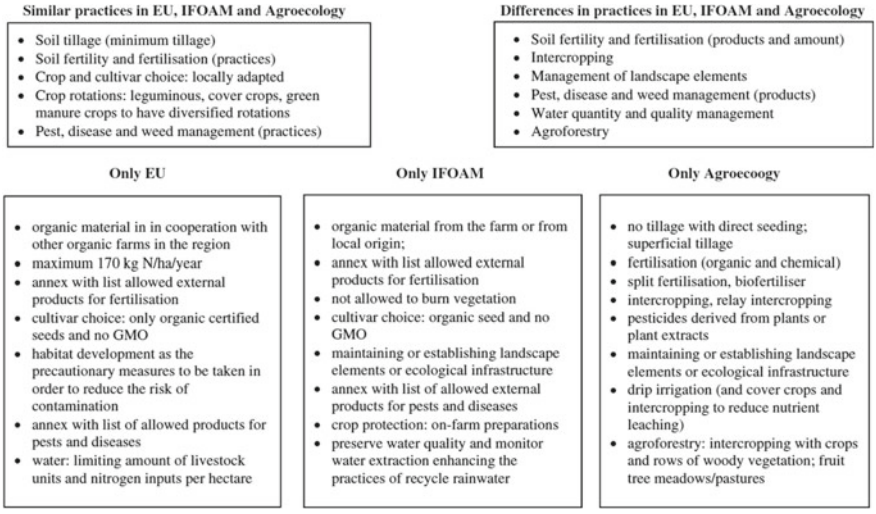

Fig. 9.10 Conformity and differences in EU organic, IFOAM, and agroecology crop production practices. From: Migliorini and Wezel (2017)

than the use of inputs with adverse effects. Organic Agriculture combines tradition, innovation and science to benefit the shared environment and promote fair relationships and a good quality of life for all involved'. (IFOAM General Assembly 2008, Vignola, Italy). However, by now, less than 1% of global farmland is managed organically and a small portion of global consumers consume organic food significantly and it appears clear that organic agriculture must always evolve to overcome emerging challenges (Wezel 2017; Willer and Lernoud 2017). In 2010, a global discussion about *Organic* 3.0 was initiated in order to identify the key questions and problems facing the current food system and where to address organic measures and strategies. *Organic* 3.0 *is about bringing organic out of its current niche into the mainstream and positioning organic systems as part of the multiple solutions needed to solve the tremendous challenges faced by our planet and our species* (Arbenz et al. 2015). With this aim, agroecology can be considered to be incorporated into organic research, reducing the trend of organic agriculture to become globally standardized and business oriented, losing the conventional input substitution approach in favour of an holistic and multidisciplinary approach, facing the global challenges of the food system (Darnhofer et al 2010; Niggli 2015). The convergence of the two concepts is strenuously institutionally pursued by the organic stakeholders (e.g. in Europe), facing the need to maintain consistency in the current phase of development of the organic sector, exposed to risks of dilution in its social and ecological mandate. The final purpose foresees agroecology and organic agriculture acting in synergy in determining the development of the global food systems, able to overcome the challenges that our society is facing, contributing to create more sustainable food system from an environmental, economic and social perspective.

9.7 Conclusion

The negative externalities derived from Green Revolution and globalized agriculture drive to the need to change both the production paradigm and food chain structure, aiming at obtaining more sustainable systems and pursuing food security all over the globe. Agroecology, as the ecology of the entire food system, aims to redesign agroecosystems scaling up to wider and more complex perspective than the plot or farm scale, considering the territorial one. Being agriculture the primary factor to produce provisions (food, fibres, energy and environmental services) for the future generations, agroecology should answer to the need to intensify agriculture in a sustainable way. Based on the above overall picture, the process of convergence of the agroecological practices towards agrobiodiversity conservation and enhancement, and the key role of biodiversity in the future of agricultural intensification appears to be clear. Research should underpin this process, studying both the effect of management strategies on agrobiodiversity and the relationship between functional traits and ecosystem services provided.

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Chapter 10
Induced Genetic Diversity in Banana

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Abstract Banana and plantains are one of the important fruit crops grown extensively in the tropical and subtropical regions of the world. The world production of banana is 145 million tons of which only a few million tons is exported, which means that most production is primarily for local consumption. The banana cultivars are derived from two diploid wild species, *Musa acuminata* (AA genome) and *Musa balbisiana* (BB genome). Majority of the edible banana cultivars are propagated vegetatively, and hence, the improvement of banana through conventional breeding methods is difficult. Attempts have been made to improve banana by inducing genetic variability by using both physical and chemical mutagens and exploiting the somaclonal variation a few varieties have been released for cultivation. Transgenic approach has also been used to incorporate the desirable traits into banana. Recent advances in genomics and the availability of genome sequence of both *Musa acuminata* and *Musa balbisiana* helps in the improvement of this fruit crop. Also the recent reports of genome editing through CRISPR-CAS9 will aid in speeding up the banana improvement programmes in the near future. This review summarizes the various advances made in inducing genetic diversity in banana.

Keywords Banana · Genetic Diversity · Mutation induction · Somaclonal variation transgenics

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10.1 Introduction

Banana and plantain (*Musa* sp.) belong to a group of edible, vegetatively propagated, monocotyledonous and herbaceous species, belonging to the Eumusa section of the genus Musa, family Musaceae and order Zingiberales (Gill 1988). Bananas are the fourth important food crop after major cereals and are consumed locally in many developing countries (FAO 2002). Banana production is around 145 million tons of which only a few million tons is exported, which means that most production is primarily for local consumption (FAO 2015). This poor man's fruit contributes very much to food and nutritional security. The fruit has significantly shares (25%) the total carbohydrate requirements of African countries (Robinson 1996). Banana fruit has majorly carbohydrates (35%) followed by fibre (6–7%), low protein and fat (1–2%), and major elements such as potassium, magnesium, phosphorus, calcium, iron, and vitamins A, B6 and C (Robinson 1996). Normally, bananas are consumed in ripe and starchy form, or boiled or cooked in different traditional cooking (Frison and Sharrock 1998). It is also an important source for making beer, wine and other products (Stover and Simmonds 1987). There are also a number of other food products including jam, juice and squashes, banana chips or crisps, sweet banana figs, banana flour, banana powder and starch (Padam et al. 2014). Banana fruit also has several valuable bioactive compounds such as phenolic compounds, carotenoids, biogenic amines and phytosterols (Singh et al. 2016), and they have antioxidant property that serves as a defence arsenal against oxidative stress.

