Compendium of Plant Genomes
Series Editor: Chittaranjan Kole

Mark A. Chapman *Editor*

The Eggplant Genome

Springer

Compendium of Plant Genomes

Series Editor

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Whole-genome sequencing is at the cutting edge of life sciences in the new millennium. Since the first genome sequencing of the model plant Arabidopsis thaliana in 2000, whole genomes of about 100 plant species have been sequenced and genome sequences of several other plants are in the pipeline. Research publications on these genome initiatives are scattered on dedicated web sites and in journals with all too brief descriptions. The individual volumes elucidate the background history of the national and international genome initiatives; public and private partners involved; strategies and genomic resources and tools utilized; enumeration on the sequences and their assembly; repetitive sequences; gene annotation and genome duplication. In addition, synteny with other sequences, comparison of gene families and most importantly potential of the genome sequence information for gene pool characterization and genetic improvement of crop plants are described.

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The Eggplant Genome

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This book series is dedicated to my wife Phullara,
and our children Sourav and Devleena

Chittaranjan Kole

Preface to the Series

Genome sequencing has emerged as the leading discipline in the plant sciences coinciding with the start of the new century. For much of the twentieth century, plant geneticists were only successful in delineating putative chromosomal location, function, and changes in genes indirectly through the use of a number of "markers" physically linked to them. These included visible or morphological, cytological, protein, and molecular or DNA markers. Among them, the first DNA marker, the RFLPs, introduced a revolutionary change in plant genetics and breeding in the mid-1980s, mainly because of their infinite number and thus potential to cover maximum chromosomal regions, phenotypic neutrality, absence of epistasis, and codominant nature. An array of other hybridization-based markers, PCR-based markers, and markers based on facilitated construction of genetic linkage maps, mapping of genes controlling simply inherited traits, and even gene clusters (QTLs) controlling polygenic traits in a large number of model and crop 88 plants have been identified. During this period, a number of new mapping populations beyond F_2 were utilized and a number of computer programs were developed for map construction, mapping of genes, and for mapping of polygenic clusters or QTLs. Molecular markers were also used in studies of evolution and phylogenetic relationship, genetic diversity, DNA fingerprinting, and map-based cloning. Markers tightly linked to the genes were used in crop improvement employing the so-called marker-assisted selection. These strategies of molecular genetic mapping and molecular breeding made a spectacular impact during the last one and a half decades of the twentieth century. But still they remained "indirect" approaches for elucidation and utilization of plant genomes since much of the chromosomes remained unknown and the complete chemical depiction of them was yet to be unraveled.

Physical mapping of genomes was the obvious consequence that facilitated the development of the "genomic resources" including BAC and YAC libraries to develop physical maps in some plant genomes. Subsequently, integrated genetic–physical maps were also developed in many plants. This led to the concept of structural genomics. Later on, emphasis was laid on EST and transcriptome analysis to decipher the function of the active gene sequences leading to another concept defined as functional genomics. The advent of techniques of bacteriophage gene and DNA sequencing in the 1970s was extended to facilitate sequencing of these genomic resources in the last decade of the twentieth century.

As expected, sequencing of chromosomal regions would have led to too much data to store, characterize, and utilize with the-then available computer software could handle. But development of information technology made the life of biologists easier by leading to a swift and sweet marriage of biology and informatics, and a new subject was born—bioinformatics.

Thus, the evolution of the concepts, strategies, and tools of sequencing and bioinformatics reinforced the subject of genomics—structural and functional. Today, genome sequencing has traveled much beyond biology and involves biophysics, biochemistry, and bioinformatics!

Thanks to the efforts of both public and private agencies, genome sequencing strategies are evolving very fast, leading to cheaper, quicker, and automated techniques right from clone-by-clone and whole-genome shotgun approaches to a succession of second-generation sequencing methods. The development of software of different generations facilitated this genome sequencing. At the same time, newer concepts and strategies were emerging to handle sequencing of the complex genomes, particularly the polyploids.

It became a reality to chemically—and so directly—define plant genomes, popularly called whole-genome sequencing or simply genome sequencing.

The history of plant genome sequencing will always cite the sequencing of the genome of the model plant Arabidopsis thaliana in 2000 that was followed by sequencing the genome of the crop and model plant rice in 2002. Since then, the number of sequenced genomes of higher plants has been increasing exponentially, mainly due to the development of cheaper and quicker genomic techniques and, most importantly, development of collaborative platforms such as national and international consortia involving partners from public and/or private agencies.

As I write this preface for the first volume of the new series "Compendium of Plant Genomes," a net search tells me that complete or nearly complete whole-genome sequencing of 45 crop plants, eight crop and model plants, eight model plants, 15 crop progenitors and relatives, and three basal plants is accomplished, the majority of which are in the public domain. This means that we nowadays know many of our model and crop plants chemically, i.e., directly, and we may depict them and utilize them precisely better than ever. Genome sequencing has covered all groups of crop plants. Hence, information on the precise depiction of plant genomes and the scope of their utilization are growing rapidly every day. However, the information is scattered in research articles and review papers in journals and dedicated Web pages of the consortia and databases. There is no compilation of plant genomes and the opportunity of using the information in sequence-assisted breeding or further genomic studies. This is the underlying rationale for starting this book series, with each volume dedicated to a particular plant.

Plant genome science has emerged as an important subject in academia, and the present compendium of plant genomes will be highly useful to both students and teaching faculties. Most importantly, research scientists involved in genomics research will have access to systematic deliberations on the plant genomes of their interest. Elucidation of plant genomes is of interest not only for the geneticists and breeders, but also for practitioners of an array

of plant science disciplines, such as taxonomy, evolution, cytology, physiology, pathology, entomology, nematology, crop production, biochemistry, and obviously bioinformatics. It must be mentioned that information regarding each plant genome is ever-growing. The contents of the volumes of this compendium are, therefore, focusing on the basic aspects of the genomes and their utility. They include information on the academic and/ or economic importance of the plants, description of their genomes from a molecular genetic and cytogenetic point of view, and the genomic resources developed. Detailed deliberations focus on the background history of the national and international genome initiatives, public and private partners involved, strategies and genomic resources and tools utilized, enumeration on the sequences and their assembly, repetitive sequences, gene annotation, and genome duplication. In addition, synteny with other sequences, comparison of gene families, and, most importantly, the potential of the genome sequence information for gene pool characterization through genotyping by sequencing (GBS) and genetic improvement of crop plants have been described. As expected, there is a lot of variation of these topics in the volumes based on the information available on the crop, model, or reference plants.

I must confess that as the series editor, it has been a daunting task for me to work on such a huge and broad knowledge base that spans so many diverse plant species. However, pioneering scientists with lifetime experience and expertise on the particular crops did excellent jobs editing the respective volumes. I myself have been a small science worker on plant genomes since the mid-1980s, and that provided me the opportunity to personally know several stalwarts of plant genomics from all over the globe. Most, if not all, of the volume editors are my longtime friends and colleagues. It has been highly comfortable and enriching for me to work with them on this book series. To be honest, while working on this series I have been and will remain a student first, a science worker second, and a series editor last. And I must express my gratitude to the volume editors and the chapter authors for providing me the opportunity to work with them on this compendium.

I also wish to mention here my thanks and gratitude to the Springer staff, Dr. Christina Eckey and Dr. Jutta Lindenborn in particular, for all their constant and cordial support right from the inception of the idea.

I always had to set aside additional hours to edit books beside my professional and personal commitments—hours I could and should have given to my wife, Phullara, and our kids, Sourav and Devleena. I must mention that they not only allowed me the freedom to take away those hours from them but also offered their support in the editing job itself. I am really not sure whether my dedication of this compendium to them will suffice to do justice to their sacrifices for the interest of science and the science community.

New Delhi, India Chittaranjan Kole

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Introduction: The Importance of Eggplant

1

Mark A. Chapman

Abstract

In this chapter, I highlight how the eggplant, whilst being globally dwarfed by other members of the Solanaceae, notably potato and tomato, offers a number of important ecological, evolutionary and agronomic features making it unique and interesting, warranting further study. It also highlights the parallels and differences between Solanaceous crops. The eggplant genome is in the process of being finalised, and once this is available to researchers, it is likely we will see a surge of papers utilising this resource for understanding the genetic basis of these important traits.

1.1 Overview

The Asian eggplant (*Solanum melongena* L.), known as aubergine in Britain and France, and brinjal in Southern Asia and South Africa, is a widely grown species from the nightshade family (Solanaceae). The fruit is popular in a range of cuisines and is an important part of the diet in many countries, especially India and Bangladesh, Southeast Asia and the Middle East (Daunay and Lester 1988). S*olanum* is a large genus of ca. 1400 species (D'Arcy 1991; Frodin 2004), several of which are poisonous to humans, most famously the nightshades (e.g. *S. dulcamara* L.). Eggplant is an Old World crop, domesticated in Asia, whereas its congeners, potato (*S. tuberosum* L.) and tomato (*S. lycopersicum* L.), are New World (South American) representatives of the genus (Daunay and Lester 1988; Weese and Bohs 2010).

The focus of this book is the Asian eggplant, *S. melongena*; however, two other *Solanum* species are known as eggplants, the Ethiopian/scarlet eggplant (*S. aethiopicum* L.) and the African/Gboma eggplant (*S. macrocarpon* L.). These two species are minor crops globally relative to Asian eggplant, but may be locally important, with the fruits and leaves of both species used for food and medicine. The similarities between the three eggplant species have previously caused taxonomic confusion; however, it is clear now they are relatively distantly related within the genus (Weese and Bohs 2010).

Several non-exclusive theories have been proposed concerning the origin of Asian eggplants, *S. melongena* (hereafter simply 'eggplant'). The general consensus (Knapp et al. 2013; Weese and Bohs 2010) is that the African/Middle Eastern species *S. incanum* L. was transported, intentionally or otherwise, into Indo-China where the true wild progenitor, *S. insanum* L., evolved, from which *S. melongena* is derived. The first domesticates are possibly

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represented now by relatively small-fruited *S. ovigerum*, and the landraces and other cultivated types are derived from this group. This is described in further detail in Chap. 12. More recent molecular evidence has suggested that eggplant was domesticated more than once (Meyer et al. 2012b); however, this remains contentious (see Chap. 12). Ancient literature suggests that eggplant has been used as a food for more than 2000 years in China (Wang et al. 2008), and traces of *Solanum* have been found in Harappan cooking vessels from ca. 4500 YBP in India (Kashyap and Weber 2013).

Traditionally the majority of research into crops of the Solanaceae has focussed on tomato and potato, presumably due to the relative economic importance of these three crops. The first potato genome was published in 2011 (Xu et al. 2011b) and tomato in 2012 (Sato et al. 2012). A draft genome of eggplant followed, however, this was incomplete, covering approximately 74% of the genome, and fragmented, being represented by 33,873 scaffolds (Hirakawa et al. 2014); see also Chap. 6). Eggplant (and wild relatives) nonetheless offer a range of features absent from potato and tomato, especially high tolerances to a number of pests and pathogens (Daunay 2008; Salgon et al. 2017) and tolerance of abiotic stresses (Fita et al. 2015; Keatinge et al. 2014).

Beyond the economic importance of eggplant, the similarities between the domestication of tomato and eggplant (and to a lesser degree peppers, *Capsicum annuum* L.) pose an interesting study system for investigating parallel and/or convergent evolution. Domestication of these members of the Solanaceae has involved an increase in fruit size and alteration in fruit colour and shape, and comparative mapping suggests these traits may be controlled (in part) by the same suite of genes (Doganlar et al. 2002). This is discussed briefly below and in more depth in Chap. 4.

For these reasons, the origin and evolution of eggplant pose some interesting and important questions for a range of researchers, which are beginning to be addressed utilising modern technologies.

1.2 Economic Importance of Eggplant

Eggplant is the third most widely grown Solanaceous vegetable after potatoes and tomatoes (Fig. 1.1a). Eggplant is especially popular in cuisines of Southeast Asia and the Mediterranean. In 2016, the area of potato and tomato harvested worldwide was greater than that of eggplant by a factor of 10 and almost three, respectively (FAO 2017). Eggplant production has increased steadily since FAOSTAT data was collected (1961) to about 1.79 M ha (51.3 M tonnes) worldwide (2016 data). Over 80% of eggplants are produced in China and India with only five other countries (Egypt, Indonesia, Turkey, Iran and the Philippines) growing more than 1% of the world's total production (Fig. 1.1b).

Whilst eggplant is not known for being high in the majority of health-related micronutrients, it is very low fat and low calorie. The Solanaceae as a whole, however, are a rich source of nutritionally and pharmaceutically useful compounds, partly

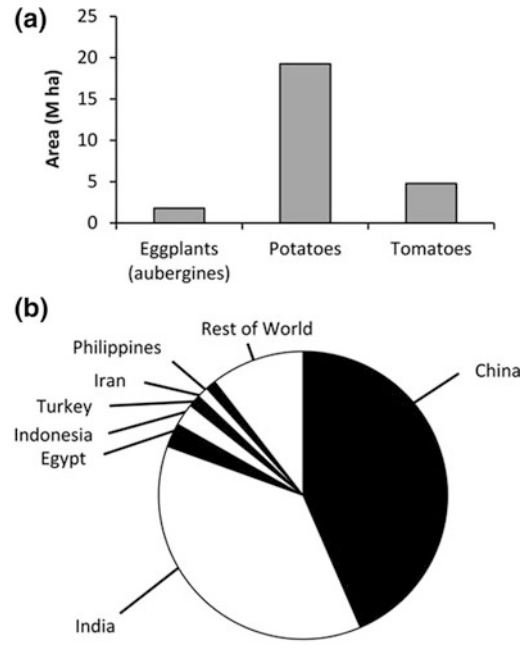

Fig. 1.1 Area of eggplant harvested in 2016. **a** Compared to congeners potato and tomato, **b** subdivided by country (only countries growing >1% of the world's eggplant are named). Data is from FAO (2017)

explaining the large number of species in the Solanaceae used for food or medicine (Hawkes 1999). In eggplant, a number of phytonutrients are present, especially hydroxycinnamic acid (HCA) conjugates, potentially involved in consumer health, fruit taste and texture (see Meyer et al. 2015, Chap. 3 and references therein). HCA conjugates are free radical scavengers and hence may play a role in mediating oxidative stress (Ma et al. 2011). A number of compounds differ in their abundance between wild and domesticated eggplant, with the domesticates containing overall a lower total abundance of HCA conjugates (Meyer et al. 2015). The quantity of total and individual HCA conjugates varies widely amongst accessions of eggplant (Stommel and Whitaker 2003). Interestingly, literature surveys and interviews demonstrate that eggplant is used as a medicine for different illnesses (especially for gastrointestinal, immune system and cardiovascular ailments) in different parts of the world (Meyer et al. 2014). This could have influenced the trajectory of eggplant domestication and improvement in different parts of the world.

Eggplant is also valued as a rootstock for tomatoes because of resistance to certain diseases and nematodes. For example, data from Vietnam suggested that grafted tomatoes (onto a range of rootstocks, including eggplant) out-yield non-grafted tomatoes by around one third (Genova et al. 2013). Eggplant rootstocks have also been shown to confer waterlogging tolerance to tomatoes (Bahadur et al. 2015), and eggplant wild relatives are also used as rootstocks because of resistance to pathogens, for example verticillium wilt (Bletsos et al. 2003).

1.3 Academic Importance of Eggplant

1.3.1 Eggplant as a Model for Parallel Evolution

As mentioned above, the domestication of multiple members of the Solanaceae has been used as a model to study convergent evolution. During domestication, human selection on fruit colour, taste, shape and size has been pervasive across many crops (Meyer et al. 2012a), and in grass crops, some traits are controlled by orthologous genes, for example loss of shattering (i.e. a loss of natural seed dispersal) in cereal crops (Lin et al. 2012). If the same genes are involved in the domestication of multiple crops, then knowledge of the genetic basis of these traits in one crop can be transferred across crops.

Tomato and eggplant exhibit a number of conspicuous similarities in their domestication syndromes, but similarities with potato and pepper in the suite of traits that were selected by humans are also evident, especially fruit shape and size in pepper, and colour, albeit tuber and flower colour, in potato. When the first genetic maps of eggplant were produced, it was noted, based on comparing genetic maps from other Solanaceae species, that a number of quantitative trait loci (QTL) for eggplant domestication traits were found in the same genomic regions as those in tomato, pepper and potato (Doganlar et al. 2002). These findings are detailed in Chap. 4.

1.3.2 Eggplant Wild Relatives for Crop Improvement

Until recently, the potential for eggplant wild relatives to improve cultivated eggplants has been discussed but little progress has been made. This is disappointing because crop wild relatives (CWRs) are often major sources of alleles for biotic and abiotic tolerances (Dempewolf et al. 2017). One major hindrance to the breeding of wild species' alleles into cultivated eggplant has been the lack of a genome sequence (Gramazio et al. 2018). This absence prevents the development of genome-anchored markers which are necessary for efficient trait transfer, e.g. through marker-assisted selection (MAS; Morrell et al. 2011). Nonetheless, success in introgressing Fusarium wilt resistance from *S. aethiopicum* and Verticillium wilt resistance from *S. linnaeanum* Hepper & P.-M.L. Jaeger into eggplant has been carried out (Toppino et al. 2008; Liu et al. 2015).

More recently, however, a large number of mapping populations from crosses between eggplant and its CWRs have been generated (Kaushik et al. 2016). This extensive work, utilising multiple cultivated eggplants and ten wild species, has seen the development of dozens of backcross populations with potential for introgressing traits of interest from wild relatives into eggplant. This, and other work (e.g. Ranil et al. 2017), also highlights the ease with which closely related *S. incanum* and *S. insanum* can be crossed with eggplant, making them important candidates for aiding eggplant improvement through introgression. Members of the secondary gene pool can be crossed with varying success to eggplant, and crosses with more distantly related species are only successful through embryo rescue (Kouassi et al. 2016). Interestingly, the cross-compatibility of different eggplant accessions with the wild species is variable, suggesting that introgression from the wild species might be easier in some eggplant varieties than in others. Extensive morphological phenotyping of the parents and F1 progeny has allowed the identification of wild species which exhibit the best potential for eggplant improvement (Kaushik et al. 2016).

Gaining a better understanding of the genetic basis of adaptive phenotypes in eggplant CWRs has been a prominent goal in eggplant breeding for years, and with the advent of high-throughput sequencing (HTS), a number of wild species have been subjected to analysis with the purpose of generating molecular markers necessary for downstream genetic mapping, diversity analysis and candidate gene analysis. Through transcriptome sequencing, i.e. sequencing the expressed portion of the genome from one or more tissue(s) at one or more time-point(s), only a subset of the genome is sequenced, decreasing the cost considerably (Ozsolak and Milos 2010). In addition, the output of a transcriptome investigation is made up primarily of coding sequences, thus enriching for regions of the genome that may code for traits of interests (although it is acknowledged that the genetic basis of some traits is not attributable to the coding portion of the genome; Wilusz et al. 2009).

Simple sequence repeat (SSR) markers (aka microsatellites) can be identified from a single transcriptome sequence (e.g. Chapman 2015; White et al. 2016), acknowledging that some proportion of these markers will not be variable when tested across multiple individuals. Single nucleotide polymorphism (SNP) markers, however, require the comparison of at least two individuals to identify polymorphisms before designing SNP assays. Through the comparison of transcriptomes from four *Solanum* species, marker databases have been developed for the wild relatives *Solanum incanum* and *S. aethiopicum* (Gramazio et al. 2016). Transcriptome sequences for *S. torvum* Sw. (Yang et al. 2014) and *S. aculeatissimum* Jacq. (Zhou et al. 2016) are available (see also below), and a comparative transcriptomic investigation could be employed to identify molecular markers.

1.3.3 Transcriptomics in Eggplant

Despite the absence of a complete genome sequence, eggplant studies have harnessed the power of HTS for a range of studies. HTS-generated molecular markers (specifically using RADseq and genotyping-by-sequencing approaches) have been employed to aid in genetic map construction and to understand genetic diversity within eggplant accessions and between eggplant and its CWRs; however, these are covered in detail in later chapters and will not be discussed here. Instead, here I focus on two set of studies which have employed transcriptomics to (1) examine the gene expression response to pathogen infection, and (2) understand the genetic basis of anthocyanin accumulation. These investigations highlight the potential for eggplant to serve as a model species for a number of adaptive traits.

1.3.3.1 Eggplant and Its Relatives as a Model for Understanding Pathogen Infection

Whilst eggplant is susceptible to several pathogens, a number of the eggplant CWRs offer

promise for resistance to these bacteria and fungi, which reduce crop yields (of eggplants and other crops) substantially if left unchecked. Efforts to understand the genetic basis of pathogen resistance are therefore of paramount importance (Piquerez et al. 2014).

Solanum torvum is an eggplant CWR and is often used as a rootstock because of strong resistance to several soil-borne pathogens, notably the root-knot nematode *Meloidogyne incognita* (Gousset et al. 2005). In order to understand more about the genes involved in root-knot nematode resistance, gene expression analysis was carried out on *S. torvum* plants with and without infection (Bagnaresi et al. 2013), revealing almost 400 genes which were differentially expressed (DE) under pathogen infection. Gene ontology analysis showed that many of these genes were related to known pathogen response genes, especially chitinases which are often upregulated in plants exposed to nematode infection (e.g. Qtu et al. 1997).

Another eggplant CWR with pathogen resistance is *Solanum aculeatissimum*, resistant to verticillium wilt which negatively affects eggplant worldwide, as well as tomato, potato and cotton (Klosterman et al. 2009). Gene expression analysis was carried out comparing verticillium-treated and verticillium-untreated *S. aculeatissimum* seedlings and revealed thousands of genes which were DE (Zhou et al. 2016). Genetic pathways relating to the biosynthesis of secondary metabolites (especially hormone signal transduction and phenylpropanoid biosynthesis) were found to be enriched in the lists of DE genes, indicating that the mechanisms of verticillium wilt resistance in *S. aculeatissimum* could be very similar to the pathways in cotton and tomato (Gayoso et al. 2010; Xu et al. 2011a).

Both of these case studies suggest that the mechanisms of pathogen resistance in *Solanum* species may be conserved across crops, with the potential for cross-crop transfer of knowledge to expedite breeding and crop improvement.

1.3.3.2 Eggplant as a Model to Understanding Anthocyanin Accumulation in Plants

Anthocyanins are red, blue and purple pigments found in some groups of plants. Anthocyanins have potent antioxidant properties in vitro (e.g. De Rosso et al. 2008); however, evidence for a positive role in humans remains elusive (Lotito and Frei 2006). Nevertheless, understanding the biosynthesis of these pigments is of interest because of the visual attractiveness, as highlighted by the popularity of recently developed blue tomatoes.

Eggplants exhibit a significant variation in fruit colours and hence pose a model for understanding anthocyanin biosynthesis. Using a cultivar in which the purple pigmentation is induced by exposure to light, recent research has identified genes which may play a role in initiating the biosynthesis of anthocyanins even before any purple pigmentation is evident (Li et al. 2017, 2018). Understandably a number of genes that were differentially expressed in the light-exposed fruit were from the anthocyanin biosynthesis pathway (Li et al. 2017); however, other genes not known to be involved in the regulation of anthocyanins were unearthed (Li et al. 2018), posing new targets for breeding both in eggplant and in other species.

Recently, successful modification of fruit colour in *S. aethiopicum* has been carried out through the transgenic expression of an eggplant gene, *SmMYB1* (Zhang et al. 2016).

1.3.3.3 Eggplant as a Model for Understanding the Wider Effects of Genetic Modification

The transgenic improvement of crops through transfer of genes from different organisms has promise for feeding the future growing population under climate change (James 2003), yet public perception is mixed, and in some cases

strongly negative. Part of this negativity comes from a lack of published knowledge about the consequences to other organisms of growing a genetically modified (GM) crop. These non-target effects, for example the effects on soil microbes (Liu et al. 2005) and food webs (Groot and Dicke 2002), remain understudied.

Several GM eggplants possessing a *Cry1Ac* toxin from *Bacillus thuringiensis* have been developed (collectively 'Bt brinjal') and were released in India in 2009, but soon a moratorium caused their use to be prohibited. This was based largely on public outcry and the insistence of anti-GM groups who called for more research (Herring 2015). In 2013, in Bangladesh, four varieties of Bt brinjal were released after seven years of trials. Putting the controversy to one side, these Bt brinjals allow the assessment of the potential for a GM crop to be effective as well as to assess their non-target effects, as follows:

Firstly, Bt brinjal (including lines developed with different Cry1 alleles) has been shown to be effective against eggplant shoot and fruit borer (ESFB) both under controlled (glasshouse) conditions (Rai et al. 2013) and in the field (Hautea et al. 2016). Infestation of Bt brinjal with ESFB was shown to be almost zero in field trials in the Philippines (Hautea et al. 2016).

Second, non-target effects have been investigated for both soil microbes and non-target arthropods. Singh et al. (2013) demonstrated that soil microbe abundance was lower in plots of Bt brinjal than non-Bt counterparts. In addition, different species of bacteria were present in soils of the two treatments (Bt brinjal and non-Bt brinjal) (Singh et al. 2013) indicating the potential for microbial non-target effects. Conversely, in field trials, there was no significant difference in the non-target arthropod communities of Bt and non-Bt brinjal indicating that the Bt brinjal is selective in its control of the ESFB (Navasero et al. 2016).

Finally, it is clear that in natural environments, there is a high potential for crop eggplant to hybridise with wild eggplants (Davidar et al. 2015; see also Chap. 12), which could be an avenue for transgene escape (Chapman and Burke 2006). The consequences for the escape of the Bt transgene into wild relatives are not known and require further research.

1.4 Conclusions

Despite eggplant being less economically important than its congeners, it serves as an important model for a number of agronomic and evolutionary processes; hence, the development of a genome sequence represents an important step forward in these fields of research. The following chapters discuss our current knowledge of eggplant as a crop in its own right as well as a model for understanding genome evolution, domestication and speciation.

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Eggplant (*Solanum melongena* L.): Taxonomy and Relationships

2

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Abstract

Solanum melongena L. (brinjal eggplant) is a member of a small monophyletic group (Eggplant clade) of mainly andromonoecious species in the large and diverse Leptostemonum clade of *Solanum* (previously referred to as subgenus *Leptostemonum* Bitter). The Leptostemonum clade (also known as the spiny solanums) is the most diverse monophyletic group in the species-rich genus *Solanum* and contains more than 500 species occurring on all continents except Antarctica. In this chapter, we summarise the current state of knowledge of the taxonomy and phylogeny of *Solanum*, the Leptostemonum clade and that of the monophyletic group of Old World taxa to which *S. melongena* belongs. We provide a species list with distributions of the currently recognised members of the Eggplant clade and discuss character evolution and biogeography in the group in the context of phylogeny.

2.1 Introduction

The brinjal eggplant (*Solanum melongena* L.) is one of approximately 1300 species in the extremely species-rich genus *Solanum* L. in the nightshade family Solanaceae. The family comprises 101 genera, including many economic and horticultural importance such as *Nicotiana* L. (the tobaccos, see Knapp et al. 2004) and *Petunia* L. (Stehmann et al. 2000). Generic diversity in the family is concentrated in the Americas, but there have been several instances of long-distance dispersal giving rise to genera and/or groups that are endemic to the Old World (Dupin et al. 2017). Generic limits in the family are under active investigation, and new genera have been included (e.g. *Nolana* L.f. and *Sclerophylax* Miers, traditionally recognised as separate families; see Olmstead et al. 2008) and segregated based on new understanding from molecular phylogenetics (e.g. *Trompettia* Dupin & S.S.Smith; Dupin and Smith 2018). With the inclusion of previously segregated genera such as *Lycopersicon* Mill., *Cyphomandra* Sendtn. and *Normania* Lowe, *Solanum* is resolved as strongly monophyletic and as sister to the genus *Jaltomata* Schltdl. (Särkinen et al. 2013).

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Solanum comprises around half of the species diversity of the family and is one of only a handful of flowering plant genera with more than 1000 species (Frodin 2004). Not only because of its large size, *Solanum* is also important for containing species of great economic importance for humans, such as potato (*S. tuberosum* L.), tomato (*S. lycopersicum* L.) and of course the eggplant, plus a host of minor fruit and leaf crops cultivated locally worldwide (see Anderson 1977; Whalen et al. 1981; Särkinen et al. 2018). Species of *Solanum* occur on all continents except Antarctica, in a wide variety of habitats from tropical rainforests to the driest deserts and have a wide range of life forms, from annual herbs to rainforest trees. The traditional view has been that the large majority of species of *Solanum* occurred in the New World, mostly in South America (e.g. see D'Arcy 1972) but recent work in Africa (Vorontsova and Knapp 2016), Asia (Aubriot et al. 2016) and Australia and New Guinea (e.g. Bean 2002, 2004, 2010, 2011, 2014, 2016; Bean and Albrecht 2008) has revealed hitherto poorly understood diversity in the Old World, especially in the spiny solanums (see below).

The genus was divided into two main groups by authors in the nineteenth and early twentieth centuries (e.g. Dunal 1852; Seithe 1962), simply described as the spiny and non-spiny solanums. These broad groups were defined on the presence or absence of prickles and anther shape (see Vorontsova and Knapp 2016 for a more complete discussion). Within those broad groups, *Solanum* was divided into a number of sections (see D'Arcy 1972, who listed many sections, subsections and series), defined largely on macro-morphological characteristics. Phylogenetic work using DNA sequences showed that most of these sectional groupings were not monophyletic (Bohs 2005); the genus can be divided into 13 major clades (Bohs 2005; Särkinen et al. 2013; Weese and Bohs 2007). Some of these (e.g. the Potato clade, including tomatoes and their relatives and a number of smaller groups such as section *Pteroidea* Dunal and the Regmandra clade; see Tepe et al. 2016) are well-supported and monophyletic, while the relationships of others are less clear (e.g. species like *S. clandestinum* Bohs and *S. mapiriense* Bohs; see Särkinen et al. 2013). Relationships between the clades are relatively stable, but a polytomy at the base of Clade 2 of Särkinen et al. (2013) means the sister group of the largest and most species-rich clade of *Solanum*—the Leptostemonum clade or the spiny solanums—is not yet clear (Särkinen et al. 2013).

2.2 The Leptostemonum Clade

The prickly solanums are the largest monophyletic group within the genus *Solanum* (Bohs 2005; Särkinen et al. 2013; Stern et al. 2011). They were traditionally referred to as subgenus *Leptostemonum* Bitter (Bitter 1919), or as "chorus subgenerum" *Stellatipilum* Seithe (Seithe 1962), highlighting the two characters whose combination defined the group—the presence of stellate trichomes and long attenuate anthers. Neither of these is unique to the Leptostemonum clade. Stellate trichomes are found in the Brevantherum clade (Stern et al. 2013; Giacomin and Stehmann 2014) and attenuate anthers in two small groups, *S. nemorense* Dunal and relatives (Bohs 2005) and the *S. wendlandii* clade (Clark et al. 2016); all of these groups are part of the polytomy at the base of Clade 2 of Särkinen et al. (2013).

The Leptostemonum clade is strongly monophyletic (Stern et al. 2011) and comprises at current estimates some 560 accepted species distributed on all continents except Antarctica (S. Knapp, unpublished). Approximately half of these occur in the New World and half in the Old World (see Aubriot et al. 2016). Using DNA sequence data to delimit monophyletic groups within the Leptostemonum clade revealed that the Old World species were a single, monophyletic clade (with a few exceptions; Levin et al. 2006; Stern et al. 2011) rather than being related to diverse groups of New World taxa as had previously been thought (e.g. D'Arcy 1972; Whalen 1984). This large Old World group was derived within the spiny solanums and was sister to a small group of taxa that exhibit an

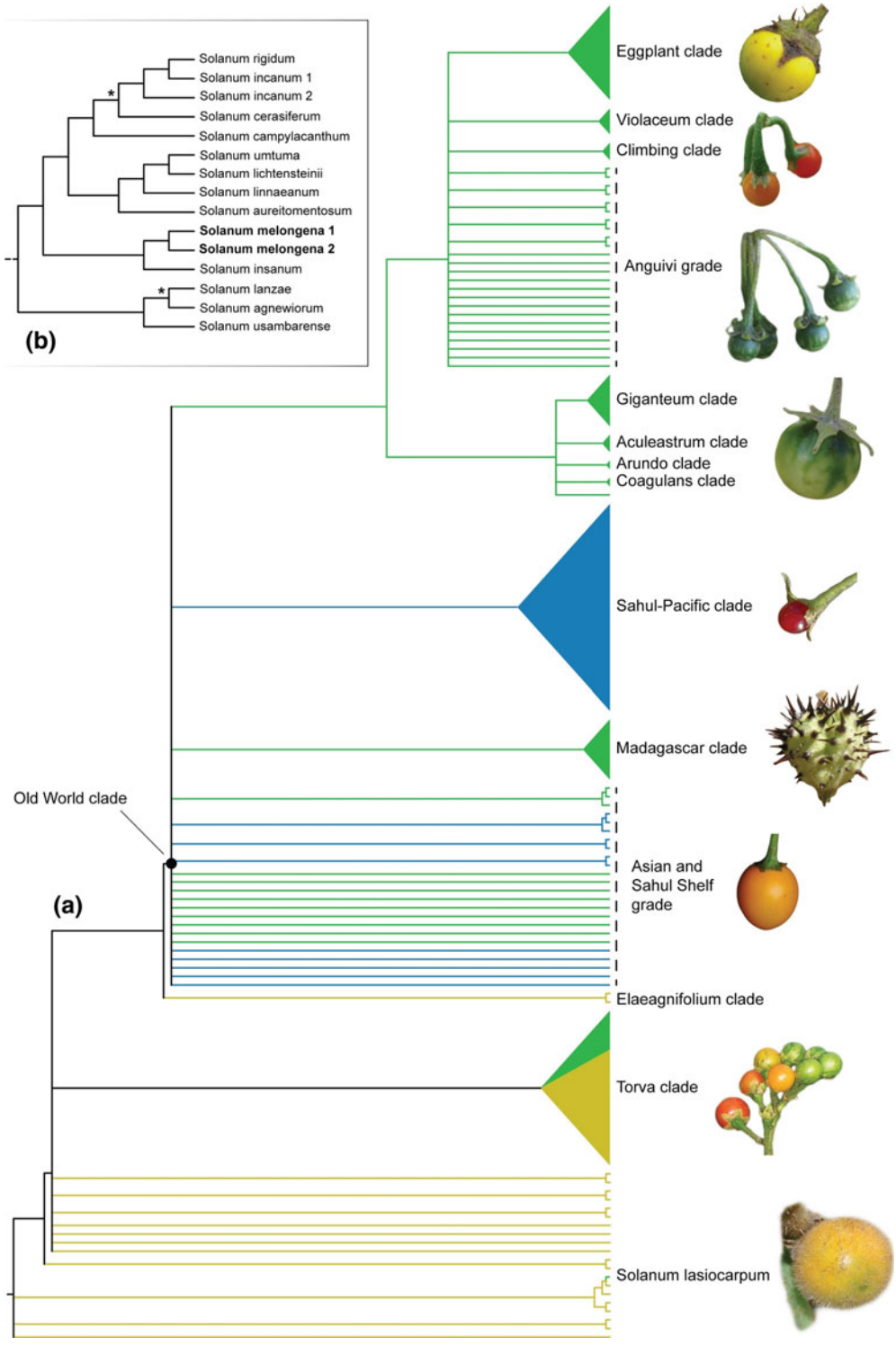
amphitropical disjunct distribution between North and South America (the *S. elaeagnifolium* Cav. clade, see Knapp et al. 2017). New World species of spiny solanums could be divided into 13 smaller monophyletic groups, some of them endemic to Brazil (e.g. Gouvêa and Stehmann 2019) while others were more widespread across the Americas (e.g. Whalen et al. 1981). The first dichotomy in the spiny solanums is between a group of taxa from Brazil and the Caribbean (the Gardneri, Thomasiifolium and Erythrotrichum clades) and the rest of group (Stern et al. 2011). The largest of the New World clades is the Torva clade with ca. 70 species of mostly Andean distribution (but see below). The continued discovery of new species of spiny solanums in the Americas, particularly in Brazil (e.g. Gouvêa and Stehmann 2016; Gouvêa et al. 2018; Ribero-Silva and Proença 2011), means that limits and composition of New World groups are both in active revision.

2.3 The Old World spiny solanums

Data from a number of studies using molecular phylogenetics showed that the Old World species of spiny solanums formed a strongly supported monophyletic group (Aubriot et al. 2016; Levin et al. 2006; Stern et al. 2011; Vorontsova et al. 2013), with the exception of a small clade of Asian species nested within the otherwise New World Torva clade (Aubriot et al.. 2016). The Fijian *S. repandum* Forst. and Asian S. *lasiocarpum* Dunal that have long been recognised as members of the largely New World Lasiocarpum clade (Whalen et al. 1981) were recovered in that position in all molecular analyses. The many species of New World solanums that are thought to have been introduced to the Old World (e.g. *S. elaeagnifolium*, *S. sisymbrifolium* Lam., *S. viarum* Dunal; see Vorontsova and Knapp 2016) are all members of New World clades.

Relationships amongst the Old World spiny solanums show an initial split between a group of species whose distributions are centred on Australia and New Guinea extending into the Pacific (the Sahul-Pacific clade of Aubriot et al. 2016) and the rest of the Old World taxa (see Fig. 2.1a for a summary of the Old World spiny solanum relationships). Monophyletic groups first recognised in the analysis of African taxa by Vorontsova et al. (2013) were for the most part upheld in an expanded analysis that included Asian and Pacific taxa (Aubriot et al. 2016), but relationships between groups along the backbone of the tree are still poorly resolved. Australian species do not comprise a single monophyletic group, but sampling of these taxa in Aubriot et al. (2016) was limited; Australian species diversity is very high (see below), and expanded sampling of these taxa is a priority for understanding relationships in the Old World spiny solanums. Previous phylogenetic work on Australian spiny solanums has focused on small groups of dioecious taxa (Martine et al. 2006, 2009). The endemic Malagasy spiny solanums are all closely related and form a monophyletic group, despite their markedly divergent morphologies (see Vorontsova and Knapp 2016). The Eggplant clade, containing *S. melongena* and its close relatives (Knapp et al. 2013), has been recovered as a strongly supported lineage in all analyses to date (Fig. 2.1b; see also summary in Aubriot et al. 2016), but with some differences in circumscription (see below). That early branching lineages in the Old World spiny solanums are all Australasian, and Pacific suggests that any long-distance dispersal from the New World was across what is now the Pacific Ocean and not the Atlantic (see Dupin et al. 2017).

Both Vorontsova et al. (2013) and Aubriot et al. (2016) recovered a large grade that has been called the "Anguivi grade"; relationships in this group are not well-defined (Fig. 2.1a). Members of this grade include the scarlet eggplant *S. aethiopicum* L. and its progenitor *S. anguivi* Lam. The widespread Asian species *S. violaceum* Ortega (often synonymised with *S. anguivi* and often called *S. indicum* L., a name suppressed [nom. utique rej.] under the *International Code of Nomenclature for algae, fungi and plants*, Turland et al. 2018) is morphologically similar to *S. anguivi*, but is part of a monophyletic and strongly supported group with four other Asian species that is sister to the Eggplant clade.


Solanum rigidum
Solanum incanum 1
Solanum incanum 2
Solanum cerasiferum
Solanum campylacanthum
Solanum umtuma
Solanum lichtensteinii
Solanum linnaeanum
Solanum aureitomentosum
Solanum melongena 1
Solanum melongena 2
Solanum insanum
Solanum lanzae
Solanum agnewiorum
Solanum usambarense
(b)
Eggplant clade
Violaceum clade
Climbing clade
Anguivi grade
Giganteum clade
Aculeastrum clade
Arundo clade
Coagulans clade
Sahul-Pacific clade
Madagascar clade
Old World clade
(a)
Asian and
Sahul Shelf
grade
Elaeagnifolium clade
Torva clade
Solanum lasiocarpum

◀ **Fig. 2.1** Outline phylograms to illustrate relationships discussed in the text. Unless otherwise noted with asterisk (*), all nodes are well-supported; for support values see the original publications. **a** The major clades of Old World spiny solanums (modified from Aubriot et al. 2016) with representative fruits to illustrate diversity. Blue branches indicate Australia/New Guinea/Pacific, green African/Middle East/Tropical Asia and yellow New World species distributions. **b** Relationships of the eggplant (*Solanum melongena*) and its relatives in the Eggplant clade (modified from Aubriot et al. 2018)

Aubriot et al. (2016) suggest this represents an instance of dispersal from Africa to Asia, since this large Asian lineage is nested within an otherwise almost exclusively African group. Within the Anguivi grade, the Macaronesian endemics *S. lidii* Sunding and S. *vespertilio* Aiton were recovered in all analyses as sister taxa, but with unresolved broader relationships. Some Indian and south-eastern Asian species are nested in otherwise African groups (i.e. *S. pubescens* Willd. in the Giganteum clade and *S. trilobatum* L. with *S. usaramense* Dammer as sister to the Gboma eggplant, *S. macrocarpon* L.; see Fig. 3 in Aubriot et al. 2016, but see below).

Species diversity in the Old World spiny solanums is not evenly distributed geographically. Vorontsova and Knapp (2016) recognised 76 native African species (including Madagascar; there are also 10 introduced taxa, all from the New World), Aubriot et al. (2016) recognised 56 native species in Asia (including New Guinea, with taxa described in Bean (2016) this number will certainly increase), and McClelland (2012) suggested there were ca. 30 species occurring in the Pacific region. Other areas have much smaller numbers of taxa. The maximum species diversity in the Old World spiny solanums occurs in Australia + New Guinea, with ca. 175 species described (see Symon 1981, 1985) many of these relatively recently (Barrett 2013; Bean 2002, 2004, 2010, 2011, 2016; Bean and Albrecht 2008; Martine et al. 2006, 2009, 2016a, b). Most of these endemic Australian species have narrow distributions (e.g. *S. watneyi* Martine & Frawley from a small area in north-western Northern Territory or *S. zoeae* R.L.Barrett from a very restricted locality in the Kimberley); some have long been recognised as distinct, but others are completely new discoveries.

The New World has been presumed to be the primary diversification "hotspot" for the genus because the species diversity is higher there, but Australia has been recognised as a secondary centre for *Solanum* diversity by many previous authors (Symon 1981; Bohs 2005). Using a variety of diversification analysis methods and a PASTIS tree corrected for taxon sampling, Echeverria-Londoño et al. (2018) showed that contrary to expectation, the Old World clade of spiny solanums exhibited the fastest diversification rate in *Solanum*, despite its lower numbers of species as compared to the New World clades. Based on the dated phylogeny published by Särkinen et al. (2013), this explosive diversification in the Old World, and more specifically in Australia, occurred after the Miocene (ca. 10 Mya) when aridification and spread of dry woodlands and deserts in the interior of Australia began. They hypothesised a long-distance dispersal event ca. 6 Mya, followed by a rapid invasion of new niches being opened up by the expansion of dry forest habitat types. Future analysis of the relationships of Australian spiny solanums in the light of these findings will certainly help explain patterns of expansion and diversification in the region. Australian solanums also share a number of drought resistance features that will be of interest to eggplant breeders in future.

2.4 The Eggplant Clade

The eggplant and its wild relatives have been the subject of considerable taxonomic confusion and controversy (e.g. Deb 1989; Lester and Hazan 1990, 1991; Meyer et al. 2012; Samuels 1996, 2010, 2012, 2013a, b, 2016) and for much of the late twentieth century, only two species (*S. melongena* and *S. incanum*) were recognised (e.g. Daunay and Hazra 2012), each with several forms or races (see Knapp et al. 2013). In part, this has been due to their morphological similarity, and the propensity of extremely closely related taxa to interbreed when in sympatry

Table 2.1 Species currently recognised as members of the Eggplant clade (Aubriot et al. 2018). For distribution maps and detailed descriptions of African species, see Vorontsova and Knapp (2016)

Species	Distribution	Breeding system	Chromosome number
Solanum agnewiorum Voronts.	Kenya	Andromonoecious	Not known
Solanum aureitomentosum Bitter	Malawi to South Africa	Andromonoecious	Not known
Solanum campylacanthum Hochst. ex A.Rich.	Widespread throughout eastern Africa; Kenya to South Africa	Andromonoecious	$n = 24$
Solanum cerasiferum Dunal	Sub-Saharan Africa; Senegal to Kenya	Andromonoecious	Not known
Solanum incanum L.	North-eastern Africa to Pakistan	Andromonoecious	$n = 12$
Solanum insanum L.	India and China, east to the Philippines (also on Madagascar)	Andromonoecious	$n = 12$
Solanum lanzae Stork &	Eastern Africa Rift valley; Ethiopia to Tanzania	Hermaphroditic	Not known
Solanum lichensteinii Willd.	South Africa to Angola, Tanzania and Democratic Republic of the Congo	Andromonoecious	Not known
Solanum linnaeanum Hepper & P.M.-L.Jaeger	South Africa (populations in northern Africa perhaps introduced)	Andromonoecious	$n = 12$
Solanum melongena L.	Cultivated	Andromonoecious	$n = 12$
Solanum rigidum Lam.	Cape Verde Islands	Andromonoecious	Not known
Solanum umtuma Voronts. & S.Knapp	Eastern South Africa	Andromonoecious	Not known
Solanum usambarense Bitter & Dammer	Tanzania and Kenya; centred on Usambara Mountains	Hermaphroditic	Not known

(Davidar et al. 2015; Hurtado et al. 2012; Mutegi et al. 2015). Detailed taxonomic work on the spiny solanums of the Old World (Ranil et al. 2017; Vorontsova and Knapp 2016), coupled with phylogenetic work more generally (Aubriot et al. 2016; Vorontsova et al. 2013), has resulted in the recognition of thirteen species in the "core" Eggplant clade (Table 2.1; Fig. 2.1b).