The genus Musa has members which are seeded (wild) and no seeded or parthenocarpic edible types (Ortiz 1995). Most cultivars are derived from two diploid wild species, *Musa acuminata* (AA genome) and *Musa balbisiana* (BB genome) (Osuji et al. 1997). Edible clones are classified based on the relative contribution of *M. acuminata* and *M. balbisiana* (Simmonds and Shepherd 1955). Around 30–40 species are present under the genus *Musa* (Simmonds 1987), and all the wild varieties are diploids with 14, 18, 20 or 22 chromosomes. On the basis of number of chromosomes, and floral arrangement in inflorescence, this genus is classified in five sections, namely Australiamusa, Callimusa, Emusa, Ingentimusa and Rhodochlamys. These include the wild varieties, i.e. seeded, marketed edible bananas, i.e. non-seeded and parthenocarpic ones (Ortiz et al. 1995). Section *Callimusa* and *Rhodochlamys* are cultivated as ornamentals and do not produce fruits, whereas *Australimusa* members are highly infertile (Jarret et al. 1992) and comprise *M. textilis Nees* (Abaca) cultivated for fibre production (Cheesman 1949), and the seedless edible *Musa Fehi* (Fe'i bananas) contain higher vitamin A content.

Eumusa (true bananas) is the largest of all, and the inflorescence is pendent or semi-pendent type. It includes 13–15 species of bananas including the ancestors of triploid bananas; *Musa acuminata* (A genome) and *balbisiana* (B genome). This is the most diversified, ancient and widely distributed section of genus *Musa*. The best examples of this group are the commercially cultivated Cavendish bananas like Grand Nain (AAA), Giant Cavendish (AAA), Williams (AAA), Robusta (AAA), Dwarf Brazilian (AAB), etc. It also contains *M. schizocarpa* (S genome). These

present-day cultivated edible bananas have chromosome numbers of 22, 33, 44 with basic number as $n = x = 11$ (Heslop-Harrison and Schwarzacher 2007). These are hybrids of A and B genome and are a result of natural crosses between two wild species, *Musa acuminata* (AA genome) and *Musa balbisiana* (BB genome) (Osuji et al. 1997). The diploid bananas grown today AA, BB or AB; however, the most prevalent and commercially grown most important bananas are the triploids, while a few tetraploids are available in nature or they are artificially developed by employing the breeding methods (Patel et al. 2016; Menon 2016).

Banana, being triploid and vegetatively propagated, has inherent constraints of low seed set and germination rates. Conventional breeding of banana has limitations of ploidy in relation to hybridisation, morphology and yield, parthenocarpy and sterility, and long time-consuming steps of hybrid development (Vuylsteke et al. 1993; Oselebe et al. 2006; Creste et al. 2004). Banana production is challenged by both biotic and abiotic stresses. Considerable attention has been paid on biotic stress factors (Kotari et al. 2016); however, research on abiotic stresses has lagged behind, and only in the past 5–10 years, there has been increasing research in this direction (Ravi and Vaganan 2016).

Banana cultivation is challenged by plant's vulnerability to pests and diseases. The most significant ones include the black Sigatoka caused by *Mycosphaerella fijiensis* Morelet, Panama disease (*Fusarium oxisporum*, race 1 and 4), bacteria *Pseudomonas solanacearum*, viruses such as the banana bunchy top virus (BBTV), cucumber mosaic virus (CMV), nematodes *Radopholus similis* and weevils *Cosmopolites sordidus*. Both conventional and biotechnological approaches are being used in improving disease/pest resistance and quality. Cavendish-type bananas exhibit susceptibility to most of these diseases. Wild species of *Musa* are known to have resistance to black Sigatoka, for example, *M. acuminata* ssp. Burmannica, ssp. Malaccensis and ssp. siamea, and diploid AA cultivars.

Most of the bananas exhibit susceptibility to abiotic stresses like drought, salinity and extreme temperature. It has been shown that different genomic groups exhibit considerable genetic variability for abiotic stress tolerance (Hu et al. 2017). The 'ABB' banana genotypes are tolerant to drought and other abiotic stresses, and hence, are a good genetic resource for use in breeding attempts to improve abiotic stress tolerance (Ravi et al. 2013). According to Simmonds (1966), bananas are grown in four different climatic zones. These include climates where bananas experience little or no seasonal growth, without much need for irrigation, climates where bananas experience seasonal drought or a combined drought and low temperature, marginal climates where bananas survive and bear fruit only under favourable conditions of soil, irrigation and genotype management, and climates where bananas are grown under irrigation throughout the growing season.