Weese and Bohs (2010) used germplasm materials and taxon circumscriptions of Lester and Hasan (1991) to test the hypothesis of stepwise migration from Africa to Asia for the origin of *S. melongena*. Their results supported this and showed that another African species, *S. linnaeanum* Hepper & P.M-L.Jaeger, was a member of the group. Vorontsova et al. (2013) expanded the data set by including many African taxa and still recovered a monophyletic Eggplant clade, but with little internal resolution. Their data showed that the narrow Kenyan endemic *S. agnewiorum* Voronts. belonged to this monophyletic Eggplant clade—a surprising result given its small fruit and weak andromonoecy (see Vorontsova and Knapp 2016). The inclusion of more species from Africa and Southeast Asia (Aubriot et al. 2016) revealed that two additional African species with hermaphroditic flowers and small fruit were members of the monophyletic Eggplant clade (*S. lanzae* J.-P. Lebrun & Stork and *S. usambarense* Bitter & Dammer). Both these studies used a combination of plastid and nuclear molecular markers, and although the circumscription of the Eggplant clade improved, relationships within it remained poorly resolved (see Fig. 2.1b).

The African taxa in Table 2.1 were recognised in previous taxonomic treatments as section *melongena* Bitter and defined by their possession of an andromonoecious breeding system (e.g. Bitter 1923), but it is clear from

recent phylogenetic work that both andromonoecious and hermaphroditic species belong to the monophyletic Eggplant clade. Andromonoecy is a derived breeding system where a single or a few flowers in an inflorescence are hermaphroditic, and the rest of the flowers are staminate and functionally male (Whalen and Costich 1986). This breeding system is common in the Leptostemonum clade (see Miller and Diggle 2003, 2007) and is thought to have originated multiple times in *Solanum* more generally (Whalen and Costich 1986). Andromonoecy is correlated with larger fruit size in *Solanum* (Miller and Diggle 2007) and is one of the characters that has been important in the domestication of the brinjal eggplant (Daunay and Janick 2007; Wang et al. 2008). None of the hermaphroditic taxa newly recognised as members of the Eggplant clade has been grown in cultivation, and both are relatively narrowly distributed in eastern Africa as is *S. agnewiorum* (see Vorontsova and Knapp 2016).

Aubriot et al. (2018) used whole plastome sequences to resolve relationships within the Eggplant clade; they also tested species-level circumscription to further refine species boundaries and definitions. They found that the widespread polymorphic species as currently circumscribed (e.g. Knapp et al. 2013; Meyer et al. 2012; Ranil et al. 2017; Vorontsova and Knapp 2016) were monophyletic; a single accession of *S. incanum* from Burkina Faso in the easternmost part of the species range did not group with the rest and was the exception. This accession may be of hybrid origin and needs further investigation. Phylogenetic reconstruction using whole plastome sequences confirmed the monophyly of the Eggplant clade including the three hermaphroditic species identified as members by Vorontsova et al. (2013) and Aubriot et al. (2016). Sister to the Eggplant clade is a lineage of two African (*S. polhillii* Voronts. and *S. supinum* Dunal) and one tropical Asian species (*S. trilobatum*).

Whole plastome sequences improved resolution of relationships within the Eggplant clade (Aubriot et al. 2018, see summary phylogram in Fig. 2.1b). The first branching lineage comprises the three hermaphroditic species: *S. agnewiorum*, *S. lanzae* and *S. usambarense*; affinities within this small group are not well-resolved. The next branching lineage is composed of the eggplant (*S. melongena*) and its wild progenitor (*S. insanum*). This lineage is sister to a monophyletic group that includes all remaining species of the Eggplant clade and is composed of two sister clades: (1) a "Southern African" group with four species from southern Africa (*S. aureitomentosum* Bitter, *S. lichtensteinii* Willd., *S. linnaeanum* and *S. umtuma* Voronts. & S.Knapp) and (2) a "Widespread" group, which includes three species with very large distribution ranges (*S. campylacanthum* Hochst. ex A.Rich., *S. cerasiferum* Dunal and *S. incanum*) and the Cape Verde islands endemic *S. rigidum* Lam. (Aubriot et al. 2018). This new phylogeny confirms taxonomic composition and phylogenetic structure of the Eggplant clade as found by Aubriot et al. (2016), but with the addition of *Solanum rigidum* and much improved resolution and support, especially amongst the African taxa sister to the eggplant and its wild progenitor.

These results suggest that the lineage from which the brinjal eggplant arose evolved after a single dispersal to Asia from Africa, but that the great diversity of eggplant wild relatives arose after that split, and in Africa itself. Biogeographic analysis showed that the origin of the Eggplant clade lies in north-eastern Africa and the Middle East, with spread both south ("Widespread" and "Southern African" groups) and east (*S. insanum* + *S. melongena*). Aubriot et al. (2018) suggest that the tropical Asian lineage of *S. insanum* did not proceed from a stepwise expansion through the Middle East, as previously thought (Lester and Hasan 1991) but instead from an early dispersal from Africa, unrelated to the southwards spread of African species.

Several African eggplant wild relatives are widespread (e.g. *S. campylacanthum*), but others, particularly those recently identified as members of the Eggplant clade (e.g. *S. agnewiorum*, *S. lanzae*, *S. usambaraense*), have more restricted distributions and are of some conservation concern (Syfert et al. 2016). Programmes to collect and conserve germplasm of these taxa

(Dempewolf et al. 2014) are focusing on these African species. Future collecting and preservation of wild relative germplasm should also sample across the range of widespread taxa like *S. campylacanthum* and *S. incanum*; their distribution has been suggested to have been influenced by the migration patterns of large mammalian herbivores like elephants and impala (Aubriot et al. 2018). Recent and ongoing range contraction of large mammalian seed dispersers could ultimately contribute to population isolation, genetic differentiation and ultimately speciation.

2.5 Other Cultivated Eggplant Species

Although we have concentrated here on the taxonomy and relationships of the brinjal eggplant *S. melongena*, two other spiny solanum species that are members of the Old World clade are cultivated and merit brief discussion here and are described in more detail in Chaps. 10 and 11. The Gboma eggplant (*S. macrocarpon*) is locally cultivated in Africa (Bukenya 1992; Bukenya and Carasco 1999) and is derived from the wild species *S. dasyphyllum* Schumach. & Thonn. In all phylogenetic studies to date, these two taxa are sisters (Aubriot et al. 2016; Vorontsova et al. 2013) and members of the poorly resolved Anguivi grade. Aubriot et al. (2016) recovered *S. trilobatum* and *S. usamarense* Dammer as sister to *S. macrocarpon* + *S. dasyphyllum*—a surprising result considering that these two taxa are scrambling vines with hermaphroditic breeding system, and the eggplants are robust, erect andromonoecious shrubs. The relationship between *S. trilobatum* and *S. usaramense* (not sampled in Aubriot et al. 2018) is strong but it seems now clear that these two species are not actually related to *S. macrocarpon* (very low support in Aubriot et al. 2016), but rather to *S. supinum* Dunal and *S. polhillii* (possibly also including *S. nigriviolaceum* Bitter but that species was not sampled in Aubriot et al. 2018). Further work with additional taxa and molecular markers will be necessary to recover the relationships of *S. macrocarpon* and its wild progenitor *S. dasyphyllum* within the Anguivi grade.

The scarlet eggplant *S. aethiopicum* L. is widely cultivated across Africa for leaves and fruit, with several fruit types recognised as distinct cultivar groups (Lester and Niakan 1986). *Solanum aethiopicum* has been taken up in cultivation much further afield than has *S. macrocarpon*; it is commonly cultivated in Asia and Brazil, where it is known as "gilo" and is usually eaten green (belying its English common name). The relationship between the African wild species *S. anguivi* and the scarlet eggplant is well established, but previous work considered *S. anguivi* to be closely related to morphologically similar species such as *S. violaceum* and *S. usambarense* (see Vorontsova and Knapp 2016). Phylogenetic analysis has shown, however, that despite morphological similarity, these taxa are not particularly closely related (Aubriot et al. 2016). *Solanum usambarense* is well supported as being a member of the Eggplant clade; *S. violaceum* and relatives (*S. deflexicarpum* C.Y. Wu & S.C.Huang of south-western China, and *S. hovei* Dunal and *S. multiflorum* Roth of southern India) are sister to the Eggplant clade (Aubriot et al. 2016; but see Aubriot et al. 2018 for an alternative position of *S. violaceum*). *Solanum anguivi* is a member of the poorly resolved Anguivi grade, along with similar *S. aldabrense* C.H.Wright (endemic to the Seychelles) and *S. platacanthum* Dunal of the Middle East (see Aubriot et al. 2016). It is clear that several dispersals from Africa to Asia have occurred in this group, and resolving relationships and biogeography of the Anguivi grade will greatly aid in identification of wild relatives for use in improvement of the scarlet eggplant.

2.6 Conclusions and Prospects for Future Understanding

Recent in-depth taxonomic work using thousands of herbarium specimens has clarified species identities and boundaries in Old World spiny solanums (Vorontsova and Knapp 2016;

X. Aubriot and S. Knapp, in prep.) and coupled with phylogenetic studies using a variety of molecular markers, eggplant relationships are now better resolved and robustly supported (Aubriot et al. 2018). Several areas for future taxonomic and phylogenetic study remain to be explored and better resolved in order to have a more complete understanding of diversity, biogeography and evolutionary history in the recently evolved and explosively radiating Old World spiny solanums.

1. Chromosome numbers are known for very few of these species; cytological investigations will greatly aid prioritisation of wild relatives for use in breeding programmes.
2. Genetic diversity across the range of widespread taxa such as *S. campylacanthum* remains to be investigated with population genetics tools (as has been done for *S. melongena* by Cericola et al. 2013; see also Chaps. 10–12).
3. Relationships of the highly diverse Australian species are poorly understood, both within the continent and to groups in Asia and Africa.
4. While the wild progenitors of *S. aethiopicum* and *S. macrocarpon* are well documented, the wider relationships of these cultivated taxa to members of the Anguivi grade are a priority; this will require new molecular markers and increased taxonomic and geographic sampling.
5. In-depth studies of morphological and molecular variation in landraces of the cultivated eggplant (*S. melongena*) and its wild progenitor (*S. insanum*) across their geographical ranges will certainly reveal pathways for domestication (e.g. Meyer et al. 2014, 2015) and new characters for crop improvement.

New genomic tools will certainly improve our ability to discover new molecular markers and ways of looking at relationships, but as ongoing taxonomic work has shown, improving taxon sampling both in terms of species and populations is equally important. The combination of the two will yield much fruit in the years to come.

Note Complete taxonomic descriptions for all species mentioned in this chapter can be found in the works cited, but also on the website Solanaceae Source (www.solanaceaesource.org).

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The Genetics of Eggplant Nutrition

3

Rachel S. Meyer, Damon P. Little, Bruce D. Whitaker and Amy Litt

Abstract

The modest genomic and genetic resources for eggplant and most fruit crops, compared to oil and starch model crops, have not tamed the interest of researchers that seek to discover and dissect the regulation of nutrition-related traits. The health-promoting, multifunctional, and sometimes toxic or antinutritional chemistry found across the supergenus *Solanum* is also present in eggplant. The international research community has explored many of the relationships among genetic variation and primary metabolites, secondary metabolites, yield, marketability, and culturally important traits, using varietal diversity and gene pools of cultivated and wild relatives. Results have opened imaginative and lucrative breeding opportunities. Eggplant is emerging as model system to demonstrate the possibility of improving health-beneficial qualities, while preserving marketable traits, through targeted introgression from related species. This chapter first describes the progress to date and illustrates the kinds of comparative questions the eggplant research community is poised to ask. Then, it presents a case study that uses a multispecies panel to identify candidate genes directing the synthesis of phenylpropanoids that offer numerous nutritional benefits.

3.1 Introduction

One aspect of plant nutrition is macro-nutrients—sugars, fiber, proteins, and fats—and their caloric value. Because macro-nutrients are essential for life, these are the nutritional metrics used for assessing how a given plant contributes to food security. Phytonutrients, also known as secondary metabolites, are the second, complementary, aspect. They have more nuanced roles in promoting health. Included with phytonutrients are the enzymes that regulate the bioavailability of nutrients. The third aspect is the situational context—economic, social, psychological, cultural, and political—that factors into nutrition access and security. The traits that relate to situational context are less well-defined, but are numerous. They include market traits such as those for sensory appeal, shelf life, and processability (e.g., canning or pickling; Hurtado

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et al. 2014) as well as agro-ecological traits such as the ability to yield fruit with limited irrigation or fertilizers—important for many of the world's subsistence farmers (FAO 1997).

Eggplant (*Solanum melongena* L.) is escalating in popularity due to its health-promoting effects (Cardoso et al. 2009). Worldwide, eggplants and their close relatives have myriad medicinal applications, with 77 unique medicinal attributes reported in Asia alone (Meyer et al. 2014). Eggplant is a 'functional food' because of both primary and secondary metabolites as well as mineral content (Caruso et al. 2017).

Genomic and genetic study of eggplant is aided by a rich written and illustrated record detailing changes in food quality that has enabled scholars to place genetic and functional diversity in an evolutionary and anthropogenic context. In China, 2000 years of historical documentation tracks increases in palatability (Wang et al. 2008). In India, there are complementary records depicting nutritional attributes: the Charaka and Sushruta Samhitas (written ~2100 years ago) describe the health-beneficial properties of eggplant. In the seventeenth century, Raghunātha described diverse fruit morphotypes with distinctive properties for satiety, weight gain, and weight loss (Raghunātha 1956 [reprint]). The historic documentation indicates that selection for nutritional qualities has always been a centerpiece of eggplant domestication. This allows researchers to use signatures of selection in the genome to pinpoint the genetic basis of nutritional trait variation and to illuminate the functional relationships among the different trait-controlling loci.

3.2 Solanaceae Biodiversity Resources: Translating Trait Variation to Nutritional Value

Understanding the genetics of plant nutrition requires the dissection of molecular evolution and function as well as the integration of results with human physiology and environment to address all three aspects of nutrition. In the realm of nutrition genetics, the scientific community is still largely at the stage of understanding simple traits and major pathways. However, eggplant presents an opportunity to drive the field forward with the availability of long scaffolds (Hirakawa et al. 2014) and a reference genome with pseudomolecules (www.eggplantgenome.org), genome-wide diversity sequencing (see Chap. 9), the many comparative genomics resources curated by the Sol Genomics Network (https://solgenomics.net/), and the international exchange of biological research collections (e.g., the germplasm bank of the Universitat Politècnica de València, Institut National de la Recherche Agronomique [Chap. 12]). Not only can these resources catalyze discoveries of genetic variation underlying traits, but they also can be used for integrated studies that translate phenotypes to nutritional value. Efforts to improve access to nutrition benefit from such improved metrics. On the flip side, a deeper, integrated understanding of the nutritional value of phenotypes can be used to better understand why nutritional traits have changed with varietal diversification over space and time.

3.2.1 Fruit Size, Antioxidants, and Shelf Life: An Entangled Relationship

Shifting fruit size preference is important for farmers and vegetable sellers to follow. Meeting demand may require changes in storage practices to minimize postharvest losses and maximize shelf life. Eggplants display a tremendous variation in fruit size, especially in Asia around primary and secondary centers of cultivation. Some popular varieties of *S. melongena* in South and Southeast Asia are small fruits often classified as *S. ovigerum*. They are larger than the wild progenitor *S. insanum* L. (see Knapp et al., 2013) and have significantly higher levels of hydroxycinnamic acid amides (HCAA; which have many health-beneficial properties) and 5-Z-caffeoylquinic acid (a chlorogenic acid; the dominant antioxidant in eggplant) than many other Asian varieties (Meyer et al. 2015). Studies have also shown a market preference for 'baby' eggplant fruit (Zaro et al. 2014). These early

development fruits also exhibit higher levels of chlorogenic acid and antioxidant compounds than fruit at later developmental stages (Zaro et al. 2014), but not necessarily HCAA.

Harvesting earlier stages means lower yield for farmers and also a shorter shelf life than more mature eggplants because respiratory rates are higher (Caruso et al. 2017). Zaro et al. determined that colder storage conditions were optimal for 'baby' eggplants. However, perpetually small eggplants, like *S. ovigerum,* may not have the same storage optima, because their cellular composition may be different: High HCAA levels cross-link cell walls and constrain cell and fruit size. Vendors may not distinguish between these two kinds of small-fruited eggplants, and researchers have yet to determine the optimal storage conditions for the perpetually small fruits. Further, fruit color may have a confounding effect: Anthocyanins are known to extend the shelf life of fruits, but Zaro et al. only tested purple fruits; thus, optimal storage conditions are unknown for small white, green, or striped fruits.

3.2.2 Exploiting the Genetic Basis of Traits to Understand Artificial Selection

The conservation of homologous gene function and similarities in synteny among eggplant and related species can be exploited to efficiently characterize genetic variation from SNPs to splice sites; and small indels to grand rearrangements. Genetics, documentation of varietal or species use, and systematic phenotyping (e.g., EGGNET; van der Weerden and Barendse 2007) of wild and domesticated relatives provide key clues to predict the phenotypic effect of genomic change. The genetic similarity among the several domesticated *Solanum* subgenus *Leptostemonum* species—*S. melongena,* gboma eggplant (*S. macrocarpon* L.), and scarlet eggplant (*S. aethiopicum* L.)—may prove useful for the discovery of candidate genes and alleles that explain their phenotypic differences. Some possible questions are: What makes leaves edible and non-toxic in the gboma eggplant? Because sugars accumulate in eggplant leaves when fruit are removed (Claussen and Lenz 1983), did artificial selection for palatable leaves lead to negative selection for fruit yield? What is the supremely bitter component in the scarlet eggplant flavor profile, and what aspect(s) of nutrition led people to maintain it over generations?

3.2.3 Introgression for Meeting Nutrition Needs

Interspecific hybridization is possible among eggplant and its relatives allowing for desirable traits in related species to be readily introgressed into the domesticated species (Kaushik et al. 2017). The Prohens lab (Valencia, Spain) argued for the value of such introgression (Plazas et al. 2013; Prohens et al. 2007, 2017) and has used relatives, such as *S. incanum* L., to increase chlorogenic acid levels. They have focused on increasing chlorogenic acid synthesis (Prohens et al. 2013) and on decreasing browning that degrades chlorogenic acid and other phenolic compounds (Gramazio et al. 2014; Kaushik et al. 2017). Chlorogenic acid has important anti-obesity (Cho et al. 2010), heart-protective, and DNA-protective functions (Wang et al. 2016). Another example is introgression from wilt resistant *S. aethiopicum* lines imparting tolerance in *S. melongena.* Remarkably, high-density genetic maps led to the discovery of an orthologous wilt resistance locus in *S. melongena* with exploitable allelic variation (Gramazio et al. 2018). Improved resistance through introgression can boost food security.

3.3 Phenotypic Similarity in *Solanum* Predicts Some Genetic Similarity

The Law of Homologous Series in Variation, first described by Nikolai Vavilov (1922), explains that genetic–phenotypic relationships are often increasingly predictable with phylogenetic closeness. This law of genetics has been

exploited for a long time, throughout the crop domestication process, and undoubtedly sometimes exploited unconsciously as humans explore and expand the phenotypic space of species (Milla et al. *in press*). The law has been the backdrop for artificial selection of nutritional qualities in eggplant over the course of domestication.

Domestication of Solanaceae fruit crops all led to larger fruits with different tastes and colors. Homology of genes/QTL, between eggplant and tomato (*S. lycopersicum* L.), has been leveraged to discover major loci controlling a range of nutrition-related traits, such as fruit color, anthocyanin intensity, and fruit size (Doganlar et al. 2002). One of the best-described tomato loci influences fruit weight (*fw2.2*, *fw2.1* in eggplant). It emerged as a major QTL in a wild-domesticated crossing experiment to control fruit size in eggplant; subsequently, it was found in bell pepper (*Capsicum annuum* L.) when markers revealed synteny with the tomato map (van der Knapp and Tanksley 2003). Later, in a much larger QTL study that identified 71 significant loci, 35% were assigned putative orthologs in tomato, potato (*S. tuberosum* L.), or pepper (Frary et al. 2014). The authors found some QTL for eggplant fruit skin anthocyanins had orthologs in other Solanaceae (De Jong et al. 2004), but that control of pigmentation was not functionally restricted to fruits: Sometimes it was in flower petals. While most eggplant QTL were unique, such as the three QTL for glossiness, or those for prickles (tomato and bell pepper do not make prickles), common patterns were reported between tomato and eggplant such as QTL hot spots for correlated traits.

Denser genome-mapping studies and genome-wide sequencing highlight the limitations of synteny for candidate gene discovery. Hirakawa et al. (2014) found synteny for 51 out of 68 tomato genes conferring disease resistance and fruit quality. The presence of hydroxycinnamic acid classes (quinic, ferulic; Wu et al. 2013) that are also present in eggplant mirrors why several candidate genes for polyphenolic content could be fine mapped using synteny with the tomato genome (*PAL*, *C4H*, *4CL*, *HCT*, *C3'H*, and *HQT*; Gramazio et al. 2014), but this still only accounts for half of the pathway genes, and one of the two *4CL* genes in eggplant could not be mapped. In a broad QTL analysis of morphological, nutritional, and antinutritional traits (Toppino et al. 2016), a major sugar content QTL was found to be orthologous between tomato (*FruE04*, Causse et al. 2004) and eggplant; however, many eggplant QTL in Toppino et al. had no corresponding QTL or candidate gene in other species.

3.3.1 From Species Comparison to Clade Comparisons

Mounting evidence suggests that secondary metabolites are under strong environmental selection and that they are less conserved across *Solanum* than regulators of primary metabolites like sugars. An illustration of this is polyphenolic oxidases (PPO) in *Solanum*. These are important for all aspects of nutrition: PPO induce unattractive browning that inhibit the marketability of fruit and have antinutritional function—inducing oxidative polymerization of flavonoids (Schijlen et al. 2004) and degradation of proteins. Gramazio et al (2014) identified five PPO genes that clustered together in the eggplant genome, suggesting recent duplication. Other *Solanum* crops have different copy numbers: naranjilla/lulo (*S. quitoense* Lam.), a domesticated crop classified like eggplant in *Solanum* subgenus *Leptostemonum* has eight copies (Arias et al. 2012). In *Solanum* subgenus *Solanum*, PPO genes are likewise clustered and present in different numbers: There are five PPO genes in potato and seven in tomato (Tran et al. 2012). The functional redundancy across PPO copies and species remains to be characterized, but it is impressive that enough genomic data exists to enable phylogenetic exploration of gene variation.

3.4 Case Study: Gene Expression Predicts the Phenylpropanoid Pathway

Meyer (2012) directly addressed the conservation of gene roles in the phenylpropanoid pathway in *Solanum*. The phenylpropanoid pathway regulates the synthesis of a multitude of specialized phenolic metabolites that play diverse ecophysiological roles in plants and contribute to the nutritional value of plant foods (Vogt 2010).

Compound profiles can distinguish among *Solanum* species, but few compounds are unique. Principal component analysis of 61 secondary metabolites places tomato (subgenus *Solanum*) in a cluster separate from all *Solanum* subgenus *Leptostemonum* species (Wu et al. 2013). These results suggest conservation of the phenolic pathway in *Solanum* subgenus *Leptostemonum* is strong. In addition, the three domesticated species in *Solanum* subgenus *Leptostemonum* appear to have undergone convergent selection for lower levels of health-beneficial phenolics (Meyer et al. 2015) effecting either taste or aesthetics. Using the Law of Homologous Series in Variation, one can predict similar genetic changes had occurred during the domestication of all three species. We hypothesize that gene function is sufficiently conserved within *Solanum* subgenus *Leptostemonum* such that correlations between gene expression and compound abundance would hold across the entire group.

3.4.1 Methods

Samples were selected for gene expression analysis from a large diversity panel that was previously characterized for genetic relatedness (Meyer et al. 2012), and phytochemistry (Wu et al. 2013; Meyer et al. 2015). Accession details as well as full compound names and structures are provided in those publications. The gene expression panel consisted of fifteen diverse *S. melongena* landraces and eight accessions of five related species in *Solanum* subgenus *Leptostemonum*. Plants were grown together in a greenhouse. Fruit set was low for some wild species. Two fruits per accession (1–2 plants) were collected, and the center 1 × 1 cm of the fruits was immediately excised, frozen with liquid nitrogen, ground to a fine powder, and split for phytochemical analysis and RNA extraction. Total RNA was extracted using the Ambion RNAqueous Kit (Ambion, Austin, TX, USA), and residual DNA was removed using the TURBO DNA-free™ Kit (Roche Diagnostics, Indianapolis, IN, USA). One microgram of total RNA template was reverse transcribed using the SuperScript III First-Strand Synthesis System (Invitrogen, Carlsbad, CA, USA) and random hexamer primers.

Primers for qRT-PCR were designed directly from eggplant ESTs from the Sol Genomics Network or from Sanger sequenced amplicons of larger genic regions (Table 3.1). Genes chosen were those posited to be involved in hydroxycinnamic acid ester and amide synthesis after the phenylalanine ammonia lyase step. *SmTIP41* was used as the endogenous control gene because previous experiments had shown it was the most stable across species (Expósito-Rodríguez et al. 2008; Meyer 2012). qRT-PCR reaction mixtures consisted of 12.5 μL FastStart Universal SYBR Green Master Mix (Roche Diagnostics, Indianapolis, IN, USA), 4 μL cDNA diluted to 3.75 ng/μL, 2.5 μL each primer (1 μM), and 3.5 μL sterile water for a total reaction volume of 25 μL. Amplification was done on an Applied Biosystems 7300 Real-Time PCR System, starting with a 2-min incubation at 50 °C followed by a 10-min incubation at 95 °C. This was followed by 40 two-step cycles, each cycle consisting of 95 °C for 5 s followed by 60 °C for 15 s. Dissociation reactions were performed to confirm that only one product was amplified. Three technical replicates were performed for each reaction. Relative quantification (RQ) of specific mRNA levels was analyzed using the cycle threshold Ct method ($2^{-\Delta\Delta Ct}$).

Table 3.1 Genes and qRT-PCR primers used in this study

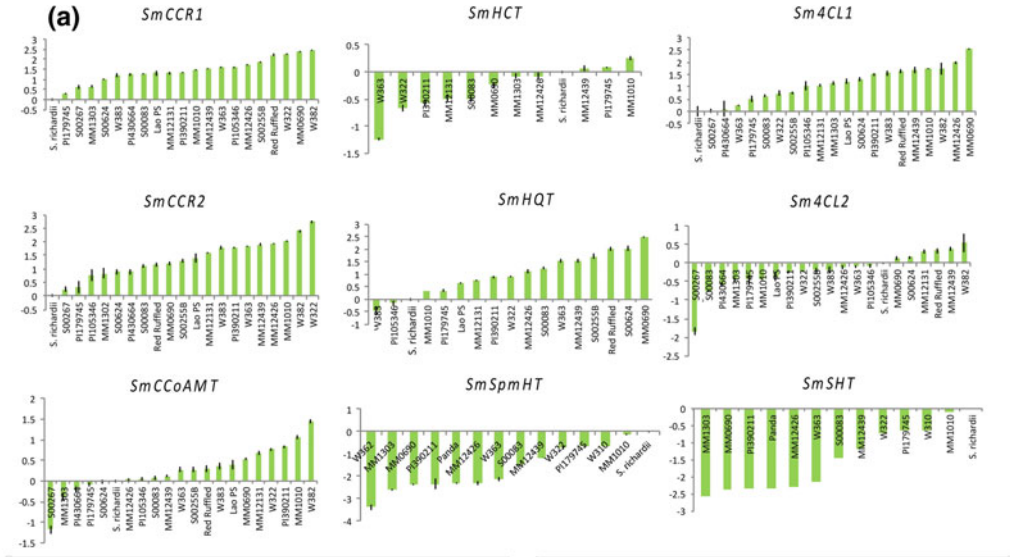
Gene	Primer	Sequence
SmCCR1	F	ACGTACGACGGGTTGTGTTC
	R	TCTCGGTTTGGGTCCATGTAC
SmCCR2	F	GCACCAACACTGGATTGATCA
	R	GATGAGTCAAGGGAAAAAGGG
Sm4CL1	F	AAGCATCGTGTGTCAGTTGC
	R	GCTTGCGGGACTCTTCTATG
Sm4CL2	F	GACGCAGTCCGAGCCAAAT
	R	CGGCTTCCGTCATTCCATAA
SmCCoAMT	F	CGGCGGCACAGGGTAAT
	R	GGCTGATCCAAGGATTGAGATT
SmSpmHT	F	CATTGAAACTTAGTGAGCTG
	R	CCTTATCAGAATTTGATCAA
SmSpdHT	F	AGTGTGTGTGATTTTTTCATTTTGGA
	R	GGGAGCTTTCTCATCCTTTCTTTATAT
HCT	F	CAACGGCTGTCGCAGGTGAT
	R	GTATGTGCACCACGAACCAATG
HQT	F	CTTGCGGCCCACATCTG
	R	CAATTGATCGTCTGGCAATCC
SmC3H	F	TTGGTGGCTACGACATTCCTAAGG
	R	GGTCTGAACTCCAATGGGTTATTCC
SmCAD	F	TGCAATGATGTCTACACTGAT
	R	ACATGATGTCCCATTGCCTTT
SmTIP41	F	TGCAGCAGAATCAGAGGGATATC
	R	ATGCTGGAGAGAAACCACATTTC

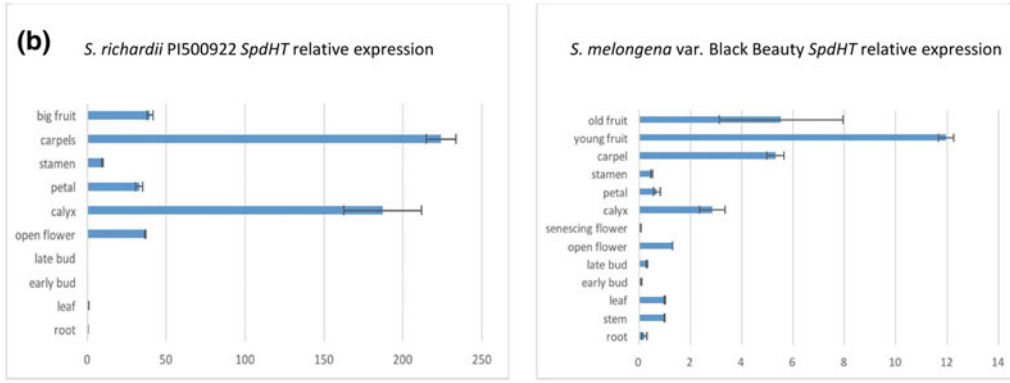
Gene abbreviations stand for CCR = cinnamoyl-CoA reductase, 4CL = 4-coumarate ligase, CCoAMT = caffeoyl-CoA 3-O-methyltransferase, SpmHT = spermine hydroxycinnamoyl transferase, SpdHT = spermidine hydroxycinnamoyl transferase, HCT = hydroxycinnamoyl transferase, HQT = hydroxycinnamoyl quinate transferase, C3H (syn C3'H) = cinnamoyl 3-hydrolase, CAD = cinnamyl alcohol dehydrogenase. *SmSpdHT* is synonymous to *SHT3b* in Meyer (2012)

RQ expression values were correlated to the abundance of phenolic constituents using Pearson's *r* correlation matrix and $\log_{10}$ RQ values.

3.4.2 Results and Discussion

Even though work included uncharacterized genes, correlation trends were largely congruent with the function of putative orthologs in other plant species. Two genes involved were identified to belong to the BAHD family of acyltransferases (*SmSpmHT* and *SmSpdHT*). BAHD proteins are known in Arabidopsis to synthesize HCAA (Handrick et al. 2010). *SmSpmHT* was hardly detectable in *S. melongena,* and often only one technical replicate produced a reading (hence no error bars in Fig. 3.1a for this gene). It was highly expressed in the eggplant relative *S. richardii*. The HCAA kukoamine A was unique to *S. richardii* in HPLC profiles; hence, correlation of 1 to *SmSpmHT*. *SmSpdHT* was expressed in all species and strongly correlated to other HCAAs that varied in abundance but not presence/absence. qRT-PCR of *SmSpdHT* in different organs showed broad expression in





(c)

Gene	3_Caff-Put	7_Fer-put	9_Caff/dhcaff-Spd-1	10_Bis-dhcaff-Spd	11_Caff/dhcaff-Spd-2	12_Bis-caff-spd	14_3-CQA	15_5-CQA	16_4-CQA	17_52-CQA	18_3,5-diCQA
Sm4CL1	-0.1064	-0.0397	-0.0284	0.0250	-0.0438	-0.0410	-0.1735	-0.3334	-0.1098	-0.0916	0.3519
Sm4CL2	-0.2885	-0.2765	-0.2281	-0.0603	-0.2277	-0.0831	-0.2322	-0.1189	0.6107	-0.1993	0.2700
SmCAD	-0.1882	-0.1357	-0.0834	0.1153	-0.1492	-0.0917	-0.1520	0.0819	-0.2191	0.0069	-0.4199
SmCCoAMT	0.8338	0.9142	0.8706	-0.2529	0.8305	0.9174	-0.1200	0.6886	0.3003	0.5596	0.6701
SmCCR1	-0.2235	-0.1268	-0.1588	-0.0977	-0.1966	-0.1364	-0.1830	-0.2903	-0.2224	-0.2226	0.2217
SmCCR2	0.4035	0.4822	0.6024	0.2949	0.5711	0.5998	-0.2520	0.4081	0.3592	0.5284	0.4412
SmHCT	0.8299	0.7784	0.8420	-0.0151	0.8833	0.8586	0.7784	0.7412	0.6055	0.6133	0.5205
SmHQT	-0.2627	-0.1972	-0.2342	-0.1804	-0.2636	-0.1937	-0.1302	-0.4098	0.0565	-0.2519	0.2361
SmSpdHT	0.9371	0.9576	0.8865	-0.3309	0.8770	0.9373	0.9576	0.8669	0.5616	0.4972	0.5878

Gene	19_4,5-diCQA	20_3,4-diCQA	21_3-M-5-CQA	22_4-C-5-MQA	23_5-p-CoQA	24_3-FQA	25_5-FQA	26_FQA-hexo	27_3,5-diFQA	32_Tryptophan	Total
Sm4CL1	0.9589	0.9589	-0.1735	-0.2857	0.0634	0.9589	-0.1828	-0.1467	-0.1272	-0.2845	-0.2418
Sm4CL2	0.2829	0.2829	0.0401	-0.4216	-0.1071	0.2829	0.2108	-0.3092	0.0061	0.1578	-0.1312
SmCAD	-0.1564	-0.1564	-0.2458	-0.0895	-0.2975	-0.1564	-0.2194	-0.1016	-0.1564	-0.0750	-0.0166
SmCCoAMT	0.1175	0.1175	0.4217	0.4489	0.2304	0.1175	-0.2159	-0.1161	-0.1254	-0.2774	0.8191
SmCCR1	0.9445	0.9445	-0.2274	-0.3157	-0.0232	0.9445	-0.1480	-0.1384	-0.0239	-0.2181	-0.2719
SmCCR2	-0.1846	-0.1846	0.4420	0.0754	-0.0066	-0.1846	-0.1239	-0.2182	-0.2737	0.1101	0.5252
SmHCT	-0.1678	-0.1678	0.3777	0.2836	-0.0477	-0.1678	-0.1480	-0.3657	NA	-0.5315	0.8130
SmHQT	0.9338	0.9338	-0.3095	-0.2919	0.0773	0.9338	-0.1717	-0.0941	-0.1532	-0.1067	-0.3646
SmSpdHT	-0.1787	-0.1787	0.5308	0.5748	0.1088	-0.1787	-0.1706	-0.1347	NA	-0.3598	0.9415

Fig. 3.1 **a**. Average Log_{10} relative quantification expression shown as the mean of two fruits. Error bars are the standard error of technical replicates. Values are normalized to *S. richardii.* The chart in blue is the same gene as in panel B. **b**. Expression of *SpdHT*. **c**. Pearson correlation between HPLC abundance data and gene expression. Compound abbreviations are defined in Meyer et al. 2015

S. richardii and *S. melongena* (Fig. 3.1b) with highest expression in floral organs and fruits. We expanded this work by forming collaboration with the USDA to functionally characterize both BAHD proteins: This corroborated that, despite the lack of qRT-PCR replicates, the associations were correct (Peng et al. 2016; Peng et al. *in review*). A synthesis of these results demonstrates HCAA abundance is limited by the expression levels of these BAHD genes and availability of specific polyamines as precursors.

HCT and HQT are similar enzymes, but their substrate preferences vary among species (Clé et al. 2008; Kim et al. 2012; Niggeweg et al. 2004). Correlation results suggest that *SmHCT* is implicated in mono-caffeoylquinic acid (CQA) synthesis (3-CQA, 4-CQA, 5Z-CQA). *SmHQT* was only implicated in diCQA synthesis. This suggests that phenylpropanoid synthesis may be regulated differently between eggplant and tomato at this step, because tomato lacks the HCT enzyme (Clé et al. 2008), and its HQT enzyme has similar function to the eggplant HCT function indicated by correlations (Fig. 3.1c). However, the CCR gene expression patterns showed correlation in accordance with their reported roles in tomato: *CCR1* shunts phenolic compounds to the lignin synthesis pathway (van der Rest et al. 2006; Escamilla-Treviño et al. 2010), whereas *CCR2* of tomato is associated with signaling pathways that broadly upregulate phenolic production, in response to factors such as stress or infection (Onkokesung et al. 2012; Prashant et al. 2011). Other gene expression patterns were in agreement with enzyme functions in Arabidopsis and other distantly related species (see Meyer 2012 for details).

In summary, this case study demonstrates that panels of accessions from within and across species can be used together to determine candidate gene function that sets up hypotheses for protein functional characterization. The variation in traits across species at the level of genus, subgenus, and section, all have value to catalyze our understanding of the myriad traits that contribute to human nutrition.

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Molecular Mapping and Synteny

4

Mark A. Chapman

Abstract

Linkage and quantitative trait locus (QTL) mapping allow regions of the genome conferring adaptive traits to be identified; this is often an early step in identifying the genetic basis of said trait. In addition, comparative mapping, i.e. using orthologous genetic markers to create linkage and QTL maps, allows genome structure (translocations, inversions and so on) to be identified as well as to provide insight into the extent to which the same genes may confer the same trait in different crops. Extensive comparative mapping in the Solanaceae (especially eggplant, tomato, potato and pepper) has revealed how genome organisation takes place during species evolution and suggests that up to 40% of agronomically important traits in eggplant may be controlled by orthologous genes in tomato, potato and/or pepper.

4.1 Introduction

Genetic mapping describes the process by which molecular markers are genotyped in a mapping population (an F2, backcross or population of recombinant inbred lines; RILs) to create a representation of the genome. Markers which are physically close to each other in the genome (as well as those in linkage disequilibrium, e.g. in regions of low recombination) are linked in the map (forming a linkage group; LG), and those unlinked in the genome are found on different LGs. Beyond simply creating a linkage map, quantitative traits can be phenotyped in the mapping population and loci controlling a portion of the variation added to the map (forming quantitative trait loci; QTL) using the statistical association between markers and phenotypes (Mauricio 2001). Identifying regions of a species' genome that confer traits of interest can assist breeders who wish to introgress adaptive alleles from one variety or species into another. The potential for this is clear, and as an example, continued backcrossing and use of linkage information was used to introgress submergence tolerance into an elite widely grown rice cultivar (Xu et al. 2006).

Knowing the map locations of loci conferring desired traits (i.e. QTL) can expedite breeding because one can identify molecular markers linked to the QTL and genotype a mapping/breeding population, discarding undesirable genotypic combinations without having to wait until the phenotype is expressed. Even rare combinations of markers (and therefore presumed phenotypes) can be identified if the mapping population is large enough. This is termed marker-assisted selection (MAS) and, with the continued reduction in the cost of

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modern sequencing and genotyping approaches (Davey et al. 2011), will likely be employed more extensively (Collard and Mackill 2008).

Comparative mapping concerns comparing genetic maps across species and analysing similarities and differences. This usually requires having orthologous markers genotyped in the different species allowing equivalent regions of the genome to be identified. From an applied point of view, if genomes exhibit extensive synteny, then data concerning the genetic basis of an adaptive trait in one species provide a good starting point to understand this trait in the second species. Further, quality and marker density of linkage maps for related crops may well vary, although for major crops there are often extensive genetic mapping resources. Comparisons between species can therefore sometimes be used to infer genetic map locations in a species with a less extensive genetic map, or to narrow down the region of the genome containing the QTL or to identify candidate genes from a related species' genome (Bajaj et al. 2015; Hiremath et al. 2012; Odonkor et al. 2018).

The information from comparative mapping also provides data on how genomes evolve during species divergence, including characterising the numbers of translocations and inversions and the rate of the occurrence of these over time. In one of the most well-known examples, extensive synteny among cereal crops (including rice, maize, sorghum, sugarcane, millet and wheat) has been reported. Despite 50 M years of independent evolution, the conservation of molecular markers is striking (Moore et al. 1995). Indeed, for an in-depth study of rice chromosome 3, marker collinearity of each chromosome arm to the equivalent maize chromosomes was almost identical (The Rice Chromosome 3 Sequencing Consortium 2005).

4.2 Genome-scale Macrosynteny in the Solanaceae

The first linkage map of eggplant was generated in the early 2000s and used an interspecific cross between the wild species *S. linnaeanum* Jaegaer & Hepper and cultivated eggplant *S. melongena* L. (Doganlar et al. 2002a). The markers used in the generation of this linkage map had been previously used to create linkage maps of tomato and potato; hence, comparative mapping was used to compare genome collinearity across these three species. In this work, Doganlar et al. (2002a) suggested a minimum of 23 inversions and five to seven translocations differentiate the genomes of eggplant and tomato.

The same eggplant linkage map was updated with the addition of further markers (Wu et al. 2009), and these same markers were mapped in multiple species from the Solanaceae (tomato, potato, pepper and tobacco; Wu and Tanksley 2010). This more comprehensive analysis allowed a more detailed assessment of genome collinearity between not only eggplant, tomato and potato, but also included pepper (*Capsicum*) and tobacco (*Nicotiana*). Since the divergence of the eggplant lineage from the tomato lineage, there have been 24 inversions and five translocations (Wu et al. 2009; Fig. 4.1). Tomato and potato are differentiated by a further six inversions, and using the eggplant map to polarise these, it can be inferred that four inversions occurred along the tomato lineage and two on the potato lineage (Wu and Tanksley 2010).

In a more recent update of the Doganlar et al. (2002a) map, ca. 600 more genetic markers were added (Doganlar et al. 2014), providing much more comprehensive information concerning genome evolution in eggplant. Several more translocations (19 instead of five) between eggplant and tomato were identified, aided by this higher resolution genetic map. This study revealed 33 conserved syntenic segments (CSSs), i.e. large regions of shared markers in the same orientation.

Following on from the comparative mapping efforts detailed above, the publication of the draft eggplant genome in 2014 (Hirakawa et al. 2014; see also Chap. 6) allowed further examination of synteny. The draft genome is highly fragmented relative to published genomes of tomato and potato, yet mapping these contigs to the tomato genome allowed for an extensive analysis of synteny. Unsurprisingly with the availability of

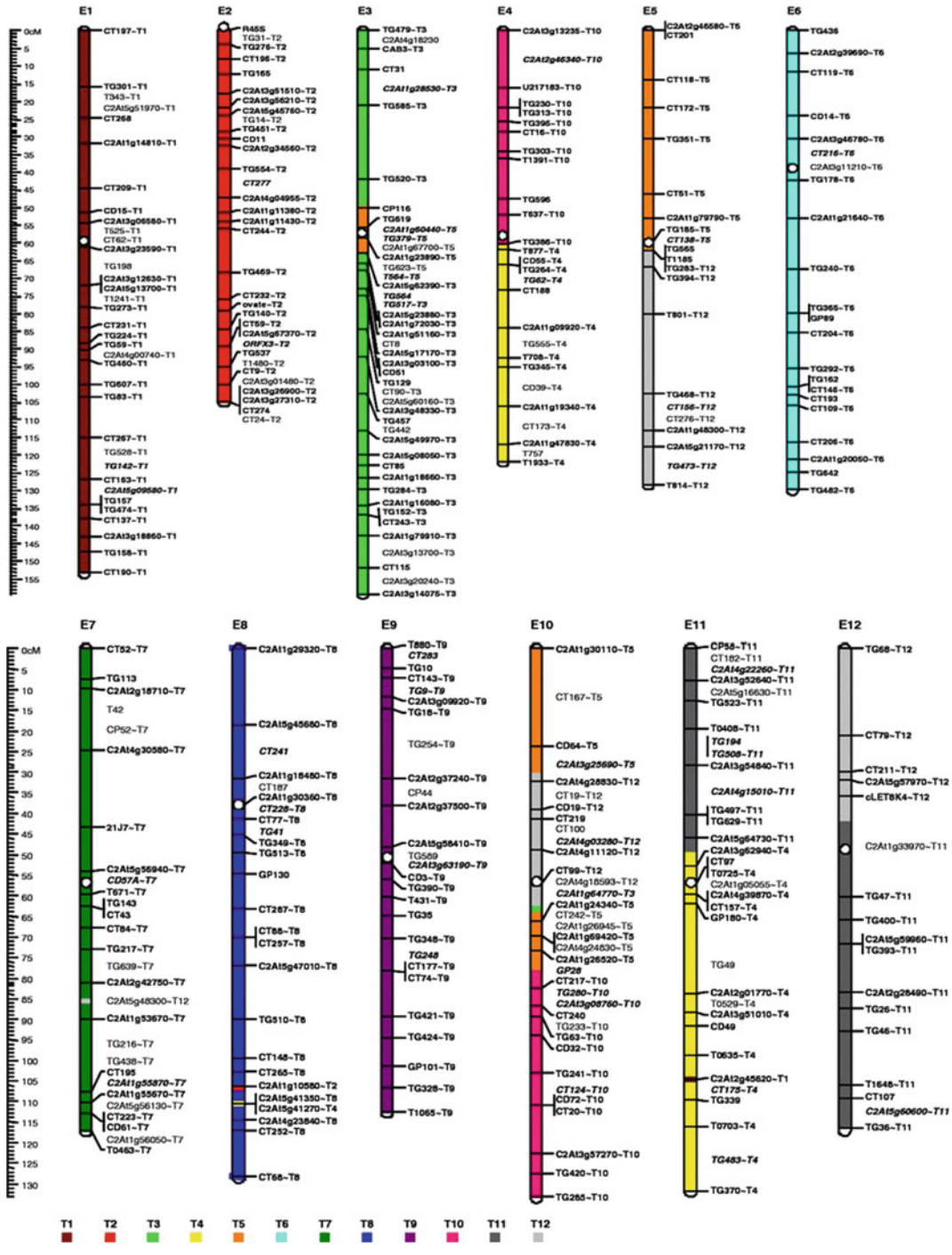

Fig. 4.1 The genetic map of eggplant. Eggplant linkage groups are designated as E1-12. Markers in bold and by tick marks are framework markers (LOD > 3); markers in bold and italic are interval markers with $2 \leq \text{LOD} < 3$; others are interval markers with LOD < 2; cosegregating markers are denoted by a vertical bar. "−Tx" following the name of a marker indicates its chromosome location on the tomato map. Each tomato chromosome is assigned a different colour (see colour codes), and the corresponding eggplant chromosome segment(s) are painted with the same colour. Putative centromere position of each eggplant linkage group is based on eggplant–tomato synteny and indicated by a white dot. Reproduced from Wu et al. (2009) with permission from Springer Nature

thousands of loci to test for synteny, additional inversions and translocations were identified on top of confirming those identified in previous studies, breaking up the previously identified CSSs into a larger number (56) of conserved segments. Further refinement of the eggplant genome (see later chapters) will allow a more rigorous test of synteny and genome evolution (Frary et al. 2016).

4.3 Microsynteny in the Solanaceae

At the sub-chromosome scale, genetic markers and loci can show extensive synteny among species, with this breaking down over evolutionary time. This microsynteny can be especially useful for comparative analysis of traits across species, especially if genomic resources are more comprehensive in one species than another. In the Solanaceae, there are several large stretches of apparent synteny based on linkage mapping (see above), but at the microsyntenic scale there has been relatively little work.

An early investigation of microsynteny used the sequence of a 105-kb gene-rich region of the tomato genome containing an agronomically important gene (*ovate*; which plays a role in fruit shape) and compared the orthologous region across other members of the family (Wang et al. 2008). Across all species, microsynteny was extensive, with only a single gene deletion (shared by tomato and pepper), two duplications (one in pepper, one in petunia) and one inversion (in petunia). This type of analysis also allows inference of evolutionary rates. For example, the species analysed by Wang et al. (2008) represent ca. 30 M years of plant evolution, with an overall d_N/d_S ratio (a measure of selection based on coding sequence polymorphisms) of 0.207, very similar to that more recently obtained for 11,751 genes across six tomato species (ca. 0.225; Koenig et al. 2013).

Little additional work has been carried out in a similar manner, but with the development of the eggplant genome (see later chapters), analyses of microsynteny will likely be carried out. It is noteworthy that whilst genetic mapping efforts show large regions of conserved marker order between Solanaceous species, individual markers are sometimes found on different linkage groups, e.g. when comparing eggplant and tomato (Barchi et al. 2012; Wu et al. 2009). This suggests that individual loci or small genomic regions have translocated, disrupting microsynteny, during the evolution of species in the Solanaceae, possibly through the movement of transposable elements (Barchi et al. 2012).

4.4 Comparative Mapping Between Eggplant and Other Members of the Solanaceae

For crops with a high level of synteny, there is great promise for using genes and loci identified in one and transferring the knowledge, expediting gene discovery and breeding, to another. This cross-crop knowledge transfer has the ability to fast-track the understanding of the genetic basis of an adaptive trait in a species with reduced genetic resources. Evidence of extensive synteny in the grasses based on genetic mapping, for example, suggested that orthologous loci may confer certain adaptive traits, including flowering time, seed size and seed dispersal in multiple crops (Paterson et al. 1995). With the availability of genome sequences and modern high-throughput technologies, it has been shown that at least a subset of potentially orthologous QTL identified by comparative mapping is indeed underscored by the same loci. In the grasses, for example, the identification of a YABBY transcription factor underlying the loss of shattering during the domestication of sorghum lead to the finding that the same locus controls, at least in part, the loss of shattering in rice and maize (Lin et al. 2012).

As mentioned in Chap. 1, eggplant has been understudied relative to its congeners tomato and potato, with genome sequences of the latter two published over five years ago (Sato et al. 2012; Xu et al. 2011). Eggplant, therefore, is a good example of how resources from more well-studied crops (i.e. tomato) can be transferred into others. Given the extensive synteny

between members of the Solanaceae (above), it is possible that, should QTL for orthologous traits in eggplant and other Solanaceae maps to the same genomic region, the genetic basis may also be the same.

Several species of the Solanaceae have been domesticated, and a number of parallels exist between these in the traits that were under selection. Most notably, parallel selection for increased fruit size and fruit colour in tomato, eggplant and pepper. The first comparative maps for the Solanaceae focussed on tomato, potato and pepper (Livingstone et al. 1999; Tanksley et al. 1992), with the first genetic map for eggplant created by Doganlar et al. (2002a). This set the stage for a companion paper which investigated the genetic basis of domestication traits in eggplant (i.e. QTL), focussing on comparisons to the tomato, potato and pepper linkage maps (Doganlar et al. 2002b). This comparative QTL mapping revealed that 40% of eggplant QTLs for domestication traits (mainly fruit weight, shape and colour QTL) have counterparts (based on overlapping QTL positions) in other Solanaceae (Doganlar et al. 2002b). As an example, fruit weight QTLs on eggplant linkage groups (LGs) 2, 9 and 11 all have counterparts from tomato, and overlapping leaf, flower and fruit anthocyanin QTL on eggplant LG 10 have equivalent loci in tomato and potato (Doganlar et al. 2002b, and references therein). As asserted by the authors (Doganlar et al. 2002b), this could mean that selection during domestication acted on orthologous loci shared across these crops. More recent genetic maps with greater marker density also confirm that several QTLs appear to be shared between eggplant and tomato (Portis et al. 2014).

With the publication of the tomato genome, the potential for orthologous loci to control the same trait could be tested, and recent genetic mapping of domestication and agronomic traits in eggplant has used the tomato genome to identify candidate genes underlying eggplant traits. Barchi et al. (2012) mapped loci controlling leaf, fruit and flower anthocyanin accumulation in eggplant and then identified genes known to be involved in anthocyanin accumulation in a variety of species in the tomato genome. Identification of marker positions from the eggplant map on the tomato genome allowed the authors to determine if the eggplant QTLs overlapped any genes known to control anthocyanin accumulation. Tomato chalcone synthase and *ant1*, as well as an orthologue of the petunia gene *an2,* are all located close to a region of the eggplant linkage map containing QTL for multiple anthocyanin-related traits (stem, calyx, leaf and fruit peduncle anthocyanin; Barchi et al. 2012). This analysis suggests that orthologous genes could confer similar traits across domesticated Solanaceae species.

In follow-up work, the same group identified the positions of yield-related QTL from the eggplant genetic map in the tomato genome (Portis et al. 2014). Instead of simply relying on candidate genes identified previously, as was done in the anthocyanin work (Barchi et al. 2012), the authors mined the tomato genome regions for genes with putative functions that could relate to the eggplant phenotypes. Although this type of analysis will only identify candidate genes, and follow-up work is required to confirm gene function, it is a useful starting point, especially because the eggplant genome is not yet published. The authors identified a member of the ferredoxin gene family which mapped to the same region of the genome as a fruit stripe QTL, as well as several genes involved in the cellulose, lignin and suberin production pathways which mapped to regions of the genome containing QTL for leaf and fruit prickles (Portis et al. 2014).

In the same study, the overlap of several eggplant fruit-shaped QTLs with tomato QTLs was found (Portis et al. 2014). A particularly promising finding was that on eggplant LG 7, QTL for fruit length and shape overlap with the tomato genome region containing the *sun* locus. The tomato *sun* locus has been cloned and is partly responsible for fruit shape differences among varieties (Xiao et al. 2008). This, along with the overlap with anthocyanin-related genes above, provides further evidence that at least some loci in tomato and eggplant are true orthologues.

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Molecular Mapping, QTL Identification, and GWA Analysis

5

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Abstract

Both inter- and intraspecific maps have been developed in eggplant. The former benefit from an enhanced frequency of marker polymorphism, but their relevance to marker-assisted crop breeding is limited. The first maps developed could be defined as 'first generation,' built up by means of pre-NGS (next-generation sequencing) molecular biology techniques (AFLP, RAPD, SSR, etc.). Unfortunately, the reduced polymorphism detected in intraspecific mapping populations in the 'first-generation' maps, along with the relatively low commercial importance in the 'seed market' of the species, hampered the construction of dense eggplant genetic maps. Recently, thanks to NGS-derived molecular markers, new marker-rich maps ('second-generation maps') were constructed. To assist selection in breeding programs, in particular to identify QTLs underlying key agronomic traits, biparental approaches as well as genome-wide association (GWA) mapping studies were conducted in this species, using the available linkage maps. Among the traits studied, great importance was given to the identification of QTLs linked to morphological and biological traits, including leaf, flower, plant, and fruit characteristics, as well as QTLs associated with parthenocarpy and to resistances to fungal (*Fusarium oxysporum* f. sp. *melongenae* and *Verticillium dahliae*) and bacterial (*Ralstonia solanacearum*) wilts. QTL studies to elucidate the genetic basis of biochemical composition, content in bioactive and antinutritional compounds, as well as other fruit quality traits were also carried out.

5.1 Linkage Map Construction

Genetic linkage maps are key tools routinely used in plant genetics and breeding to carry out genome analysis as well as to identify genomic regions associated with agronomic and qualitative traits by means of quantitative trait loci (QTL) mapping. The construction of linkage maps in eggplant can be divided into two main groups: the ones constructed by means of pre-NGS (next-generation sequencing) molecular biology techniques (AFLP, RAPD, SSR, etc.), later referred to as 'first-generation maps,' and the ones constructed by means of NGS-derived molecular markers ('second-generation maps'). In that respect, genetic maps for *Solanum melongena* (eggplant) were constructed using plant populations from both intra- and inter-specific hybridizations.

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5.1.1 First-Generation Maps

Among the interspecific linkage maps, the earliest constructed was based on the RFLP genotyping of 58 F_2 individuals bred from the interspecific cross *S. linneanum* × *S. melongena* (Doganlar et al. 2002a). This map contains 233 markers distributed over 12 linkage groups, spanning 1480 cM, and was used for comparative analysis between eggplant and tomato. Subsequently, the previously mentioned map was improved by Wu et al. (2009). In particular, a total of 110 COSII markers (Conserved Ortholog Set; Wu et al. 2006), previously mapped in the tomato genome, and five tomato-derived markers were selected primarily in the regions with identified chromosome rearrangements between the genomes of eggplant and tomato. The obtained genetic map contains 347 markers, assigned to the 12 chromosomes in the haploid chromosome set of eggplant; it spans 1535 cM, with a framework marker density of about 6 cM, and the size of the chromosomes ranging from 105 to 159 cM. According to synteny between the tomato and eggplant genomes, the locations of additional 522 COSII markers on the eggplant linkage map were deduced, bringing the total number of RFLP and COSII markers of known position in this interspecific eggplant population to 869. The improvement of the interspecific map previously established has been reported by Doganlar et al. (2014), essentially achieved by increasing the number of individuals used (from 58 to 108 F_2 individuals), as well as by adding AFLP and 117 previously unmapped RFLP and COSII markers. Overall, the newly developed map is constituted of 400 AFLPs, 348 RFLPs, and 116 COSII for a total of 864 markers, spanning 1518 cM, with the size of the chromosomes ranging from 93 to 152 cM.

Gramazio et al. (2014) developed a new interspecific (*S. melongena* × *S. incanum*) linkage map based on a first backcross (BC1) generation (91 plants) toward the cultivated accession of eggplant *AN-S-26*, as a tool for introgressing *S. incanum* alleles involved in the biosynthesis of chlorogenic acid in the genetic background of *S. melongena*. The mapping population was genotyped with 243 molecular markers comprising 42 COSII, 99 SSRs, 88 AFLPs, nine CAPS, four SNPs and the morphological marker PRICKLINESS. The linkage map covers 1085 cM, with linkage groups length comprised between 58.6 and 132.9 cM, number of markers for each LG comprised between 16 and 27, and an average marker density of 4.46 cM.

The first true intraspecific map of eggplant was built up in 2001 by Nunome et al. (2001) by using 168 F_2 individuals. A combination of 88 random amplified polymorphic DNA (RAPD) and 93 amplified fragment length polymorphism (AFLP) markers allowed identification of 21 LGs with a length comprised between 27 and 95.6 cM and covering a total of 779.2 cM. The same map was improved in 2003 (Nunome et al. 2003a) with the addition of seven SSR markers, resulting in 17 LGs, for a total of 162 markers (some previous markers were excluded) and spanning 716.9 cM. Later, Nunome et al. (2009) used a population of 94 F_2 individuals to improve the already available map, by using 214 newly identified genomic and seven EST SSRs, together with 15 published SSRs (for a total of 236 markers). A new map was so established, covering 959 cM in 14 LGs, which ranged in size from 11 to 120 cM, including between 2 and 37 markers. Furthermore, distances between markers varied from 0 to 32.6 cM, with an average value of 4.3 cM.

Barchi et al. (2010) developed two intraspecific mapping populations [a doubled haploid (DH) and an F_2] from the cross between the breeding lines '305E40' and '67/3'. Both the populations were screened with 170 AFLP markers to check their suitability for linkage analysis. As an extensive segregation distortion was observed in the DH population, only the F_2 population was, later on, used for mapping purposes. A total of 141 F_2 individuals were genotyped using 406 AFLP informative fragments, together with 22 SSRs, 1 RFLP, and 3 CAPS markers linked to the *Rfosa1* locus conferring resistance against *Fusarium oxysporum* f.sp. *melongenae* (Toppino et al. 2008). The framework map consists of

238 markers (212 AFLPs, 22 SSRs, 1 RFLP, and 3 CAPS), spanning 718.7 cM, an LG length comprised between 27.3 and 82.2 cM, with a mean intermarker distance of 3 cM.

Subsequently, a great improvement in eggplant intraspecific map was performed by Fukuoka et al. (2012). Two intraspecific F_2 mapping populations, LWF2 (n = 90) and ALF2 (n = 93), were used for constructing two separated linkage maps, which were subsequently combined into one by means of common markers in each linkage group. A total of 952 DNA markers, including 623 single-nucleotide polymorphisms (SNPs) and Insertion/Deletion polymorphisms (InDels) found in eggplant-expressed sequence tags (ESTs) and related genomic sequences [introns and untranslated regions (UTRs)], and 313 genomic SSR markers previously developed were used. The final integrated map covers 1285.5 cM in 12 LGs, with an average interval between markers of 1.4 cM. The map was used to carry out macrosyntenic relationships between eggplant and tomato, as well as for QTL analysis of parthenocarpy in eggplant (Miyatake et al. 2012).

5.1.2 Second-Generation Maps

In the era of next-generation sequencing (NGS), it is possible to identify and exploit hundreds of polymorphic molecular markers well distributed all over the genome in a single experiment, also for species missing genomic information. In particular, SNPs markers are developed based on the so-called genotyping-by-sequencing (GBS) approach, which included reduced representation sequencing (RRS) as well as whole genome resequencing (WGR) techniques. Among them, the restriction site-associated DNA sequencing (RAD-seq) was first set up by Baird et al. (2008). Briefly, genomic DNA is digested with a single restriction enzyme and, to proceed with multiplexing (the sequencing of several samples on a single lane), each digested fragment is linked to a barcoded adapter. After sonication, an additional adapter is attached to the free end of each fragment. In the last step, the library is selected based on the size, and the fragments with both linked adapters are subjected to PCR amplification.

Barchi et al. (2011) applied the protocol from Baird et al. (2008) to the genomic DNA of the F2 segregating population obtained by crossing '305E40' and '67/3', used as female and male mapping parents, respectively. The resulting non-redundant genomic sequence dataset allowed the discovery of ~10,000 SNPs and nearly 1000 InDels. Furthermore, more than 2000 of the SNPs were found to be potentially genotyped using Illumina GoldenGate© assay. Subsequently, Barchi et al. (2012) developed a new intraspecific map by using 156 F_2 plants from the cross between '305E40' × '67/3,' which comprises 415 markers assigned to the 12 chromosomes (Fig. 5.1). The map is mainly composed of SNPs [339 markers from the set previously identified (Barchi et al. 2011)] and genotyped via Illumina GoldenGate©. Furthermore, two SNPs identified within sequences that were differentially expressed following inoculation with the fungal pathogen *Fusarium oxysporum* f.sp. *melongenae* (Barbierato et al. 2016) were genotyped via high-resolution melting (Wittwer et al. 2003, HRM technique). In addition, 33 SSRs already available (Nunome et al. 2003a; b; Frary et al. 2005; Stàgel et al. 2008; Vilanova et al. 2012) were mapped to the 12 chromosomes, together with three CAPS (Toppino et al. 2008), 11 RFLPs (Bernatzky and Tanksley 1986; Doganlar et al. 2002a), and 27 COSII markers (Wu et al. 2006). The overall length of the map was 1390 cM, with individual chromosomes ranging in length between 80.2 and 136.5 cM. The genome-wide mean interlocus separation (discounting completely co-segregating ones) was 3.8 cM.

A new intraspecific map was developed by Hirakawa et al. (2014) for the anchoring of the draft genome sequence of the species and includes both SNPs and SSRs markers. Firstly, a custom oligonucleotide sequence capture array (Roche Applied Science, Mannheim, Germany) was designed based on more than 43 k EST assembly. The 'probable' SNPs were located in the reference tomato genome SL2.40, and marker

Fig. 5.1 Eggplant intraspecific linkage map developed by Barchi et al. (2012) and graphical representation of QTL locations (Barchi et al. 2012, Portis et al. 2014) in comparison to the genomic regions identified by GWA approach (Cericola et al. 2014; Portis et al. 2015). Marker names are shown to the right of each chromosome, with map distances (in cM) shown on the left. Map positions of the QTLs identified are shown to the right of each chromosome, while GWA outcome is given to the left of each chromosome (the vertical bars represent a ± 3.4 cM interval around the position of the associated SNP loci)

candidates evenly distributed over the eggplant genome were selected according to eggplant–tomato macrosynteny. The GoldenGate array (Illumina) was then used for genotyping a subset of these SNPs. SSRs located in probable euchromatic regions in the eggplant genome, identified following genome sequencing, were selected and used for mapping purpose. Then two linkage maps based on two independent interspecific F_2 mapping populations were constructed using 574 SNPs and 221 SSRs, and a new integrated map was established, incorporating data also from the previously map published by Fukuoka et al. (2010). This integrated map covers 1280.6 cM for a total of 1745 loci, with an average interval between markers of 0.73 cM and a maximum gap of 9.6 cM.

More recently, Salgon et al. (2017) developed a high-density intraspecific genetic map using a population of 180 RILs, derived from the cross between *S. melongena* MM738 (susceptible to bacterial wilt) and *S. melongena* AG91-25 (resistant to bacterial wilt) using single-nucleotide polymorphisms (SNPs) developed from genotyping-by-sequencing together with 168 molecular markers (AFLPs, SSRs, and SRAPs) previously developed (Lebeau et al. 2013). The genetic map includes 867 SNPs, 139 AFLPs, 28 SSRs, and 1 SRAP in 14 linkage groups. Lengths of linkage groups ranged from 37.7 to 156.5 cM, and the total length of the map is 1518.1 cM, with an average marker density of 1.47 cM and 22–138 markers per linkage group. Finally, Salgon et al. (2018) used a DH population of 123 lines from the intraspecific cross EG203 × MM738 to map 1170 SNPs over the 12 eggplant chromosomes, spanning 1461 cM. The size of the linkage groups ranges from 91.39 to 167.34 cM, containing from 53 to 141 SNPs. The map has a high density with an average of 1 SNP every 1.25 cM.

5.2 QTL Mapping

Although the huge phenotypic variability available in *S. melongena* accessions, the reduced polymorphism detected in intraspecific mapping populations with the markers used (i.e., RFLP, RAPD, AFLP) in the 'first-generation' maps, along with the relatively low commercial importance in the 'seed market' of the species, hampered the construction of dense eggplant genetic maps. As a result, the identification of QTLs in this species was limited, especially when compared to the other Solanaceae crops as tomato, potato, and pepper.

The first QTLs mapped in eggplant were related to fruit shape and color of fruit, stem, and calyx (Table 5.1) and were spotted, respectively, on LG2 and LG7 among the 21 LGs of the intraspecific map developed by Nunome et al. (2001). Doganlar et al. (2002b) phenotyped for 22 traits, including those related to fruit weight, fruit shape, and color, the interspecific population of 58 F_2 lines developed from the cross between *S. melongena* and *S. linneanum*, and a total of 47 unique QTLs were identified. Later, Frary et al. (2003) used the same population to map chromosomal regions associated with 19 morphological and biological traits, including leaf, flower, fruit characteristics, day to flowering, and fruit set. Overall, 63 unique QTLs were identified, as well as potential tomato and potato ortholog QTLs. This work suggested that *S. linneanum* may be a source of superior alleles for some agronomical traits (e.g., calyx size, fruit set) in addition to resistance to abiotic and biotic stresses (Frary et al. 2003). The same domestication and morphological trait data for the *S. linnaeanum* × *S. melongena* F_2 population were re-analyzed (Frary et al. 2014) using a more performant method (CIM, composite interval mapping Jansen and Stam 1994; Zeng 1994) and a more dense map (Doğanlar et al. 2014). Overall, 71 QTLs were identified, of which 22 were novel ones while 49 already detected by Doganlar et al. (2002b) and Frary et al. (2003) (Table 5.1).

QTLs associated with parthenocarpy, a very important fruit qualitative trait, were identified by Miyatake et al. (2012), by exploiting the improved maps of Fukuoka et al. (2012). Two populations of F_2 plants derived from the crosses between two non-parthenocarpic eggplant lines, LS1934 and Nakate-Shinkuro, with a parthenocarpic line, AE-P03, were used, allowing to identify two main QTLs (*Cop3.1* and *Cop8.1*) spotted in both maps (Table 5.1). The percentage

Table 5.1 Overview of the QTLs identified in eggplant not included in Fig. 5.1. For each study, the traits are reported, together with the chromosomal position of the QTLs in parentheses

References	Traits (Eggplant Chromosome)
Nunome et al. (2001)	Fruit shape (2)—Fruit color (7)—Stem color (7)—Calyx color (7)
Doganlar et al. (2002b)	Fruit weight (2,9,11)—Fruit length (2,9,11)—Fruit diameter (1,11)—Fruit shape (2,7)—Ovary length (1,4,9)—Ovary diameter (9)—Ovary shape (4)—Ovary locule number (5)—Fruit anthocyanin presence (10)—Fruit anthocyanin intensity (1,10,12)—Fruit color (8,10)—Leaf lamina anthocyanin (2,6,9,10)—Leaf rib anthocyanin (10)—Stem anthocyanin (6,10,12)—Prickle anthocyanin (10)—Corolla anthocyanin (3,5,6,10)—Fruit stripe (4,10)—Leaf prickle (6,10)—Stem prickle (6)—Flower calyx prickle (6)—Fruit calyx prickle (6,9,11)—Petiole prickle (6)
Frary et al. (2003)	Leaf length (11,12)—Leaf width (1,3,7)—Leaf shape (1,5,7,8)—Leaf lobing (6,10)—Leaf surface (4)—Flower diameter (7)—Flower shape (1,7,9)—Days to flowering (1,2,3,5,9,11)—Flowers/inflorescence (3,4)—Fruit/infructescence (3,4,7,10)—Fruit set (4,7)—Fruit calyx size (2,9)—Fruit glossiness (1,6,8,9,12)—Plant height (2,5,10,12)—Apex hairs (3,4,5,10)—Leaf hairs (1,2,3,5,6,8,10)—Stem hairs (2,3,10)—Ovary hairs (1,3,6,10)
Miyatake et al. (2012)	Controlling parthenocarpy (3,8)
Lebeau et al. (2013)	*Ralstonia solanacearum* resistance (2,3,13)
Frary et al. (2014)	Leaf length (11)—Leaf width (1,4)—Leaf shape (1,5)—Leaf lobing (5,6,7)—Ovary length (1,9)—Ovary diameter (9)—Ovary area (6,11)—Locule number (5)—Fruit length (1,2,7,9)—Fruit shape index (7)—Fruit weight (1,2,9)—Fruit stripe (4,10)—Fruit chlorophyll netting (3,4)—Fruit glossiness (1,6,9)—Flowers/fln (3,4)—Fruit/ftn (3,9-Apex hairs (2,3,7)—Stem hairs (3,10)—Leaf hairs (3,9,10)—Ovary hairs (10)—Stem prickle (1,3,6)—Leaf prickle (2,3,6)—Petiole prickle (2,6)—Flower calyx prickle (6)—Fruit calyx prickle (6)—Stem anthocyanin (6,10)—Prickle anthocyanin (10)—Leaf rib anthocyanin (10)—Leaf lamina anthocyanin (10)—Corolla anthocyanin (5)—Fruit anthocyanin intensity (11,12)—Fruit anthocyanin presence (10)
Miyatake et al. (2016)	*Fusarium oxysporum* f. sp. *melongenae* resistance (2, 4)
Toppino et al. (2016)	Fruit color (5,8)—Under calyx color (5,10)—Peel next to calyx color (10)—D3R (5)—Nasunin (5)—Dry matter (2)—Soluble solid content (3,4, 11)—Solamargine (6)—Fructose (4)—Glucose (4)—Quinic acid (1,9)—Shikimic acid (2,9)—Chlorogenic acid (4,6)
Salgon et al. (2017)	*Ralstonia solanacearum* species complex (RSSC) resistance (2,9,14)
Salgon et al. (2018)	*Ralstonia pseudosolanacearum* resistance (1,2,3,4,6,7,8,9)
Barchi et al. (2018)	*Fusarium oxysporum* f. sp. *melongenae* resistance (2,11)—*Verticillium dahliae* resistance (5,8,9)

of phenotypic variance explained (PVE) was different in the two populations (45.7 and 29.7%) for *Cop8.1*, whose involvement in parthenocarpy, contrarily to *Cop3.1,* was confirmed by using a population of backcrossed inbred lines.

The first intraspecific map developed by Barchi et al. (2010) allowed the positioning of the *Rfo-sa1* locus for the resistance trait to *Fusarium oxysporum* in the upper part of chromosome 2. The second-generation map constructed using the same F_2 population (Barchi et al. 2011) was validated by mapping QTLs linked to seven traits (Table 5.2) related to anthocyanins pigmentation and distribution on leaf, stem, flower, and fruit peduncle. To increase data reliability, the F_2 population of 156 individuals was replicated by means of cuttings and the obtained plantlets were phenotyped in

Table 5.2 Codes used to identify the traits measured by Barchi et al. (2012), Portis et al. (2014, 2015), Cericola et al. (2014)

Trait	Code	Trait	Code
Adaxial leaf lamina anthocyanin	*adlan*	Peduncle length	*pedl*
Stem anthocyanin	*stean*	Fruit calyx prickliness	*fcpri*
Abaxial leaf lamina anthocyanin	*ablan*	Fruit calyx removal	*fcr*
Calyx anthocyanin	*calan*	Calyx coverage	*cacov*
Corolla color	*corcol*	Outer fruit firmness	*outfir*
Adaxial leaf venation anthocyanin	*adlvean*	Flesh color	*flcol*
Abaxial leaf venation anthocyanin	*ablvean*	Flesh green ring	*gring*
Fruit peduncle anthocyanin	*pedan*	Plant growth habit	*hab*
Fruit color	*fcol*	Number of branches	*br*
Fruit glossiness (scale 0–3)	*fglo*	Leaf width	*lw*
Fruit weight	*fw*	Leaf length	*lle*
Fruit length	*fl*	Adaxial leaf central ven. prickl.	*adlcevepri*
Fruit diameter 1/4	*fd1/4*	Adaxial leaf lateral ven. prickl.	*adllavepri*
Fruit diameter 1/2	*fd1/2*	Abaxial leaf central ven. prickl.	*ablcevepri*
Fruit diameter 3/4	*fd3/4*	Abaxial leaf lateral ven. prickl.	*abllavepri*
Fruit diameter max	*fdmax*	Stem prickliness	*stpri*
Fruit diameter max position	*fdmaxp*	Abaxial leaf prickles number	*ablprin*
Fruit shape	*fs*	Adaxial leaf prickles number	*adlprin*
Fruit curvature	*fcur*	Leaf hairiness	*lha*
Fruit apex shape	*fas*	Number of flowers/inflorescence	*flwin*
Inner fruit firmness	*intfir*	Flowering time	*flwt*
Number of locules	*slon*		

replicated blocks in two locations. Twenty-six major (PVE > 10%) and minor QTLs were spotted on chromosome E02, E05, E06, E08, and E10 (Fig. 5.1); major QTLs were stable in the two locations, while location-specific QTLs were solely represented by minor QTLs. Finally, putative orthologous genes syntenic with other Solanaceous species were identified.

The same population was phenotyped in two locations for further twenty traits (Table 5.2) including the agronomical relevant features early and total yield, fruit weight and shape, prickliness in calyx and leaf (Portis et al. 2014). A total of 34 major QTLs (Fig. 5.1) were identified, of which 24 were in common between the two locations while eight and two were location-specific; furthermore, seven appeared as major in one of the sites but were found as minor in the other. Among the QTLs identified, those controlling fruit production, as early and total yield (PVE ranging from 24 to 53%), as well as fruit weight, co-localized onto E02, with a confidence interval of just 0.3 cM in one location and 2.1 cM in the other. This outcome was also confirmed by the high correlation among these traits. Furthermore, both parents contributed positively to these traits, with alleles coming from '67/3' having a higher effect. Other major QTLs were identified for fruit dimension, shape and firmness, number of seed locules, length of the peduncle, prickliness, and plant growth habit.

The F_2 population was, later on, used to perform a QTL search for biochemical composition and qualitative traits of fruit, including fruit coloration (Toppino et al. 2016). In particular, a biochemical characterization for both fruit

qualitative traits, including dry matter, brix, sugars, and organic acids, as well as for health-related compounds, including chlorogenic acid, the two peel anthocyanins (i.e., delphinidin-3-rutinoside and delphinidin-3-(p-coumaroylrutinoside)-5-glucoside [nasunin]), and the two main steroidal glycoalkaloids (solasonine and solamargine) was carried out. For most of the traits, one major QTL was identified (Table 5.1) and putative orthologies with other Solanaceae crops were discovered. As an example, we mention a solid and stable QTL identified for fruit skin coloration on E05 (*Frucol E05*) which shows a PVE in the two locations of 56 and 70%, and maps in the same position of QTLs controlling the trait *Undcal* (under calyx coloration), the anthocyanins delphinidin-3-rutinoside (present in 305E40 parent), and nasunin (present in 67/3).

Resistances to pest and diseases have been the targets of several QTLs studied in the Solanaceae (Gebhardt 2016). Despite the importance of resistance to biotic and abiotic stresses in eggplant, only in the twenty-first century were the first QTLs for fungal and bacterial wilts identified, mainly using second-generation maps. An intraspecific population of recombinant inbred lines (RILs) was developed for mapping a major resistant locus to the bacterial wilt (BW) caused by *Ralstonia solanacearum* (Lebeau et al. 2013). The resistant parent of this map combined the resistance from a Turkish line with that of an introgression line from *S. aethiopicum* gr. *aculeatum*, while the susceptible parent was the line MM738, already used as parent in a previous mapping population (Doganlar et al. 2002a). A major gene (*ERs1*) was mapped on E02 (Table 5.1), although the map had only 119 markers which were spread across 18 LGS. This gene was effective in controlling three strains and fully susceptible to a virulent strain from phylotype I of *R. solanacearum*. The same population was used for mapping the resistance against eight strains by using a high-density GBS-based map with 1035 markers and anchored on eggplant, tomato, and potato genomes (Salgon et al. 2017). The major QTL *EBWR9* was identified at the bottom of chromosome 9 and flanked the previously identified *ERs1* resistance gene. Furthermore, two other QTLs (on Ch 2 and 5) were discovered conferring specific partial resistance to other strains (Table 5.1).

Recently, a new mapping population constituted by 123 doubled haploid lines from the cross MM 738 (susceptible) × AG91-25 (resistant to a broad range of *R. solanacearum* strains) was genotyped by GBS and a high-density map of 1170 markers generated (Salgon et al. 2018). The population was infected with two phylotype strains (PSS4 and R3598), able to overcome the resistance of *EBWR9* in two environments (Reunion Island and Cameroon). Ten and three resistance QTLs were detected and mapped as specifically resistant to PSS4 and R3598, respectively (Table 5.1); these QTLs also showed a heavy influence by environmental conditions. The most stable QTLs were found on chromosomes 3 and 6 and resulted syntenic with those conferring BW resistance in tomato. Epistatic analysis showed a possible digenic interaction between the identified QTLs. These works offer breeders the possibility to cumulate specific and non-specific resistance QTLs with single major gene (i.e., *ER-bw*; Xi'ou et al. 2015) to establish a more durable resistance against BW.

Fusarium oxysporum f. sp. *melongenae* and *Verticillium* spp. are the most dangerous soil-borne fungal wilt disease causing heavy yield losses in eggplant cultivation. Markers associated with full resistance to *Fusarium* were discovered through bulked segregant analysis by Mutlu et al. (2008) in the Malaysian *S. melongena* line LS2436 and by Toppino et al. (2008) in resistant lines containing introgression from *S. aethiopicum* gr. *gilo* and gr. *aculeatum*. The map position of the resistant locus *Rfo-sa1* was firstly established onto LG 1 (Barchi et al. 2011) which, then, was demonstrated to be correspondent to E02 using the second-generation map of the same F_2 population (Barchi et al. 2012; Portis et al. 2014). Another work on *Fusarium* wilt resistance was performed using the *S. melongena* resistant source lines LS1934, LS174, and LS2436 (Miyatake et al. 2016). Precise mapping was accomplished by using three genetic maps from F_2 and F_3 populations as well as backcross inbred lines populations. A resistant semi-dominantly

inherited QTL locus (*FM1*) was significantly associated with LS1934 and LS174 resistant sources (PVE 66 and 75% according to the populations) and was positioned on chromosome 2 at the same location of *Rfo-sa1*, suggesting they might be orthologous (Table 5.1). The resistance locus from LS2436 was instead mapped in the middle of chromosome 4.

A recent study reported on the use of $F_{2:3}$ progenies from the cross between '305E40' (carrying the resistant locus *Rfo-sa1* to *Fusarium* and tolerance to *Verticillium*) and '67/3' lines, for QTL study after inoculation with *Fusarium* and *Verticillium dahliae* (Barchi et al. 2018). A major QTL (~70% PVE, Table 5.1) for complete resistance to *Fusarium oxysporum* was located onto the E02 in the same position of the *Rfo-sa1* locus, introgressed from *S. aethiopicum*, but also another QTL on E11, conferring partial resistance to *Fusarium* and deriving from the parent 67/3 was identified. Inoculation in growth chambers with *Verticillium* enabled the localization of a major QTL acting at 20 days after inoculation (DAI) on E08, while at 40 DAI a major (E05) and a minor QTL (E09) were identified. All these QTLs derived from the female parent '305E40' and represent the first QTLs associated with *Verticillium* wilt resistance.

5.3 Genome-Wide Association Mapping

The genome-wide association (GWA) mapping approach represents an alternative to biparental linkage mapping for determining the genetic basis of trait variation. Both approaches rely on recombination to re-arrange the genome, and seek to establish correlations between phenotype and genotype, based on the non-random association of alleles at two or more loci, termed linkage disequilibrium (LD). The major advantages of GWA mapping lie in being able to sample a much wider range of the phenotypic and genotypic variation present in many different lineages, in exploiting multiple rounds of historical recombination, and in including multiple accessions of direct relevance to crop improvement (Yu et al. 2006).

In a pioneering attempt to apply a GWA approach in eggplant, Ge et al. (2013) were able to identify a number of phenotype/genotype associations related to eight fruit-related traits. Subsequently, the analyses of a large association panel and SNP data set were performed to identify and position marker/trait associations related to fruit, plant, and leaf morphological traits relevant for eggplant breeding (Cericola et al. 2014; Portis et al. 2015). An eggplant association panel of 191 accessions (Cericola et al. 2013), comprising a mixture of breeding lines, old varieties, and landrace selections originating from Asia and the Mediterranean Basin, was SNP genotyped and phenotyped for key breeding traits (relating to either anthocyanins pigmentation, fruit morphology, plant and leaf morphology, listed in Table 5.2) at two Italian locations over two years. Each accession was genotyped at 384 SNP loci, as reported by Barchi et al. (2011), 339 of these being previously genetically mapped (Barchi et al. 2012).

The STRUCTURE analysis of the collection resulted in a prediction for K of either 1 or 2 (Fig. 5.2a). The UPGMA-based dendrogram (Fig. 5.2a) and the PCoA (Fig. 5.2b) show the genetic relationships between the 191 accessions. Their form suggested a population structure comprising two subgroups. According to the level of membership provided by STRUCTURE, cluster A contained 91% of the Asian accessions, while cluster B comprised 96% of the Mediterranean accessions. The remaining 35 accessions (18%) had ambiguous membership and were thus classified as admixed. An r^2 threshold of 0.15 was applied to define which SNP loci were significantly associated with one another. On the basis of a r^2_{sv} model (taking into account both the STRUCTURE output and the phylogenetic relationship, Fig. 5.2c), LD extended over 3.4 cM, which matches reasonably well with the level reported for eggplant by Ge et al. (2013) and also with those documented in other self-pollinating species such as the close relative tomato (Robbins et al. 2011).

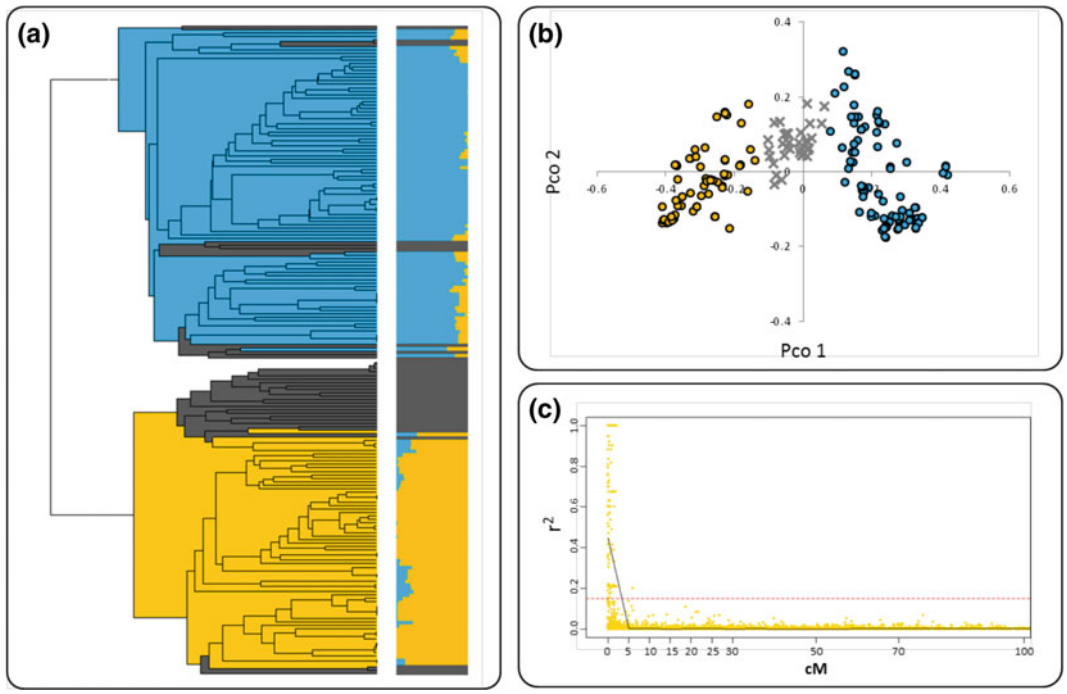

Fig. 5.2 Genetic architecture of the full germplasm panel. **a** UPGMA dendrogram derived after taking account of the STRUCTURE analysis. **b** PCoA visualization of the genetic relationships between members of the association panel. **c** LD decay. The curve was fitted using a locally weighted scatterplot smooth regression with the threshold set at 0.15

Regions carrying presumed genes/QTLs affecting 38 of the 43 traits (with the exception of *ablan, corcol, slon, hab,* and *flwin*) were identified on each of the 12 chromosomes. The number of associations per trait ranged from two (*fcr, cacov, outfir, br, lha,* and *flwt*) to 17 (*intfir* and *stpri*). To correlate the associations with known QTLs, SNP loci separated from another by <6.8 cM (double the global estimate for LD) were considered as a unit and their genomic location was obtained from Barchi et al. (2012) map. Overall, 44 regions were defined, involving 1-7 SNP loci each. The most prominent trait clusters were found on chromosomes E01, E06, E07, E08, and E10 (Fig. 5.1). Chromosome E10 proved to harbor the most genes/QTL underlying variation in anthocyanin content and fruit color, including a cluster of genetic factors for *adlan, stean, calan*, *adlevean, ablevean, pedan,* and *fcol* and another one for *stean, ablevean*, *adlevean, pedan, fcol,* and *fglo.*

The most important regions influencing variation in fruit morphology were on E01 (*fw, fl, fs, fcur, intfir,* and the fruit diameter traits) and E10 (*fw, fl, fd1/4, fdmax, fs, fcur, outfir, intfir,* and *flcol*). Two regions, one on chromosome E01 and one in the distal part of E02 were associated with variation for fruit diameter and shape. E03 was associated with variation for fruit diameter, weight, and apex shape, while the distally located segment on E08 harbored genes affecting fruit diameter and *fas* as well as *fs*. Four regions of chromosome E06 were associated with variation for prickliness (*adllavepri, abllavepri, adlcevepri, ablcevepri, stpri, ablprin,* and *adlprin*), as were regions on E07 and E08. Genes determining *fd1/4* were also located to E07 and those influencing *gring* on E08.

Comparative mapping exposed the high degree of synteny retained between the tomato and eggplant genomes (Wu et al. 2009; Portis et al. 2014; Rinaldi et al. 2016; see also Chap. 4).