Musa gene pool consisting of important wild species can be exploited for genetic improvement of certain traits which include tolerance to cold (*Musa sikkimensis* Kurz, Musa basjoo P. F. (B.) von Siebold ex Iinuma, Musa thomsonii (King ex Schumann) Cowan and Cowan), waterlogging (*Musa itinerans* Cheesman) and drought (*Musa balbisiana* Colla, Musa nagensium Prain) (INIBAP 2006; Oselebe et al. 2006). Several banana-growing countries have crop improvement programmes to

improve local germplasm for biotic and abiotic stress tolerance and improved fruit quality. A global initiative, International Musa Testing Program (IMTP; http://www.promusa.org/IMTP), has become significant for evaluating and release (through trials across counties and locations) of different cultivars, landraces, selections and hybrids for agronomy, pests and diseases, post-harvest traits, for adoption to different geographical zones and local conditions (Orjeda 2000). Molecular characterization of *Musa* genetic diversity assumes significance for exploring genetic markers in *Musa* improvement programmes (Till et al. 2010). In a first report, these authors found Ecotilling method to be effective for the discovery of nucleotide polymorphisms in diploid and polyploid accessions of Musa. The results revealed more than 800 novel alleles in 80 accessions suggesting that the method of Ecotilling can be useful for investigating into genetic polymorphisms in banana. The meristematic tissues of banana are treated with a mutagenic agent, and then plants are regenerated. The regenerated plants are multiplied, and the meristematic cells are successively isolated and bisected to obtain tissues devoid of chimeras. The problem of chimeras can be eliminated by optimizing protocol for mutagenesis or using single-cell cultures for mutagenesis. However, development of cell suspension cultures and regeneration of plant from a single cell is very established in banana. Above all, since banana is vegetatively propagated and do not set seeds, the induced mutations will be fixed in the clonal plants (Jankowicz-Cieslak et al. 2012).

Banana is vegetatively propagated, and hence, there is a greater need to induce additional genetic variability to enable selection for improved agronomic and quality traits. Biotechnological methods such as organogenesis, plant regeneration, mutagenesis, transgenic methods and molecular markers have been deployed (Jain and Swennen 2004). In banana, in vitro shoot-tip culture is most practised for micropropagation of commercial and novel genotypes. It has been very well established that plant developed through micropropagation grow better than those grown via suckers and have uniform growth (Robinson 1996). In vitro culture also guarantees safe collection, exchange and conservation of germplasm required for identification of breeding traits, and facilitates dissemination and propagation of newly selected cultivars or hybrids. The method of meristem culture is also very useful for the production of disease-free planting material and for preservation of novel banana genotypes (Cronauer and Krikorian 1984; Hwang et al. 1994; Helliot et al. 2002).

Somatic embryogenesis and high-frequency plant regeneration from commercial varieties of banana are prerequisites for realizing the potential for large-scale production of planting material and cellular and molecular approaches for crop improvement. Although different explants like scalps (proliferating meristems), rhizomes, leaf bases, immature zygotic embryos and young male flowers can be used for induction of somatic embryogenesis, immature male inflorescences and proliferating meristem sections have been successfully employed to initiate embryogenic cultures of several banana and plantain cultivars (Escalant et al. 1994; Navarro et al. 1997; Ganapathi et al. 1999; Suprasanna et al. 2001; Kulkarni et al. 2006; Sidha et al. 2011). Embryogenic cell suspensions (ECS) have become useful for use in genetic manipulation using different biotechnological tools, and relative success in genetic engineering of bananas is often dependent on this ECS system (Ghag and Ganapathi

2017). The method involves initiation of embryogenic callus, induction of cell suspension and assessment of regeneration ability. There has been good progress in the past 5–10 years, and for a wide range of banana genotypes, embryogenic callus can be induced and embryogenic cell suspensions (ECS) can be routinely established from embryogenic calluses of different cultivars of banana (Strosse et al. 2006).

10.2 Mutagenesis and Induction of Novel Genetic Variation

In vitro mutagenesis followed by in vitro selection is a very useful method as different cell, organ and tissue cultures can be mutagenized, uniform mutagen treatment can be given, large number of samples can be handled in a short span of time, large mutant population can be raised to separate chimeras, and it has options for including selection agents in culture media for in vitro selection (Van Harten 1998). In vitro mutagenesis is an effective method for the induction, and the selection of somatic mutations and increased mutant recovery may be possible through lower somatic competition by modifying culture conditions (Suprasanna et al. 2012). Plant growth regulators, and in particular a cytokinin, can increase the recovery rate of mutated cells. Hence, the combined use of mutation induction and in vitro technology is more efficient because it speeds up the production of mutants as a result of an increased propagation rate and a greater number of in vitro generations (Morpurgo et al. 1997).

Mutation induction using physical and chemical mutagens has been practised for generating useful mutations in banana. Physical mutagens like gamma rays have high and uniform penetration of multicellular system. Gamma irradiation results in small deletions (1–10 bp) while neutrons cause 300 bp to 12 kbp deletions, and chemical mutagens result in point mutations, mainly G/C-to-A/T transitions (Morita et al. 2009). On the other hand, ion beams have high linear energy transfer (LET) ranging from 22.5 to 4000 keV μm^{-1} compared to 0.2–2 keV μm^{-1} LET of γ-rays and X-rays (Ryuto et al. 2008). Heavy-ion-beam (HIB) irradiation is shown to be superior for mutation breeding as higher rate of mutations can be obtained at low doses (Hirano et al. 2015). Ion-beam techniques have also been used as they frequently produce large DNA alterations such as inversions, translocations and large deletions rather than point mutations. Reyes-Borja et al. (2007) reported use of ion-beam irradiation for mutation breeding in banana in selecting lines tolerant to black Sigatoka. The effect of irradiation doses on the regeneration of plantlets was investigated in Japan, and the variation in black Sigatoka response under field condition was evaluated in Ecuador.

For mutagenesis, both types of in vivo and in vitro explants are used in banana (Jain et al. 2011). During the early sixties, seeds and suckers of *Musa balbisiana* were used for gamma-ray mutagenesis, and it was observed that rate of seed germination and seedling survival were affected by the radiation dose (Stotzky et al. 1964). In a further study, Menendez (1973) treated seeds of diploid *Musa acuminata* with ethyl methanesulphonate (EMS). Irradiation of suckers prior to isolation of shoot-tip explants and in vitro culture gave a low yield of mutagenized material for further

screening (De Guzman et al. 1976). Irradiation of suckers prior to tissue culture initiation is not effective and can only yield low number of plantlets in the M1V1 generation because of the large sucker size, and their number is often critical for managing either physical or chemical mutagenesis. Karmarkar et al. (2001) compared radiosensitivity of different in vivo and in vitro planting materials and observed a decrease in per cent survival of irradiated material with increasing irradiation dose. In terms of radiosensitivity, hardened plants were most sensitive followed by suckers and in vitro shoots. Multiple shoot cultures of banana variety 'Giant Cavendish' were gamma irradiated at different doses (5, 10 and 30 Gy), and the irradiated population was field evaluated for different morphological and yield contributing traits. From one of the mutant lines (a 10 Gy mutant), dwarf mutant was identified (Fig. 1) and multiplied for further evaluation (Ganapathi et al. 2016).

In vitro shoot-tip cultures have been most commonly employed for chemical mutagenesis especially with ethyl methanesulphonate (EMS). Omar et al. (1989) observed that fresh weight and number of newly initiated adventitious buds from shoot tips of banana clones SH-3362 (AA) and GN-60A. For banana, EMS dose suggested for shoot tips was 12.41–37.23 mM with 1–3 h duration. Bhagwat and Duncan (1998a, b) compared the effects of sodium azide (NaN_3), diethyl sulphate (DES) and EMS on in vitro shoot tips of banana (Musa spp., AAA Group cv. Highgate) and found that the mutagens differed in their mutation induction efficiency. While NaN_3 showed the highest effectiveness (7.8%) resulting in 63.3% explant survival and 58.9% shoot regeneration, DES yielded 65.5% survival with 38.2% shoot regeneration and EMS

Fig. 1. Isolation of dwarf mutant in banana. **a** Giant cavendish control plant **b** 10 Gy gamma ray derived dwarf mutant

gave 5.8% effectiveness, 80% explant survival and 31.6% shoot regeneration. Bidabadi et al. (2012) treated shoot-tip explants with EMS and found 10-14% increase in phenotypic variation with time and dose of treatment. Pestanana et al. (2011) applied gamma rays and evaluated genetic variability for short height in putative banana 'Pacovan' (AAB genome, subgroup Prata type) mutants. Saraswathi et al. (2016) have successfully isolated three putative mutants resistant to Fusarium wilt. In vitro proliferating buds of Rasthali (Silk, AAB) were treated with EMS, NaN3 and DES (2, 0.02 and 0.15%), and then the mutated explants were screened in vitro against Fusarium wilt using fusaric acid and culture filtrate, followed by pot screening which led to isolation of resistant mutants.

Banana embryogenic cell suspension cultures are considered a good system for in vitro mutagenesis (Kulkarni et al. 2004; Jankowicz-Cieslak et al. 2012). Embryogenic cell suspension is of single-cell origin, and hence, the number of regenerated of chimeric plants can be reduced allowing rapid generation of homo-histonts or non-chimeric plants. Thus, ECS-based mutagenesis system should be the right choice for accelerating induction, selection and recovery of mutations (Suprasanna et al. 2008, 2012). Mutagenesis of shoot tips of banana cv. Nanicao (*Musa* cv. AAA group, Cavendish subgroup) with gamma rays followed by in vitro selection with 10 mM aluminium chloride resulted in the isolation of aluminium-tolerant banana plants (Matsumoto and Yamaguchi 1990). Roux and Toloza (2002) gamma-irradiated shoot cultures and selected plants for resistance to black Sigatoka. Banana cv. Grande Naine were irradiated at 35 Gy gamma rays, and after four in vitro passages were subjected to early mass screening by using juglone which is a toxic metabolite of *Mycosphaerella fijiensis*. The authors isolated 15 putative mutants showing black Sigatoka tolerance. Chen et al. (2013) developed a microcross section (MCS) culture system for EMS mutagenesis of Brazilian banana and selected banana plants for Fusarium wilt resistance. Five wilt-resistant lines were isolated suggesting that MCS system has good potential for use in mutagenesis and in vitro propagation. There are several successful examples on the isolation of mutants of different genomic groups for various agronomic traits (Table 10.1). Despite significant outcome on mutagenesis and mutant isolation, there have been few commercial releases of mutants as varieties (Table 10.2, Mutiara and Novaria by United Plantation Bhd in Malaysia) which may be due to long generation time of the crop, availability of a good in vitro system, handling of chimeras, non-stable genetic variability and continued efforts for field evaluation of mutant clones. Sustained research is warranted to realize the potential of mutation breeding to isolate, select, evaluate and develop commercially useful mutants in banana.

10.3 Somaclonal Variation

Edible banana and plantains are propagated vegetatively, and most of the genotypes are sterile (Heslop-Harrison and Schwarzacher 2007). Banana crop is severely affected by abiotic and biotic factors which limit its full production capability.

Table 10.1 Studies on mutation induction using in vitro cultured shoot tips in treatment with chemical or physical mutagens in banana (modified after Jain et al. (2011))

Cultivar/genomic group	Mutagen dose	Modification of trait(s)	Mutant/clones developed	Reference
Chemical mutagens				
SH-3362 (AA); GN-60A (mutant of Grande Naine-AAA)	EMS 24.69 mM	Number of newly initiated adventitious buds decreased with increased EMS concentrations	–	Omar et al. (1989)
Highgate AAA Group	– Sodium azide (NaN_3) 2.3 mM – Diethyl sulphate (DES)-20 mM – EMS 200 mM	Tolerance to *Fusarium oxysporum* f.sp. *cubense*	Tolerant clones for field screening	Bhagwat and Duncan (1998a)
Brazilian banana	EMS-microcross section culture system	Tolerance to *Fusarium oxysporum* f.sp. *cubense*	Five wilt-resistant lines	Chen et al. (2013)
'Berangan Intan', 'Berangan' (AAA group) and 'Rasthali' (AAB group)	EMS 200 mM EMS	Morphological variations	Morphological variations	Bidabadi et al. (2012)
Rasthali (Silk, AAB)	2% EMS, 0.02% NaN_3 and 0.15% DES	Fungal disease resistance	Fusarium wilt-resistant mutants	Saraswathi et al. (2016)
Physical mutagens				
Grande Naine AAA	Gamma rays 25 Gy	Bunch size and cylindrical shape	Klue Hom Thong KU1	Anonymous (1990)
Nanicao AAA	Gamma rays 2kR	Aluminium tolerance	Tolerant lines	Matsumoto and Yamaguchi (1990)
Diploid and tetraploid clones	Gamma rays 25 Gy	Diploid clones were more sensitive than tetraploids	Plants with morphological and physiological traits	Novak et al. (1990)
Grande Naine AAA	Gamma rays	Earliness	Early flowering putative mutant designated 'GN-60A'	Roux (2004)