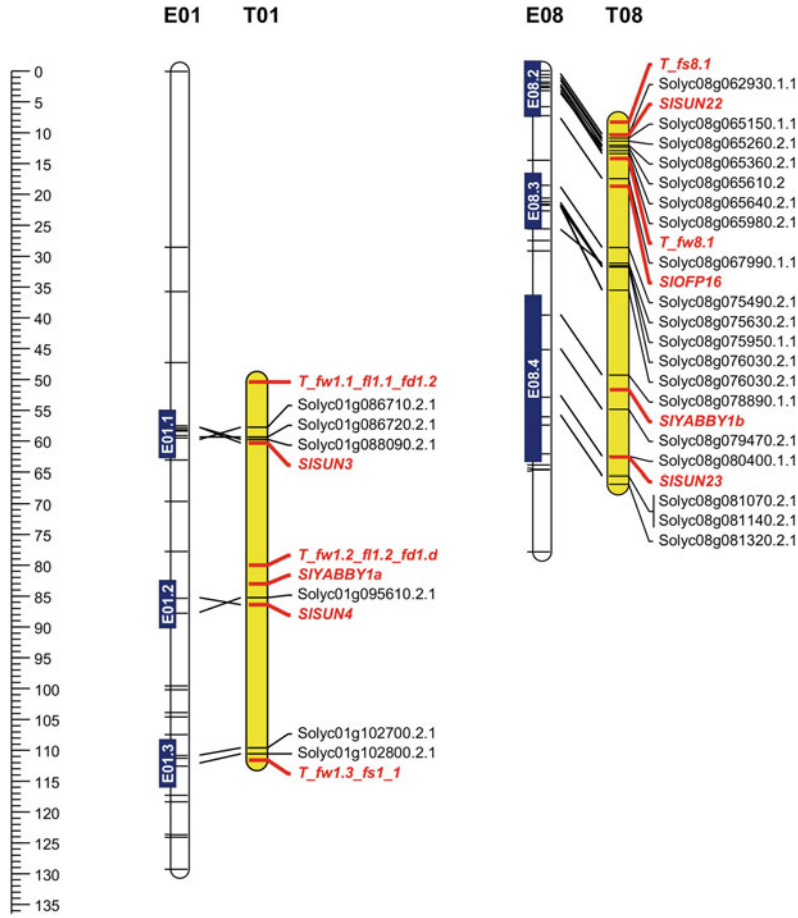

Fig. 5.3 Synteny in the Solanaceae. Eggplant chromosomes E01 and E08 are represented by white bars, and the site of QTL detected by GWA analysis is indicated; tomato chromosomes are represented on the right, along with the position of candidate genes (see Portis et al. 2015 for details)

Specifically, the gene content of a genomic region in eggplant harboring a particular set of trait/marker associations has a good chance of being replicated in the orthologous segments of tomato and pepper. The presence of regions syntenic with either tomato or pepper was here identified on ten of the 12 eggplant chromosomes (Cericola et al. 2014; Portis et al. 2015). For example, the syntenic regions on E01 and T01 both harbor genes/QTLs associated with fruit size, weight and shape, and a similar relationship holds between E08 (fruit shape and size) and T08 (Fig. 5.3). On the other hand, synteny-based comparisons between eggplant and tomato were not informative for the genetic basis of plant and leaf morphology, as these traits (e.g., prickliness) are of no relevance to either tomato or pepper.

The genetic variability captured by the association germplasm panel, which includes contrasting morphology for most of the studied traits, proved to be a great source of allelic variation. The described GWA approach successfully validated a number of previously detected QTLs, thereby providing the potential for applying a marker-assisted selection strategy for improving some key breeders' traits. At the same time, it identified the location of a number of yet unknown genes/QTL (fully described by Portis et al. 2015). The study has also demonstrated that a comparative genetic approach,

relying on the much larger knowledge base associated with tomato, provides a useful shortcut for identifying candidate genes. The sequences of such genes can readily provide the materials necessary to develop marker-assisted selection assays, while also advancing the understanding of synteny in the Solanaceae.

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The Draft Genome of Eggplant

6

Hideki Hirakawa

Abstract

The genome sequences of many plant species have been determined using next-generation sequencing (NGS) data. In the Solanaceae, the genome sequences of species such as potato, tomato, wild tomato, and pepper have been determined. To determine the genome sequence of eggplant, several kinds of linkage maps were constructed using random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), expressed sequence tag (EST), simple sequence repeat (SSR), single nucleotide polymorphism (SNP), and conserved ortholog set (COS) markers. To clarify the genome structure and complex traits, the draft genome sequence SME_r2.5.1 of the purebred cultivar Nakate-Shinkuro was determined in 2014. SME_r2.5.1 consisted of 33,873 sequences with a total length of 833.1 Mb. Gene prediction identified 85,446 coding sequences (CDSs) (SME_r2.5.1_cds). According to similarity and domain searches, 41,048 transposable elements, 1714 pseudogenes, and 649 short genes were excluded, and the remaining 42,035 CDSs constituted the final subset of genes (SME_r2.5.1_cds_ip). Annotation was performed by BLAST searches against NCBI's NR and TAIR10 databases and InterProScan searches against the Pfam database. Genes related to the phenylpropanoid pathway producing the antioxidant chlorogenic acid (CGA) were searched by their annotation (hydroxycinnamoyl-CoA shikimate hydroxycinnamoyl transferase [HCT], hydroxycinnamoyl-CoA quinate hydroxycinnamoyl transferase [HQT], and *p*-coumarate 3-hydroxylase [C3H]). The information about the genome, genes, and annotation is available at the Eggplant Genome Database (http://eggplant.kazusa.or.jp). The DNA markers of eggplant have been manually curated from the literature and deposited in the Plant Genome Database Japan (http://pgdbj.jp). Collectively, the use of this genomic information is expected to accelerate the breeding efficiency of eggplant.

6.1 Introduction

As a result of the development of next-generation sequencing (NGS), the genome sequences of many plant species have been determined. In the Solanaceae family, the genome sequence of the *Solanum lycopersicum* L. (tomato) cultivar Heinz, which is closely related to eggplant (*S. melongena* L.), was determined by a combination of Sanger and NGS data in 2012 (The Tomato Genome Consortium 2012). The total

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length of the 12 chromosome sequences of SL2.40 was 759.9 Mb, and 34,727 genes were predicted in ITAG 2.3. The genome and gene annotation have since been updated to SL3.0 and ITAG 3.20, respectively. The total length of the 12 chromosome sequences in SL3.0 was 807.2 Mb, and the number of genes in ITAG 3.20 was 35,768. In the case of eggplant, the linkage map for an F2 population (168 individuals) derived from an intraspecific cross of the cultivated lines, EPL-1 and WCGR112-8, was first constructed by using random amplified polymorphic DNA (RAPD) and amplified fragment length polymorphism (AFLP) markers (Nunome et al. 2001). Since then, more detailed linkage maps were constructed based on expressed sequence tags (ESTs) (Fukuoka et al. 2010), simple sequence repeats (SSRs; Nunome et al. 2003a, b) and single nucleotide polymorphisms (SNPs; Barchi et al. 2011). The orthologous gene-based markers, such as conserved ortholog set II (COSII) markers were also used for detailed synteny map construction (Wu et al. 2009). The *Solanum* orthologous (SOL) gene sets (Fukuoka et al. 2012) have been developed, and macro-syntenic relationships among the Solanaceae species, including *S. lycopersicum* (tomato), *S. tuberosum* (potato), *S. melongena* (eggplant), *Capsium annuum* (pepper), and *Nicotiana*, have been investigated (Wu and Tanksley 2010).

To clarify the genome structure and to aid in the identification of complex traits, the draft genome sequence of eggplant was determined in 2014 (Hirakawa et al. 2014) by using NGS data obtained by the 454 GS FLX Titanium platform (Roche Diagnostics, Basel, Switzerland) and HiSeq 2000 platform (Illumina, San Diego, CA). In the Solanaceae, the genome sequences of *S. tuberosum* (potato; Potato Genome Sequencing Consortium 2011), *S. pimpinellifolium* (wild tomato; The Tomato Genome Consortium 2012), *Nicotiana benthamiana* (wild tobacco; Bombarely et al. 2012), *S. pennellii* (wild tomato; Bolger et al. 2014), and *C. annuum* (pepper; Kim et al. 2014) have all been determined. In this chapter, the genome sequencing, gene prediction, and annotation of the first eggplant genome will be described.

6.2 Genome Assembly of Eggplant

6.2.1 Sequencing of the Eggplant Genome

The genome sequencing was conducted for purebred cultivar Nakate-Shinkuro. Total DNA was extracted from leaves. The total Illumina reads of the paired-end (PE) library with an insert size of 200–300 bp and the mate-pair (MP) library with an insert size of 2 kbp were obtained using the Illumina HiSeq 2000 platform (Illumina). In addition, the sequence capture analysis of genomic DNA was performed for the cultivar Nakate-Shinkuro, LS1934 (germplasm), WCGR112-8 (germplasm), and AE-P03 (purebred breeding line) using 454 GS FLX Titanium platform (Roche Diagnostics).

6.2.2 Genome Assembly

The k-mer frequency distribution plot of the Illumina PE reads is shown in Fig. 6.1. The eggplant cultivar Nakate-Shinkuro was revealed to have a low-heterozygosity genome due to the large peak in the k-mer frequency distribution plot. The genome size was estimated as 1.127 Gb by using the KmerFreq_AR in SOAPec v2.0.1 package (http://soap.genomics.org.cn). The assembly procedure conducted is shown in Fig. 6.2. The Illumina PE and MP reads were trimmed by the fastq_quality_filter and fastq_quality_trimmer tools in the FASTX-Toolkit (http://hannonlab.cshl.edu/fastx_toolkit/). The artifacts were removed by using fastx_artifacts_filter, and adaptor sequences were also removed by using fastx_clipper in the FASTX-Toolkit. The trimmed PE reads (total length: 85.4 Gb) and MP reads (29.9 Gb) were then assembled de novo by using SOAPdenovo (Li et al. 2010) using a k-mer size of 51 with default parameters, and 9,708,734 contigs (total length: 1.423 Gb; N50 length: 329 bases) and 1,321,157 scaffolds (hereafter Illumina consensus sequences) (total length: 1.093 Gb; N50 length: 30,558 bases) were obtained (Table 6.1). The gapped regions on the scaffolds were closed by GapCloser 1.10

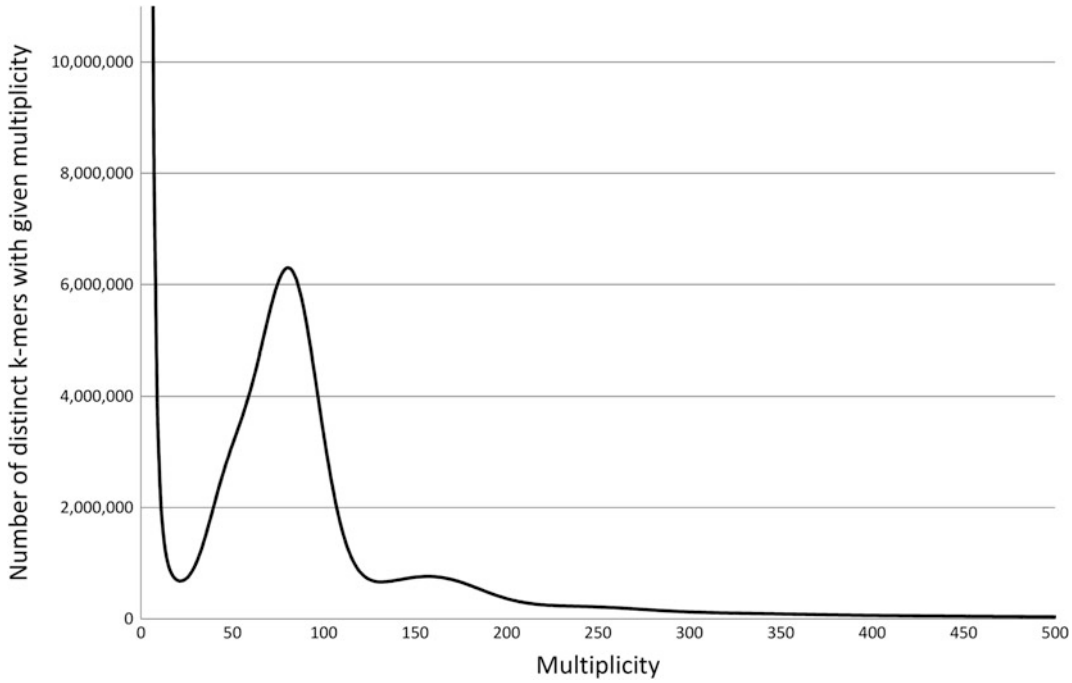

Fig. 6.1 K-mer frequency distribution plot of the eggplant cultivar Nakate-Shinkuro. The k-mer distribution was calculated by Jellyfish with k-mer size 17

(http://soap.genomics.org.cn), and 1,321,157 scaffolds (total length: 1.093 Gb; N50 length: 28,984 bases) were obtained. The 454 reads obtained from DNA capture of the four cultivars were assembled into 45,786 contigs (total length: 38.3 Mb; N50 length: 1128 bases) by Newbler 2.7 in genomic mode (Roche Diagnostics). The trimmed Illumina PE reads were mapped onto the contigs by BWA v0.6.2 (Li and Durbin 2009), resulting in 45,729 contigs (hereafter 454 consensus sequences) (total length: 38.2 Mb; N50 length: 1127 bases). The 454 consensus sequences and Illumina consensus sequences were merged by PCAP.rep (Huang et al. 2006) with 98% identity, and 81,273 hybrid-scaffolds (total length: 836.8 Mb; N50 length: 49,406 bases) were obtained. The probable contaminated scaffolds were excluded by BLAST (Altschul et al. 1990) searches against the dataset of bacterial genome sequences deposited in NCBI (http://www.ncbi.nlm.nih.gov), the chloroplast genome sequences of tomato (accession number: NC_007943) and *Arabidopsis thaliana* (NC_000932), and the mitochondrial genome sequences of tomato (SOLYC_MT_v1.50, http://www.mitochondrial-genome.org), tobacco (NC_006581) and *A. thaliana* (NC_001284), with an *E*-value cutoff of 1E-10 and length coverage of $\geq$90%. The hybrid-scaffolds were further connected by MP reads using SSPACE2.0 (Boetzer et al. 2011), and 33,873 super-scaffolds were obtained (total length: 833.1 Mb; N50 length: 64,536 bases). The super-scaffolds covered 74% of the estimated genome size of eggplant and were designated as draft genome sequence SME_r2.5.1. The statistics of the draft genome sequence SME_r2.5.1 are shown in Table 6.1 (Hirakawa et al. 2014).

6.2.3 Repetitive Sequences

Repetitive sequences in the draft genome sequence SME_r2.5.1 were detected by using RepeatMasker (http://www.repeatmasker.org) and RepeatScout (Price et al. 2005). The total length of repeats was 586.8 Mb (70.4% of the

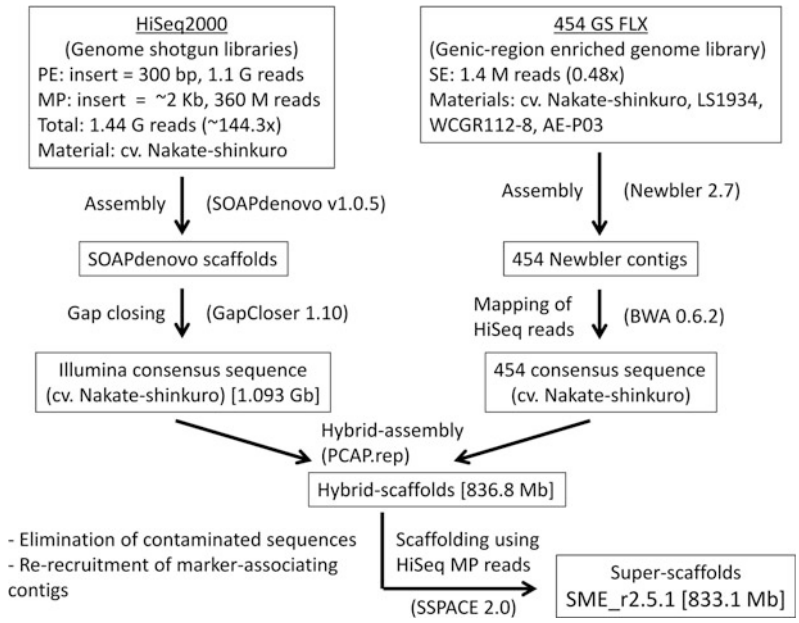

Fig. 6.2 Assembly procedure of the eggplant genome. The Illumina reads obtained from the cultivar Nakate-Shinkuro were assembled into scaffolds by SOAPdenovo, and Illumina consensus sequences were constructed. The 454 reads obtained from the cultivars, Nakate-Shinkuro, LS1934, WCGR112-8, and AE-P03, were assembled by Newbler 2.7, and Illumina paired-end (PE) reads were mapped onto the contigs assembled by 454 reads (454 Newbler contigs), and 454 consensus sequences were constructed. The Illumina consensus sequences and 454 consensus sequences were assembled by PCAP.rep. The hybrid-scaffolds were connected by Illumina mate-pair reads (MP) by SSPACE 2.0, and the super-scaffolds, SME_r2.5.1, were constructed

draft genome sequence), of which the subtotal of known repeats defined in Repbase (Jurka 1998) and the length of the unknown repeats were 298.1 Mb (35.8%) and 288.6 Mb (34.6%), respectively. The percentages of the repeats were almost the same as those of tomato SL2.40 (68.3%), *S. pimpinellifolium* A-1.0 (68.2%), potato PGSC DM v3 (64.2%), and *N. benthamiana* v0.4.4 (72.6%). The repetitive sequence element most found was the Gypsy class retrotransposon element (total length: 212.0 Mb (25.4%)), which was found with almost the same frequency as those of tomato (220.1 Mb; 28.2%), *S. pimpinellifolium* (185.8 Mb; 27.0%), potato (209.3 Mb; 28.8%), and *N. benthamiana* (321.6 Mb; 12.4%).

6.2.4 Gene Prediction

Gene prediction was performed by Augustus v2.7 (Stanke and Waack 2003). By using a training set of tomato ITAG 2.3, 85,446 genes were predicted and named SME_r2.5.1_cds. The genes were searched against NCBI's NR database (https://www.ncbi.nlm.nih.gov) using BLAST and GyDB 2.0 (Llorens et al. 2011) with HMMER v3.0 (Eddy 2011). According to the definition of top hits, 41,048 genes were excluded as transposable elements (TEs), 1714 genes were excluded as pseudogenes (with in-frame stop codons) and 649 were short genes (<50 amino acids). Of the remaining 42,035 genes, 39,498 genes were classified into intrinsic genes

Table 6.1 Statistics of the draft genome and CDS sequences

		Genome	CDS	
		SME_r2.5.1	SME_r2.5.1_cds	SME_r2.5.1_cds_ip
Total	Number of sequences	33,873	85,446	42,035
	Total length (bases)	833,108,131	93,189,508	36,732,556
	Average length (bases)	24,595	1091	874
	Max length (bases)	629,958	15,414	15,243
	N50 length (bases)	64,536	1515	1212
	A	255,484,950	26,748,727	10,647,950
	T	254,643,398	25,973,395	10,073,020
	G	141,325,886	23,229,019	8,840,588
	C	142,070,567	17,234,544	7,168,841
	N	39,583,330	3823	2157
	G+C%	35.7	43	44
≥ 500 b	Number of sequences	33,872	60,892	25,288
	Total length (bases)	833,107,658	84,705,534	30,997,288
	Average length (bases)	24,596	1391	1226
≥ 1 kb	Number of sequences	30,983	34,709	12,493
	Total length (bases)	831,088,565	65,691,231	21,850,950
	Average length (bases)	26,824	1893	1749
≥ 5 kb	Number of sequences	21,443	466	97
	Total length (bases)	804,313,164	2,945,437	594,980
	Average length (bases)	37,509	6321	6134

(with start and stop codons), and 3537 genes were classified into partial genes (without start and/or stop codons). The 42,035 genes were named SME_r2.5.1_cds_ip and were applied to further analyses. They were classified into KOG (Tatusov et al. 2003) and Gene Ontology (GO) categories (The Gene Ontology Consortium 2000), and mapped onto KEGG metabolic pathways (Ogata et al. 1999).

6.2.5 Gene Annotation

The genes were applied to similarity searches against NCBI's NR and TAIR10 (Lamesch et al. 2012) databases by BLAST with an *E*-value cutoff of 1E-10 and domain searches against the Pfam database (Finn et al. 2016) by InterProScan (Quevillon et al. 2005) with an *E*-value cutoff of 1.0. Comparative analysis of the genes among tomato, potato, *N. benthamiana*, and *A. thaliana* revealed 6780 common orthologous groups, which, respectively, consisted of 21,445, 23,548, 35,235, 39,629, and 23,834 genes of eggplant, tomato, potato, *N. benthamiana*, and *A. thaliana*, respectively (Hirakawa et al. 2014). According to the annotation, we identified genes related to the phenylpropanoid pathway, including hydroxycinnamoyl-CoA shikimate hydroxycinnamoyl transferase (HCT), hydroxycinnamoyl-CoA quinate hydroxycinnamoyl transferase (HQT), and *p*-coumarate 3-hydroxylase (C3H), all of which are considered to be related to chlorogenic acid (CGA), which is a known antioxidant (Cao et al. 1996).

Among the predicted genes, only one putative gene encoding HCT (Sme2.5_04555.1_g00001.1 in SME_r2.5.1_cds) and one putative gene encoding HQT (Sme2.5_00673.1_g00011.1) were found to be similar to those of AtHCT (NP_199704.1) in

A. thaliana and LeHQT (CAE46933.1) in tomato. Regarding C3H, five closely related homologs (Sme2.5_06006.1_g00001.1, Sme2.5_00085.1_g00033.1, g00034.1, g00035.1, and g00036.1) were found in the predicted genes of eggplant and a phylogenetic analysis resolved the relationships for the CH3 paralogs and orthologs among eggplant, tomato, potato, *N. benthamiana,* and *A. thaliana* (Hirakawa et al. 2014).

6.3 Databases

6.3.1 The Eggplant Genome DataBase

The draft genome and predicted genes in SME_r2.5.1 are available from the Eggplant Genome Database (http://eggplant.kazusa.or.jp). On the "BLAST" page, BLAST searches against the genome sequence (SME_r2.5.1), CDSs (SME_r2.5.1_cds) and protein sequences (SME_r2.5.1_pep) are available. On the "KEYWORD" page, keyword searches against the definition of the top hit of BLAST searches in each gene for NCBI's NR (https://www.ncbi.nlm.nih.gov), TAIR10 (https://www.arabidopsis.org), and Swiss-Prot (https://www.ebi.ac.uk/uniprot) are available. On the "DOWNLOAD" page, the genome sequence (SME_r2.5.1.fa), CDSs (all sequences: SME_r2.5.1_cds.fa; sequences without TEs and pseudogenes: SME_r2.5.1_cds_ip.fa), protein sequences (all sequences: SME_r2.5.1_pep.fa; sequences without TEs and pseudogenes: SME_r2.5.1_pep_ip.fa), tables displaying the top hit against NCBI's NR (SME_r2.5.1_pep_vs_NR_bp_E-10_top.txt), TAIR10 pep (SME_r2.5.1_pep_vs_TAIR10_bp_E-10_top.txt), the amino acid sequences of tomato ITAG2.3 (SME_r2.5.1_pep_vs_ITAG2.3_bp_E-10_top.txt), and the GFF file (SME_r2.5.1.genes.gff) are available.

6.3.2 The Plant Genome DataBase Japan (PGDBj)

To integrate the plant genome information, a portal site, the Plant Genome Database Japan (PGDBj), has been developed and is available at http://pgdbj.jp (Asamizu et al. 2014, Nakaya et al. 2017). DNA markers of CAPS (number of entries: 6), InDel (874), SCAR (2), SNP (7542), SSR (1990), and AFLP (1) of eggplant have been curated manually from the literature, and are available at the PGDBj. Primer sets that can be used for amplification of the DNA markers are also available at PGDBj. In addition, quantitative trait loci (QTL) information that has been curated manually from the literature is also available. Currently, 606 QTLs related to parthenocarpy, resistance to *Ralstonia solanacearum*, venation anthocyanin, and so on have been released. In addition, the QTL information related to the significant markers, location on linkage groups, LOD peak, LOD score, P-value, and so on can be browsed at the PGDBj.

6.4 Genome Sequencing Projects by Other Research Groups

6.4.1 Genome Sequencing of Eggplant

The Sol Genomics Network (SGN; https://solgenomics.net) has released a pre-publication version of the eggplant genome sequenced by the Eggplant Genome Project. Genome sequencing of the inbred eggplant line "67/3" was performed at the University of Verona, the University of Turin, the Italian Council for Agricultural Research and Economics, and the Italian National Agency for New Technologies, Energy and Sustainable Development. A summary of the current status of genome assembly for the inbred

eggplant line 67/3 is available at the Eggplant Genome Project website (http://www.eggplantgenome.org). The genome sequence was determined by means of Illumina sequencing, optical mapping, and genetic mapping to build at the chromosome level. The total length of the genome is currently 1.06 Gb, and it consists of 10,383 scaffolds. A total of 34,916 genes have been predicted on the scaffolds. In the analysis, for assessing genome assembly and annotation completeness, 96.3% of the BUSCOs were annotated. At the Eggplant Genome Project Web site, BLAST searches against the genome sequences and genome browser are available. This is the genome sequence that is discussed in later chapters.

6.4.2 Complete Chloroplast Genome Sequence

The complete chloroplast genome sequencing of the eggplant cultivar Nakate-Shinkuro has been assembled (Ding et al. 2016) using the genome sequencing reads (NCBI SRA database BioProject: PRJDB1505, Hirakawa et al. 2014). About 813.5 Mb of Illumina reads were selected from the SRA data by BLASTN searches against the chloroplast genome sequence of *S. nigrum* (NC_028070) and assembled using SOAPdenovo (Li et al. 2010). The annotation of the chloroplast genome was performed by Dual Organellar GenoMe Annotator (DOGMA, https://dogma.ccbb.utexas.edu). The complete chloroplast genome sequence is available under the accession number KU682719.

6.5 Conclusion

The eggplant genome sequence SME_r2.5.1 has been considered to be a draft level sequence, because there are many scaffolds and many gaps (the gaps alone account for 4.75% of the total length) (Hirakawa et al. 2014). As described above, only a PE library with an insert size of 200–300 bp and an MP library with an insert size of 2 kb were used for the de novo assembly. In addition, the genomic regions sequenced by DNA capture by 454 GS FLX Titanium were used to connect the scaffolds in the hybrid assembly. If MP reads with a range of insert sizes were used, longer scaffolds would be obtained. In this context, it is interesting to note that the long-read sequencers, Sequel (Pacific Biosciences) and MinION/GridION/PromethION (Oxford Nanopore), are becoming widely used for de novo assembly in plant species (Schmidt et al. 2017). The total length of the reads obtained by Sequel is about 5–7 Gb per cell, and that of the reads obtained by MinION is over 10 Gb per flowcell. If the amount of the long-reads is sufficient (>50× in depth), de novo assembly using only long-reads can be performed, and then the connectivity of contigs could be considerably improved. In addition, several technologies have been developed to connect the contigs or scaffolds to determine the sequences at the pseudomolecule level. Of these, the libraries constructed by chromatin proximity ligation methods, such as the Chicago library developed by Dovetail Genomics (https://dovetailgenomics.com/) and the Hi-C (chromatin conformation capture sequencing) library (Belton et al. 2012), are used for connecting the scaffolds or contigs to determine the sequences at the pseudomolecule level. The programs SALSA (Ghurye et al. 2017) and 3D-DNA (Dudchenko et al. 2017) have been developed for connecting the contigs or scaffolds by using Hi-C data. An optical mapping approach developed by BioNano Genomics (https://bionanogenomics.com) is also useful for connecting the contigs or scaffolds along with the positions of the restriction sites. In the Eggplant Genome Project (http://www.eggplantgenome.org), optical mapping was applied to the genome assembly of eggplant line 67/3 using Illumina reads, and the resultant sequences have been determined at the pseudomolecule level (see Chap. 7). In addition, haploid-resolved de novo assembly of diploid genomes can be conducted by several approaches, including by using FALCON-Phase (https://github.com/phasegenomics/FALCON-Phase) with the long-read PacBio and Hi-C data. The chromium system using GemCode

Technology developed by 10× Genomics (https://www.10xgenomics.com) can also be applied to haploid-resolved de novo assembly of diploid by using supernova (https://github.com/10XGenomics/supernova), and this method is less expensive than the approach using PacBio data. Thus, high-quality genome sequences can be constructed at the pseudomolecule level by using the technologies described above. Using this broad range of techniques, the genome sequence of eggplant, including many different cultivars and lines, can be determined with high accuracy. Through the application of this genomic information, it is expected that the breeding efficiency of eggplant will be accelerated.

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Advances in Eggplant Genome Sequencing

7

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Abstract

Thanks to the recent development of NGS technologies, draft genomes are at present available for many crops. However, most of the genome sequences are incomplete and highly fragmented because they rely on the alignment of billions of short-sequence reads and do not comprehensively report on genomic elements such as highly variable regions and DNA repeats that are difficult to map using short reads. The Italian Eggplant Genome Consortium (IEGC) has recently developed a high-quality and anchored genome assembly of the eggplant line 67/3, which is the male parent of an F6 RIL (Recombinant Inbred Line) mapping population. The hybrid assembly, obtained by merging the sequence assembly obtained with SOAPdenovo2 and the optical map, covered 1.22 Gb. The newly developed eggplant genome sequence represents an improvement in respect to the previous one published in 2104 by Hirakawa and co-authors, both in terms of projected genome size and lower number of larger scaffolds (N50 of 2.0 Mb in respect to 64.5 Kb). Optical mapping demonstrated the ability to facilitate the assembly of super-scaffolds and made it possible to correct misassembly and scaffolding errors. The female parent of the RIL mapping population (inbred line '305E40') was also sequenced (coverage of 34×), and following the low-coverage resequencing (coverage 1×) of the F6 RIL population, the genome assembly was anchored to the 12 chromosomes by applying the SoiLoCo pipeline. Recently, the complete chloroplast (cp) genome of *S. melongena* has been also assembled and characterized. It exhibited a circular DNA molecule of 154,289 bp and displayed a typical quadripartite structure including a pair of inverted repeats (IR) as well as one large single-copy (LSC) and one small single-copy (SSC) region for a whole of 125 unique functional genes.

7.1 Development of a New Eggplant Genome Sequence

7.1.1 Genome Sequencing and Assembly of the Eggplant Inbred Line 67/3

As reported in the previous chapter, Hirakawa et al. (2014) produced the first unanchored draft of *S. melongena* genome sequence, which covered about 70% of its projected 1.2 Gb genome size.

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Recently, an Italian Eggplant Genome Consortium (IEGC), which includes the Department of Agricultural, Forestry and Food Science (DISAFA) of the University of Torino, the Biotechnology Department of the University of Verona, the CREA (Consiglio per la Ricerca in Agricoltura e l'Analisi dell'Economia Agraria) Research Centre for Genomics and Bioinformatics of Montanaso Lombardo (LO) and the Italian National Agency for New Technologies, Energy and Sustainable Economic Development (ENEA) in Rome, has obtained an high-quality eggplant genome sequence of *S. melongena*, which is available in the public domain at www.eggplantgenome.org. The project was also funded by the seed companies Vilmorin & Cie, Rijk Zwaan and Enza Zaden Research and Development.

Nuclear DNA was extracted from young leaf tissues of the eggplant inbred eggplant line (67/3), which produces round and violet coloured fruits (Fig. 7.1) and which was developed from an intra-specific cross between 'Purpura' × 'CIN2' followed by nine cycles of selfing. Small-insert libraries of 400–500 and 600 bp as well as long-insert mate-pair libraries, whose insert ranged from 3.4 to 20 Kb, were sequenced as 2 × 100 nt runs on a HiSeq 1000 instrument. A whole of 153 and 135 Gb was obtained from standard paired-end (PE) and mate-pair (MP) libraries, respectively.

Raw reads were subjected to a quality-filtering process and their assembly and scaffolding are performed using a multiple k-mer strategy in SOAPdenovo2 (Luo et al. 2012). A k-mer value ranging from 83 to 95 was chosen on the basis of maximized N50 scaffold value and total assembly length. The detection of contaminating sequence segments of foreign origin was performed following BLAST searches against RefSeq (O'Leary et al. 2016) bacterial, fungi and oomycetes databases. The assembly length was estimated about 1163 Gb and included more than 11 k sequences. The N50 and N90 lengths were 678,719 and 151,506 bp, respectively. The assembly metrics are reported in Table 7.1.

The completeness and accuracy of gene regions were assessed with the CEGMA pipeline

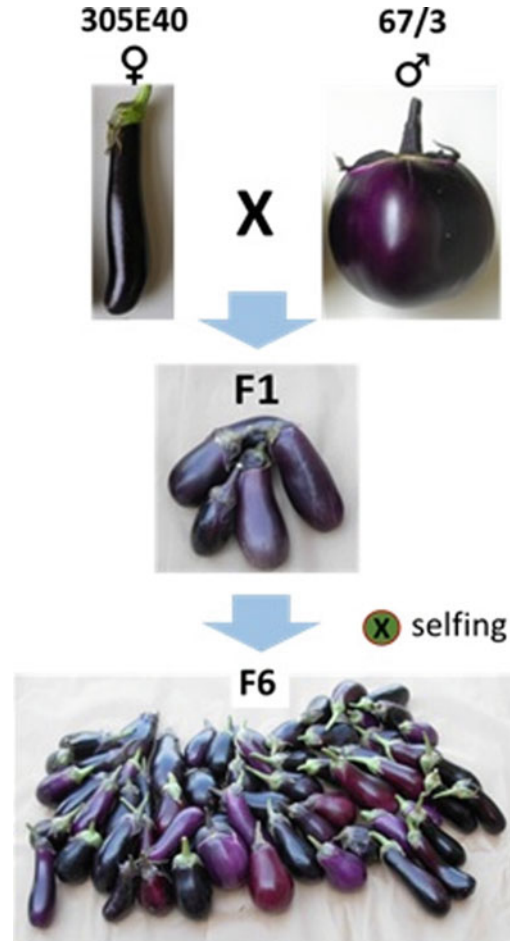

Fig. 7.1 Phenotype of the two parental lines, their F1 and the F6 RIL mapping population obtained through the SSD approach

(Parra et al. 2007). In total, 248 CEGs (ultra-conserved eukaryotic genes) were compared to the genome assembly, of which 83.87% highlighted a complete match. Since CEGMA is limited to conserved genes, the assembled genome was also aligned with 98,089 EST of *S. melongena* downloaded from NCBI, and 93.5% matches were identified.

7.1.2 Optical Mapping of the Line 67/3

The construction of an optical map consists in establishing an enzymatic profile of genomic

Table 7.1 Assembly metrics obtained using SOAPdenovo2

Assembly length	1,163,300,423 bp
Number of sequences	11,204
Average Scaffold length	103,829 bp
Maximum length of Scaffolds	5,035,223 bp
GC percentage	35%
Gap cumulative length	104,848,488
Number of gaps	138,537
Average gap length	7568 bp
Number of contigs	149,741
Maximum contig length	135,467 bp
Median contig length	3770 bp
N50	678,719 bp
N90	151,506 bp

Table 7.2 Statistics of hybrid scaffolding of the *S. melongena* genome sequence reconstructed using NGS assembly and optical mapping

Assembly length	1,220,547,359 bp
Number of sequences	469
Average Scaffold length	2,602,450 bp
Maximum length of Scaffolds	15,811,610 bp
GC percentage	35%
Gap cumulative length	299,612,751 bp
Number of gaps	96,679
Average gap length	3099 bp
Number of contigs	96,801
Maximum contig length	135,467 bp
Median contig length	4930 bp
N50	3,586,651 bp
N90	1,295,900 bp

DNA molecules based on the size and order of the fragments obtained after digestion with restriction enzymes, which cleave in specific digestion site (Neely et al. 2011). By compiling several profiles from several molecules, it is possible to obtain a complete map of the restriction of a genome (whole genome optical map) on which an assembly can be both templated and validated, thus facilitating the large-scale assembly of the genome.

A next-generation genome map of the line '67/3' was obtained with BioNano technology. High-molecular-weight DNA was extracted from leaves, labelled and stained using the IrysPrep Kit. The data collection and the de novo assembly were performed at BioNano Genomics in San Diego.

The result of the final de novo assembly was 1.186 Gbp with a map N50 of 2.56 Mb. The hybrid assembly, obtained by merging the sequence assembly and the optical map, covered 1.22 Gb in 469 scaffolds with an N50 and N90 of about 3.58 and 1.30 Mb, respectively. The optical mapping made it possible the correction of more than one hundred errors in the scaffolding of the Illumina assembly. Statistics of hybrid scaffolding are reported in Table 7.2.

7.1.3 Eggplant Genome Anchoring

Assigning chromosomal locations to the genome sequence requires the construction and integration of genome-wide physical maps and dense genetic linkage maps. The ultimate goal of the process is to establish pseudomolecules, which is single accurately ordered sequence scaffolds for each chromosome with as little gaps as possible. Methods to rapidly construct ultradense linkage maps including millions of genetic markers from WGS sequencing data of segregating populations allow the direct assignment of genetic positions to scaffolds (Mascher and Stein 2014).

The eggplant inbred line '67/3' is the male parent of a RIL (F6) mapping population whose female parent is the inbred line '305E40' (Fig. 7.1). The latter produces long, highly pigmented dark purple fruit and it was obtained, through anther culture, from an inter-specific

somatic hybrid between *Solanum aethiopicum* gr. *Gilo* and *S. melongena* cv. Dourga, with the goal to introgress from the former the gene *Rfo-sa1* which confers resistance to *Fusarium oxysporum* f.sp *melongena* (Toppino et al. 2008). The line '305E40' also includes in its pedigree the inbred lines 'DR2' and 'Tal1/1'. High-quality DNA of the line '305E40' was obtained from leaves, and small-insert libraries (400–600 bp) were sequenced on a HiSeq 1000 and assembly and scaffolding of filtered reads performed as previously described for the line 67/3. The total size of the line '305E40' genome sequence was 1.09 Gb with an N50 of 6.9 Kb. The residual heterozygosity of both parental inbred was estimated, being 0.027% for '67/3' line and 0.067% for the '305E40' line, confirming the high homozygosity of both parental lines.

Following low-coverage resequencing ($\sim 1\times$) of 157 individuals of the RIL population, the genome assembly was anchored to the 12 chromosomes. The progeny reads were aligned against the 67/3 genome sequence, SNPs called by applying the SoiLoCo pipeline (Scaglione et al. 2016), and a set of 17,688 markers was generated and used for mapping. A total of 5964 markers were positioned on the genetic linkage map, covering 2666 cM and corresponding to 847.5 Mb of anchored sequence, e.g. about 73% of the genome.

In the previous work, Barchi et al. (2012) developed a RAD Tag-derived marker-based eggplant linkage map on an F_2 progeny, which was obtained by crossing the breeding lines '67/3' and '305E40', and from which the RIL mapping population was obtained through the single seed descent (SSD) method. The blasting of scaffolds against the previously developed RAD markers together with syntenic and optical mapping information allowed to assign all the LGs to the eggplant chromosomes, although the chromosomes 2, 8 and 11 included each two non-joined portions. A total of 1062 NGS scaffolds were included in 376 hybrid scaffolds, and assigned to chromosomes thanks to the optical mapping information, while other 1120 NGS scaffolds were assigned to chromosomes on the basis of their linkage map position. The unanchored portion of eggplant genome corresponded to about 300 Mb (28% of the genome sequence).

7.2 The Chloroplast Genome of Eggplant

Chloroplasts and mitochondria are multifunctional organelles possessing their own genetic material and originated from ancient eubacterial invasions. However, if genomic analyses indicate that specific endosymbionts gave birth to these organelles, proteomics reveals a surprisingly large contribution from the host, multiple symbioses and/or horizontal gene transfers (Dyall et al. 2004). The non-recombining, uniparentally inherited nature of chloroplast (cp) genomes makes them tools of election for evolutionary studies and barcoding. In higher plants, the double-stranded and circular cp DNA genome ranges from 120 to 250 Kb, but over time it tends to decrease because of extensive gene losses and/or gene transfer to mitochondrial and/or nucleus genomes (Sheppard et al. 2008; Xu et al. 2015). The plant cp genomes typically harbour a quadripartite structure consisting of two inverted repeats (IRs) separated by two regions of unique DNA, the large (LSC) and small (SSC) single-copy regions (Jansen et al. 2005).

Recently, Ding et al. (2016) assembled and characterized the complete cp genome of *S. melongena* from the whole genome sequence of the eggplant Japanese cv 'Nakate-Shinkuro' published by Hirakawa et al. (2014). The cp genome readings were filtered using the sequence of *Solanum nigrum* chloroplast genome as a reference, and the 813.5 Mb chloroplast readings obtained assembled with SOAPdenovo (Li et al. 2010). The *S. melongena* complete cp exhibited a circular DNA molecule of 154,289 bp. As expected, it displayed a typical quadripartite structure which included a pair of

IR regions of 25,566 bp, one large single-copy (LSC) of 84,749 bp and a small single-copy (SSC) of 18,408 bp. In total, 125 unique functional genes were detected which included tRNA genes, protein-coding and rRNA genes while its GC content was 37.86%. Ding et al. (2016) also performed a phylogenetic analysis by including, together the cp genome of *S. melongena*, the cp genomes of 12 *Solanum* species as well as *Capsicum annuum* used as an outgroup. The phylogenetic tree obtained following ML analysis clustered *S. melongena* with *S. nigrum* and confirmed genetic relationships previously reported (Wu 2016).

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Genome Annotation

8

Sergio Lanteri and Lorenzo Barchi

Abstract

Genome annotation makes it possible to identify the coding and non-coding regions of a genome, such as exons-introns, regulatory elements, repeats as well as gene functions and locations. The newly developed eggplant genome sequence (see Chap. 7) was masked using RepeatMasker, by combining homology-based and de novo approaches, and ~73% of the eggplant genome was found to include transposable elements (TEs). In total, 34,916 protein-coding genes were predicted, confirming that the diploid gene number in the Solanaceae is around 35,000, as previously reported for tomato (*Solanum lycopersicum* L.), potato (*S. tuberosum* L.) and pepper (*Capsicum* spp.). A total of 108,360 protein sequences from eggplant, pepper and potato were clustered into 22,337 gene families (excluding singletons) using OrthoMCL, with 12,568 gene families (comprising 76,920 genes) in common between the four Solanaceae crops, while 674 eggplant-specific clusters containing 1999 genes were identified. The high-quality eggplant genome sequence offers the possibility to perform comparative genomic studies within species, in order to find variation across individuals for genetic association and linkage analyses, as well as between species, with the goal to perform evolutionary studies. Furthermore, it provides a key resource for the understanding the Solanaceae biology and a key tool for future breeding programmes. The newly developed eggplant genome was also surveyed for the identification of single-locus SSR markers and nearly 133,000 perfect SSRs, a density of 125.5 SSRs/Mbp, as well as about 178,400 imperfect SSRs were identified. Using these data, a public dynamic microsatellite database was developed (www.eggplantmicrosatellite.org), which represents a one-stop resource for the global community of scientists and breeders.

8.1 Identification of Transposable Elements (TEs)

TEs can be grouped into two classes, based upon their manner of transposition: Class I elements or retrotransposons moving via a 'copy-and-paste' manner, and Class II elements moving via a 'cut-and-paste' manner. They can also be classified as autonomous elements, which move by themselves because equipped with requisite molecular features for transposition, or non-autonomous elements, which usually are derived from the former through mutations and cannot move unless they are provided with proteins for their mobility in trans (Kim 2017).

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Before performing gene prediction, the reference genome was masked using RepeatMasker (Smit, AFA, Hubley, R & Green P; http://www.repeatmasker.org) by combining homology-based and de novo approaches. The identified TEs were classified in Class I elements, which primarily consisted of long terminal repeat (LTR) retroelements, known to be predominant in plant genomes (Wicker et al. 2007), short interspersed nuclear elements (SINEs) and long interspersed nuclear elements (LINEs) as well as Class II, which included hAT, En-Spm, Mudra and related sequences. Globally, ~73% of the eggplant genome was masked (Fig. 8.1).