(continued)

Table 10.1 (continued)

Cultivar/genomic group	Mutagen dose	Modification of trait(s)	Mutant/clones developed	Reference
Highgate (AAA)	Gamma rays 8-20 Gy	Tolerance to *Fusarium oxysporum* f. sp. *cubense*	Tolerant clones for field screening	Bhagwat and Duncan (1998b)
Basrai AAA	Gamma rays	Morphological traits	Clones for different morphological traits	Kulkarni et al. (1997)
Grande Naine AAA	Gamma rays 35 Gy	Resistance to black Sigatoka	15 putative mutants	Roux and Toloza (2002)
Dwarf Parfitt, an extra dwarf Cavendish banana	Gamma rays 20 Gy	Improved agronomic characteristics (taller plant size, increased yield and no choking)	Improved lines with productivity and resistance	Smith et al. (2006)
Williams' and 'Cavendish Enano'	Carbon ion beam 0, 0.5, 1, 2, 4, 8, 16, 32, 64 and 128 Gy	Fungal disease resistance	Resistant plants to black Sigatoka	Reyes-Borja et al. (2007)
Basari (AAA), Chakkarakela (AAB) and Rasthali (AAB)	Gamma rays 30 Gy (recurrent dose)	Morphological traits	Thick shiny dark green leaves, ovate leaves and a dwarf with a rosette of leaves	Mishra et al. (2007)
AAA group Giant Cavendish	Gamma rays (10, 30 Gy)	Morphological traits	Short height, early maturity	Ganapathi et al. (2008, 2016)
Dwarf Cavendish (AAA)	Gamma rays 8–20 Gy	Morphological traits	22 clones for different morphological traits	Miri et al. (2009)
Banana 'Pacovan' (AAB genome, subgroup Prata	Gamma rays 20 Gy	Morphological traits	Short height	Pestanana et al. (2011)

This has resulted in a concerted effort to genetically improve this crop. Conventional breeding is difficult in banana owing to its low female fertility and complex ploidy (Bakry et al. 2009). Biotechnological approaches complement the conventional breeding techniques for genetic enhancement of this crop. This includes in vitro multiplication, somatic embryogenesis, somaclonal variation, mutation breeding and genetic manipulation. Somaclonal variation has been quite effective approach in generating useful variations in vegetatively propagated plants such as banana, papaya, strawberry and watermelon (Jain et al. 2013; Krishna et al. 2016). Somaclonal vari-

Table 10.2 Examples of desirable variants/putative mutants identified for release or further confirmation trials (Jain et al. 2011)

Country	Parent/selection	Traits	Technique	Place of induction
Cuba	SH3436 (AAAB)/SH3436	Reduced height	Gamma rays	Cuba
	Parecido al Rey (AAA)/Parecido al Rey 6.44	Reduced height	Gamma rays	IAEA
Malaysia	Pisang Rasthali (AAB)/Mutiara	Tolerance to Foc race 4	Somaclones	United Plantation Bhd., Malaysia
	Grande Naine GN-GoA (AAA)/Novaria	Tolerance to Foc race 4	Somaclones	
	Pisang Berangan	Early flowering and reduced height	Somaclones	
	Pisang Berangan	Tolerance to Foc race 4	Gamma rays	IAEA
	Pisang Mas	Tetraploid	Colchicine	
Philippines	Lakatan (AAA)	Reduced height and earliness	Gamma 40 Gy	IAEA
	Latundan (AAB)	Large fruit size and reduced height	3 Gy fast neutrons	
Sri Lanka	Embul (AAB)/Embul	Earliness and reduced height	Gamma rays	Sri Lanka

ations are either genetic or epigenetic that are observed in plants regenerated from tissue culture (Morrison et al. 1988; Evans 1989; Karp 1995; Brar and Jain 1998; Kaeppler et al. 2000). These variations occur due to changes in chromosome number, insertions, deletions, mutations, translocations, transposon activity and changes in the DNA methylation profile which can eventually lead to increased or decreased vigour and changes in qualitative or quantitative characteristics (Bairu et al. 2011; Krishna et al. 2016). Nevertheless, the epigenetic changes are lost after transfer from the culture conditions or multiplication after a few generations (Smulders and de Klerk 2011). Thus, the success of somaclonal variations in crop improvement is completely dependent on the genetic stability of the clones.

Somaclonal variations are quite common in banana with an average observed frequency of 6% at the phenotypic level (Vuylsteke et al. 1991). While some of the banana genotypes show higher rate of somaclonal variation, other genotypes have low rate of variation (Côte et al. 1995; Smith 1988; Vuylsteke et al. 1991) However, Hwang and Tang (2000) reported a somaclonal variation frequency as high as 69% in various banana and plantain cultivars. Various factors during tissue culture phase affect the incidence of somaclonal variations that includes genotype, type of explant, number of subculture cycles and plant growth regulators in the medium (Bairu et al.

2006). Several somaclones of banana have been isolated with varying visible morphological characters such as size and colour of pseudostem, reduced height, varying leaf size and variegation, bunch length and bunch mass (Israeli et al. 1991). Dwarf off-types are quite common among the tissue culture-derived Cavendish cultivars (Reuveni et al. 1986; Walduck et al. 1988; Hwang and Ko 1987; Damasco et al. 1997). In banana, dwarf mutants showed reduced height, thicker pseudostem and shorter but wider leaves (e.g. 'Cachaco Enano', 'Prataana' and 'Figue Rose naine') (Daniells et al. 2001). Drew and Smith (1990) observed that dwarf off-types in the tissue culture-regenerated banana cultivar New Guinea Cavendish (*Musa* sp., AAA Group, Cavendish subgroup) were stable up to five generations. However, in case of micropropagated 'False Horn' plantains, morphological variation in inflorescence type in the form of reversion to a typical 'French' plantain bunch type was variable from 0.4 to 100% of the total variability (Vuylsteke et al. 1988) which explains epigenetic variation. It is clearly observed that somaclonal variations are more profound in plants originated from differentiated tissues such as stem, leaves, roots or flowers and less when meristematic tissues were used as explants (De Klerk 1990; Skirvin et al. 1994).

Detection of somaclonal variation can be done using different morphological, cytological markers or molecular markers such as randomly amplified polymorphic DNA (Damasco 1997; Grajal-Martin et al. 1998; Giménez et al. 2001; Sheidai et al. 2008), amplified fragment length polymorphism (Engelborghs et al. 1998) and inter-simple sequence repeats (Lakshmanan et al. 2007). Somaclonal variation has dual considerations: it is not advantageous for micropropagation and germplasm preservation since maintenance of genetic uniformity is a must, whereas it can be advantageous for creating additional genetic variability for use in genetic improvement of banana. A somaclone CIEN BTA-03 was obtained from cultivar Williams (susceptible to black Sigatoka) and was micropropagated via apical shoot culture for five multiplication cycles. The CIEN BTA-03 clones demonstrated resistance to black Sigatoka and showed infection indexes similar to those of the resistant cultivar Yangambi Km5 (Trujillo and Garcia 1996; Giménez et al. 2008). Similarly, many somaclonal variants were developed having moderate to complete resistance to Fusarium wilt disease caused by *Fusarium oxysporum* f. sp. *cubense* (Foc). Taiwan Banana Research Institute (TBRI) developed somaclonal variants of the Giant Cavendish clone Pei Chiao and Tai Chiao No. 2 by using tissue culture. The mass-produced plantlets (called GCTCV) were tested for resistance against Fusarium wilt disease strain tropical race 4 (TR4) by planting them in TR4-infested fields and monitored for symptoms of Fusarium wilt. Although around 12 GCTCV clones were found to be more resistant to TR4 than Pei Chiao, these clones had poor agronomic traits making them unsuitable for the export trade. Since TBRI continuously supplied clones to farmers for plantation, a farmer from south Taiwan identified a high-yielding cultivar which was resistant to Foc TR4. This clone was labelled as GCTCV 218 and commercialized in Taiwan in the year 2001 under the name formosana (Tang 2001; Hwang and Ko 2004). In yet another study, somatic embryo-derived banana plants cv. Rasthali were maintained under tissue culture conditions for more than ten years (Ghag et al. 2014d). These plants were tested for resistance to Foc race 1, and five of

them did not show any Fusarium wilt symptoms after repeated bioassay. Molecular basis of resistance was investigated using cDNA-RAPD, and the differential bands were identified and characterized by quantitative RT-PCR. It was observed that a gene involved in jasmonic acid pathway, namely lipoxygenase, was downregulated in these resistant plants as compared to the susceptible controls indicating that lipoxygenase negatively regulates resistance response (Ghag et al. 2014d). These plants are currently being multiplied for field studies.

Banana and plantains are susceptible to low temperatures, nutrition deficiency, drought and poor light. However, some banana somaclones were also developed and tested for tolerance to low temperature and better yield under poor light conditions (Damasco et al. 1997). Several agronomically important characters go unnoticed when tissue culture plants are raised for a different purpose where somaclonal variation is undesirable for clonal propagation. However, somaclonal variation is a novel source for use in crop improvement in vegetatively propagated plants such as banana. Extensive studies are needed for the selection of desirable characters and field evaluation to prove their stability over multiple generations with superior performance.

10.4 Novel Genetic Variation Through Transgenics

Development of efficient cell and tissue culture protocols and successful transformation methods has enabled genetic engineering a possible reality in banana. Genetic transformation has been optimized in different banana and plantain cultivars using different methods. First transformation of banana (*Musa* spp., cv. 'Bluggoe', ABB group) was performed by electroporating protoplast cultures obtained from embryogenic cell suspension cultures (Sagi et al. 1994). First transgenic banana plants were generated from embryogenic cell suspension cultures using the particle gun method (Côte et al. 1995; Sagi et al. 1995; Escalant et al. 1995). But the most popular method of transformation of different banana tissues has been the *Agrobacterium*-mediated transformation (May et al. 1995; Ganapathi et al. 2001; Khanna et al. 2004; Tripathi et al. 2005).