The same approach was used to mask the tomato (v2.5), potato (ITAG v1.0) and pepper genomes which resulted in 67.74, 45.59 and 74.51% masking of ungapped genomic sequences, respectively, in substantial agreement with what was previously reported for these crops

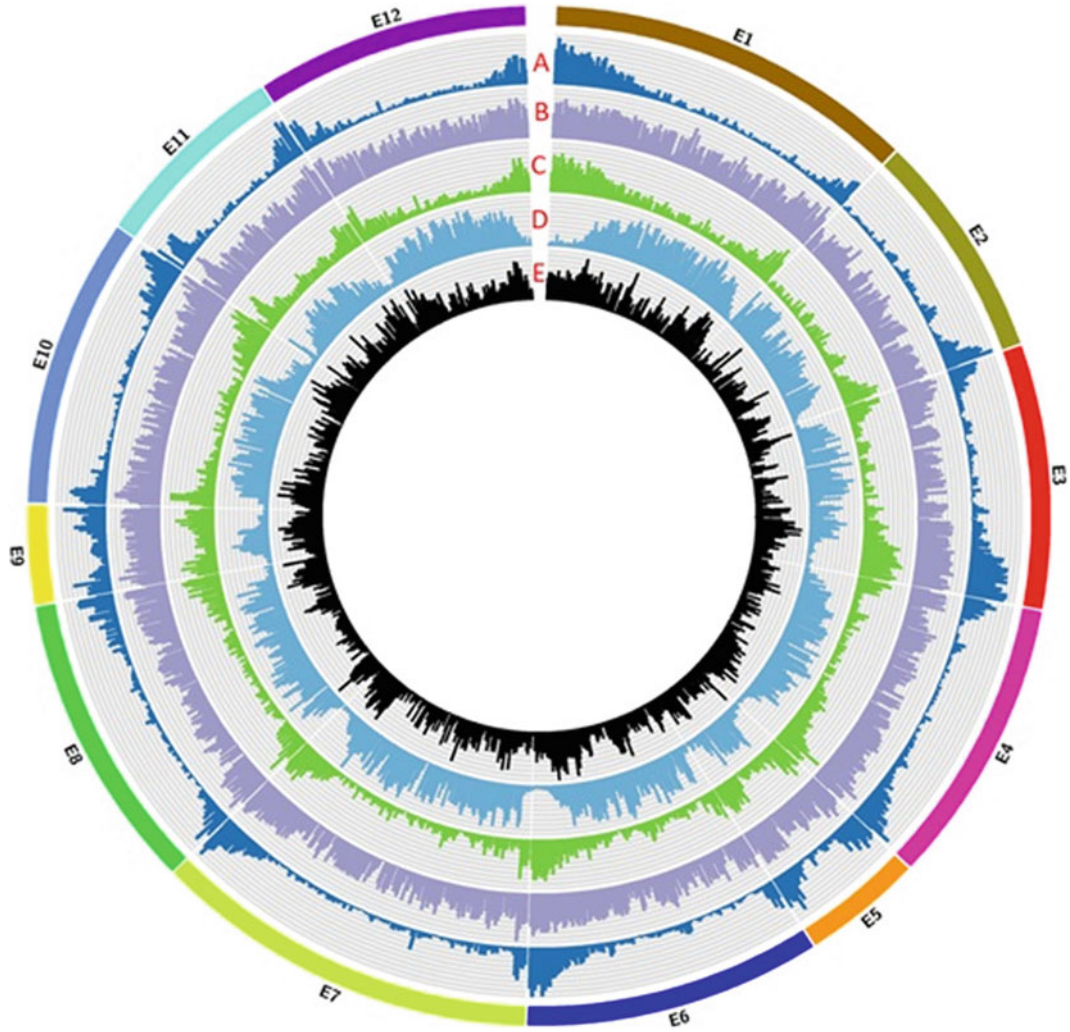

Fig. 8.1 Topography of the eggplant genome. Track A: gene density; Track B: overall repeat density; Track C: DNA repeat density; Track D: LTR-*Gypsy* transposon density; Track E: LTR-*Copia* transposon density. Densities are presented in 1-Mb intervals

(Kim et al. 2014; Lu et al. 2011; Qin et al. 2014; Sato et al. 2012; Xu et al. 2011).

8.2 Gene Prediction and Annotation

Total RNA was isolated from 19 tissues of the inbred line '67/3', including roots, cotyledons, flowers, leaves, stems and fruits at various developmental stages and pooled.

Putative transcripts were constructed from the data obtained from 19 RNA-Seq libraries using the Velvet+Oases pipeline (Schulz et al. 2012), producing 127,244 putative transcripts which were then merged and the redundant sequences removed leading to the identification of 39,408 primary isoforms, each putatively corresponding to a single gene.

The primary transcripts were compared with the proteomes of *Nicotiana benthamiana* (Bombarely et al. 2012), *S. lycopersicum* (Sato et al. 2012), *S. tuberosum* (Xu et al. 2011) and *Arabidopsis thaliana* (Berardini et al. 2015), and their filtering, based on 50% identity and 99% reciprocal coverage with at least one known protein, resulted in 14,353 sequences. The latter were once again compared with the four above-mentioned proteomes to eliminate potential artefacts and then aligned against the reference genome using the software MAKER-P (Campbell et al. 2014). This led to retain a total of 8751 sequences.

Prior to ab initio prediction, a set of 111 control genes was defined from manually curated coding sequences or from full-length cDNA alignments. Out of the 8751 high-quality gene models previously defined from the assembly and analysis of RNA-Seq data, 70 matched genes in the control gene set were excluded. The remaining 8681 gene models were used for the first round of training of three ab initio predictors: (i) GeneID (Parra et al. 2000), (ii) Augustus (Stanke et al. 2006) and (iii) TwinScan (Korf et al. 2001).

The MAKER-P (Campbell et al. 2014) pipeline was applied to integrate the gene models from ab initio prediction and the protein and EST sequences of eggplant, other Solanaceae including tomato and potato as well as other well-annotated species such as *A. thaliana*. In addition, 127,244 contigs obtained by de novo assembly of RNA-Seq were also provided to MAKER-P pipeline. The annotation produced 48,412 transcripts in 44,618 gene loci. For the final annotation, only gene models with an AED (Annotation Edit Distance) of 0.48 or lower were retained, resulting in a final dataset of 34,916 genes.

The assignment of gene functions to protein sequences was performed via phmmer from HMMER package (hmmer.org), on Swiss-Prot and TrEMBL (Trapnell et al. 2012; The UniProt Consortium 2014) databases. In addition, InterProScan (Jones et al. 2014) was used to scan protein sequences against the protein signatures from InterPro (Jones et al. 2014). Based on Hmmer UniProtKB/Swiss-Prot/TrEMBL search, a description of 28,858 genes of the 34,916 predicted eggplant genes was assigned, while InterProScan identified 193,950 protein domains and, on the whole, ~76% of the genes (26,411 out of 34,916) were assigned with at least one domain (6408 unique IPR domain).

In order to evaluate the quality of the annotation, in terms of both completeness and accuracy, the predicted protein sets were validated with different approaches, including by using single-copy orthologs, which relies on the near-universal single-copy orthologs selected from OrthoDB (Waterhouse et al. 2013) and exploited in the software BUSCO (Simão et al. 2015). This algorithm performs a quantitative assessment of transcriptome completeness based on evolutionarily informed expectations of the presence of clade-specific sets of core genes and is based on a more recent datasets than CEGMA (Parra et al. 2007). Eggplant proteins from the Italian consortium (34,916 loci) alongside with those of tomato (ITAG v2.4, 34,725 proteins; Sato et al. 2012), potato (ITAG v1, 35,004 proteins; Xu et al. 2011), pepper (PGA v1.55, 34,899 proteins; Kim et al. 2014) and the eggplant annotation generated by Hirakawa et al. (2014; SME_r2.5.1, 42,035 proteins) were compared with the *Arabidopsis* nuclear proteome (TAIR10, 27,206 proteins; Lamesch et al. 2012).

The results highlighted a higher number of complete single-copy proteins in the annotation of the eggplant genome sequence developed by IGSC (i.e. 1387), with respect to the one also previously detected in eggplant (e.g. 1080) by Hirakawa et al. (2014). The number of annotated complete single-copy proteins was close to those detected in potato and tomato (1396 and 1416, respectively), and higher than detected in pepper (e.g. 1175).

8.3 Comparison Among Solanaceae Genome Sequences

In spite of the very similar number of genes found in the four Solanaceae, the eggplant and pepper genomes are, respectively, ≈1.3-fold and ≈3.5-fold larger than those of tomato and potato, mainly as a consequence of the amplification of *Gypsy* and *Copia* retrotransposons. As evidenced by the data reported in Table 8.1, the quality of the newly developed eggplant genome sequence is comparable to the ones of the already available genome sequences of tomato, potato and pepper and represents a significant improvements in metrics if compared to the one previously published by Hirakawa et al (2014).

On the whole, the genomic landscape of the 12 eggplant chromosomes is similar to the one of the other sequenced Solanaceae, with gene-rich distal chromosome arms and gene-poor pericentromeric heterochromatin (Fig. 8.1).

8.4 Gene Families

A total of 108,360 protein sequences from eggplant, pepper (Kim et al. 2014), tomato (Sato et al. 2012) and potato (Xu et al. 2011) were clustered into 22,337 gene families (except singletons) using OrthoMCL (Li et al. 2003) version 2.0.9 (Fig. 8.2). Among 34,916 protein-coding sequences predicted for eggplant, 27,009 genes were clustered in 17,945 families. A total of 12,568 gene families (including 76,920 genes) were in common between the four Solanaceae crops, of which 606 families (2480 genes) shared among the berry-producing species (eggplant, tomato and pepper), and 2378 families (7864 genes) among the *Solanum* species (eggplant, tomato and potato). All in all 674, 422, 533 and 821 gene families were unique to eggplant, tomato, potato and pepper, respectively. The 674 eggplant-specific clusters contained 1999 genes of which 867 have at least one InterPro domain. The eggplant sequences which did not fall into any

Table 8.1 Assembly and annotation metrics of the eggplant genome and its comparison with previous eggplant, potato, tomato and pepper genomes

	Eggplant (Italian Cons.)	Eggplant (Hirakawa)	Potato (ITAG v1.0)	Tomato (ITAG v2.4)	Pepper (PGA v1.55)
Projected genome size	1.2 Gb	1.13 Gb	844 Mb	900 Mb	3.3 Gb
Number of scaffolds	10,383	33,873	66,254	3,223	37,989
Ungapped length of scaffolds	1.06 Gb (88.3%)	780.2 Mb	585.8 Mb (69%)	737.6 Mb (82%)	2.96 Gb (90%)
Ungapped length of anchored scaffolds	825.5 Mb (69%)	–	585.8 Mb (69%)	719 Mb (80%)	2.67 Gb (81%)
N50 of anchored scaffolds	2.9 Mb	64.5 Kb	1.3 Mb	16.5 Mb	2.4 Mb
Protein-coding genes	34,916	85,446	35,004	34,725	34,899
BUSCO genes present in the annotation	1387 (96.3%)	1080 (75.0%)	1396 (96.9%)	1416 (98.3%)	1175 (81.6%)

BUSCO—Benchmarking Universal Single-Copy Orthologs

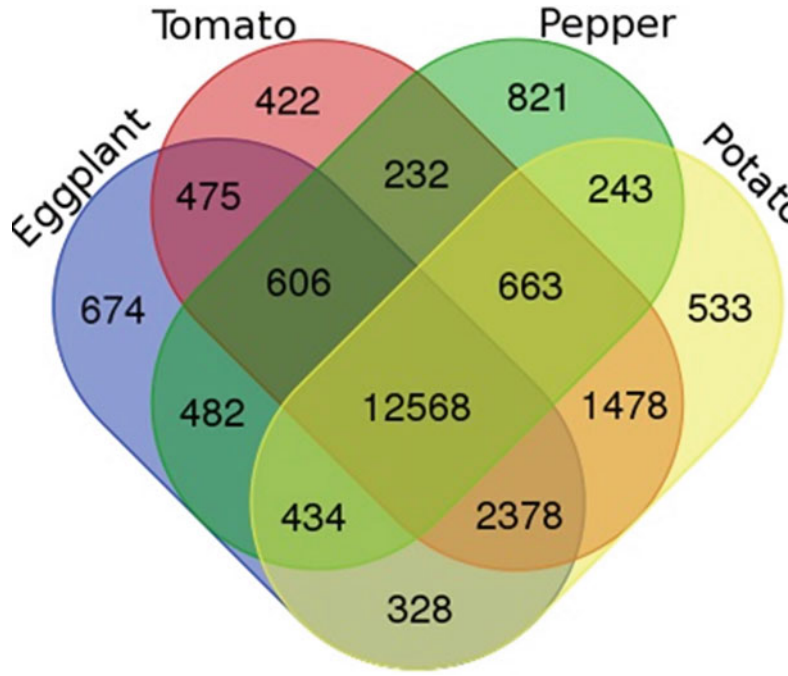

Fig. 8.2 Distribution of orthologous gene families in eggplant, tomato, potato and pepper, calculated with OrthoMCL

cluster (singletons) included 7907 genes, of which 4177 have at least one InterPro domain.

8.5 Discovery of *Solanum melongena* Genome-Wide Microsatellite Markers

Microsatellites (1–10 nucleotides) and minisatellites (>10 nucleotides) are subcategories of tandem repeats (TRs) that, together with the predominant interspersed repeats (or remnants of transposable elements), make up genomic repetitive regions (Vieira et al. 2016). Microsatellites are present in both protein-coding and non-coding regions of the genome, although their occurrence is lower in gene regions as their high mutation rate may compromise gene expression.

Microsatellites or simple sequence repeats (SSRs) have become a common tool in plant genetics analysis and breeding programmes, due to their characteristics of abundance, ubiquity, variation, co-dominance, multi-allelism as well as presumed neutrality. The polymorphism of SSRs generated from the number of repeat units can easily be detected by PCR using primers designed according to the flanking sequences.

The first set of eggplant microsatellites was developed from the screening of small-insert genomic libraries with di- and trinucleotide probes (Nunome et al. 2003a, b). Later, a small set of SSR markers from genic DNA sequence available in public databases was developed by Stàgel et al. (2008), while Nunome et al. (2009) identified over 1000 SSR markers by screening gDNA and cDNA libraries. Barchi et al. (2011) isolated about 2000 putative eggplant SSRs from RAD (restriction site-associated DNA) tags, of which a subset was assessed for polymorphism among the parents of mapping populations. Further, SSR markers were also developed from eggplant genomic libraries enriched for AG/CT motif by Vilanova et al. (2012) and by Gramazio et al. (2016) from transcriptome analysis of scarlet eggplant (*S. aethiopicum*) and *S. incanum*, the wild progenitor of *S. melongena*.

The availability of the high-quality genome sequence of the eggplant inbred line '67/3' has provided the opportunity for identifying single-locus SSR markers following a genome-wide

survey. The 12 pseudomolecules obtained following genome anchoring, as well as the unmapped scaffolds, were used by Portis et al. (2018) for the bulk mining of SSR markers. Perfect, imperfect and compound SSRs were in silico mined using the SciRoKo SSR-search module (Kofler et al. 2007). Microsatellite sequences were considered as a perfect SSR when a motif was repeated at least 15 times (1nt motif), eight times (2nt), five times (3nt) or four times (4–6nt), allowing for only one mismatch. For compound microsatellites, the maximum default interruption (spacer) length was set at 100 bp. In total, 133,831 perfect SSR motifs (density of 125.5 SSR/Mb), which included about 15.5% of compound SSRs, as well as more than 178,000 imperfect SSR motifs, were identified. Table 8.2 reports the distribution of the SSR markers along the 12 eggplant chromosomes. About 21% SSR were present in unanchored scaffolds (chromosome E00).

Dinucleotides were found to be the most common SSR type (42.8%), followed by tri- (37%), mono- (8.4%) and tetranucleotides (7.1%). Penta- and hexanucleotide repeats just represented less than 5% of the set of perfect SSRs (Fig. 8.3).

The average number of SSRs detected on pseudomolecules E01–E12 was 8746 and 11,673 for perfect and imperfect SSRs, respectively. When considered the distribution of different motifs on a chromosome basis, the percentages of the most frequent mono-, di- and trinucleotides were similar to those detected in the whole genome, while the relative contributions of the tetra-, penta- and hexanucleotides varied between chromosomes.

Using the same procedures adopted for the eggplant line 67/3, the presence of perfect SSRs was also scanned in the genome sequences of the Asian eggplant cultivars 'Nakate-Shinkuro' (Hirakawa et al. 2014), and other 13 plant species including 12 members of the Solanaceae family (*S. lycopersicum, S. pimpinellifolium, S. pennellii, S. tuberosum, Capsicum annuum, C. chinense, C. baccatum, Nicotiana tabacum, N. attenuata, N. benthamiana, Petunia axillaris, P. inflata*) and their closely related species *Coffea*

Table 8.2 Chromosome-by-chromosome distribution of perfect, compound and imperfect SSRs. E00 are the sequences not anchored to the 12 eggplant chromosomes

Chromosome	Perfect							Compound	Imperfect
	Mono	Di	Tri	Tetra	Penta	Hexa	Total		
E01	1256	5604	4811	916	415	242	13,244	2078	17,432
E02	514	3006	2764	449	191	137	7061	1223	9453
E03	1045	3458	2856	760	356	212	8687	1165	12,004
E04	799	4023	3184	661	284	147	9098	1271	12,525
E05	508	2027	1522	383	163	87	4690	473	6722
E06	965	4285	3913	819	268	194	10,444	1874	13,540
E07	847	5330	4461	799	297	214	11948	2130	15,617
E08	801	4056	3108	669	267	155	9056	1325	12,356
E09	551	1809	1719	381	156	91	4707	875	5994
E10	792	4186	3959	760	271	246	10,214	1834	13,465
E11	576	3069	2711	489	184	139	7168	1081	9423
E12	673	3521	3466	559	246	168	8633	1471	11,545
E00	1825	12,506	10,614	1783	652	501	27,881	3870	38,331
Total	11,152	56,880	49,088	9428	3750	2533	132,831	20,670	178,407

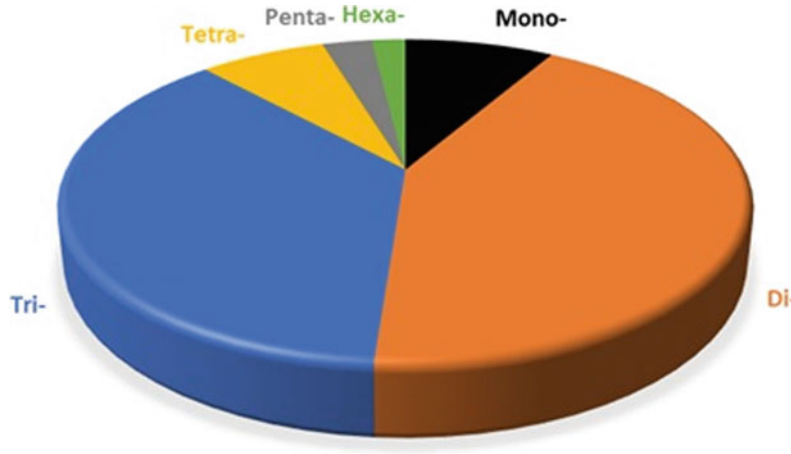

Fig. 8.3 Distribution of the major repeat types in the eggplant genome

canephora. The number of perfect SSRs found in the genome sequence of the eggplant line '67/3' was more than one-third higher than the one detectable in the previously developed genome sequence of the cultivar 'Nakate-Shinkuro' (Hirakawa et al. 2014), and this was attributed to a better quality of the genomic sequence of the former. Considering all 14 species, the genome size was found to be positively associated with the number of identified SSR motifs; however, species possessing larger genomes, such as *Capsicum* species, showed lower SSR density (SSRs/Mb). Indeed, in *Capsicum* species, the larger genome size has been found mainly attributable to an accumulation of *Gypsy* and *Caulimoviridae* family elements (Kim et al. 2014).

With the goal to provide browsable access to the SSR data, Portis et al. (2018) developed a user-friendly tool, Eggplant Microsatellite DataBase (EgMiDB, which is available, at www.eggplantmicrosatellite.org). The EgMiDB makes it possible to identify SSR markers in terms of their location on the genome, type of repeat (perfect vs. imperfect), motif type, sequence, repeat number and genomic/gene context. Furthermore, it also suggests forward and reverse primers for PCR amplification and identifies markers with thermodynamic compatibility for multiplex designing. At last, taking advantage of transcriptome/genomic resources available in databases, an in silico validation of SSR markers available in the database demonstrated that they provide key information for studies of population structure, genetic mapping and evolutionary processes.

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Resequencing

9

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Abstract

The next-generation sequencing revolution is allowing the whole-genome resequencing (WGRS) of hundreds or even thousands of accessions for staple crops and model species. With the release of their reference genome, progressively also other plants, species are undertaking WGRS projects for a broad variety of studies. In common eggplant (*Solanum melongena* L.), although a first draft of the reference genome sequence has been published, no resequencing studies have been performed so far. In this chapter, we present the first results of the resequencing of eight accessions, seven of common eggplant and one of the wild relative *S. incanum* L., that correspond to the parents of a multi-parent advanced generation inter-cross (MAGIC) population that is currently under development using the newly developed eggplant genome sequence presented in Chap. 7 of this book. Over ten million polymorphisms were identified among the accessions, 90% of them in the wild related *S. incanum*, confirming the genetic erosion of the cultivated common eggplant. Among the MAGIC population parents, the common polymorphism distribution pattern along the chromosomes has revealed possible footprints of ancestral introgression from interspecific crosses. The set of polymorphisms has been extensively annotated and currently is being used for further analyses in order to efficiently genotype the ongoing MAGIC population and to dissect important agronomic and morphological traits. The information provided in this first resequencing study in eggplant will be extremely helpful to assist plant breeding to develop new improved and resilient varieties to face future threats and challenges.

9.1 Introduction

The release of the first drafts and complete genomes of the most important cultivated crops has represented a scientific milestone in plant biology. For the first time ever, genome sequences have provided a vast amount of information for a comprehensive analysis of the genome structures, genes and repetitive elements, among others (Huang et al. 2013). However, the information retrieved from the reference genome sequence is not sufficient to provide a comprehensive picture of the structural and allelic variation of a species or of its related genepool materials (Schatz et al. 2014). The continuing improvements in sequencing technologies coupled with the significant decrease of the

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sequencing costs have opened the way for the whole-genome resequencing (WGRS) of hundreds of cultivated accessions and wild relatives for model crops and the most important economically cultivated crops such as *Arabidopsis thaliana*, tomato, rice, soybean, and cotton, among others (Weigel and Mott 2009; Xu et al. 2012; Aflitos et al. 2014; Zhou et al. 2015; Du et al. 2018). In fact, WGRS can extremely speed up the challenging task of reconstructing the 'pan-genome puzzle' of a species through the identification of global polymorphisms and gene variations, genomic structural variations, gene copy number, or copy number variation (Jha et al. 2016).

With WGRS, the natural variation of a crop can be easily captured through the identification of millions of robust polymorphisms among accessions, allowing researchers to perform forward genetic techniques and genome-wide association studies, and thus unravelling the genetic basis of complex traits of agronomic importance (Ogura and Busch 2015). WGRS can also shed light on the history of a crop and identify the genetic diversity bottlenecks that occurred during domestication, and the genes that are associated with this process. For example, Zhou et al. (2015) were able to detect 230 selective sweeps and 162 selected copy number variants associated with ten genomic regions and nine domestication traits in addition to the identification of 13 previously uncharacterized loci for agronomic traits in soybean including oil content, plant height and pubescence form. Moreover, using this approach, it is possible to associate genes and traits with geographical areas revealing how populations and subpopulations have adapted to a specific geographic area (Qi et al. 2013). Ultimately, the reconstruction and identification of the different stages of breeding history and artificial selection by WGRS provide new and most efficient tools and strategies for future crop breeding and biotechnology (Jiao et al. 2012).

9.2 Resequencing in Eggplant

To our knowledge, no resequencing studies have been published in eggplant (*Solanum melongena* L.) so far. In fact, despite the economic importance of this crop, which ranks fifth among vegetables in total worldwide production (Faostat 2016), and its role to guarantee food security in tropical and subtropical regions, few genomic studies have been performed in eggplant and its wild relatives (Gramazio et al. 2018). The disparity between eggplant and other important cultivated crops for genomic data and information is still large, although some efforts are being done to narrow the gap. In comparison, in tomato, several resequencing studies have been published, including the resequencing of 360 cultivated and wild relative accessions representing several geographical origins, consumption types and improvement statuses (Lin et al. 2014), 84 tomato accessions and related wild species to explore genetic variation (Aflitos et al. 2014), experimental populations (Causse et al. 2013; Kevei et al. 2015; Zhang et al. 2018), elite cultivars (Kobayashi et al. 2014; Jung et al. 2016), mutants (Shirasawa et al. 2016), abiotic stress tolerance (Tranchida-Lombardo et al. 2018), among others.

The lack of resequencing studies in eggplant might be, in part, due to the unavailability of a high-quality reference genome sequence and the corresponding annotation. Up to now, the eggplant research community can rely on just a draft eggplant genome published in 2014, which is fragmented in 33,873 scaffolds covering 833.1 Mb (ca. 74% of the eggplant genome) and where 85,446 genes were predicted (Hirakawa et al. 2014). Thus, mapping a WGRS dataset onto this eggplant genome sequence could lead to a loss of valuable information about the target of the study. A new high-quality eggplant genome sequence, obtained by the 'Italian Eggplant Genome Sequencing Consortium' (http://www.eggplantgenome.org/), has been presented and

will soon be released (see Chap. 7 in this book, Barchi et al. 2016). Based on the statistics presented, this new reference genome is much less fragmented and the number of genes annotated is about 35,000, very similar to those described in the close relative tomato (Sato et al. 2012). The imminent availability of this high-quality reference genome will foster genomic studies as has occurred in other cultivated species. In fact, our group, which had access to this improved genome sequence thanks to fruitful collaborations with the members of the 'Italian Eggplant Genome Sequencing Consortium', took the opportunity to use this valuable information to assist several research lines, including a resequencing study. Among them, we have performed the WGRS of eight accessions that correspond to the parents of a multi-parent advanced generation inter-cross (MAGIC) population that we are currently developing. Seven out of the eight parents correspond to common eggplants (*S. melongena*) from different geographic areas. These accessions are phenotypically very diverse, showing substantial differences in fruit size, fruit shape, fruit colour, calyx prickliness, and many other agronomic and morphological traits. The eighth parent is a *S. incanum* accession, a wild species from the secondary genepool of common eggplant (Syfert et al. 2016). *Solanum incanum* is very interesting for eggplant breeding since has been reported as a powerful source of phenolic compounds, showing contents several times higher than common eggplant (Stommel and Whitaker 2003; Prohens et al. 2013), and is tolerant to some biotic and abiotic stresses, mainly drought (Knapp et al. 2013). The specific *S. incanum* accession (MM577) used for this WGRS has been extensively characterized in other studies for several traits (Stommel and Whitaker 2003; Gisbert et al. 2011; Salas et al. 2011; Meyer et al. 2015). In addition, MM577 and one of the seven MAGIC parents, AN-S-26, have been used to build an interspecific genetic linkage map to locate the candidate genes involved in the chlorogenic acid biosynthesis pathway and other candidate genes of agronomic interests, as well as, the candidate genes involved in the fruit flesh browning (Gramazio et al. 2014). Subsequently, this mapping population was used to develop the first introgression line population in eggplant genepool (Gramazio et al. 2017).

The main goal of this WGRS project was to provide a large set of molecular markers among the eight founder lines to efficiently assist the genotyping of the first MAGIC population in eggplant, as well as, to dissect the genetic base of complex traits of agronomic importance in eggplant, detect potential introgressions associated to domestication and geographical areas and ultimately provide tools to clarify the eggplant evolutionary history and enhance eggplant breeding.

9.3 High-Throughput Sequencing and Mapping

To generate a large set of high-resolution SNPs distributed throughout the genome, a whole-genome sequencing approach was adopted. After preparing the Illumina paired-end libraries of 300 bp, the MAGIC parents were sequenced on two lines of an Illumina HiSeq 4000 sequencer. The sequencing produced over 100 Gb of data with a range of 150–220 million raw reads per sample (Fig. 9.1). Less than 3% of the raw reads were discarded after the trimming and cleaning process and the remaining clean reads were mapped onto the high-quality reference genome (Barchi et al. 2016). Over 80% of the reads were successfully mapped with an overall coverage of around 20×. Thanks to the newly improved Illumina platforms, it becomes affordable to have a good coverage also for genomes of medium size as *S. melongena* (around 1.2 Gb). In fact, just a few years ago, the most common mapping coverage in WGRS projects was around 10× or less for small- to medium-sized genomes such as rice, tomato or soybean (Causse et al. 2013; Zhou et al. 2015; Xu et al. 2012). Although single-molecule real-time sequencing (SMRT) platforms are becoming more popular for de novo whole-genome sequencing, the Illumina platform is by far the most frequently selected sequencing

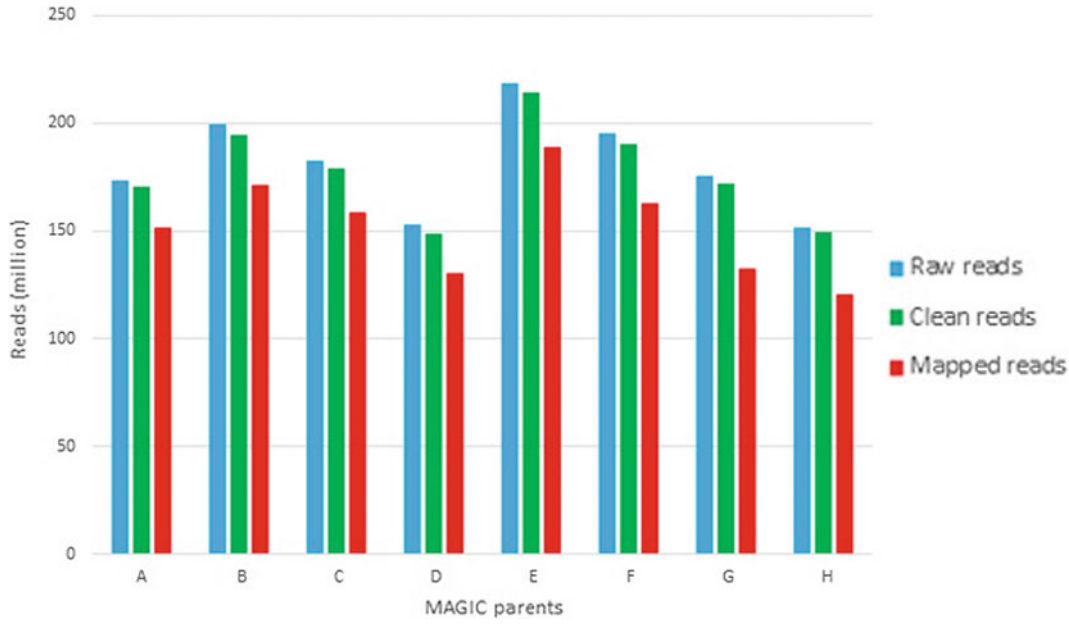

Fig. 9.1 Statistics of the sequencing of the eight MAGIC parents and the read mapping onto the eggplant reference genome. The codes A to G correspond to *S. melongena*, while the code H to *S. incanum*

technology for WGRS studies, especially for high-quality and completed genomes, since in this scenario the short read length is not a limitation and the higher throughput compared to other technologies is preferred. New Illumina platforms, like NovaSeq 6000 System, can give an impressive sequencing output up to 6 Tb of data that correspond to around 20 billion paired-end reads and thus may further decrease sequencing costs, which may foster resequencing studies in eggplant.

9.4 Variant Calling, Distribution and Annotation

Over ten million polymorphisms were identified among the eight MAGIC parents resequenced, most of which were SNPs. While among the *S. melongena* accessions, the variants identified were around one million per accession, for the *S. incanum* accession the number of variants was over nine million (Fig. 9.2). This large difference in polymorphisms between cultivated and wild relative species is quite common for the most economically important and staple crops, where artificial selection for important breeding traits and the seeking to uniformity for commercial varieties have dramatically increased their genetic erosion (Aflitos et al. 2014; Zhou et al. 2015). Before the advent of the next-generation sequencing era, the development of reliable molecular markers was not an easy and inexpensive task. In consequence, many crops, in particular non-model crops, had been neglected from research studies and molecular-marker-assisted selection (MAS). Common eggplant was one of them and just a few years ago the gap with other economic important crops has been narrowed thanks to the first genomic studies performed (Gramazio et al. 2018).

The variants detected were divided into 10-Mbp-sized bins in order to identify similar patterns of polymorphisms distribution and associate them with potential common ancestral introgressions. Figure 9.3 shows an example of the distribution of homozygous SNPs for eggplant chromosome 6. It is very clear that the common eggplant accessions C, D, E and G presented a similar SNP distribution pattern from

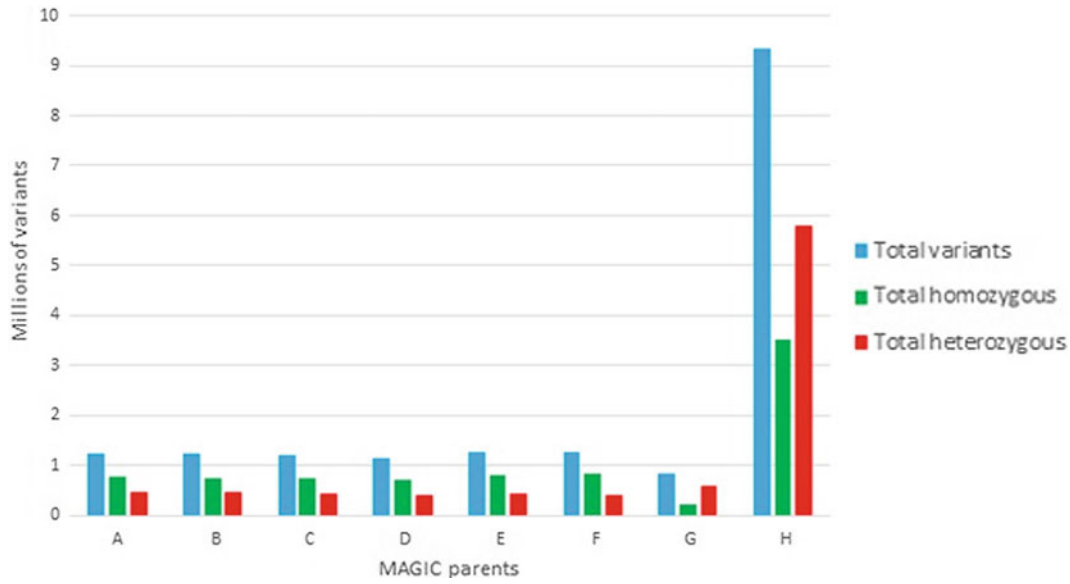

Fig. 9.2 Statistics of the variants identified in the eight MAGIC parents. The codes A to G correspond to *S. melongena*, while the code H to *S. incanum*

the beginning of the chromosome 6 to about 25 Mbp and then until 60 Mbp the accessions C and D shared other common peaks while the accessions E and G did not present the same ones. This similar SNPs distribution represented by these peaks may be a footprint of an old interspecific introgression from a common eggplant relative. Then, the accessions C and D, which are from the same geographical area and probably shared a recent ancestor, could have incorporated an additional introgression resulted from another hybridization event. An alternative hypothesis could be that the accessions E and G might have lost part of the introgression during the domestication events.

In addition, the variants were annotated and classified by impact (high, low, moderate or modifier), by functional class (missense, nonsense or silent mutation), by the type (start lost, stop gained, stop lost, and others) and region affected (intergenic, intron, exon, and others), as well as, DNA substitution mutations (transitions and transversions) and amino acids changes. At the time this chapter is being written many analyses are being performed using the information generated in this WGRS study, including repetitive elements, copy number variations (CNVs), relationship analyses among the accessions, and the search for candidate genes underlying important agronomic traits.

9.5 Conclusions

The combination of the decreasing cost of sequencing and the availability of high-quality genome sequences are boosting resequencing studies even for non-model plant species, including eggplant. Although for model plants like *Arabidopsis thaliana* (Weigel and Mott 2009) or important staple crops like rice (Guo et al. 2014) thousands of accessions have been resequenced during the last decade, the first resequencing studies in other species of scientific or economic interest are, little by little, being published. The potential of resequencing to interrogate the whole genome of eggplant and identify structural and functional variation among accessions makes it a great powerful analysis and inquiry strength. Furthermore, its high versatility of approaches and strategies allows answering many scientific and technical

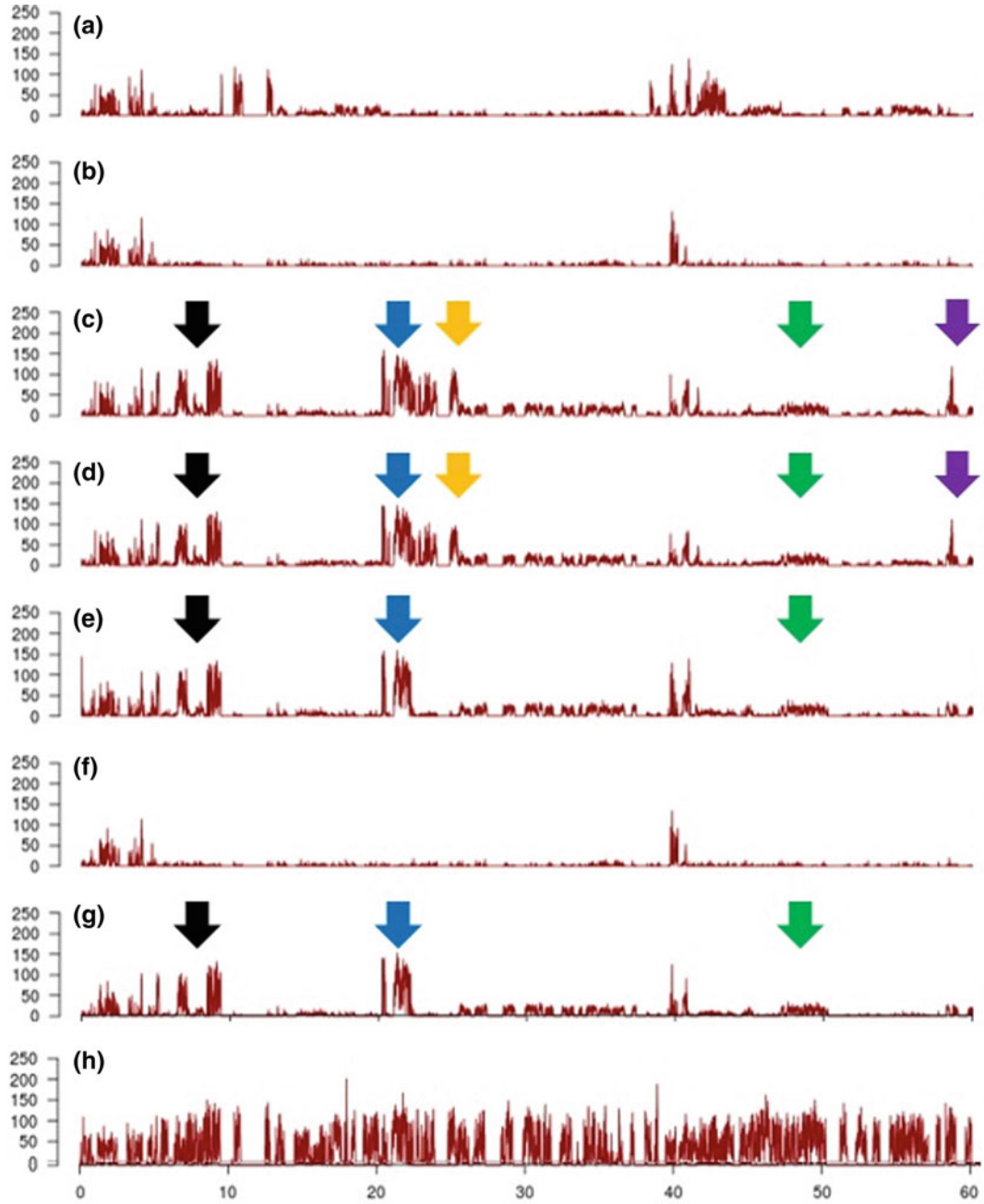

Fig. 9.3 Distribution of homozygous variants along the first part of chromosome 6 divided into 10-Mbp-sized bins (in red). The *x*-axis represents the Mbp of chromosome 6 and *y*-axis the number of homozygous SNPs identified. The arrows of the same colour indicate the similar SNP distribution pattern. The codes A to G correspond to *S. melongena*, while the code H to *S. incanum*

questions, including allele and variants discovery, germplasm genomic characterization, domestication history, or dissecting agronomic-associated loci for plant breeding, among others. The first resequencing efforts performed in eggplant can boost the gathering of genomic data from the germplasm of this species and wild relatives, which may be pivotal to develop a new generation of improved eggplant varieties adapted to present and future challenges in eggplant production and fruit quality.

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10 Eggplants and Relatives: From Exploring Their Diversity and Phylogenetic Relationships to Conservation Challenges

Xavier Aubriot and Marie-Christine Daunay

Abstract

Eggplant is a generic name encompassing several species of *Solanum* cultivated for their fruits, in particular the aubergine (*S. melongena* L.) of East Asian origin, and the two African indigenous Scarlet (*S. aethiopicum* L.) and Gboma (*S. macrocarpon* L.) eggplants. These three species are closely related to each other and share a common set of numerous wild relatives, all belonging to the megadiverse subgenus *Leptostemonum* Bitter (also known as the spiny solanums). As a whole, the taxonomic and phylogenetic treatment of these wild species is arduous and unstable, mostly due to their high number and the rapid evolution of the technical tools and conceptual approaches used for assessing their relationships. Large scale and carefully sampled phylogenetic studies of the last decade have dramatically improved our in-depth understanding of genus *Solanum*. Eggplant's closest relatives are African and Asian species belonging to subgenus *Leptostemonum*; but species of the same subgenus that originate from Australia, the Pacific and the Americas also deserve attention. Here, we provide a historical survey of the last taxonomic treatments and phylogenetic analyses for genus *Solanum*. We hope that this will familiarise the reader with this wide topic and will ease up his/her orientation through the specialised and extensive literature. Updated inventories of eggplants and related species organised by geographical areas of origin are completed with information as to whether the species are maintained in ex situ collections. Challenges associated to ex situ eggplants germplasm conservation are also discussed. To complete the picture, other *Solanum* species that are sometimes allocated the generic term of eggplants are briefly mentioned.

10.1 Introduction

Eggplant is a name commonly applied to at least three species of *Solanum*: *Solanum melongena* L. also known as aubergine, a globally cultivated crop of Asian origin, as well as the African indigenous scarlet (*S. aethiopicum* L.) and Gboma (*S. macrocarpon* L.) eggplants. Outside Africa, *S. aethiopicum* is grown commercially in Brazil and has some local developments such as in Southern Italy. Both species have spread in Asia. *Solanum melongena* is by far the most economically important eggplant, grown at large scales on all continents, and bred for decades by

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many research institutes, universities and seed companies. Accordingly, the scientific literature on its genetics is much richer than that of African eggplants. Common eggplants are diploid with $2n = 24$ chromosomes and share a similar floral biology: their reproduction regime is partially autogamous, with an allogamy rate depending on insect pollinator's frequency. In their respective areas of origin and domestication, wild forms grow in sympatry with landraces and cultivars. Natural gene flows between wild and cultivated materials, and subsequent natural and human selection, gave rise to intermediate phenotypes bearing variable associations of wild and domesticated traits, in particular for *S. melongena* and *S. macrocarpon*.

Eggplants share a common and wide set of wild relatives of Old World and New World origins, the inventory and classification of which evolved strongly from the 1970s onwards, first thanks to the scientific inputs of the University of Birmingham (UK), then via several NSF-funded projects (USA). From the 1990s onwards, the use of chloroplast and nuclear DNA markers for phylogenetic purposes, combined to the development of statistical algorithms, rocked the systematics of genus *Solanum*. Revamped species delimitations for African and Asian material, together with identification of a significant number of new species from Australia and New Guinea, have resulted into important taxonomic changes, with significant reshuffling of scientific names and addition of new names.

This chapter starts with an overall picture of the genus *Solanum* and its taxonomic treatment, before focusing on subgenus *Leptostemonum* Bitter. These backgrounds provide a practical baseline for understanding the variation of, and matches between, species names and ranking categories, along the successive publications on eggplants and their relatives. The readers in a hurry can jump directly to the geographically structured inventories of the species related to eggplants. These are followed by a brief outline of the challenges faced by germplasm holders and breeders in this context.

We hope that the reader will find hereafter a useful and practical body of information on the diversity of eggplants and their wild relatives which will ideally inspire and guide future research programmes.

10.2 The Genus *Solanum* and Its Taxonomic Treatment

The majority of *Solanum* species are native to the New World. They are characterised by flattened seeds and curved embryos, traits that are shared by all members of the Solanoideae subfamily (Hunziker 1979). Diploidy and self-compatibility are common features in *Solanum* species, with the exception of (1) the groups of nightshades and potatoes that include many polyploid species, and (2) the groups of pepinos, potatoes and tomatoes where gametophytic self-incompatibility is commonly found (Correll 1962; Edmonds 1977; Whalen and Anderson 1981). *Solanum* species sexuality is mostly andromonoecious and hermaphroditic (Symon 1979). The genus *Solanum* is one of the largest genera of vascular plants, with a number of species estimated between 1000 (D'Arcy 1991) and 1400 species (D'Arcy 1979). The combination of high species richness, considerable phenotypic plasticity of the species, and their presence in a wide range of climatic and ecological conditions on all continents (except Antarctica), explain why botanists struggled, and still struggle, to study, circumscribe and classify the genus. Over time, generations of botanists created an even greater wealth of species names, estimated between 3600 (D'Arcy 1979) to over 5000 (Child and Lester 2001), with the consecutive results of complicating the situation with numerous synonymies and homonymies.

As a caveat, it should be noticed that this chapter provides a state of the art. As botanical and taxonomic researches are still actively ongoing, we thus advise the reader to constantly refer to the most up-to-date publications on the subject.

10.2.1 Classifications Based on Phenotypic Similarities Between Species

Initial circumscription of the genus *Solanum* (Linnaeus 1753) included 23 species. It was divided into two groups, "Inermia" and "Spinosa", on the basis of the presence of "spines".[1] The French botanist Michel Félix Dunal later described a number of new species (Dunal 1813, 1816); his work ended up with a monograph that included 901 species of *Solanum* (Dunal 1852); it is the last time *Solanum* was revised in its entirety. Dunal's groupings were based on prickliness and anther morphology (Bohs 2005). These artificial groupings were further adjusted with the progresses in regional inventories along the twentieth century mostly on the basis of taxa similarities for hair type, branching patterns and shoot morphology. Development of scientific techniques led to the use of more detailed criteria (e.g. trichome morphology, ultrastructure of seed teguments, ploidy level, chromosomes, secondary metabolites, seed proteins and enzymes) and contributed to stepwise refinements of the classification.

In 1972, D'Arcy divided *Solanum* into seven subgenera, viz. *Archaesolanum* Marzell, *Bassovia* (Aubl.) Bitter, *Brevantherum* (Seithe) D'Arcy, *Leptostemonum*, *Lyciosolanum* Bitter, *Potatoe* (G.Don) D'Arcy and *Solanum*. Each of these subgenera was subdivided into sections, reaching a total of 60–70 sections (D'Arcy 1972), themselves subdivided into subsections, series and subseries. Later on, the total number of sections was decreased to 62, and subgenus *Brevantherum* was reduced to sectional rank and placed into the newly circumscribed subgenus *Minon* Raf. (D'Arcy 1991). This instability in infrageneric rankings and names was discussed in several papers (Knapp 1983; Weese and Bohs 2007), but these subdivisions stayed in use in many of the synopsis and taxonomic revisions that were later on published. Among these works, we can cite the synoptic treatments (1) for ca. 850 New World *Solanum* species (Nee 1999), and (2) for 110 species originating or introduced in Africa (Jaeger and Hepper 1986), as well as the taxonomic revisions of Australian and New Guinean spiny solanums (Symon 1981, 1985). The last synopsis of the entirety of genus *Solanum* dates from 2001 (Child and Lester 2001). At the beginning of the rise of phylogenetic approaches in the 1990s, these morphological classifications had ended up with several well-defined subgenera and sections coexisting with many poorly circumscribed groups (Bohs and Olmstead 1997). For the readers interested in getting more thorough information, we recommend reviews of historical systematics of Solanaceae (D'Arcy 1979, 1991; Nee 2001), as well as a detailed survey of the whole family (Hunziker 2001).

10.2.2 Classifications Based on Phylogenetic Relationships Between Species

Within the last ca. thirty years, the use of phylogenetic methods spread among taxonomists and revolutionised scientific classifications. Thanks to the combined improvement of phylogenetic algorithms, and development of affordable DNA-sequencing techniques, taxonomists now aim at building classifications that reflect species relationships, i.e. phylogenetic classifications. Although phylogenetic inferences can be carried out on any type of biological data (e.g. phenotypic or genetic), molecular data soon became the overwhelming source of phylogenetic hypotheses. This is due to several intrinsic and extrinsic advantages of molecular over morphological data. Molecular data can be represented as a string of four discrete states, viz. the four nucleotide bases of DNA. This enables (1) systematic comparisons between closely or distantly related species, and (2) implementation of more or less complex statistical evolutionary models that take into account various properties of DNA—e.g. variability in substitution rates. Also, with the

[1] Anatomically speaking, *Solanum* species produce prickles which derive from epidermis tissues; they can be found anywhere on the aerial parts of the plant.

continual improvement of sequencing techniques, molecular data have become increasingly straightforward to generate, even for a large number of taxa or DNA region. Systematics relatively rapidly adapted to the DNA revolution; chloroplast DNA, because of its highly conserved structure and gene content across taxa, was preferentially used for phylogenetic reconstruction in land plants. Solanaceae systematics benefited from a number of large scale phylogenetic studies that included an ever-increasing taxonomic sampling (Olmstead and Palmer 1992; Olmstead and Bohs 2007; Olmstead et al. 2008; Särkinen et al. 2013). This led to drastic classification changes, such as the inclusion of the former genera *Lycopersicon* Mill. and *Cyphomandra* Mart. ex Sendtn. within genus *Solanum* (Spooner et al. 1993; Bohs 1995; Bohs and Olmstead 1997).

The first phylogenetic analysis focused on phylogenetic relationships within genus *Solanum* (Bohs and Olmstead 1997) was based on the sole chloroplast gene *ndh*F and was carried out on 25 species, representing all of the seven recognised subgenera, with the exception of subg. *Bassovia* and subg. *Lyciosolanum*. Four major monophyletic units, or clades (groups that include a hypothetical common ancestor and all of its descendants), were identified, but their relationships were poorly resolved. This was followed by a study based on chloroplast restriction site variation that used an enlarged taxonomic sampling of 49 *Solanum* species, but *Bassovia* and *Lyciosolanum* were still not included (Olmstead and Palmer 1997). This later analysis yielded three clades; their taxonomic compositions were partly consistent with the ones of the clades obtained by Bohs and Olmstead (1997). The correspondence between subgenera and sections on one hand and clades on the other hand was partial.

The next phylogenetic study included a broad sampling of *Solanum* subgroups and again used the plastid gene *ndh*F (Bohs 2005). It included 112 species of *Solanum* that belonged to the seven subgenera defined by D'Arcy (1972). It revealed 12 major and statistically well-supported clades within *Solanum* (Bohs 2005). These clades were allocated informal taxonomic designations, some being synonyms of historical names of subgenera and sections (e.g. Leptostemonum clade, Dulcamara clade). Some other clades (Geminata clade and Cyphomandra clade) bore names of taxa that were not included in the formal ranks of D'Arcy (1972). This nomenclatural heterogeneity closed the era of almost clear cut and stable ranking categories within *Solanum*. Most clades within *Solanum* were found in a large basal polytomy; this clearly revealed a lack of overall phylogenetic resolution.

Later on, a set of three DNA regions, the plastid gene *ndh*F, the intergenic spacer *trn*T-F and the nuclear gene *waxy*, were sequenced for a total of 102 species in order to assemble a sampling that was consistent with previous analyses, as well as representative of the systematic and morphological diversity of the genus; seven additional Solanaceae taxa served as outgroup (Weese and Bohs 2007). Maximum parsimony and Bayesian methods were run for separate and combined data sets. The resulting consensus tree displayed the same global architecture than the one of Bohs (2005), but with much greater resolution and statistical support for the internal nodes of the phylogeny. The eggplants and their wild relatives were shown to pertain to the largest monophyletic group within *Solanum*, i.e. the Leptostemonum clade. Several major instances of polytomies were still recovered, but in smaller proportion than in Bohs (2005).

The last global picture of Solanaceae and *Solanum* (Fig. 10.1) carried out with a very dense species sampling (Särkinen et al. 2013) confirmed the monophyly of subgenus *Leptostemonum* and was the first attempt to provide a robust framework for estimating divergence time in Solanaceae and *Solanum*. The genus was found to be of early Miocene origin, and subgenus *Leptostemonum* was hypothesised to have diversified within the last 10 million years.

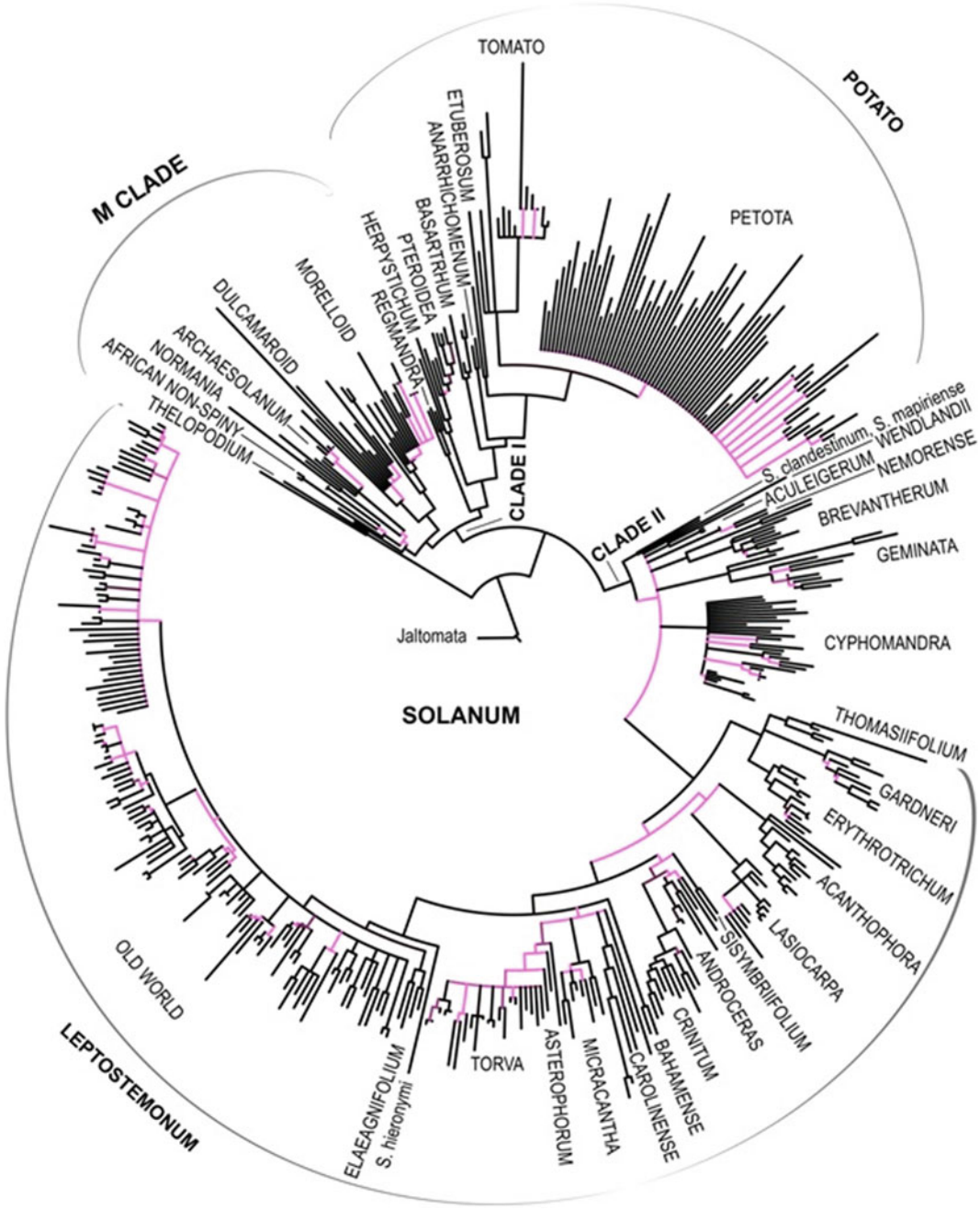

Fig. 10.1 Phylogenetic relationships between major clades of *Solanum*. Major clades recovered by previous phylogenetic studies are labelled; the M Clade is identified for the first time here. Clades with low bootstrap support (60–79%) are shown in pink, while strongly supported clades (boostrap support 80–100%) are in black. Modified from Särkinen et al. (2013) under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/2.0)

10.2.3 Phylogenetic Relationships Within Subgenus *Leptostemonum* (spiny solanums)

The species belonging to subgenus *Leptostemonum* can be found across the Southern Hemisphere, both in the Neotropics and Paleotropics. Their growth habits vary from annual herbs to shrubs (occasionally small trees) and are found in "montane and open seasonal forest, dry scrub woodland, savannas and successional formations associated with disturbance" (Whalen 1984). Species are considered as diploid and self-compatible (Whalen and Anderson 1981) although these traits have not been checked exhaustively, and exceptions are mentioned here and there in the literature. Sexuality is dominantly andromonoecious or hermaphroditic, with few cases of androdioecy (Symon 1979) or dioecy (Martine et al. 2006, 2009) for Australian species. Species of this subgenus are characterised by a combination of stellate trichomes, long tapering anthers with distal pores of dehiscence and usually the presence of prickles. This latter characteristic explains why species of subgenus *Leptostemonum* are commonly referred to as "spiny solanums", although anatomically speaking their "spines" are prickles.

Whalen (1984) published the first phylogenetic classification of subgenus *Leptostemonum*. He compiled morphological and biogeographical elements to suggest hypotheses on species relationships and used these hypotheses to provide an informal classification scheme. Around 450 species were listed and organised into a system of 33 groups, with a "catch-all" 34th group composed of unallocated species.

Phylogenetic relationships across the whole spiny solanums (Levin et al. 2006) were first investigated with three DNA regions—the chloroplast intergenic spacer *trn*S-G and two nuclear markers (*waxy* and ITS). A set of 112 species, representative of previous systems of classification (D'Arcy's sections and Whalen's groups), were sampled. The results confirmed the monophyly of subgenus *Leptostemonum* and split the subgenus into ten major clades. Nine of these clades were named after species names, viz. Acanthophora, Androceras/Crinitum, Bahamense, Carolinense, Eleagnifolium, Lasiocarpa, Micracantha, Robustum and Torva clades. The tenth one, the most species rich that includes the cultivated eggplants, was named after the native geographical distribution of the species, viz. the Old World Clade. Relationships between clades and among clades were, however, often moderately to poorly resolved; in particular, the Old World clade displayed a very low level of resolution, most of the species being part of a large polytomy.

Difficulties to unravel spiny solanum relationships at the species level have been tackled by later phylogenetic analyses by (1) focusing on geographically defined species subgroups, and (2) using a more representative sampling. Major studies were dedicated to the resolution of phylogenetic relationships among New World (Stern et al. 2011) and Old World spiny solanums (Vorontsova et al. 2013; Aubriot et al. 2016b). For Old World species, the sampling concentrated on spiny solanums native to Africa (Vorontsova et al. 2013) and from tropical Asia (Aubriot et al. 2016b). An account of these phylogenetical works is provided below.

10.2.3.1 New World spiny solanums

The sampling assembled by Stern et al. (2011) accounted for 102 taxa; it aimed at correcting the sampling limits of previous studies (Levin et al. 2006; Weese and Bohs 2007), by (1) including missing sections, and (2) completing insufficiently represented sections and geographical origins. Non-spiny solanums were used as outgroups, and *Solanum laciniatum* G.Forst., a member of the Archaesolanum clade, was used to root all analyses. Stern et al. (2011) selected the same markers as those used in Levin et al. (2006), with the exception of *trn*S-G; this later region was replaced by the longer and more variable chloroplast marker *trn*T-F. Parsimony and Bayesian analyses yielded a strict consensus in which subgenus *Leptostemonum* was divided into 14 clades, pertaining to two big clades (Stern

et al. 2011). The largest one included 11 clades, viz. Acanthophora, Androceras/Crinitum, Asterophorum, Bahamense, Carolinense, Eleagnifolium, Lasiocarpa, Micracantha, Old World, Sisymbriifolium and Torva clades. The other major clade included three New World clades, viz. Erythrotrichum, Gardneri and Thomasifolium clades. With its enlarged taxonomic sampling and improved resolution, this study was the first to provide a reliable phylogenetic framework for subgenus *Leptostemonum*. However, the phylogenies still suffered from a number of irresolution, in particular for the relationships between the clades Asterophorum, Bahamense, Carolinense, Micracantha and Torva.

10.2.3.2 Old World spiny solanums

a. **African spiny solanums**

In their synoptic overview of African solanums, Jaeger and Hepper (1986) estimated the number of *Solanum* species native to Africa and the adjacent islands to be about 90, and those introduced to be about 20. They suggested that these 110 species belonged to subgenera *Leptostemonum*, *Lyciosolanum* and *Solanum* and noticed that the hot spots of endemicity were located on the Eastern side of Africa.

Vorontsova et al. (2013) were the first to investigate the systematics of African spiny solanums using phylogenetical methods. They sampled 62 out of the 76 native species inventoried and used the chloroplast intergenic spacer *trn*T-F and two nuclear markers, ITS and *waxy*, to decipher the phylogenetic relationships among African and Malagasy spiny solanums. Nine species distributed throughout Asia and Australia, together with 10 New World species, completed the sampling. The trees were rooted with *Solanum betaceum* Cav. (Cyphomandra clade). Old World *Leptostemonum* species emerged again as a monophyletic grouping (Fig. 10.2). Seven clades, named after species names, plant habit or geographical distribution were identified, viz. Aculeastrum, Arundo, Climbing, Coagulans, Eggplant, Giganteum and Madagascar clades (Vorontsova et al. 2013). Some of these clades corresponded to units that had previously been recognised as sections (e.g. Arundo and Coagulans clades previously recognised as sections *Ischyracanthum* Bitter and *Monodolichopus* Bitter, respectively) or as groupings of closely related species (e.g. Giganteum clade). Interestingly, the Madagascar clade only included Malagasy endemic species. It is in this publication that the term "Eggplant clade" was coined for the first time; this clade included *S. melongena* and eight close relatives. Matches between clades on one hand, and species morphological features and eco-geographical characteristics on the other hand, are discussed in detail by Vorontsova et al. (2013).

In addition to these clades, Vorontsova et al. (2013) recognised a cluster of mostly African species that they called the "Anguivi Grade". Contrary to a clade that is, by definition, a monophyletic grouping, a grade is a paraphyletic assemblage of taxa. This type of grouping is often used to designate parts of phylogenetic trees that are poorly resolved. The Anguivi grade included the two cultivated African eggplants, *Solanum aethiopicum* and *S. macrocarpon*; it also accounted for two Asian species, *S. platacanthum* Dunal and *S. violaceum* Ortega.

Although species from Madagascar emerged as a distinct clade, Asian and African species were still intermingled into an unresolved pattern. The numerous members of former sections *Melongena* Dunal and *Oliganthes* (Dunal) Bitter were located in several non-directly related lineages which indicated their artificial status. Interestingly, *Solanum elaeagnifolium* Cav. and *S. hieronymi* Kuntze were the two New World species that emerged as sister to the Old World clade (see also Särkinen et al. 2013).

On the whole, the results of Vorontsova et al. (2013) suffered, as previous studies, from many poorly resolved or unresolved relationships. The authors concluded with the need to gain a better understanding of the complex phylogenetic relationships among Old World *Solanum* species. They indicated that a larger sampling was required, both in terms of number of species and

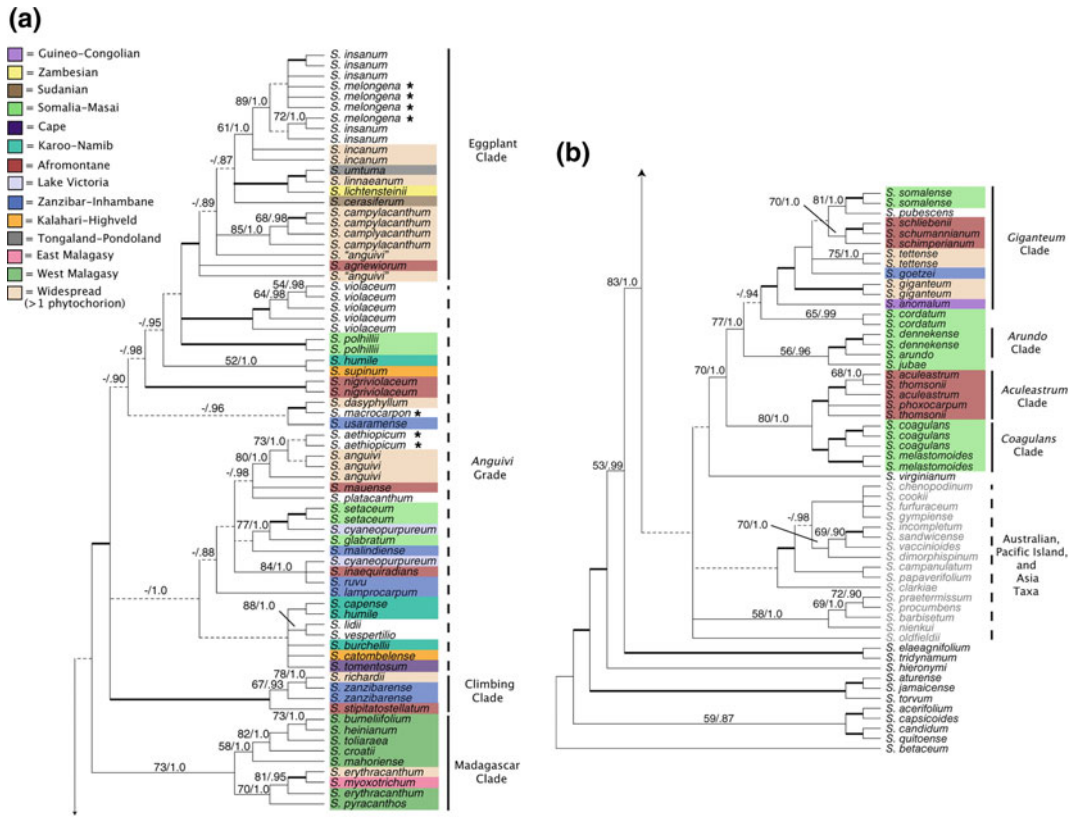

Fig. 10.2 A, B 50% majority rule tree from the Bayesian analysis of the combined data set including ITS, *waxy* and *trn*T-F regions The first number on each branch indicated bootstrap values over 50% and the second number indicated posterior probabilities (PPs) from the Bayesian analysis. Only branches with >0.80 PP are shown. Branches with >90% bootstrap support (BS) and >0.95 PP are marked in bold. Broken lines represent branches that are collapsed in the parsimony strict consensus tree. Species vouchered from cultivated plants are marked with a star. Wild accessions from continental Africa and Madagascar are marked in colour to indicate the phytochorion of the species. The recognised clades are marked with a full line on the right-hand side of the figure and named. The Australasian, Pacific islands and Asian group are marked with a broken line. Modified from Vorontsova et al. (2013) with permission from Oxford University Press

in terms of geographical representativeness. In particular, they insisted on the inclusion of Asian taxa and on the use of additional DNA regions.

b. Tropical Asian spiny solanums

Building on the final recommendations of Vorontsova et al. (2013), Aubriot et al. (2016b) considerably enlarged the sampling of Asian material for Old World Leptostemonum. In an attempt to obtain a better resolution of phylogenetic relationships, the core set of DNA regions traditionally used for *Solanum* phylogenetics (ITS, *waxy* and *trn*T-F) was completed by two intergenic spacer, *ndh*F-*rpl*32 and *trn*S-G.

With the exception of taxonomical investigations of New Guinean *Solanum* species (Symon 1985, 1986), tropical Asian spiny *Solanum* were particularly understudied. They had only been incorporated in small scale floristic (Zhang et al. 1994; Hul and Dy Phon 2014; Aubriot et al. 2016a) and few of these species (16 out of 56 recognised species) had been included in molecular phylogenetic studies prior to that of Aubriot et al. (2016b). The authors sampled 42 out of the 56 species native to Tropical Asia.

They completed their Old World dataset by sampling members of groups formerly identified, including 57 African and Malagasy species, 18 Australian species, and some others from the Arabian Peninsula (1 species), Seychelles (1) and Pacific region (3) (see Aubriot et al. 2016b for details). In addition to these 122 Old World species, 34 New World Leptostemonum species were sampled. The tree was rooted with *Solanum betaceum* of the Cyphomandra clade, sister to the Leptostemonum clade (Särkinen et al. 2013).

On the whole, the consensus tree obtained with a Bayesian analysis of the combined data set—157 species and five DNA regions—resolved the Old World spiny solanums as a polyphyletic assemblage, with clades composed of species of intermingled geographical origins (Fig. 10.3). This result is not completely congruent with former findings based on narrower sets of species and markers (Levin et al. 2006; Stern et al. 2011; Vorontsova et al. 2013). These later studies identified a large monophyletic grouping that accounted for all Old World spiny solanums, with the exception of two species (*Solanum lasiocarpum* Dunal from tropical Asia and *S. repandum* G.Forst. from the Pacific region) that were found to be more closely related to New World species.

In details, the results show that the vast majority of Old World species are part of a large monophyletic grouping, the Old World clade. But in addition to *Solanum lasiocarpum* and *S. repandum*, there is a set of five Old World spiny solanums species (*S. dammerianum* Lauterb. & K. Schum, *S. peikuoense* S.S.Ying, *S. poka* Dunal, *S. pseudosaponaceum* Blume and *S. torvoideum* Merr. & L.M.Perry) that fall outside of the Old World clade. These five species are resolved as closely related to the New World species of the Torva clade, confirming former morphological hypotheses (Symon 1985, 1986; Whalen 1984) of a close relationship between some of these species (*S. dammerianum*, *S. pseudosaponaceum* and *S. torvoideum*) and New World Torva clade species. Within the Old World clade, a few Asian species are placed within two previously described clades (Eggplant and Giganteum clade), but most of the tropical Asian taxa are part of large polytomies. The Anguivi grade described by Vorontsova et al. (2013) was again poorly resolved, and no major well-supported clade emerged. The Climbing clade, Anguivi grade, and the bulk of two sister groups identified as "*S. violaceum* and relatives" and the Eggplant clade were found to be phylogenetically closely related. However, the resolution of their relationships was still insufficient, since a bunch of polytomy cases remained, between and within these four "items".

Apart from a newly defined Sahul-Pacific clade (material from New Guinea, Australia and Pacific islands), Aubriot et al. (2016b) refrained from naming new clades to reflect the insufficient level of confidence in the species relationships that had been inferred. This research detected close relationships between the Eggplant clade and its Asian sister group named "S. violaceum and relatives" (*Solanum deflexicarpum* C.Y.Wu & S.C.Huang, *S. hovei* Dunal, *S. multiflorum* Roth and *S. violaceum*), albeit this result was poorly supported (but see Aubriot et al. 2018 for an alternative phylogenetic position of *S. violaceum*). Another interesting result is the confirmation of close relationships, previously suggested by the results of Vorontsova et al. (2013), between the cultivated *S. macrocarpon* and its wild form *S. dasyphyllum* Schumach. & Thonn.

Finally, the authors suggested that spiny solanum underwent at least three independent dispersal events from the New World to the Old World, the consequences of which yielded contrasted patterns in species richness, morphological diversity and spatial distribution. One pattern relates to the few Old World members of the Torva clade (*Solanum dammerianum*, *S. peikuoense*, *S. poka*, *S. pseudosaponaceum* and *S. torvoideum*, all gathered as "Old World torvoids") and Lasiocarpa clade (*S. lasiocarpum* and *S. repandum*). These lineages likely underwent a low level of diversification in tropical Asia. The second pattern is exemplified by the Old World clade; this later encompasses a large number of highly variable species that can be found from Macaronesia to Hawaii, in the wide area encompassing continental Africa and Asia, as well as

Fig. 10.3 A, B 50% majority rule tree from the Bayesian partitioned analysis of the combined data set (ITS, *waxy*, *ndh*F-*rpL*32, *trn*S-*trn*G and *trn*T-*trn*F) Numbers above each branch are bootstrap values >50% followed by posterior probabilities from the Bayesian analysis. Clades discussed in the manuscript are labelled. Cultivated species are indicated with an asterisk (*). Species names are in black for tropical Asia; green for Africa, Madagascar, Seychelles (*S. aldabrense*), Canary Islands (*S. lidii* and *S. verspertilio*) and western Asia (*S. platacanthum*); blue for Australia; red for the Pacific archipelagos (Hawaii for *S. incompletum* and *S. sandwicense*, New Caledonia for *S. pancheri*); grey for New World species. Modified from Aubriot et al. (2016b)

Australia and New Guinea. It has been found recently that this Old World clade results from a recent long-distance dispersal event from the Neotropics, with recent and rapid diversification within the most arid parts of Australia and Africa (Echeverría-Londoño et al. 2018).

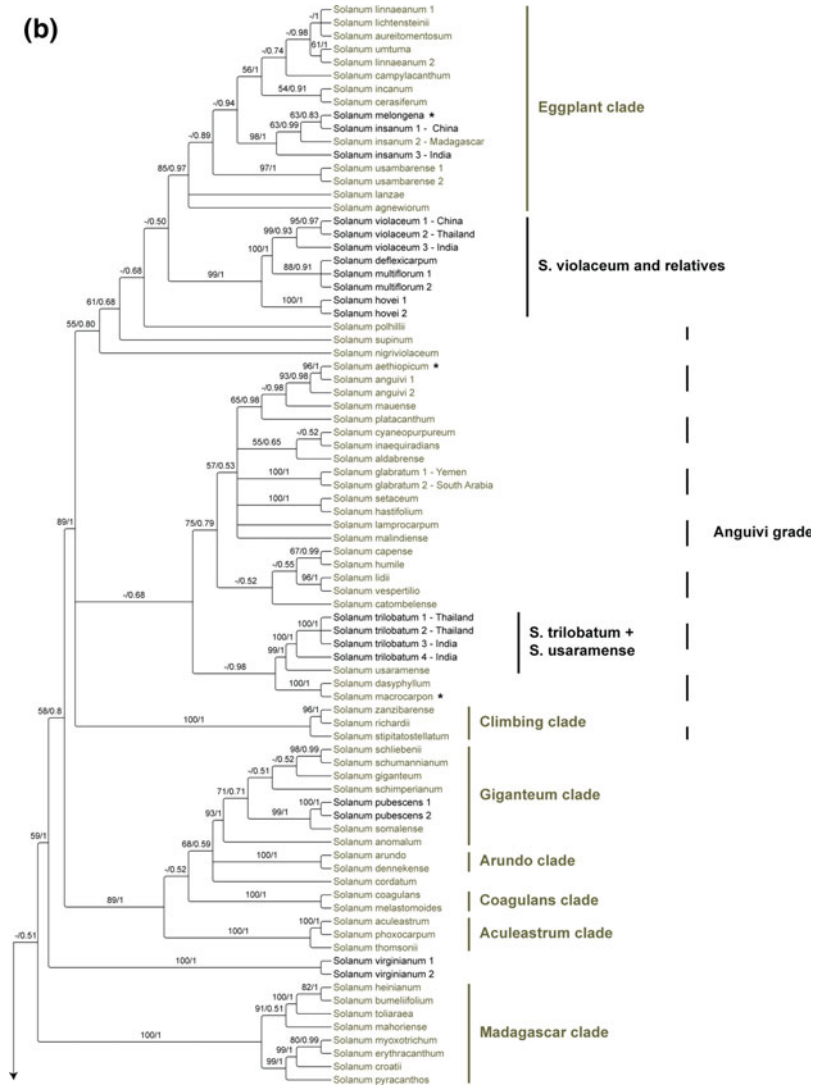

Fig. 10.3 (continued)

c. **Australian spiny solanums**

According to the last complete inventory of Australian *Solanum* (Symon 1981), Australia accounts for 120 *Solanum* species, of which 94 are native. Most of these 94 native species were classified as spiny solanums. In particular, 19 species were allocated to section *Melongena* by Symon, which was subdivided into two groups, according to their sexuality (Anderson and Symon 1989).

DNA-based phylogenetic studies of Australian spiny solanums are scarce. The Australian species that had been recognised as members of section Melongena were analysed by Martine et al. (2006) using the nuclear DNA region ITS. The authors sampled 18 out of the 19 Australian species pertaining to section Melongena together with three common representatives of this section (*Solanum linnaeanum* Hepper & P.-M.L.Jaeger, *S. macrocarpon* and *S. melongena*). The African *S. aculeastrum* Dunal and the pan-tropical *S. torvum* Sw. were used as outgroups. Although several polytomies appeared in the resulting trees, preliminary phylogenetic information was

obtained. The non-Australian species of section *Melongena* were bulked together, their grouping being sister to several of the Australian species.

The phylogeny of the Australian members of section *Melongena* was reassessed three years later using the markers ITS and *trn*K-*mat*K (Martine et al. 2009). The authors identified the dioecious Australian species as a monophyletic group, with a unique origin of dioecy derived from andromonoecy (in *Solanum* dioecy has evolved independently several times; Knapp et al. 1998). High-rank polytomies still persisted, and the relationships between Australian and out-of-Australia members of section *Melongena* remained unclear.

Much wider sets of markers and species sampling have shown that section *Melongena*, like many other former sections, was polyphyletic (Levin et al. 2006; Stern et al. 2011; Vorontsova et al. 2013; Aubriot et al. 2016b; Vorontsova and Knapp 2016; Aubriot et al. 2018). Recently, close relationship between Australian and New Guinean spiny solanums was suggested, together with their distant relationship to eggplants closest relatives (Aubriot et al. 2016b). A large-scale phylogenetic study of Australian spiny solanums is still lacking and would benefit the entire "*Solanum* community".

10.2.3.3 The Cultivated Eggplants and Their Closest Relatives: From Morphology to Genomics

Before phylogenetic analyses started to be used, eggplants and relatives were dispatched among 22 sections of subgenus *Leptostemonum*, and most of them belonged to sections *Melongena* and *Oliganthes* (D'Arcy 1972); e.g. *Solanum melongena* and *S. macrocarpon* were part of section *Melongena*, whereas *S. aethiopicum* was allocated to section *Oliganthes*. Phylogenetic studies (Aubriot et al. 2016b, 2018; Levin et al. 2006; Stern et al. 2011; Vorontsova and Knapp 2016) have demonstrated the artificial status of both sections and have dispatched eggplants and relatives throughout ca. 13 clades for the New World material and ca. 15 clades for the Old World material; many species are still either allocated to poorly supported clades and groups or not allocated at all. For end-users such as breeders, the understanding of eggplants relatives is therefore not a simple task.

The complex question of *Solanum melongena* direct wild relatives has concentrated much research efforts. The first molecular phylogeny specifically focused on *S. melongena* and its direct wild relatives (Weese and Bohs 2010) used germplasm accessions and informal classification of Lester (Lester and Hasan 1991). This study included also *S. aethiopicum*, *S. macrocarpon* and their direct wild relatives, *S. anguivi* Lam. and *S. dasyphyllum*, respectively. Two nuclear markers (ITS and *waxy*) and two chloroplast intergenic spacers (*trn*T-L and *trn*L-F) were sequenced for a sampling of 43 *Solanum* species, 40 of which were Old World material. The eight eggplant groups of Lester's classification, *S. incanum* A-B-C-D and *S. melongena* E-F-G-H (Lester and Hasan 1991; Daunay et al. 2001; Daunay and Hazra 2012), were sampled on the basis of two accessions per group, with the exception of group F (one accession only) and group G (three accessions). The strict consensus tree that resulted from a maximum parsimony analysis grouped these 16 individuals together with two accessions of the andromonoecious African species, *S. linnaeanum*. Based on the phylogenetic pattern they obtained, the authors agreed with the biogeographic hypothesis previously suggested by Lester and Hasan (1991), which explained the actual distribution of Lester's groups A, B, C and D, in Africa, and E, F, G and H in Asia. Following this scenario, the origin of *S. melongena* lies in Africa, and its ancestor(s) would have dispersed to the Middle East and Asia. However, if phylogenetic relationships between the groupings are relatively well resolved, they are often poorly supported. Also, the absence of formal species delimitation and naming, as well as the reduced taxonomical sampling were motivations towards further and more densely sampled and resolved phylogenies.

The effort to provide a revamped taxonomical framework for the whole genus *Solanum* impacted the taxonomy and nomenclature of the direct relatives of *Solanum melongena* as well.

Lester's groups were replaced by a set of species that followed the traditional Linnaean nomenclature and that referred to formal species delimitations based on a set of distinctive morphological features and on explicit type specimens (Knapp et al. 2013). The correspondence between Lester's groups and species names, now in use, is as follows:

Solanum incanum group A & B	*Solanum campylacanthum* Hochst. ex A.Rich.
Solanum incanum group C	*Solanum incanum* L.
Solanum incanum group D	*Solanum lichtensteinii* Willd.
Solanum melongena group E & F	*Solanum insanum* L.
Solanum melongena group G	*Solanum melongena* L. (primitive cultivars)
Solanum melongena group H	*Solanum melongena* L. (advanced cultivars)

This updated taxonomical framework was used in subsequent taxonomical and phylogenetic publications (Vorontsova et al. 2013; Aubriot et al. 2016b; Vorontsova and Knapp 2016; Aubriot et al. 2018). In particular, they were used to reassess phylogenetic relationships between *S. melongena* and its direct wild relatives.

The two studies recently published on the phylogenetics of native African and Asian spiny solanums (Vorontsova et al. 2013; Aubriot et al. 2016b) both incorporated *Solanum melongena* with a much denser sampling of its direct wild relatives. Vorontsova et al. (2013) were the first to delimit an "Eggplant clade" that included (1) the five newly delimited species (Knapp et al. 2013), viz. *S. campylacanthum* Hochst. ex A.Rich., *S. incanum* L., *S. insanum* L., *S. lichtensteinii* Willd., and *S. melongena*, (2) *S. linnaeanum* a species previously recognised as very close to the eggplant (Weese and Bohs 2010) and (3) three species (*S. agnewiorum* Voronts., *S. cerasiferum* Dunal and *S. umtuma* Voronts. & S.Knapp) not previously recognised as direct relatives to *S. melongena*. Surprisingly, *S. agnewiorum* and *S. umtuma* were until then unknown to botanists and plant breeders. They had been described very recently from a limited number of collections (Vorontsova et al. 2010; Vorontsova and Knapp 2012). This is also the first phylogenetic analysis that used the updated taxonomic circumscription for the wild progenitor of the eggplant, *S. insanum*. This publication and all the subsequent ones unambiguously resolved *S. insanum* as sister to the cultivated brinjal eggplant.

The research on tropical Asian spiny solanums (Aubriot et al. 2016b) extended the general sampling of Old World spiny solanums. Surprisingly, two African species fell into the Eggplant clade, viz. *S. lanzae* J.-P.Lebrun & Stork and *S. usambarense* Bitter & Dammer. Phylogenetic results confirmed also that *S. aureitomentosum* Bitter, a species that had been expected to pertain to the Eggplant clade on the basis of its morphology (Knapp et al. 2013), was closely related to the eggplant. In the end, twelve species were recognised as members of the Eggplant clade, viz. *S. agnewiorum*, *S. aureitomentosum*, *S. campylacanthum*, *S. cerasiferum*, *S. incanum*, *S. insanum*, *S. lanzae*, *S. lichtensteinii*, *S. linnaeanum*, *S. melongena*, *S. umtuma* and *S. usambarense*. However, a Cape Verdean species, *S. rigidum* Lam., considered a member of the Eggplant clade on the basis of its morphology (Knapp and Vorontsova 2013; Knapp et al. 2013) was still missing from phylogenetic analyses. Also, despite the use of a set of five DNA regions (Aubriot et al. 2016b), phylogenetic resolution within the Eggplant clade was still poor and statistical support for the few resolved nodes were rather low. This limited evolutionary insights for the Eggplant clade.

To tackle this situation, a combination of analyses based on traditional genetic data (amplification and Sanger sequencing of DNA regions) and genomic data (next-generation sequencing of whole chloroplast genomes) was used (Aubriot et al. 2018). The authors used two

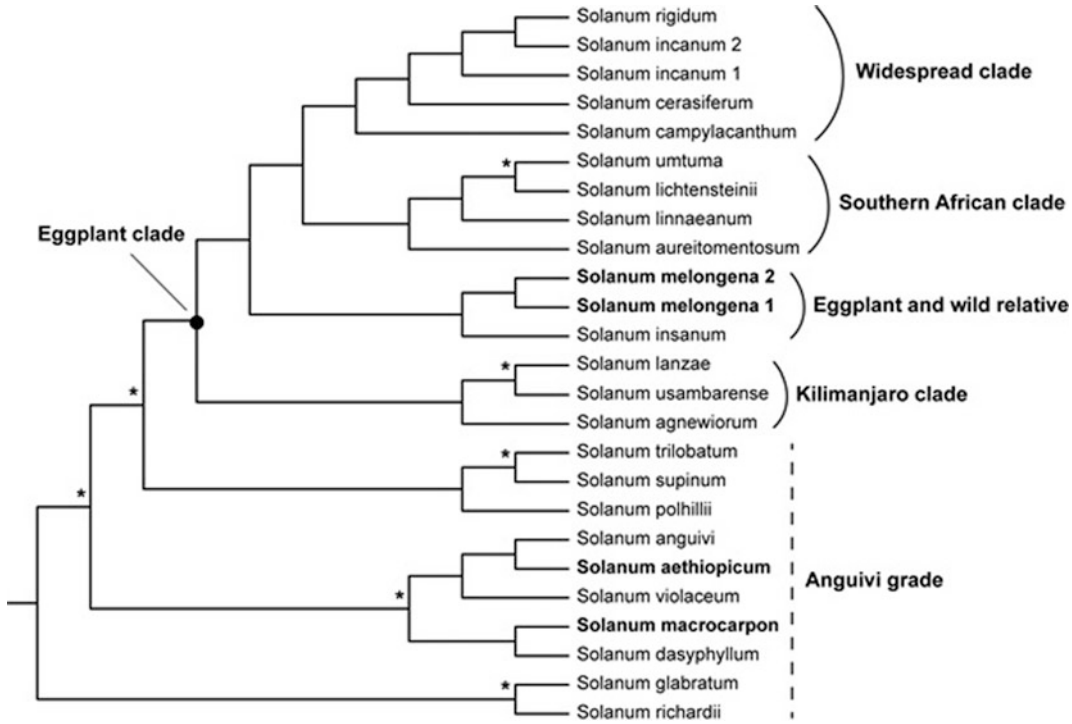

Fig. 10.4 Full plastome phylogeny of the Eggplant clade (consensus of 4 BEAST analyses; 159, 227 bp matrix) All nodes are well supported except for the nodes designated with * (for support values see the original publication). Names of cultivated species are in bold. Modified from Aubriot et al. (2018)

nuclear regions (ITS, *waxy*), as well as a chloroplast intergenic spacer (*ndh*F-*rpl*32), to check species delimitations within the Eggplant clade. They used whole chloroplast genomes analyses to build a sound phylogenetic framework in order to reconstruct the biogeographical history of the Eggplant clade. The sampling included the twelve species formerly identified as members of the Eggplant clade (see above, and Aubriot et al. 2016b), with the addition of the previously unaccounted-for Cape Verdean species, *Solanum rigidum*. In addition to the Eggplant clade, sampling included representatives of the Anguivi grade (*S. aethiopicum*, *S. anguivi*, *S. dasyphyllum*, *S. glabratum* Dunal, *S. macrocarpon*, *S. polhillii* Voronts., *S. supinum* Dunal and *S. trilobatum* L.), and of the phylogenetic unit "*S. violaceum*, and relatives" (*S. violaceum*) (Aubriot et al. 2018). An African species pertaining to the Climbing clade, *S. richardii* Dunal, served as an outgroup for the analyses. To test species delimitations, several accessions from geographically widespread species (*S. campylacanthum*, *S. cerasiferum*, *S. incanum* and *S. insanum*) were included in the sampling.

Combined results for the three DNA regions used in the preliminary analysis confirmed the monophyly of the Eggplant clade, and that *S. rigidum* belonged to that clade. *Solanum campylacanthum* and *S. cerasiferum* species concepts (sensu Knapp et al. 2013) were shown to be monophyletic; this contrasted with *S. incanum* that was identified as a potentially paraphyletic species. Unsurprisingly, *S. melongena* and its wild progenitor, *S. insanum* were assembled within the same polytomy. Phylogenetic relationships within the Eggplant clade were still insufficiently resolved, apart from the case of the strongly supported group formed by *S. aureitomentosum*, *S. lichtensteinii*, *S. linnaeanum* and *S. umtuma*.