Genetic modification has always been a lucrative strategy for banana improvement because most edible elite cultivars of bananas have poor male and female fertility hindering conventional breeding, thereby preventing introgression of desired characters in these cultivars. On the other hand, transgenic technology provides a wide gene pool for choosing the desired genes which can be selectively transferred to the banana cultivar. Several laboratories across the world, namely BARC (India), QUT (Australia), IITA (Kenya), NARO (Uganda) and KU Leuven (Belgium) have developed transgenic banana plants with improved character/s and demonstrated their potential under greenhouse or even in field trials. Plant-based vaccines are another application of plant genetic engineering for human health (Gujjula et al. 2004, 2007). In a first report, Kumar et al. (2005) reported expression of hepatitis B surface antigen in transgenic banana fruits. Transgenic banana plants have been developed targeting resistance to pest and pathogens, abiotic stress tolerance and delayed fruit ripening

(Ghag and Ganapathi 2017). The gene/s used to impart these traits into transgenic banana plants were either from banana plants or identified from other sources (Tripathi et al. 2010; Paul et al. 2011; Ghag et al. 2012, 2014a; Shekhawat et al. 2011, 2013; Sreedharan et al. 2013a, b). Large expanse of information has now become available to us due to the genome sequencing of important banana cultivars such as *Musa acuminata* (D'Hont et al. 2012), *Musa balbisiana* (Davey et al. 2013) and *Musa itinerans* (Wu et al. 2016). Concurrently, transcriptome and proteome data in response to resistance–susceptibility (Li et al. 2012; Bai et al. 2013), abiotic stress sensitivity tolerance (Yang et al. 2015; Muthusamy et al. 2016) and fruit ripening (Asif et al. 2014) has also been generated. All these sequencing programmes will have to provide better understanding and identification of gene/s present in banana genome which can be used to impart characteristic traits in transgenic banana plants. Table 10.3 lists some of the developments on genetically modified banana for improved traits.

Recent technologies such as RNA interference (Ghag et al. 2014b; Elitzur et al. 2016) and metabolic engineering (Paul et al. 2017) have been successfully performed in banana using different genes targeting important traits, whereas the latest genome-editing technology CRISPR-Cas is currently under research. Host-induced gene silencing, an approach which employs RNA interference technology, was used to develop resistance to one of the most devastating diseases of banana, the Fusarium wilt. DNA fragments of some vital genes (Velvet protein gene and FTF1 gene) from Fusarium wilt pathogen were identified and transferred to banana plants using *Agrobacterium*-mediated genetic transformation. These fragments were oriented in the construct such a way that resulted in generation of double stranded small RNA molecules which inhibited the pathogen growth and development in susceptible banana cultivars imparting high-level resistance (Ghag et al. 2014b). Moreover, RNA interference was also used to confer resistance in transgenic banana plants to Banana bunchy top disease (Shekhawat et al. 2013). But these studies were restricted to the greenhouse. Few studies did advance to the field trials including resistance to bacterial wilt and nematodes, biofortification and delayed ripening.

Banana Xanthomonas wilt is yet another problem constraining banana production, and resistance to this disease was demonstrated in transgenic banana plants by expressing two genes, namely the hypersensitive response-assisting protein (*Hrap*) and the plant ferredoxin-like protein gene (*Pflp*). These transgenic banana lines showed complete resistance under glasshouse as well as field conditions (Tripathi et al. 2014a, b). Transgenic plantains expressing cysteine proteinase inhibitor and an anti-root invasion, non-lethal synthetic peptide demonstrated 99% resistant to banana nematodes *Radopholus similis* and *Helicotylenchus multicinctus,* and the transgenic plants had equivalent agronomic performance as compared to the non-transgenic counterparts (Tripathi et al. 2015).

Biofortification programmes have always been forerunners in developing regions as alternatives to adequate food supply and supplementation. Very recently, provitamin A biofortified bananas were developed by metabolic engineering using different constructs having phytoene synthase gene (Paul et al. 2017). Checking postharvest losses by controlling ripening and extending shelf-life in banana is one of the approaches to improve food security. In this regard, transgenic Cavendish bananas

Table 10.3 List of transgenic banana plants generated with improved characters

Target gene	Transgenic plant/cultivar	Improved character	References
Abiotic stress tolerance			
Musa-DHN-1	*Musa* spp. *Rasthali*	Drought, salt tolerance	Shekhawat et al. (2011)
MusaSAP1	*Musa* spp. *Rasthali*	Drought, salt, oxidative stress tolerance	Sreedharan et al. (2012)
MusaPIP1;2	*Musa* spp. *Rasthali*	Cold, salt, drought tolerance	Shreedharan et al. (2013a, b)
MusaWRKY71	*Musa* spp. *Rasthali*	Salt, oxidative stress tolerance	Shekhawat et al. (2013)
MusabZIP53	*Musa* spp. *Rasthali*	Cold, drought, salt tolerance	Shekhawat et al. (2014)
MaPIP1;1	*Arabidopsis*	Salt, drought tolerance	Xu et al. (2014)
AhSIPR10	*M. acuminata* cv. *Matti*	Salt, drought tolerance	Rustagi et al. (2015)
MusaPIP2;6	*Musa* spp. *Rasthali*	Salt tolerance	Sreedharan et al. (2015)
MpMYBS3	*Musa* spp. cv. *Brazil*	Cold tolerance	Dou et al. (2016)
MusaNAC042	*Musa* spp. *Rasthali*	Salt, drought tolerance	Tak et al. (2017)
MusaNAC68	*Musa* spp. *Rasthali*	Salt, drought tolerance	Negi et al. (2016)
Biotic stress tolerance			
Magainin	*Musa* spp.cv. *Rasthali*	Resistance to Foc race 1	Chakrabarti et al. (2003)
HL	*M.* spp. cv. *Taijiao*	Resistance to Foc race 4	Pei et al. (2005)
Endo β-1,3-glucanase gene	*M.* spp.cv. *Rasthali*	Resistance to Foc race 1	Maziah et al. (2007)
pflp	*M. acuminata* cv. *Pei Chiao* *M. acuminate* cv. *Gros Michel*	Resistance to Foc race 4	Yip et al. (2011)
Bcl-xL, Ced-9 and Bcl-2 3'UTR	*M.* spp. cv. *Lady Finger*	Resistance to Foc race 1	Paul et al. (2011)
tlp	*M.* spp. cv. Nangka	Resistance to Foc race 4	Mahdavi et al. (2012)
PhDef1, PhDef2	*M.* spp. cv. *Rasthali*	Resistance to Foc race 1	Ghag et al. (2012)

(continued)

Table 10.3 (continued)