Based on this Sanger-sequencing phylogeny, a more resolved and dated phylogeny of the Eggplant clade was achieved with whole chloroplast

genome analyses (Fig. 10.4). The clade formed by *S. melongena* and its wild progenitor *S. insanum* ("Eggplant and wild relative") was shown to be sister to a pair of clades: the Southern African clade (*S. aureitomentosum*, *S. lichtensteinii*, *S. linnaeanum* and *S. umtuma*) and the Widespread clade (*S. campylacanthum*, *S. cerasiferum*, *S. incanum*, and the Cape Verdean endemic *S. rigidum*). These three clades are sisters to a clade of three African species (*S. agnewiorum*, *S. lanzae* and *S. usambarense*) whose geographical distribution is centred on the Kilimanjaro region, whence the name of the clade. Representatives of the Anguivi grade, including the two other cultivated eggplants (*S. aethiopicum* and *S. macrocarpon*) and their respective wild progenitor (*S. anguivi* and *S. dasyphyllum*), were more distantly related. Interestingly, *S. violaceum* once thought to be among the closest relatives to the Eggplant clade (together with the four other species that pertain to "*S. violaceum* and relatives" lineage; see Aubriot et al. 2016b), unambiguously branched with *S. aethiopicum* in the full plastome tree. Additional molecular data, and in particular high number of low copy nuclear genes, are now needed to fine-tune these phylogenetic hypotheses.

Using this revamped phylogenetic framework, Aubriot et al. (2018) reassessed the biogeographical hypothesis of Lester on the dispersion of the *S. melongena* group (Lester and Hasan, 1991). The biogeographical analyses showed that the Eggplant clade originated in the region encompassing North-eastern Africa and West Asia; but in contrast to the hypotheses developed by Weese and Bohs (2010), the dispersion of the Eggplant clade in tropical Asia did not seem to proceed from a stepwise expansion through the Middle East. The biogeographic results that were obtained were more consistent with an early dispersal from Northern Africa and/or West Asia, unrelated to the southwards and eastwards spread of the Widespread and Southern African clades. The authors related the diversification of the Eggplant clade into southern and western Africa to the large savannah mammals that disperse the seeds, the African elephants and impalas. They did not exclude the possibility of early interactions between these species and members of the human lineages. However, the absence of fossil records that could definitely attest of the deep historical link between the eggplant direct relatives and potential seed dispersers are still lacking. It is clear that knowledge of the identity and historical affinities of eggplant direct relatives is a research field of ever-growing interest. The very rapid progresses made in the management and analysis of large amount of genomic data is likely to provide new insights into the complex history of *S. melongena* relatives.

10.2.4 Species Relationships: A Breeder's Look at the Crossroads Between Approaches and Criteria

The wealthy taxonomic literature on *Solanum* species is difficult to handle for eggplants breeders, because of its continuing evolution, the use of various criteria, concepts and statistical models that they are not familiar with, combined to the high number of species involved. Although the mainstream of information is now provided by DNA-based phylogenetic approaches, one cannot ignore (1) the publications using phenetic approaches, (2) the still ongoing use of non-DNA criteria for assessing relationships between species, and (3) the interest of features linked to ploidy level, chromosomes and their meiotic behaviour. Indeed, on the whole, the literature indicates that waving between molecular and other types of informative criteria seems to be the route taxonomist's ride, for progressively reaching a better understanding of the complex evolutionary history of *Solanum*.

10.2.4.1 DNA-Based Similarities Between Species

We have seen that from the 1990s onward taxonomic initiatives aimed towards a global treatment of genus *Solanum* with an exclusively phylogenetic approach. However, in the meantime, another wealth of results, these ones based on similarities between eggplants and other

Solanum species, bloomed in the literature. These researches were often carried out without interaction with taxonomists and without taking in account evolutionary hypotheses. Along with technological progresses, they used successive types of markers throughout the 1990s, 2000s and till now; first, seed proteins and allozymes (Isshiki et al. 1994; Karihaloo et al. 2002; Ahmed and Fadl 2015); then, nuclear DNA markers such as RAPD (Karihaloo et al. 1995; Singh et al. 2006; Ahmed and Fadl 2015), ISSR (Isshiki et al. 2008), STMS (Behera et al. 2006), AFLP (Mace et al. 1999; Furini and Wunder 2004), SSR (Stagel et al. 2008; Gramazio et al. 2017), SNP (Acquadro et al. 2017; Gramazio et al. 2017); but also, chloroplast DNA markers (Sakata et al. 1991; Sakata and Lester 1994, 1997; Isshiki et al. 1998) as well as mitochondrial DNA markers (Isshiki et al. 2003). Genetic similarities were calculated with various methods (Nei pairwise similarity coefficient, Nei and Li similarity/Dice coefficient, Jaccard coefficient, and/or simple matching coefficient), dendrograms were generated with unweighted pair-group method (UPGMA), weighted pair-group method (WPGMA), group average method or neighbour-joining algorithm (NJ); bootstraps and principal coordinates analyses were occasionally used to validate the results.

These papers compared *Solanum melongena* with various small sets of commonly known related spiny solanums: *S. aculeastrum*, *S. anguivi*, *S. aethiopicum*, Gilo (= *S. gilo* Raddi, *S. olivare* Pailleux & Bois), Kumba or Shum Groups, *S. cerasiferum*, *S. dasyphyllum*, *S. elaeagnifolium*, *S. forskalii* Dunal (= *S. albicaule* Kotschy ex Dunal), *S. insanum*, *S. lidii* Sunding, *S. linnaeanum*, *S. macrocarpon*, *S. mammosum* L., *S. marginatum* L.f., *S. rostratum* Dunal, *S. schimperianum* Hochst. ex A.Rich., *S. nigriviolaceum* Bitter (= *S. sessilistellatum* Bitter), *S. sisymbriifolium* Lam., *S. tomentosum* L., *S. torvum*, *S. viarum* Dunal, *S. violaceum* (= *S. indicum* L., *S. kurzii* Brace ex Prain, *S. sanitwongsei* Craib), *S. virginianum* L. (= *S. surattense* Burm.f.). As indicated in parentheses, nomenclatures used by these various authors were not consistent; and this arguably reflects the ever-changing taxonomy and nomenclature of *Solanum* as well as the rather poor interactions between the authors of these studies and *Solanum* taxonomists. Sometimes the papers included more distantly related material belonging to subgenus *Solanum* (*S. nigrum* L., *S. dulcamara* L., *S. americanum* Mill.) and in one case (Ahmed and Fadl 2015) even tomato!

Although comparisons among papers are difficult because of the variable sets of species and markers used, the clusterings produced in these papers are sometimes rather consistent between each other; this is the case for the close relationships between eggplants and their respective closest wild relatives (*Solanum melongena* with *S. insanum* and *S. incanum*; *S. aethiopicum* with *S. anguivi*, and *S. macrocarpon* with *S. dasyphyllum*). But there are also cases of conflicting results such as *S. aethiopicum* and *S. violaceum* found closer to *S. melongena* than *S. incanum* (Isshiki et al. 2008), although *S. incanum* was generally found closer to *S. melongena* than any other taxon. Another example is *S. torvum* either found to be the most distant species to *S. melongena* (Isshiki et al. 1994; Isshiki et al. 2008; Acquadro et al. 2017) or to be closer to *S. melongena* than the usually closely related *S. aethiopicum* (Stagel et al. 2008). Different markers applied to a same set of species can also provide different grouping patterns, such as in the case of *S. aethiopicum* and *S. anguivi* found closer to *S. melongena* than *S. macrocarpon* and *S. dasyphyllum* with SSRs and as distant with SNP (Gramazio et al. 2017).

10.2.4.2 DNA-Based Phylogeny Combined to Other Criteria

Most Solanaceae phylogenetic studies based on DNA polymorphism (Bohs 2005; Levin et al. 2005; Levin et al. 2006; Weese and Bohs 2007; Miz et al. 2008; Stern et al. 2011; Vorontsova et al. 2013; Aubriot et al. 2016b) discuss clades composition in relation to (1) geographical and/or ecological ranges, (2) the historical systems of infrageneric classifications, and (3) morphological similarities between species. Common phytogeographical features sometimes match DNA-based phylogenetic relationships.

Thus, Malagasy endemic *Solanum* species are gathered within a single clade (Vorontsova et al. 2013). This is also the case for spiny solanums originating from Eastern Asia, Australia and Oceania, that form a clade distinct from the species originating from Western Asia and Africa (Aubriot, unpub.). On the contrary, the Torva clade includes both New World and Old World (tropical Asian) species (Aubriot et al. 2016b).

Phenotypic resemblance between taxa that share common morphological features has traditionally been the main basis for defining formal infrageneric ranks. However, resemblance can only be interpreted in a phylogenetic perspective if there is a linear relationship between the time of divergence between taxa and the degree of their morphological (or molecular) differences. Hence, in many cases, the link between morphological resemblance on one side and phylogenetic relationship on the other side is a deceptive shortcut. For instance, members of Acanthophora (Levin et al. 2005), Lasiocarpa (Whalen and Caruso 1983; Bohs 2004) and Torva (Miz et al. 2008) clades illustrate a good (although not fully complete) match with the corresponding sections. Similarly, common morphological features match sometimes DNA-based phylogenetic relationships, as exemplified by the morphologically consistent unit "*S. violaceum* and relatives", the members of which share long inflorescences with many hermaphrodite deeply stellate flowers, and small berries orange or red at maturity (Aubriot et al. 2016b). Another example is that of the Old World torvoids, nested within New World members of Torva Clade, which all share, among other morphological traits, an erected shrubby growth habit, straight prickles, many branched inflorescences and small leathery berries (Aubriot et al. 2016b). However, generally only loose or no correspondence is found between sections or morphological similarities and molecular phylogeny. For instance, the species of sections *Dunaliana* (Bitter) Seithe and *Graciliflorum* (Dunal) Seithe are spread in various branches of the phylogenetic tree (Aubriot et al. 2016b). Such lack of match is also found for members of the Elaeagnifolium Clade, which were formerly dispatched in sections *Leprophora* Dunal, *Nycterium* (Ventenat) Dunal and *Lathyrocarpum* G.Don (Knapp et al. 2017). Another example is that of the Madagascar clade composed of species which are morphologically very divergent (Vorontsova et al. 2013).

This lack of match between morphological similarities and phylogenetic relationships is arguably due to common homoplasy. Indeed, morphological similarities between species can originate from different evolutionary routes (convergence, parallelism, reversion) or from the implementation of the same biological function (analogy) in different taxa. Homoplasy exists also for molecular criteria used in phylogenetic studies, but at a lesser scale and frequency than for morphological features, since the markers used for phylogenetic purposes are intentionally chosen for their appropriate conservativeness or diversity between taxa of different hierarchical ranks (Olmstead and Palmer 1994). Homoplastic traits in solanums include andromonoecy (Whalen and Costish 1986; Vorontsova et al. 2013), dioecy (Knapp et al. 1998), zygomorphy and heterandry (Knapp 2001; Bohs et al. 2007) and vegetative features (Vorontsova et al. 2013; Aubriot et al. 2016b). As such traits are found in unrelated lineages, they must be considered with caution when assessing relationships between species.

Handling molecular phylogeny together with morphology is sufficiently frequently conflictual for having been thought over thoroughly. Knapp (2001) surveyed half a dozen of traits of different scales, from global plant architecture to detailed morphology of pollen and trichomes that deserve future thorough investigation. Such traits still have a scanning role to play at different hierarchical levels of phylogenetic trees and are complementary to molecular scanning. More generally, inferring phylogenetic relationships on the basis of one criterion alone is hazardous for several reasons. First, a few molecular markers cannot summarise the complexity of the evolutionary history of organisms that have evolved along huge periods of time. Second, solanums display an extreme morphological variability between species, and sometimes within a single

species (e.g. *Solanum campylacanthum*; see Knapp et al. 2013). Third, solanums display contrasting geographical and ecological ranges, from very narrow distribution ranges (e.g. all Malagasy species, *S. rigidum*, the recently defined *S. agnewiorum* and *S. umtuma*) to very wide ones (e.g. *S. anguivi* and *S. giganteum* Jacq. in Africa; *S. elaegnifolium* and *S. torvum* which are now widespread in all tropics). Finally, despite the large collecting efforts developed within the last decade, species and accession level samplings are still insufficient to fully unravel the complexity of the phylogenetic relationships between many Old World solanums. This situation is complicated by the fact that (1) herbarium specimens are dispersed in many herbaria worldwide, (2) it is increasingly difficult to access in situ diversity in many countries, and (3) the living seed material in germplasm collections are scarce.

10.2.4.3 Ploidy, Chromosomes and Meiotic Behaviour

Diploidy and a chromosome number of $n = 12$ are common features for *Leptostemonum* species, to which eggplants wild relatives belong (Whalen 1984). However, some exceptions are mentioned such as $n = 11$ for two species of the Acanthophora clade—*S. mammosum* and *S. platense* Diekm. (Chiarini and Bernadello 2006). Diploidy ($2n = 24$) together with tetraploidy ($2n = 48$) and hexaploidy ($2n = 72$) was found in different populations of *S. elaeagnifolium* (Scaldaferro et al. 2012), among species of section *Lathyrocarpum* (Wahlert et al. 2015), and the presence of tetraploidy among accessions of *S. campylacanthum* or *S. incanum*—two direct wild relatives of the eggplant—is questioned (Knapp et al. 2013). These few examples point out that cytogenetic information is actually scarce for *Leptostemonum* species, although karyotypic features are potentially informative about evolutionary processes (Chiarini and Bernadello 2006). Investigations about ploidy levels in eggplants wild relatives are also considered as a priority for facilitating the use of wild relatives in eggplant(s) breeding (Knapp et al. 2013).

Chromosomes meiotic behaviour is commonly looked at for understanding late post-zygotic barriers contributing to interspecific hybrids pollen sterility (Chap. 11). In publications dealing with this topic, chromosomes morphology and meiotic behaviour of the species themselves are also considered as a source of information about species origin, distinction and relationships. Differences were observed for chromosome size between *S. anguivi*, *S. aethiopicum* and *S. torvum* (small chromosomes), and *S. melongena* and *S. macrocarpon*, two species characterised by longer chromosomes (Oyelana 2005). Oyelana (2005) further noticed that the symmetrical chromosomes of *S. torvum* contrast with the unequal arms of some chromosomes of *S. melongena* and *S. macrocarpon*, which suggests centromere reposition shift due to breaks and rearrangements. On the basis of this example, the author suggested that genomic evolution among *Solanum* species could result from structural chromosome changes, with metacentric chromosomes as a plesiomorphic trait and sub-metacentric and sub-telocentric chromosomes as derived traits. Meiotic abnormalities (clumps, univalents, multivalents, bridges, lagging chromosomes etc.) observed in *S. aethiopicum*, *S. macrocarpon* and *S. melongena* have been interpreted as a possible trace of a hybrid origin of these species (Omidiji 1983; Oyelana and Ugborogho 2008). A higher rate of meiotic aberrations in *S. aethiopicum* Gilo and Shum cultigroups (12.5 and 10.6%, respectively), compared to their wild progenitor *S. anguivi* (2.5%), inversely mirrored in pollen stainability (88% for the cultigroup vs. 97% for the wild species), was interpreted as the cultigroups being translocation heterozygotes of hybrid origin, still enduring chromosomal evolution (Anaso 1991). Although literature is rather scarce on chromosome shapes and meiotic behaviour in spiny solanums, these examples invite to allocate future attention to these types of features.

10.3 Old World Subgenus *Leptostemonum*: Inventory and Conservation

10.3.1 Preliminary Inventory

The estimated number of species belonging to *Leptostemonum* was loosed for a long time, with estimations varying from 250 to 450 (Whalen 1984; Child and Lester 2001; Bohs 2005; Levin et al. 2006). Recent taxonomic progresses have identified over 500 recognised species for *Leptostemonum*, out of which the number of native Old World species is provisionally estimated and split as follow (Aubriot et al. 2016b):

- Three species are endemic to Macaronesia (two from the Canary Islands and one from the Cape Verde archipelago) (Anderson et al. 2006),
- 76 originate from continental Africa and Madagascar—including the eggplant and its wild progenitor, *Solanum melongena* and *S. insanum*, respectively (Vorontsova and Knapp 2016),
- 56 from tropical Asia—including 29 from New Guinea (Aubriot et al. 2016b),
- ca. 90 to ca. 120 are native to Australia (Symon 1981),
- ca. 30 from the Pacific (Mc Clelland 2012).

On the whole, *Leptostemonum* is estimated to account for ca. 250–280 Old World species and ca. 270–300 New World species. These numbers are still approximate; every year new species are described and a number of taxonomic treatments are in preparation, in particular for Tropical Asian, Australian and New World species.

Although the number of spiny solanums is still an approximation, we provide here preliminary inventories that are structured in accordance with the native geographical distribution of the species. For those phylogenetically closest to eggplants (African and Asian species), our inventories are meant to be comprehensive; they gather species originating from Africa, Macaronesia, Madagascar, Western Asia (Appendix 1[2]) and tropical Asia (Appendix 2). The lists of other spiny solanums, more distantly related, originating from Australia (Appendix 3) and from New World (Appendix 4) are not comprehensive and have been intentionally restricted to the taxa (i) mentioned in publications concerning eggplants taxonomy[3] and/or crossability, or (ii) present in germplasm collections.

The reader must be aware that species belonging to other clades (or subgenera) than *Leptostemonum*, and that are found in various publications concerning eggplants or mentioned in this text, are not listed in the Appendices. This is for instance the case of *S. scabrum* Mill. (Morelloids clade), *S. pseudocapsicum* L. (subgenus *Solanum*), *S. aviculare* G.Forst. and *S. laciniatum* Aiton (Archaesolanum clade), *S. muricatum* Aiton (Basarthrum clade), as well as *S. erianthum* D.Don (Brevantherum clade) and *S. betaceum* Cav. (Cyphomandra clade) (see Fig. 10.1 for phylogenetic position).

10.3.2 From Nature to Genebanks: Opportunities and Threats

10.3.2.1 Ex Situ Collections of Cultivated and Wild Eggplants

a. **Cultivated eggplants**

Under the impulsion of the International Board for Plant Genetic Resources[4] (IBPGR), eggplants were identified as crops of economic importance

[2]**Appendix** 1 also includes three species that were not treated by Vorontsova and Knapp (2016): *S. platacanthum* from the Arabian Peninsula, *S. aldabrense* from the Seychelles islands, and *S. rigidum* from the Cape Verde archipelago.

[3]Also, given the high number of spiny solanum species originating from Australia and the New World, their still ongoing taxonomic treatment, and their frequent absence from publications relating to eggplants, it is useless (and arduous) to provide here full-length lists of them.

[4]Renamed International Plant Genetic Resources Institute (IPGRI) in 1991, and Bioversity International in 2006.

in the tropics and in danger of suffering genetic erosion (Grubben 1977). IBPGR sponsored several eggplants collecting missions, specifically Africa during the 1980s (Lester et al. 1990). IBPGR also backed up a series of national initiatives for collecting *S. melongena* in Asia, for instance in Thailand (Wivutvongvana et al. 1984). A historical and analytical overview of eggplant germplasm collections, backed up by IBPGR, remains to be assembled. National collecting initiatives focusing mostly on cultivated material were also organised, in particular in India and China, two important centres of diversity for *S. melongena*.

A recent worldwide survey of the germplasm collections identified 6632 accessions for the cultivated eggplants, with 5665 accessions for *S. melongena*, 798 for *S. aethiopicum* and 169 for *S. macrocarpon* (Taher et al. 2017). These numbers originate from the global gateway for genetic resources (GENESYS[5]) and from the genetic resources information system of the World Vegetable Center (WorldVeg, Tainan, Taiwan). While these numbers are imperfect because it is extremely difficult to compile data at the worldwide level, they clearly indicate that if substantial germplasm is available in genebanks for *S. melongena*, further collecting efforts are necessary for *S. aethiopicum* and even more for *S. macrocarpon*, as well as for the wild material.

b. **Wild species**

The first collection of wild species related to eggplants, particularly rich in species native to Africa, was set up at the University of Birmingham (UK) as a basis for the taxonomic researches on eggplants relatives carried out from the 1970s to the 1990s; this collection also covered broad diversity in the Solanaceae family (Lester et al. 2001). It was split during the 2000s between several research partners of the European Union "EGGNET" project (1999–2004), in particular between the Institut National de la Recherche Agronomique (INRA, France) for eggplants and their Old World relatives, and the Radboud University of Nijmegen for most other Solanaceae genera and species (Barendse et al. 2001). The material related to eggplants was shared with EGGNET partners during the course of the project. In 2001, in junction with EGGNET, the European cooperative programme on plant genetic resources (ECPGR) started a collaborative initiative on Solanaceae genetic resources (including eggplant).[6] This initiative aims at extending collaborations at the European continental scale in order to harmonise the practices, rationalise the collections and complete an eggplant centralised passport database[7] (Daunay et al. 2011); that work is still in progress.

Availability of wild spiny solanums germplasms is a crucial challenge for future research projects. Only part of the wild spiny solanum species diversity is included in ex situ collections, in particular at INRA[8] (Montfavet, France), Radboud University[9] (Nijmegen, The Netherlands), and other genebanks in Europe such as CGN[10] (Wageningen, The Netherlands), IPK[11] (Gatersleben, Germany), COMAV[12] (Valencia, Spain), and other places such as the Kew Millennium Seed Bank[13] (Wakehurst, UK). The material held in worldwide ex situ collections was estimated to account for 33 species and 1304 accessions (Taher et al. 2017). Below we provide compiled information, which includes also wild material kept in INRA germplasm collection[14] and matches the ex situ material together with the estimated total number of wild (and cultivated) species, gathered by geographical distribution.

[5]https://www.genesys-pgr.org/fr/welcome.

[6]http://www.ecpgr.cgiar.org/working-groups/solanaceae/.

[7]http://www.ecpgr.cgiar.org/Resources/germplasm_databases/list_of_germplasm_databases/crop_databases/crop_database_windows/eggplant.html.

[8]https://www6.paca.inra.fr/gafl_eng/Vegetables-GRC.

[9]https://www.ru.nl/bgard/solanaceae-collection/databases/solanaceae-database/.

[10]https://www.wur.nl/en/Research-Results/Statutory-research-tasks/Centre-for-Genetic-Resources-the-Netherlands-1.htm.

[11]https://www.ipk-gatersleben.de/en/genebank/.

[12]https://www.comav.upv.es/index.php/databasesgermplasm/bancoger.

[13]https://www.kew.org/science/collections/seed-collection.

[14]Center for vegetables genetic resources (https://www6.paca.inra.fr/gafl_eng/Vegetables-GRC/Our-Collections).

The result is the flabbergasting under-representation of spiny solanum in genebanks.

African, Malagasy and West Asia species	79 estimated species	39 in ex situ collections	49%
Tropical Asian species	56	6	7%
Australian species	120	15	12.5%
New world species	300	23	7.7%

For the African species, the ones that are the most closely related to the eggplant, only half of the species (49%) are available as seeds; hence, in order to enlarge the material available for research and breeding, a considerable effort of collecting is necessary. The situation is much worse for the tropical Asian species (7%), as well as for the Australian and New World species.

Spiny solanums high species richness, their almost worldwide distribution, together with their difficult taxonomic treatment, have hampered so far the setting up of a global strategy for securing their wild genetic diversity. The recent progress in the taxonomy of African (Vorontsova and Knapp 2016) and tropical Asian (Aubriot et al. 2016b; Aubriot unpub. data) spiny solanums lay the basis for multilateral initiatives for enriching the existing collections with missing taxa and with further accessions of species already present in some national genebanks. Such initiatives are all the more urgent that many African species have a restricted area of distribution (Vorontsova and Knapp 2016) and many are under threat of extinction (Syfert et al. 2016). Hence, it is essential to organise ex situ safeguarding of African spiny solanums and to make all efforts to promote long term in situ conservation.

10.3.2.2 Ex Situ Regeneration of Eggplants and Relatives Germplasm

a. **Cultivated germplasm**

Before the rise of scientific breeding several decades ago, cultivated eggplants were landraces bred by peasants. As eggplants are partially autogamous, landraces are roughly homogeneous. The commercial material of *Solanum melongena* is currently mostly compound of hybrids, whereas it mostly consists of landraces or lines for *S. aethiopicum*[15] and *S. macrocarpon*. Hybrids are generally not included in genebank collections because it is impossible to carry out conservative selection for them, given their F2 progeny segregates. Germplasms collected in fields or markets are generally heterogeneous because they originate from open pollination, i.e. from selfing to outcrossing, with an outcrossing rate that depends from the frequency of local pollinators visits. Hence, the homozygosity of introduced material is variable from one accession to another.

Seed production is a key step in the management of the genetic quality of the germplasms held ex situ. Generally, this quality is understood as keeping the initial (morphological) homogeneity or heterogeneity of the material along successive regeneration cycles. When a given accession is heterogeneous and kept as such, like in the case of landraces or mixed up material, the number of plants used for seed production has a direct influence on the genetic diversity of the offspring: the less the plants set seeds, the stronger the genetic drift and then the reduction of genetic diversity. Another germplasm holder's practice is to homogenise heterogeneous material by successive selfing of selected plants and to end up with one or several lines issued from an initial accession. This strategy is relevant when the material is directly used for inheritance studies, but has the inconvenient of losing some of the initial diversity. On the whole, both methods have their pros and cons.

Depending on genebanks policy and facilities, regeneration of the material in collection is carried out either in controlled (insect proof greenhouses) or uncontrolled (open field) conditions. In the first case, seeds are issued from each individual plant selfing, whereas in the

[15]Hybrids of *Solanum aethiopicum* are currently being developed by several European seed companies.

second case seeds will be a mixture of selfing, sister x brother plants crosses and uncontrolled hybridization with neighbouring accessions. Allopollination rate in *Solanum melongena* was estimated as rocketing up to 47% (Daunay and Hazra 2012); hence, the risk of pollen pollution in uncontrolled conditions is far from negligible.

b. **Wild germplasm**

Managing ex situ collections of wild *Solanum* germplasm is a difficult task for several reasons (Daunay et al. 1999). First, the correct botanical identification of most of the wild species is a challenge for germplasm curators. This is due to a series of factors such as (1) the widespread use of erroneous names, synonyms or homonyms, (2) the constant taxonomic changes, and (3) the scarcity of scientists that are able to allocate a proper species name to a given accession. As a result, many accessions in germplasm collections either bear outdated species names or are misidentified or not identified at all. Seed dormancy, frequent in wild material, is the next obstacle germplasm holders have to face; however, this can be managed with gibberellic acid treatment, alternate temperatures and other treatments (Daunay et al. 1999; Gisbert et al. 2011; Ranil et al. 2015).

The next difficulty is due to the variable and poorly known biology of spiny solanums; these solanums display a wide range of physiological diversity such as adaptation to dry to humid climates, day length sensitivity or not, short to long life cycles and short to long duration of fruit maturation. The environmental requirements specific to each species and appropriate for a good seed set are not documented other than through the scarce knowledge of their in situ ecological conditions or through local ex situ observations. Ex situ natural environmental conditions such as seasonality, day length and thermoperiod, together with cultivation calendars, protocols and techniques (greenhouses, fields, irrigation, mulching[16]) are inadequate for several wild species and impact negatively their seed production; this latter can be null or poor, as well as erratically variable from one year to another. The seed production system developed at INRA consists in growing sets of a priori non-intercrossable species in isolation open fields, on the basis of a dozens of plants per species (one accession per species). This method was the best among the others tested, but the unsuitability of several species to temperate and agricultural conditions remains a severe issue. Finally, ex situ seed production of wild material is generally difficult and seeds are often unavailable for distribution, although the species appear in genebank catalogues (Daunay et al. 1999).

The last category of burden ex situ collections suffer is of genetic nature and is insidious. Floral biology and consecutive genetic characteristics of the wild species are mostly unknown and this can impact ex situ regeneration. Natural allogamy versus autogamy rate, presence of auto-incompatibility, degree of sensitivity to inbreeding, specific to each *Solanum* species, are mostly unknown characteristics (Daunay et al. 1999; Barendse et al. 2001). The genetic profile of the material that has just been collected from the wild, in terms of genetic diversity and degree of heterozygosity, is neither assessed at the time of its introduction in any collection, nor after each regeneration cycle. This is important as these latter genetic parameters are most probably strongly impacted in ex situ conditions, where populations sizes suffer a major bottleneck and are sometimes reduced down to a few or a single surviving or seed setting individual. Field observations (M.-C. Daunay, pers. obs.) suggest for several species (e.g. *Solanum burchellii* Dunal, *S. campylacantum*, *S. humile* Lam., *S. incanum*, *S. tomentosum*) a fruit and seed set decrease along successive regeneration cycles and hence a potential sensitiveness to inbreeding. The maintenance of the largest amount of genetic integrity for wild accessions along regeneration cycles is therefore a crucial challenge in the long-term run; unfortunately, this is not sufficiently taken into consideration by germplasm holders, because they have more immediate

[16]For instance, Australian materials adapted to arid conditions do not stand drip irrigation under plastic mulch and wither away.

constraints of experimental and financial nature to compose with. Unquantified and uncontrolled loss of heterozygosity, loss of allelic richness and genetic drift along successive ex situ regeneration cycles are not problems specific to spiny solanums. They should also be a concern for other allogamous or partially allogamous solanaceous crops wild relatives, such as the ones of the tomato. Such critical topics need to be addressed in the future by germplasm holders in collaboration with geneticists in order to (1) characterise the genetic diversity loss and its progression along regeneration cycles, and (2) adapt regeneration protocols for limiting the loss. End-users should also feel concerned about the way the germplasm they access was regenerated (e.g. degree of homogenisation, number of ex situ generations), because it can skew the interpretation of their experimental results.

If germplasm collections are essential for research and rescue of endangered material, we must not forget that crop wild relatives also require in situ conservation. Indeed, evolution and diversification are permanent biological processes that ensure, in a changing environment, the long-term propagation of living beings, including eggplants wild relatives. Hence, geneticists, germplasm holders, ECPGR crop wild relatives working group[17] and other international organisations must mobilise and use their synergic forces towards improved in situ and ex situ management of eggplant wild relatives.

10.4 Other "Eggplant" Species

The genus *Solanum* includes a wide number of edible species, cultivated or spontaneous, that are poorly known outside of their areas of cultivation; their fruits and/or leaves are used for various food preparations and beverages, and/or for medicinal purposes. Partial reviews on the edible species are available (Lawrence 1960; Heiser 1969; Nee 1991; Daunay et al. 1995), together with thorough surveys focused on South American (Council 1989) and African species (Schippers 2002). Here, we provide a glimpse at these edible species for two main reasons. First, they appear for diverse purposes in publications concerning eggplants (*S. aethiopicum*, *S. macrocarpon*, *S. melongena*). Second, in the near future, the genomic knowledge of an increasing number of *Solanum* species will provide access to (1) new allelic variation for traits of interest for eggplants (e.g. for pests and disease resistance, biochemical composition), and (2) access to genes controlling new traits of potential interest for eggplants (e.g. aerial and root architecture, fruit texture, fragrances, volatiles and secondary metabolites). For the sake of consistency, the following overview of these species is limited to the cultivated ones; clade names follow the nomenclature used by Särkinen et al. (2013).

10.4.1 Lasiocarpa Clade (Leptostemonum Clade)

The Lasiocarpa clade includes a dozen species, cultivated or wild. Their fruit, sweetish and aromatic, are well known in different South American countries. These species are perennials and are easily recognisable by their large and woolly leaves, as well as by their fruits, fuzzy when immature. All the Lasiocarpa clade species are diploid (Bernardello et al. 1994). *Solanum quitoense*, known as naranjilla or lulo, is a high-altitude crop, enjoyed in Ecuador and Columbia for its fragrant juice. *Solanum sessiliflorum*, known as cocona in Spanish speaking countries, and cubiu in Brazil, is native to the humid areas of the upper Amazon basin; its large (up to 9 cm in diameter) acidic berries is used either raw (salad, juice) or cooked (preserves, sauces, pies). Fruits of the tropical Asian *S. lasiocarpum* are used for flavouring curries in Thailand (Heiser 1985) and as food in China and the Philippines (Meyer et al. 2014).

[17] http://www.ecpgr.cgiar.org/working-groups/wild-species-conservation/.

10.4.2 Morelloid Clade (M Clade)

There are many edible nightshade species, the taxonomy of which is the subject of numerous successive clarifications (e.g. Edmonds 1972, 1977; Manoko 2007; Särkinen et al. 2018). These species are widespread in temperate and tropical regions of the world. Several species are common and popular leafy vegetables in Africa, such as the diploid *Solanum americanum* ($2n = 24$), the tetraploid *S. villosum* Mill. and *S. "eldoretii"*[18] ($2n = 48$), the hexaploid *S. scabrum* ($2n = 72$) (Schippers 2002; Fontem and Schippers 2004) and some other species (Ojiewo et al. 2013). Fruits of *S. retroflexum* Dunal, a tetraploid species, are used for tarts and jam. Philippines, Chinese and Indian ways of preparing *S.* "*americanum/nigrum*" are reported in Meyer et al. (2014).

Solanum nigrum, a widespread wild species, native to Europe, Asia and probably to Africa, is often confused with other nightshades (Schippers 2002). *Solanum nigrum* is rarely collected for consuming its young shoots and leaves, and its unripe fruits are considered poisonous (Jansen 2008).

10.4.3 Archaesolanum Clade (M Clade)

The so lovely called kangaroo apples were reported as being cultivated and consumed by Maoris in the past (Symon 1994). Endemic to New Guinea, Australia and New Zealand, they include species possessing a basic chromosome number $x = 23$ (Poczai et al. 2011a, b) among which *Solanum aviculare* ($2n = 2x = 46$) and *S. laciniatum* ($2n = 4x = 92$) are well known for their high alkaloid content and their pharmaceutical use (Symon 1994). Besides their ornamental interest, both species are grown industrially for alkaloid production. An additional peculiarity of the kangaroo apples is the presence of abundant stone cell aggregates within the fruits.

[18]This species could be *S. tarderemotum* Bitter (Manoko 2007)—also named *S. florulentum* Bitter (Ojiewo et al. 2013).

10.4.4 Potato Clade

Solanum sect. *Basarthrum* (Bitter) Bitter includes the domesticated *Solanum muricatum* (pepino), and a tenth of wild species that also produce edible fruits (Riley 1983); all these species are diploid (Anderson 1979). Native to Peru and Chile, *S. muricatum* was a prominent crop in the Andes before Hispanic conquests (Prohens et al. 1996). This vegetatively propagated crop has been bred and developed for commercial production in countries outside South America (Prohens et al. 2005; Herraiz et al. 2015). Fruits are used fresh or cooked or fried as vegetable. Further horticultural information is available in Council (1989).

10.4.5 Cyphomandra Clade

The 32 recognised species of the American Cyphomandra clade are diploid ($2n = 24$) woody shrubs or small trees without spines; their distinctiveness lies in their large chromosome size, high amounts of nuclear DNA, and in the presence of self-incompatibility for part of the species (Bohs 2007). In many species, sclerotic concretions are present in fruit mesocarp (as in Archaesolanum clade). This clade includes *Solanum betaceum* (tree tomato), a well-known cultivated taxon, but some wild species of the clade also produce edible fruits (Riley 1983; Bohs 1989). Fruits are eaten in similar way to tomatoes. Further information on Cyphomandra species traits, cultivation, genetic diversity and various uses are available in Bohs (1989).

10.5 Conclusion

Several major publications during the last 20 years have turned upside down the classification of the genus *Solanum*, including the particularly species-rich and challenging subgenus *Leptostemonum*. The transition between the old and new taxonomical systems, the first one mainly based on morphological similarities, the second on molecular phylogenetics, is visible in most publications,

where both former hierarchical ranks (e.g. subgenera, sections) and newly defined ones (e.g. clades, grades) are used. In addition to this, species names are continually stabilised among their many synonyms, and new species are defined or former species are lumped together as a single taxon. Hence, species names are in many cases misleading, in particular throughout papers published over a large span of time, even within the last ten years. As an end-user statement, eggplant breeders have no other choice but to adapt to a flexible naming and ranking of taxa; the evolutionary trees should be looked at as "flexible scaffolds", depending on the species sampling and set of markers used throughout the specialised literature. Some of the difficulties any neophyte interested in solanums has to face have been summarised (here and in Daunay et al. 2008). In the world of solanums, "nothing is as constant as change" (Knapp 2008). For now, many high-rank relationships in spiny solanum are still unresolved; the few deep nodes often suffer from weak statistical support. Therefore, further investigations are needed at various hierarchical ranks of the spiny solanums, by enlarging the molecular criteria used and the taxonomical sampling. However, strongly supported phylogenetic clades have also been identified within spiny solanums. In the case of the eggplant (*S. melongena*) and its closest relatives, the various phylogenetic efforts (Aubriot et al. 2018; Aubriot et al. 2016b; Vorontsova et al. 2013) have ended up in the delimitation of an Eggplant clade that groups the brinjal eggplant, with its 12 closest African and Asian relatives (Fig. 10.4). On a broader scale, investigations on phylogenetic relationships between intercontinental representatives of subgenus *Leptostemonum* are still ongoing. If a set of monophyletic clades has been identified so far, they only include a fraction of the concerned species. Hence, ongoing and future phylogenetic studies should provide within the next decade a clearer picture of this large group of species. Germplasm holders and eggplants breeders should be conscious that taxonomists are developing a sort of "species library" that they must take into consideration and use, for the sake of biodiversity rescue and use in breeding for sustainable food production in a changing environment.

Relatively good collections of cultivated eggplants exist in several genebanks, although *Solanum aethiopicum* and *S. macrocarpon* deserve further collecting efforts. The challenge for eggplants breeding in the future lies mostly in the capacity of breeders to get familiar with their wild related species. The EGGNET project was the first international attempt to create collaboration between taxonomists, breeders, germplasm holders and geneticists on the topic of eggplants wild relatives. This experience has shown that the transfer of botanical knowledge to end-users is arduous. Further efforts are needed before genebanks and breeders become familiar with the complex world of spiny solanums. The very large number of species related to eggplants is indeed both an outstanding opportunity for breeders to access a large phenotypic and genetic diversity, but is also a great knowledge-related obstacle. Availability of wild spiny solanums in ex situ collections (genebanks) is limited, even for the most represented species which are those originating from Africa, Madagascar and West Asia (49%). The Eggplant clade itself is so far poorly represented, since six (*S. agnewiorum*, *S. aureitomentosum*, *S. lanzae*, *S. rigidum*, *S. umtuma* and *S. usambarense*) out of the 13 species are missing in genebanks. This statement indicates that forces need to be combined for completing collections (more species, more accessions per species), because ex situ collections are the base and the long-term insurance of eggplants breeding. Collecting efforts are all the more urgent that many East African spiny solanums are endangered. Given the wealth of species concerned, the legal regulations about germplasm exchange, and the difficulty to collect in the wild in Africa, a collaborative initiative, headed by international bodies such as Biodiversity International or the Global Crop Diversity trust, seems desirable. However, ex situ collections are not the ultimate solution to crop wild relatives saving and conservation, in particular because their genetic diversity is inevitably negatively affected by ex situ regeneration. Also, ex situ conservation in cold rooms with few regeneration cycles per decade stops otherwise natural and continuous evolution events within a changing environment. Therefore, in situ actions are crucial for effective and long-term species conservation and should be prioritised.