Target gene	Transgenic plant/cultivar	Improved character	References
chit42	*M.* spp. cv. *Furenzhi*	Resistance to Foc race 4	Hu et al. (2013)
Ace-AMP1	*M.* spp. cv. *Rasthali*	Resistance to Foc race 1	Mohandas et al. (2013)
VEL and *FTF1*	*M.* spp. cv. *Rasthali*	Resistance to Foc race 1	Ghag et al. (2014a)
Sm-AMP-D1	*M.* spp. cv. *Rasthali*	Resistance to Foc race 1	Ghag et al. (2014b)
MusaDAD1, *MusaBAG1* and *MusaBI1*	*M.* spp. cv. *Rasthali*	Resistance to Foc race 1	Ghag et al. (2014c)
ThEn-42 and *StSy*	*M.* spp. cv. *Grand Nain*	Resistance to *Mycosphaerella fijiensis*	Vishnevetsky et al. (2011)
rcc2, rcg3	*M.* spp. cv. *Gros Michel*	Resistance to *Mycosphaerella fijiensis*	Kovács et al. (2013)
Pflp, *hrap*	*M.* spp. cv. *Sukali Ndizi* and *M.* spp. cv. *Nakinyika*	Resistance to *X. campestris* pv. *musacearum*	Tripathi et al. (2014a)
Xa21	*M.* spp. cv. *Gonja manjaya*	Resistance to *X. campestris* pv. *musacearum*	Tripathi et al. (2014b)
Rep	*M.* spp. cv. *Dwarf Brazilian*	Resistance to *BBTV*	Cheah et al. (2009)
BBTV-G- cp	*M.* spp. cv. Williams	Resistance to *BBTV*	Ismail et al. (2011)
Rep, ProRep	*M.* spp. cv. *Rasthali*	Resistance to *BBTV*	Shekhawat et al. (2013)
Cystatin	*M.* spp.	Resistance to *Radopholus similis*	Atkinson et al. (2003)
Cystatin, synthetic peptide	*M.* spp. cv. *Gonja manjaya*	*R. similis, Helicotylenchus multicinctus* and *Meloidogyne spp*	Roderick et al. (2012)
Biofortification			
Soyferritin	*M.* spp. cv. *Rasthali*	Enhanced iron content in leaves of transgenic plants	Sunil Kumar et al. (2011)
MtPsy2a/ZmPsy1	*M. acuminata* cv. *Dwarf Cavendish*	Enhanced levels of pro-vitamin A	Paul et al. (2017)

[a] *Foc*—Fusarium oxysporum f. sp. cubense, *BBTV*—banana bunchy top virus

were generated by repressing the MADS box genes *MaMADS*1/*MaMADS*2 using RNAi technology. These plants were evaluated under field conditions and displayed delayed ripening and extended shelf-life phenotypes that include delayed colour development and softening (Elitzur et al. 2016). Although most of these studies have undergone field trials and have proved their excellence, none of them are yet commercialized. This is because of the scare spawned among the general public about transgenics or genetically modified foods. Some of the transgenic bananas have already been field tested for its improved traits, nutritional and agronomic performance (Ghag and Ganapathi 2017). The recent technologies such as CRISPR-Cas9 are useful in editing genome for value-added traits without introgression of foreign genes.

10.5 Banana Genomics

The draft genome sequence of banana has been made available through the sequencing efforts of the doubled haploid genome of *Musa acuminate*—DH Pahang (D'Hont et al. 2012). The sequence represents 90% of the genome and has 36542 protein-coding genes and 37 microRNA families, and half of the sequence composed of transposable elements. Davey et al. (2013) reported the draft genome for *Musa balbisiana* Pisang Klutuk Wulung (PKW). This group has used Illumina HiSeq 2000 II technology and generated 281 million, 100 bp paired-end Illumina reads, and the reads were assembled using the already available reference A genome. The group has identified 36638 protein-coding genes and 3276 transposable elements. The available sequence data can be effectively used for genome editing/manipulation for abiotic and biotic stress tolerance as well as for fruit quality improvement in banana. The data revealed that banana genome had most genes located in the distal part of its chromosomes, and akin to other plant genomes, banana also has major chunk (50%) of transposable elements. D'Hont et al. (2012) also observed highest number (3155) of transcription factors of which 759 (MYB and AP2/ERF type) are specific to banana. Ghag et al. (2015) conducted small RNA expression profiling in two commercially important banana cultivars and identified several cultivar specific miRNAs along with putative target transcripts. In a further study, Harikrishna et al. (2016) analysed stress-related miRNA, and their predicted targets in the banana A and B genomes and their results suggest that siRNA expression patterns change in response to salt stress. The post-genomics research is poised to provide greater insights and boost to Musa genetic engineering and improvement through the identification, cloning and functional characterization of useful genes for key agronomic traits (Dash and Rai 2016; Ghag and Ganapathi 2017).

10.6 Conclusions

Banana is an important food crop after the major cereals like rice, wheat and maize. Currently, several biotechnological tools are being applied for improving the *Musa*

germplasm. In the early 2000, the Global Musa Genomics Consortium was established with the prime focus on efficient use of Musa biodiversity for sustainability through the integrated approaches of genetic and genomic resources, precision breeding and genetic transformation. The crop is also susceptible to biotic and abiotic stress factors, and hence, there is a greater need to aim for improvement to better adapt to the changing climatic environment. For vegetatively propagated plant like banana, induced mutations offer as a potential for generating novel variability. In vitro mutagenesis has been successfully adopted followed by production of mutant population, mutation screening and phenotype characterization. The establishment of appropriate transgenic methods has contributed banana improvement. Several useful gene constructs, promoters and other regulatory elements have been made available for achieving stress-resistant/tolerant plants. Current and near-future improvement strategies for developing cold, drought-tolerant bananas can augment efforts for growing under the climate change challenges and thus can contribute substantially to food security.

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