Appendix 1

Inventory of African, Malagasy and West Asian spiny solanums

Species	Species names, when mentioned additionally or differently in Daunay and Hazra (2012)	Geographical distribution	Phylogenetic grouping (clade or grade)	Historical section	Exist in ex situ collections?
S. aculeastrum Dunal	–	Africa, down 10°N	Aculeastrum	Melongena Dunal	INRA
S. adoense Hochst. ex A.Rich.	S. piperiferum A. Rich.	North-Eastern Africa	Anguivi grade	Oliganthes (Dunal) Bitter	–
S. aethiopicum L.	S. gilo Raddi, S. zuccagnianum Dunal	Tropical subsaharan Africa & Madagascar	Anguivi grade	Oliganthes (Dunal) Bitter	INRA and others
S. agnewiorum Voronts.	–	Kenya	Eggplant	Melongena Dunal	–
S. aldabrense C.H. Wright	–	Seychelles islands	Anguivi grade	Oliganthes (Dunal) Bitter	–
S. anguivi Lam.	S. indicum L., S. rohrii C.H. Wright	Africa (mostly between 15 °N and 25 °S)	Anguivi grade	Oliganthes (Dunal) Bitter	INRA and others
S. anomalum Thonn.	–	Central West Africa	Giganteum	Torva Nees	–
S. arundo Mattei	S. diplacanthum Dammer	Somalia, Kenya, Tanzania	Arundo	Ischyracanthum Bitter	INRA
S. aureitomentosum Bitter	–	Africa: spread from 5° to 20° latitude S	Eggplant	Melongena Dunal	–
S. batoides D'Arcy & Rakotozafy	–	Madagascar	Madagascar	Croatianum D'Arcy & Keating	–
S. bumeliifolium Dunal	–	Madagascar	Madagascar	Croatianum D'Arcy & Keating	–
S. burchellii Dunal	–	South Africa and Namibia	Anguivi grade	Oliganthes (Dunal) Bitter	INRA
S. campylacanthum Hochst. ex A.Rich.	S. incanum group A and B, S. delagoense Dunal, S. panduriforme Drège ex Dunal	Southern and eastern Africa	Eggplant	Melongena Dunal	INRA and others
S. capense L.	S. dinteri Bitter, S. namaquense Dammer	Namibia and South Africa	Anguivi grade	Oliganthes (Dunal) Bitter	INRA and others
S. catombelense Peyr.	S. rautanenii Schinz	Southern Africa	Anguivi grade	Oliganthes (Dunal) Bitter	INRA
S. cerasiferum Dunal	–	Subsaharan Africa, from Senegal to Sudan	Eggplant	Melongena Dunal	INRA

(continued)

Species	Species names, when mentioned additionally or differently in Daunay and Hazra (2012)	Geographical distribution	Phylogenetic grouping (clade or grade)	Historical section	Exist in ex situ collections?
S. coagulans Forsskal	S. dubium Fresen., S. thruppii C.H. Wright	Eastern corner of Africa; limited occurrence on Arabian peninsula	Coagulans	Monodolichopus Bitter	INRA
S. cordatum Forsskal	S. darassumense Dammer, S. gracilipes Decne.	Africa horn, Arabian peninsula east to Northern India	–	Oliganthes (Dunal) Bitter	–
S. croatii D'Arcy & Keating	–	Madagascar	Madagascar	Croatianum D'Arcy & Keating	–
S. cyaneopurpureum De Willd.	–	Central Africa	Anguivi grade	Oliganthes (Dunal) Bitter	INRA
S. cymbalariifolium Chiov.	–	Somalia	–	Oliganthes (Dunal) Bitter	–
S. dasyphyllum Schumach. & Thonn.	S. acanthoideum Drège ex Dunal	Africa: common between ca. 15 °N and ca. 10 °S	Anguivi grade	Melongena Dunal	INRA & others
S. dennekense Dammer	S. ogadense Bitter	Somalia, Ethiopia, Kenya, Tanzania	Arundo	Ischyracanthum Bitter	INRA
S. erythracanthum Dunal	S. flagelliferum Baker, S. nossibeense Vatke	Madagascar	Madagascar	Oliganthes (Dunal) Bitter	–
S. forskalii Dunal	S. albicaule Kotschy ex Dunal	Common in Eastern Africa and Arabian peninsula	–	Oliganthes (Dunal) Bitter	INRA
S. giganteum Jacq.	–	Tropical and southeastern Africa, eastwards to India	Giganteum	Torva Nees	INRA
S. glabratum Dunal	S. sepicula Dunal	North-eastern Africa and Arabia	Anguivi grade	Oliganthes (Dunal) Bitter	INRA
S. goetzii Dammer	–	Southeastern Africa	Giganteum	Torva Nees	INRA
S. hastifolium Hochst. ex Dunal	–	Tropical Eastern Africa	Anguivi grade	Oliganthes (Dunal) Bitter	INRA
S. heinianum D'Arcy & Keating	–	Madagascar	Madagascar	Croatianum D'Arcy & Keating	INRA
S. humile Lam.	S. giftbergense Dunal, S. rigescens Jacq., S. rigescentoides Hutch., S. subrectimunitum Bitter	Southwestern Africa (Angola to South Africa)	Anguivi grade	Oliganthes (Dunal) Bitter	INRA

(continued)

Species	Species names, when mentioned additionally or differently in Daunay and Hazra (2012)	Geographical distribution	Phylogenetic grouping (clade or grade)	Historical section	Exist in ex situ collections?
S. inaequiradians Werderm.	–	Tanzania	Anguivi grade	Oliganthes (Dunal) Bitter	–
S. incanum L.	–	Subsaharan Africa; north of equator, eastwards to western Pakistan	Eggplant	Melongena Dunal	INRA & others
S. jubae Bitter	–	Horn of Africa	–	Somalanum Bitter	–
S. lamprocarpum Bitter	S. zanzibarense Vatke	Costal Tanzania and northern Mozambique	Anguivi grade	Oliganthes (Dunal) Bitter	INRA
S. lanzae J.P.Lebrun & Stork	–	From Ethiopia to Tanzania	Eggplant	Oliganthes (Dunal) Bitter	–
S. lichtensteinii Willd.	S. incanum group D	Southern Africa	Eggplant	Melongena Dunal	INRA
S. lidii Sunding	–	Canary island	Anguivi grade	Nycterium (Vent.) Walp.	INRA
S. linnaeanum Hepper & P.M.L. Jaeger	S. sodomeum Dunal (non L.)	South Africa, Mediterranean	Eggplant	Melongena Dunal	INRA
S. litoraneum A.E. Gonç.	–	South Mozambique	Anguivi grade	Oliganthes (Dunal) Bitter	–
S. macracanthum A. Richard	S. bellicosum Bitter	Ethiopia	Anguivi grade	Oliganthes (Dunal) Bitter	–
S. macrocarpon L.	–	Cultivated across tropical Africa	Anguivi grade	Melongena Dunal	INRA and others
S. mahoriense D'Arcy & Rakotozafy	–	Madagascar	Madagascar	Cryptocarpum Dunal	INRA
S. malindiense Voronts.	–	Kenya (coastal)	Anguivi grade	Oliganthes (Dunal) Bitter	–
S. marginatum L.f.	–	Ethiopia and Erithrea	–	Melongena Dunal	INRA
S. mauense Bitter	–	Kenya, Tanzania	Anguivi grade	Oliganthes (Dunal) Bitter	–
S. melastomoides C.H.Wright	–	Horn of Africa	Coagulans	Monodolichopus Bitter	–
S. myoxotrichum Baker	–	Madagascar	Madagascar	Oliganthes (Dunal) Bitter	INRA
S. nigriviolaceum Bitter	S. sessilistellatum Bitter	Kenya	Anguivi grade	Melongena Dunal	INRA
S. pampaninii Chiov.	S. robecchii Bitter & Dammer	Somalia to Kenya	–	Somalanum Bitter	–

(continued)

Species	Species names, when mentioned additionally or differently in Daunay and Hazra (2012)	Geographical distribution	Phylogenetic grouping (clade or grade)	Historical section	Exist in ex situ collections?
S. pauperum C.H. Wright	–	Angola	Giganteum	Torva Nees	–
S. phoxocarpum Voronts.	–	Kenya, Tanzania	Aculeastrum	Melongena Dunal	–
S. platacanthum Dunal	–	Arabian peninsula	Anguivi grade	–	–
S. polhillii Voronts.	–	Kenya, Tanzania	Anguivi grade	Oliganthes (Dunal) Bitter	–
S. pyracanthos Lam.	–	Madagascar	Madagascar	Oliganthes (Dunal) Bitter	INRA
S. richardii Dunal	–	Southeastern Africa and Madagascar	Climbing	Melongena Dunal	INRA
S. rigidum Lam.	–	Cape verde islands	Eggplant	–	–
S. rubetorum Dunal	S. rigescens Dunal (non Jacq.)	South Africa	Anguivi grade	Oliganthes (Dunal) Bitter	INRA
S. ruvu Voronts.	–	Tanzania	Anguivi grade	Oliganthes (Dunal) Bitter	–
S. schimperianum Hochst. ex A.Rich.	–	North-eastern Africa, across the Middle East to India	Giganteum	Torva Nees	INRA
S. schliebenii Werderm.	–	Tanzania	Giganteum	Torva Nees	–
S. schumannianum Dammer	S. kagehense Dammer	Central eastern Africa	Giganteum	Torva Nees	–
S. setaceum Dammer	–	Northern Tanzania and southern Kenya	Anguivi grade	Oliganthes (Dunal) Bitter	–
S. sodomaeodes Kuntze	–	South Africa	Anguivi grade	Oliganthes (Dunal) Bitter	–
S. somalense Franch.	–	Horn of Africa	Giganteum	Anisantherum Bitter	INRA
S. stipitatostellatum Dammer	S. kitivuense Dammer	Tanzania, Kenya, Mozambique	Climbing	Oliganthes (Dunal) Bitter	–
S. supinum Dunal	S. leucophaeum Dunal	Southern Africa	Anguivi grade	Oliganthes (Dunal) Bitter	INRA and others
S. taitense Vatke	–	Kenya, Tanzania	Anguivi grade	Oliganthes (Dunal) Bitter	–
S. tettense Klotzsch	S. renschii Vatke, S. kwebense N.E.Br.	Widespread in Eastern Africa (down Somalia), and southern Africa (up to Angola)	Giganteum	Torva Nees	INRA

(continued)

Species	Species names, when mentioned additionally or differently in Daunay and Hazra (2012)	Geographical distribution	Phylogenetic grouping (clade or grade)	Historical section	Exist in ex situ collections?
S. thomsonii C.H. Wright	–	Tanzania	Aculeastrum	Melongena Dunal	–
S. toliaraea D'Arcy & Rakotozafy	–	Madagascar	Madagascar	Oliganthes (Dunal) Bitter	–
S. tomentosum L.	S. coccineum Jacq.	Southern South Africa	Anguivi grade	Oliganthes (Dunal) Bitter	INRA
S. torreanum A.E. Gonçalves	–	Area at the junction of Mozambique, South Africa, Swaziland	Anguivi grade	Oliganthes (Dunal) Bitter	–
S. umtuma Voronts. & S.Knapp	–	KwaZulu Natal and Eastern South Africa	Eggplant	Melongena Dunal	–
S. usambarense Bitter & Dammer	–	Tanzania and Kenya	Eggplant	Oliganthes (Dunal) Bitter	–
S. usaramense Dammer	–	Southern Kenya to Mozambique	Anguivi grade	Oliganthes (Dunal) Bitter	–
S. vespertilio Aiton	–	Canary island	Anguivi grade	Nycterium (Vent.) Walp.	INRA
S. wittei Robyns	–	Uganda to Tanzania	Giganteum	Torva Nees	–
S. zanzibarense Vatke	S. monotanthum Dammer, S. vagans C.H.Wright	Coastal areas of Kenya, Tanzania and Mozambique	Climbing	Oliganthes (Dunal) Bitter	–

Appendix 2

Inventory of tropical Asian spiny solanums

Species	Geographical distribution	Phylogenetic grouping (clade or grade)	Historical section	Exist in ex situ collections?
S. abortivum Symon	Papua New Guinea	–	Graciliflorum (Dunal) Seithe	–
S. anfractum Symon	Indonesia (West Papua), Papua New Guinea	Sahul-Pacific	Graciliflorum (Dunal) Seithe	–
S. athenae Symon	Papua New Guinea	S. athenae and relatives	Lasiocarpa Dunal (D'Arcy)	–
S. barbisetum Nees	China, Indian subcontinent, Indochinese Peninsula	–	–	–
S. borgmannii Symon	Papua New Guinea	Sahul-Pacific	Graciliflorum (Dunal) Seithe	–

(continued)

Species	Geographical distribution	Phylogenetic grouping (clade or grade)	Historical section	Exist in ex situ collections?
S. camranhense Dy Phon & Hul.	Vietnam	S. camranhense and relatives	–	–
S. comitis Dunal	Indonesia (Java)	–	–	–
S. cyanocarphium Blume	Indonesia (Borneo, Java, Sumatra), Philippines	S. cyanocarphium and S. sakhanii	–	–
S. dallmannianum Warb.	Papua New Guinea	Sahul-Pacific	Graciliflorum (Dunal) Seithe	–
S. dammerianum Lauterb. & K. Schum	Indonesia (West Papua), Papua New Guinea	Old World torvoids	Torvum Nees	–
S. deflexicarpum C.Y.Wu and S.C. Huang	China (Yunnan)	S. violaceum and relatives	–	–
S. denseaculeatum Symon	Papua New Guinea	Sahul-Pacific	Graciliflorum (Dunal) Seithe	–
S. dunalianum Gaudich.	Indonesia, Papua New Guinea	Sahul-Pacific	Dunalianum (Bitter) Symon	–
S. expedunculatum Symon	Papua New Guinea	S. expedunculatum and relatives	Graciliflorum (Dunal) Seithe	–
S. gibbsiae J. Drumm.	Indonesia, Papua New Guinea	–	Graciliflorum (Dunal) Seithe	–
S. graciliflorum Dunal	Indonesia	Sahul-Pacific	Graciliflorum (Dunal) Seithe	–
S. harmandii Bonati	Cambodia	–	–	–
S. heteracanthum Merr. & L.M.Perry	Indonesia (West Papua), Papua New Guinea	S. expedunculatum and relatives	Graciliflorum (Dunal) Seithe	–
S. hovei Dunal	India (Goa, Gujarat, Maharashtra, Kerala)	S. violaceum and relatives	Oliganthes (Dunal) Bitter	–
S. incanoalabastrum Symon	Papua New Guinea	–	Dunalianum (Bitter) Symon	–
S. infuscatum Symon	Papua New Guinea	–	Graciliflorum (Dunal) Seithe	–
S. insanum L. (1)	China, Indian subcontinent, Indochinese Peninsula, Indonesia, Madagascar, Malesia	Eggplant	Melongena Dunal	INRA & others
S. involucratum Blume	Indochinese Peninsula, Indonesia	S. expedunculatum and relatives	–	–
S. lasiocarpum Dunal	China, Indian subcontinent, Indochinese Peninsula, Indonesia, Malesia, Papua New Guinea	Lasiocarpa	Lasiocarpa (Dunal) D'Arcy	INRA & others
S. leptacanthum Merr. & L.M.Perry	Papua New Guinea	S. athenae and relatives	Graciliflorum (Dunal) Seithe	–
S. lianoides Elmer	Philipines (Sibuyan)	Sahul-Pacific	Micracantha Dunal	–
S. melongena L. (2)	Cultivated worldwide	Eggplant	Melongena Dunal	many genebanks

(continued)

Species	Geographical distribution	Phylogenetic grouping (clade or grade)	Historical section	Exist in ex situ collections?
S. missimense Symon	Papua New Guinea	Sahul-Pacific	Graciliflorum (Dunal) Seithe	–
S. multiflorum Roth	Indian subcontinent (Kerala, Tamil Nadu)	S. violaceum and relatives	–	–
S. nienkui Merr. & Chun	China (Hainan), Viet Nam	S. camranhense and relatives	–	–
S. nolense Symon	Papua New Guinea	–	Graciliflorum (Dunal) Seithe	–
S. papuanum Symon	Papua New Guinea	Sahul-Pacific	Graciliflorum (Dunal) Seithe	–
S. peekelii Bitter	Papua New Guinea	–	Dunalianum (Bitter) Symon	–
S. peikuoense S.S. Ying	Taiwan	Old World torvoids	–	–
S. platacanthum Dunal	Arabian peninsula	Anguivi Grade	–	–
S. poka Dunal	Indonesia	Old World torvoids	–	–
S. praetermissum Kerr	China, Indian subcontinent, Indochinese Peninsula	–	–	–
S. procumbens Lour.	China, Indochinese Peninsula	S. expedunculatum and relatives	–	–
S. pseudosaponaceum Blume	China, Indonesia, Japan, Laos, Philipines, Taiwan	Old World torvoids	Torvum Nees	–
S. pubescens Willd.	Indian subcontinent, Saudi Arabia, Sri Lanka, Yemen	Giganteum	Anisantherum Bitter	–
S. putii Kerr ex Barnett	Thailand	S. camranhense and relatives	–	–
S. retrorsum Elmer	Philippines (Luzon)	–	–	–
S. rivicola Symon	Papua New Guinea	Sahul-Pacific	Graciliflorum (Dunal) Seithe	–
S. robinsonii Bonati	Vietnam (Cam Ranh)	–	–	–
S. saruwagedensis Symon	Papua New Guinea	–	Graciliflorum (Dunal) Seithe	–
S. schefferi F.Muell.	Papua New Guinea	S. athenae and relatives	Micracantha Dunal	–
S. tetrandrum R.Br.	Papua New Guinea	–	Dunalianum (Bitter) Symon	–
S. torricellense Bitter	Papua New Guinea	–	Dunalianum (Bitter) Symon	–
S. torvoideum Merr. & L.M.Perry	Australia, Indonesia, Papua New Guinea, Philippines, Taiwan	Old World torvoids	Torvum Nees	–
S. trichostylum Merr. & L.M.Perry	Indonesia (West Papua), Papua New Guinea	Sahul-Pacific	Graciliflorum (Dunal) Seithe	–
S. trilobatum L.	Indian subcontinent, Indochinese Peninsula, Sri Lanka	Anguivi grade	Oliganthes (Dunal) Bitter	INRA & others

(continued)

Species	Geographical distribution	Phylogenetic grouping (clade or grade)	Historical section	Exist in ex situ collections?
S. turraeaefolium S. Moore	Papua New Guinea	Sahul-Pacific	Graciliflorum (Dunal) Seithe	–
S. violaceum Ortega (3)	China, Indian subcontinent, Indochinese Peninsula, Indonesia, Madagascar, Malesia, Mauritius	S. violaceum and relatives	Oliganthes (Dunal) Bitter	INRA & others
S. virginianum L. (4)	Afghanistan, China, Indian subcontinent, Iran, Yemen	–	Melongena Dunal	INRA & others
S. viridifolium Dunal	Australia, Papua New Guinea	–	Dunalianum (Bitter) Symon	–
S. wightii Nees	India (Tamil Nadu)	–	–	–

(1) S. melongena L. group E (S. insanum L.), group F (S. cumingii Dunal, S. undatum Poir.) in Daunay and Hazra (2012); (2) S. melongena L group G (S. ovigerum Dunal), group H (S. melongena advanced cultivars) in Daunay and Hazra (2012); (3) S. indicum L. in Daunay and Hazra (2012). The prickleless form of S. violaceum is found under the name of S. kurzii Brace ex Prain and S. sanitwongsei Craib; (4) S. xanthocarpum Schrad. & J.C.Wendl., and S. surattense Burm.f. in Daunay and Hazra (2012)

Appendix 3

Partial inventory of Australian spiny solanums

Species	Phylogenetic grouping	Historical section	Exist in ex situ collection?
S. asymmetriphyllum Specht	–	Melongena Dunal	–
S. beaugleholei Symon	–	Melongena Dunal	INRA
S. campanulatum Symon	Sahul-Pacific clade	Campanulata Symon	INRA
S. carduiforme F.Muell.	–	Melongena Dunal	–
S. cataphractum A.Cunn. ex Benth.	–	Melongena Dunal	–
S. centrale J.M.Black	–	Leprophora Dunal	INRA
S. chenopodinum F.Muell.	Sahul-Pacific clade	Graciliflorum (Dunal) Seithe	–
S. chippendalei Symon	–	Melongena Dunal	INRA
S. cinereum R.Br.	Sahul-Pacific clade	Melongena Dunal	INRA
S. clarkiae Symon	–	Melongena Dunal	INRA
S. cleistogamum Symon	–	Oliganthes (Dunal) Bitter	–
S. cookii Symon	–	–	–
S. cunninghamii Benth.	–	Melongena Dunal	INRA
S. densevestitum F.Muell. ex Benth.	Sahul-Pacific clade	–	–
S. dimorphospinum C.T.White	–	–	–
S. dioicum W.Fitzg.	–	Melongena Dunal	INRA
S. diversiflorum F.Muell.	–	Melongena Dunal	INRA
S. eburneum Symon	–	Melongena Dunal	INRA
S. echinatum R.Br.	–	Leprophora Dunal	–

(continued)

Species	Phylogenetic grouping	Historical section	Exist in ex situ collection?
S. ellipticum R.Br.	–	Leprophora Dunal	–
S. esuriale Lindl.	–	Leprophora Dunal	INRA
S. ferocissimum Lindl.	Sahul-Pacific clade	Graciliflorum (Dunal) Seithe	–
S. furfuraceum R.Br.	Sahul-Pacific clade	–	–
S. gympiense Symon	–	–	–
S. heteropodium Symon	–	Melongena Dunal	–
S. hoplopetalum Bitter & Summerh.	–	–	–
S. hystrix R.Br.	–	–	–
S. leopoldensis Symon	–	Melongena Dunal	–
S. melanospermum F.Muell.	–	Melongena Dunal	INRA
S. nemophilum F.Muell.	–	–	–
S. nummularium S.Moore	–	–	–
S. oedipus Symon	–	Melongena Dunal	–
S. oldfieldii F.Muell.	–	–	–
S. oligandrum Symon	–	–	–
S. papaverifolium Symon	Sahul-Pacific clade	–	–
S. petraeum Symon	–	Melongena Dunal	INRA
S. phlomoides A.Cunn. ex. Benth.	–	Melongena Dunal	INRA
S. prinophyllum Dunal	Sahul-Pacific clade	Oliganthes (Dunal) Bitter	INRA
S. pugiunculiferum C.T.White	–	–	–
S. sejunctum Brennan, Martine & Symon	–	–	–
S. stelligerum Sm.	Sahul-Pacific clade	–	–
S. stupefactum Symon	–	–	–
S. tudununggae Symon	–	Melongena Dunal	–
S. vansittartensis C.A.Gardner	–	Melongena Dunal	–

Appendix 4

Partial inventory of New World spiny solanums

Species	Native to	Phylogenetic grouping (clade)	Historical sections	Exist in ex situ collections?
S. absconditum Agra	Brazil	Erytrotrychum	–	–
S. accrescens Standl. & C.V.Morton	Costa Rica	Erytrotrychum	Erythrotrichum (Whalen) Child	–
S. acerifolium Dunal	Central and South America	Acanthophora	Acanthophora Dunal	–

(continued)

Species	Native to	Phylogenetic grouping (clade)	Historical sections	Exist in ex situ collections?
S. aculeatissimum Jacq.	South America (introduced in the Old World tropics; widespread in tropical Africa)	Acanthophora	Acanthophora Dunal	INRA
S. acutilobum Dunal	Bolivia	Torva	–	–
S. affine Sendtn. (1)	Brazil	Acanthophora	Acanthophora Dunal	INRA
S. agrarium Sendtn.	Central and South America	Gardneri	Acanthophora Dunal	–
S. albidum Dunal	South America	Torva	Torva Nees	–
S. arachnidanthum Rusby	Bolivia, Brazil	Micracantha	Micracantha Dunal	–
S. aridum Morong	Argentina, Bolivia, Paraguay	Carolinense	–	–
S. asperolanatum Ruiz & Pav.	South America	Torva	Torva Nees	–
S. asterophorum Mart.	Brazil	Asterophorum	Polytrichum (Whalen) Child	–
S. asteropilodes Bitter	Ecuador	Torva	Torva Nees	–
S. atropurpureum Schrank	South America	Acanthophora	Acanthophora Dunal	INRA
S. aturense Dunal	Central and South America	Micracantha	Micracantha Dunal	–
S. bahamense L.	Carribean islands	Bahamense	Persicariae Dunal	–
S. bolivianum Britton ex Risby	Bolivia	Torva	Torva Nees	–
S. bonariense L.	South America (introduced in Asia and Europe)	Torva	Torva Nees	–
S. buddleifolium Sendtn.	South America	Thomasiifolium	Persicariae Dunal	–
S. campechiense L.	Central and South America, Carribean islands	–	Melongena subsect. Cryptocarpum (Dunal) G. Don	–
S. candidum Lindl. (2)	Central and South America	Lasiocarpa	Lasiocarpa (Dunal) D'Arcy	INRA
S. capsicoides All.	South America (introduced in tropical Asia)	Acanthophora	Acanthophora Dunal	INRA
S. caricaefolium Rusby	Bolivia, Peru	Torva	Torva Nees	–
S. carolinense L.	United States of America	Carolinense	Melongena subsect. Lathyrocarpum G. Don	–
S. chrysotrichum Schltdl. (3)	Central and South America	Torva	Torva Nees	INRA
S. citrullifolium A. Braun	Central America	Androceras/crinitum	Melongena series Violaceiflorum Whalen	INRA
S. comarapanum M. Nee	Bolivia	Torva	–	–
S. comptum C.V. Morton	Argentina, Bolivia, Paraguay	Carolinense	Melongena subsect. Lathyrocarpum G. Don	–

(continued)

Species	Native to	Phylogenetic grouping (clade)	Historical sections	Exist in ex situ collections?
S. coriaceum Dunal	South America	Androceras/Crinitum	Micracantha Dunal	–
S. crinitipes Dunal	South America	Torva	Torva Nees	–
S. crinitum Lam.	South America	Androceras/Crinitum	Crinitum (Whalen) Child	–
S. crotonoides Lam.	Carribean islands	–	Persicariae Dunal	–
S. decompositiflorum Sendtn.	Brazil	Erythrotrichum	–	–
S. decorum Sendtn.	Brazil	Erythrotrichum	–	–
S. donianum Walp.	Central America	Torva	Torva Nees	–
S. elaeagnifolium Cav.	North and Southern America (amphitropical distribution)	Elaegnifolium	Melongena subsect. Lathyrocarpum G. Don	INRA & others
S. ensifolium Dunal	Puerto Rico	Bahamense	–	–
S. felinum Bitter ex Whalen	Venezuela	Lasiocarpa	Lasiocarpa (Dunal) D'Arcy	–
S. gardneri Sendtn.	Brazil, Colombia, Venezuela	Gardneri	Persicariae Dunal	–
S. glutinosum Dunal	Colombia, Ecuador, Peru	Torva	Torva Nees	–
S. grayi Rose	Mexico	Androceras/Crinitum	Melongena series Pacificum Whalen	–
S. hasslerianum Chodat	Paraguay	Sisymbriifolium	Melongena subsect. Lathyrocarpum G. Don	–
S. hexandrum Vell.	Brazil	Erythrotrichum	Polytrichum (Whalen) Child	–
S. hieronymi Kuntze	Argentina, Bolivia, Paraguay	–	Melongena subsect. Lathyrocarpum G. Don	–
S. hindsianum Benth.	Mexico, United States of America	Elaegnifolium	Melongena subsect. Lathyrocarpum G. Don	–
S. hirtum Vahl	Central and South America	Lasiocarpa	Lasiocarpa (Dunal) D'Arcy	INRA
S. homalospermum Chiarini	Argentina	Elaegnifolium	–	–
S. houstonii Martyn (4)	Mexico	Elaegnifolium	Melongena subsect. Lathyrocarpum G. Don	INRA
S. hyporhodium A. Braun & C.D. Bouché	Venezuela	Lasiocarpa	Lasiocarpa (Dunal) D'Arcy	–
S. incarceratum Ruiz & Pav.	South America	Acanthophora	Acanthophora Dunal	–
S. jabrense Agra & M.Nee	Brazil	Erythrotrichum	–	–
S. jamaicense Mill.	Central and South America, Carribean islands	Micracantha	Micracantha Dunal	INRA
S. juvenale Thell.	Argentina	Carolinense	Melongena subsect. Lathyrocarpum G. Don	–

(continued)

Species	Native to	Phylogenetic grouping (clade)	Historical sections	Exist in ex situ collections?
S. lanceifolium Jacq.	Central America, Carribean islands	Micracantha	Micracantha Dunal	–
S. lanceolatum Cav.	Central and South America	Torva	Torva Nees	–
S. leucopogon Huber	South America	Micracantha	Micracantha Dunal	–
S. lycocarpum A.St.-Hil.	Brazil, Paraguay	Androceras/Crinitum	Crinitum (Whalen) Child	–
S. mammosum L.	Central and South America, Carribean islands (introduced in the Old World tropics)	Acanthophora	Acanthophora Dunal	INRA
S. megalonyx Sendtn.	Brazil	Erythrotrichum	Erythrotrichum (Whalen) Child	–
S. metrobotryon Dunal	Brazil	Torva	–	–
S. microphyllum (Lam.) Dunal	Carribean islands	Gardneri	Persicariae Dunal	–
S. mitlense Dunal	Mexico	Androceras/critum	Crinitum (Whalen) Child	–
S. monachophyllum Dunal	South America	Micracantha	Micracantha Dunal	–
S. mortonii Hunz.	Argentina	Elaegnifolium	Melongena subsect. Lathyrocarpum G. Don	–
S. moxosense M.Nee	Bolivia	Carolinense	–	–
S. multispinum N.E. Br.	Argentina, Paraguay	–	Melongena subsect. Lathyrocarpum G. Don	–
S. myriacanthum Dunal	Central America	Acanthophora	Acanthophora Dunal	–
S. palinacanthum Dunal	South America	Acanthophora	Acanthophora Dunal	INRA
S. paniculatum L.	Brazil, Paraguay	Torva	Torva Nees	–
S. paraibanum Agra	Brazil	Thomasiifolium	Micracantha Dunal	–
S. pectinatum Dunal	Central and South America	Lasiocarpa	Lasiocarpa (Dunal) D'Arcy	INRA & others
S. pedemontanum M.Nee	South America	Micracantha	–	–
S. piluliferum Dunal	Brazil	Asterophorum	–	–
S. platense Diekm.	Brazil, Uruguay	Acanthophora	–	–
S. pluviale Standl.	Costa Rica, Panama	Torva	–	–
S. poinsettiifolium Rusby	Bolivia, Brazil, Peru	Torva	Micracantha Dunal subsect. Subinermia (Dunal) G. Don.	–
S. polygamum Vahl	Carribean islands	–	Persicariae Dunal	–
S. polytrichum Moric.	Brazil	Gardneri	Polytrichum (Whalen) Child	–
S. pseudolulo Heiser	Colombia, Ecuador	Lasiocarpa	Lasiocarpa (Dunal) D'Arcy	INRA

(continued)

Species	Native to	Phylogenetic grouping (clade)	Historical sections	Exist in ex situ collections?
S. quitoense Lam.	South America	Lasiocarpa	Lasiocarpa (Dunal) D'Arcy	INRA & others
S. reflexiflorum Moric. ex Dunal	Brazil	Erythrotrichum	–	–
S. rhytidoandrum Sentdn.	Bolivia, Brazil, Paraguay	Erythrotrichum	–	–
S. robustum H.L. Wendl.	South America (introduced in Old World tropics)	Erythrotrichum	Erythrotrichum (Whalen) Child	–
S. rostratum Dunal	Mexico, United States of America	Androceras/crinitum	Melongena series Androceras (Nutt.) Whalen	INRA & others
S. rudepannum Dunal	Central and South America	Torva	Torva Nees	–
S. rupincola Sendtn.	Brazil	Thomasiifolium	–	–
S. schomburghii Sendtn.	South America	Gardneri	Persicariae Dunal	–
S. scuticum M.Nee	Bolivia, Brazil, Paraguay	Torva	–	–
S. sendtnerianum Van Heurck & Müll. Arg.	Brazil, French Guiana	Androceras/Crinitum	Micracantha Dunal	–
S. sessiliflorum Dunal (5)	South America	Lasiocarpa	Lasiocarpa (Dunal) D'Arcy	INRA & others
S. sisymbriifolium Lam.	South America (introduced in the Old World tropics)	Sisymbriifolium	Melongena subsect. Cryptocarpum (Dunal) G. Don	INRA & others
S. stagnale Moric.	Brazil	Erythrotrichum	Polytrichum (Whalen) Child	–
S. stellativelutinum Bitter	Bolivia	Torva	Torva Nees	–
S. stenandrum Sendtn.	Brazil	Gardneri	Acanthophora Dunal	–
S. stramoniifolium Jacq.	Central and South America, Carribean islands	Lasiocarpa	Lasiocarpa (Dunal) D'Arcy	INRA & others
S. subinerme Jacq.	Central and South America, Carribean islands	Torva	Micracantha Dunal subsect. Subinermia (Dunal) G. Don.	–
S. subumbellatum Vell.	Brazil	Torva	Torva Nees	–
S. talarense Svenson	Peru	Gardneri	Acanthophora Dunal	–
S. tampicense Dunal	Central America, Carribean islands	Micracantha	Micracantha Dunal	–
S. tenuispinum Rusby	Argentina, Bolivia, Peru	Acanthophora	Acanthophora Dunal	–
S. tetramerum Dunal	Carribean islands	Gardneri	–	–
S. thomasiifolium Sendtn.	Brazil	Thomasiifolium	Persicariae Dunal	–
S. torvum Sw.	Carribean and Central America (probably introduced and then naturalized in the Old World tropics)	Torva	Torva Nees	INRA & others
S. ursinum Rusby	Bolivia, Peru	Torva	Torva Nees	–

(continued)

Species	Native to	Phylogenetic grouping (clade)	Historical sections	Exist in ex situ collections?
S. urticans Dunal	Bolivia	Androceras/Crinitum	Crinitum (Whalen) Child	–
S. vaillantii Dunal	Brazil	Acanthophora	Acanthophora Dunal	–
S. vestissimum Dunal	Colombia, Venezuela	Lasiocarpa	Lasiocarpa (Dunal) D'Arcy	–
S. viarum Dunal	South America (introduced in tropical Asia)	Acanthophora	Acanthophora Dunal	INRA & others
S. whalenii M.Nee	Bolivia	Torva	–	–
S. wrightii Benth. (6)	Central and South America (introduced in the Old World tropics)	Androceras/Crinitum	Crinitum (Whalen) Child	INRA

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Crossability and Diversity of Eggplants and Their Wild Relatives

11

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Abstract

Eggplants and related germplasm are a barely unveiled genetic treasure, for reasons developed in Chap. 10. Diversity and interspecific crossability researches focused so far on *Solanum melongena* L., the economic importance of which towers that of the indigenous African *S. aethiopicum* L. and *S. macrocarpon* L. and which consequently attracted most of geneticists' and breeders' attention. However, as *S. melongena* shares many connections with eggplant germplasm as a whole, this chapter pays as much attention to this species as to the other cultivated and wild ones. Their genetic and phenotypic diversity is surveyed and critically analysed in order to place the reader at the crossroads between the present knowledge and desirable future researches in terms of both traits of interest to breeders and methods for assessing the diversity. The dense corpus of information about interspecific crossability is organised across several axes. Conventional sexual crosses and somatic hybridisations are presented separately, given both methods yield genetically different interspecific material. The section devoted to sexual crosses begins with a survey of the interspecific barriers, and with an overview of the crossing results that are discussed in their methodological dimensions, in particular the criteria assessing the success or failure of the crossing experiments. Then, the crossing results are structured according to the combinations of crosses within and between cultivated and wild material. Species crossability is discussed with regard to the genepool concept and to relationship between species assessed by phylogenetics. The section ends up with interspecific hybrid by-products such as male sterilities and information on traits genetics. The chapter turns then to somatic hybridisations; this part is structured according to groups of species (e.g. New World species) used as fusion partners of *S. melongena*, the pivotal taxon for most of the fusion experiments. The conclusions outline the limits of the present knowledge on eggplants germplasm diversity and crossability and suggest potential new research routes on these topics.

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11.1 Introduction

Most diversity and crossability researches have focused so far on *Solanum melongena* L., the worldwide economically most important eggplant, for which a wide germplasm is available in

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several genebanks (c.f. Chap. 10); eggplant breeding is rather dynamic in public as well as in seed companies. The mostly indigenous African germplasm of *S. aethiopicum* L. and *S. macrocarpon* L., less collected and less available in genebanks, has been characterised and bred to a much lesser extent than in the case of *S. melongena*. However, this situation is evolving, given that European and Asian seed companies are beginning to focus on the African vegetable market; also, researchers of the public sector are getting increasingly conscious of the potential of this wide source of poorly known diversity. Until now, crossability between cultivated eggplants and relatives has been focused on crosses involving *S. melongena*; the material was chosen mostly on the basis of criteria such as (1) known or expected relationship with *S. melongena*, and/or (2) resistance to various pests and diseases affecting *S. melongena*. The blurred understanding by geneticists and breeders of the complex world of eggplants relatives in terms of range and identity of the species involved, as well as in terms of relatedness degree with the cultivated eggplants, has strongly limited so far the characterisation of wild species and their use in breeding programmes. As seen in Chap. 10, even taxonomists and phylogeneticists had and still have the utmost difficulties to outline a general picture of the part of genus *Solanum* eggplants belong to, i.e. the subgenus *Leptostemonum*, also known as "spiny solanums". Luckily enough and also as seen in Chap. 10, the botanical background is on the way of stepwise clarification and the phylogenetic progresses pave the way for carrying out enlarged and better directed (1) characterisations of eggplants and relative diversity, and (2) investigations of their inter crossability.

First, this chapter summarises the current knowledge on diversity of eggplants and their relatives, from the genetic and phenotypic point of view. We restricted the phenotype to the major morphological and horticultural traits of special interest to breeders. Phenotypic diversity of traits impacted by domestication of *Solanum aethiopicum*, *S. macrocarpon* and *S. melongena* is mentioned in Chap. 12. The second part of the chapter unfolds the rich information provided by interspecific crosses results. Sexual and somatic crosses are analysed separately; sexual crosses results are structured by species groups involving (1) only cultivated eggplants, (2) cultivated eggplants × wild progenitors, (3) cultivated × other wild species, and (4) only wild species. Results are also analysed across several axes including (1) crossability barriers, criteria and predictability, (2) exploitation of male sterilities produced by interspecific crosses, and (3) access to trait genetics. Somatic hybridisation results are summarised and gathered by types of partners, (1) *S. melongena* + New World *Leptostemonum* species, (2) *S. melongena* + Old World *Leptostemonum* species, (3) other combinations of *Leptostemonum* species, and (4) *S. melongena* + distantly related Solanaceae.

11.2 Diversity of Cultivated and Wild Germplasm

Characterisation of diversity is only possible when representative germplasm collections are available in genebanks. As far as eggplants and related species are concerned, several good collections are available for *Solanum melongena*, whereas those including the African eggplants and wild *Leptostemonum* species are less numerous and poorly representative of the existing diversity. This is particularly true for the wild species (c.f. Chap. 10). Further, research on germplasm is driven by the economic importance of the crops and consequently by the requirements of breeders which are continuously looking for new traits to be incorporated into their elite germplasm. As a consequence, most available information on diversity is anchored to *S. melongena*. African eggplants and wild *Leptostemonum* species have been so far characterised only for a restricted range of traits of interest,

mostly disease resistance and fruit biochemical constituents. Here, we limit ourselves to a global survey of the information, in order to indicate the major achievements, as well as the missing information that deserves further research.

11.2.1 Morphological and Genetic Diversity

11.2.1.1 Cultivated Germplasm

Phenotypic diversity for fruit, plant and other traits of interest is described in many papers for *Solanum melongena* (Prohens et al. 2005; Kumar et al. 2008; Tümbilen et al. 2011b; Cericola et al. 2013), *S. aethiopicum* (Adeniji et al. 2012; Kouassi et al. 2014) or for two or more eggplant species (Osei et al. 2010; Polignano et al. 2010; Plazas et al. 2014). Morphological diversity of *S. melongena*, *S. aethiopicum* and *S. macrocarpon* has been recently revisited on the basis of large sets of accessions (Kumar et al. 2008; Osei et al. 2010; Polignano et al. 2010; Sunseri et al. 2010; Adeniji et al. 2012; Kouassi et al. 2014; Plazas et al. 2014; Taher et al. 2017). The contribution to the diversity is unequal between traits of breeding interest. On a set of 33 Indian landraces of *S. melongena*, yield per plant, fruit width, number of long styled flowers per plant, flowering earliness, total phenolic content and ascorbic acid content were the traits which contributed the most to the divergence between accessions (Prabakaran et al. 2015). Of course, the results depend on the set of accessions used and so far no wide range study including accessions representative of the full phenotypical diversity of each cultivated eggplant was carried out. Summaries of the phenotypic diversity of eggplants, together with the Mendelian or quantitative heredity patterns of traits of interest, are available in various chapters (Daunay et al. 2001; Daunay 2008; Daunay and Hazra 2012).

Analyses of the genetic diversity of *Solanum melongena* using molecular markers provided insights in allelic richness and diversity, for instance among Jordanian (Sadder et al. 2006), Spanish (Prohens et al. 2005), Turkish (Tümbilen et al. 2011b; Demir et al. 2010) and Chinese accessions (Ali et al. 2011). Sampling of *S. melongena* accessions that originate from wider distribution areas was also used for investigating possible relations between molecular diversity on one hand, and geographical origin, morphological traits or cultivar types on the other hand (Hurtado et al. 2012; Vilanova et al. 2012; Cericola et al. 2013; Naegele et al. 2014). African eggplants' genetic diversity was also investigated with molecular markers, but to a lesser extent than *S. melongena* (Sunseri et al. 2010; Tümbilen et al. 2011a). On the whole these publications indicate that molecular markers and morphological traits are complementary tools for assessing diversity.

11.2.1.2 Wild Germplasm

Morphological characterisation of wild *Solanum* species is common in botanical publications which provide very detailed conventional information, e.g. (Vorontsova and Knapp 2016). Less detailed descriptions can be found in papers comparing parents to their interspecific hybrids (Sect. 11.8). Descriptors derived from IPGRI recommendations for *Solanum melongena* (IBPGR 1990) were used for comparing morphological traits between *S. incanumL.*, *S. insanum* L. and *S. melongena* (Ranil et al. 2017). Phenotypic comparison between accessions of a given wild species of interest is rarely assessed, probably because of the difficulty to access different accessions. However, some examples are available. Indonesian accessions of *S. torvum* Sw. were compared for morphological traits and resistance to two soil-borne vascular diseases (Gousset et al. 2005). *Solanum elaeagnifolium* Cav. is mentioned as morphologically variable through its distribution area, in particular for prickliness and leaf shape (Scaldaferro et al. 2012). Genetic diversity for molecular markers between *Solanum* species has been analysed with the aim to assess (1) genetic distances or (2) phylogenetic relationships between species; only a few publications compared accessions

within a single species such as for *S. torvum* (Clain et al. 2004), and for *S. incanum* and *S. insanum* (Tümbilen et al. 2011a).

11.2.2 Pest and Disease Resistances

Pests and disease resistances have a major interest in plant breeding, and resistances have been identified within the cultivated species, as well as among several wild species; see Daunay (2008) for an overview. Pests with major economic importance are root knot nematodes (*Meloidogyne* spp.), soil-borne diseases (*Verticillium dahliae*, *Fusarium oxysporum* f. sp. *melongenae* and *Ralstonia solanacearum* species complex-RSSC[1] (Safni et al. 2014), insects (fruit and shoot borer *Leucinodes orbonalis*, leaf hopper *Amrasca biguttula bigutulla*) and mites (*Tetranychus* spp. and *Polyphagotarsonemus latus*). The incidence of these pests and diseases on each eggplant species depends on the geographical areas and climatic conditions, but on the whole all cultivated eggplants are susceptible to a similar range of pests and pathogens.

11.2.2.1 Cultivated Germplasm

Resistances to *Fusarium* wilt (Hébert 1985; Boyaci et al. 2012), bacterial wilt (Daunay 2008; Lebeau et al. 2011) and both pathogens (Daunay et al. 2016) have been identified within *Solanum melongena* and *S. aethiopicum* germplasm. Monogenic dominant control has been identified for *Fusarium* wilt resistance originating from *S. melongena* (Mutlu et al. 2008; Boyaci et al. 2011) and from *S. aethiopicum* (Toppino et al. 2008b). Genetic control of resistances to RSSC is very variable (monogenic or polygenic, recessive or dominant) depending on *S. melongena* accessions (Daunay 2008) and on bacterial strains (Salgon et al. 2017; Salgon et al. 2018). Monogenic dominant resistances to this disease have been recently mapped (Lebeau et al. 2013; Salgon et al. 2017), and their functional characterisation is ongoing (Xiao et al. 2015; Morel et al. 2018). A monogenic resistance of *S. melongena* to *Colletotrichum gloeosporioides* (which causes fruit anthracnosis) was also described (Kaan 1973). Search for resistance to viruses has so far concerned a narrow range of viruses towards which some resistances have been identified (Daunay 2008). Resistance to *Verticillium* wilt (*Verticillium dahliae*) and root knot nematodes (*Meloidogyne* spp.) have not been found so far within cultivated eggplant germplasm.

The dense hairiness of some accessions of *S. melongena* was suggested to be at the origin of their partial resistance to leaf hopper (Daunay 2008). Hairiness of *S. aethiopicum* Gilo and Aculeatum groups was given as explaining their resistance by antixenosis to mites, whereas the glabrous Kumba group is susceptible (Seck 1997). Contrastingly (and counter-intuitively), the absence of hairs on vegetative parts would confer resistance to leaf hopper and red mites of *S. macrocarpon* (Daunay 2008) as well as to white fly *Trialeurodes vaporariorum* (Malausa et al. 1988). Fruit epidermis thickness and biochemical compounds (in sap, glandular hairs or fruits) are also mentioned as possibly interacting with resistance to some pests (Daunay 2008). The publications concerning eggplants resistance to insects and mites are mostly field observations where antixenosis is observable. Very few quantified details on the life cycle of the pests are available; one study revealed the existence of antibiosis towards white fly in *S. melongena* germplasm (Malausa et al. 1988).

11.2.2.2 Wild Germplasm

Many publications mention the resistance of *Solanum* species to various pests and pathogens, but the main difficulty in handling the detailed literature on the subject is the frequent unreliability of species identifications. Recent progresses concerning the taxonomy of spiny solanums, together with a better interaction between taxonomists and the community of germplasm holders and geneticists, should solve this issue. Attempts of summing up information are available for instance in (Collonnier et al. 2001a; Robinson et al. 2001; Kashyap et al. 2003; Daunay 2008). Global information

[1]Agents of the bacterial wilt.

indicates that high resistance to major pathogens that are not controlled by *Solanum melongena* germplasm are available in species so far not crossable (*S. sisymbriifolium* Lam.) or very difficult to cross with *S. melongena* (*S. torvum*); *Solanum sisymbriifolium* and *S. torvum* are in particular resistant to *Verticillium* wilt and to several root knot nematodes.

11.2.3 Diversity for Other Traits

For wild germplasm as well as for cultivated eggplants, much less characterisation researches are focused on other traits than crossability and pest and disease resistance. Graft affinity between cultivated eggplants (scion) and wild species (rootstock) is continually evaluated (Gisbert et al. 2011a, b; Villeneuve et al. 2016). This field of research is of the utmost interest given that grafting is a common worldwide practice for *Solanum melongena* cultivation. Rootstocks are indeed precious alternatives when resistance to soil-borne pests and diseases is not available in the cultivated germplasm or is not transferable from a resistant wild species because of interspecific cross failure. However, rootstocks may transfer alkaloids to the scion (Villeneuve et al. unpub.) and may also modify soil pathogenic profile (Villeneuve et al. 2014); given their potential side effects, these aspects need to be taken into account in parallel with the evaluation of wild germplasm for graft affinity with cultivated eggplants.

Phenolic acids were analysed in relation to health value (Stommel and Whitaker 2003; Mennella et al. 2010; Plazas et al. 2013; Meyer et al. 2015; Jose et al. 2016; Kaushik et al. 2017) or pest resistance (Prabhu et al. 2009). Glycoalkaloids and furostanol-type steroidal saponins are the major compounds responsible for eggplants bitterness (Aubert et al. 2009a) and diversity among *Solanum melongena*, *S. aethiopicum* and *S. macrocarpon* genotypes is being investigated (Aubert et al. 2009b; Mennella et al. 2010; Sanchez-Mata et al. 2010). Among wild *Solanum* species, the diversity of alkaloids, both in terms of molecules and content, is wide (Jayakumar and Murugan 2016). These compounds have a strong medicinal and pharmaceutical (Gurbuz et al. 2015; Jayakumar and Murugan 2016), as well as bio-insecticidal interest (Chowanski et al. 2016). Interspecific diversity for phenolic acids and glycoalkaloids was also characterised in order to generate a *Solanum* metabolic database and look at evolutionary patterns (Wu et al. 2013).

Other wild traits of strong interest, such as root vigour and architecture (Garcia-Fortea et al. 2019) and resistance to drought (Gramazio et al. 2017b), are being looked at, although this approach is so far limited to particular interspecific crosses, between *Solanum melongena* on one hand and *S. elaeagnifolium* or *S. incanum* on the other hand. A detailed phenotyping methodology has been used for a first investigation of root system diversity among accessions of Solanaceae including *S. melongena* (Bui et al. 2015). Such characterisation should be extended in the future to the cultivated eggplants germplasm and the related wild species, given that climatic changes will unarguably impact yield. Breeders should find a way to face this challenge, in particular by creating varieties (and rootstocks) with vigourous root systems. The many spiny solanums originating from dry (and hot) areas of Africa (Vorontsova and Knapp 2016), Asia (Aubriot et al. 2016) and Australia (Echeverría-Londoño et al. 2018) constitute to this respect an inestimable potential resource of adaptation to dry conditions.

11.3 Crossability Between Eggplants and Relatives

This field of research has attracted many dispersed efforts, limited in many publications to a single or to a few cross partner's couples, except studies carried out within the frame of taxonomic researches for investigating relationships between species which generally encompass many partner's couples. Crossability between species has the double interest of (1) informing about their phylogenetic and/or genetic relationships, and (2) identifying germplasm potentially usable as a source of genes controlling traits of

interest to be introgressed from one species to another.[2] The first attempts of interspecific crosses between spiny solanums started from the 1930s and were carried out in particular by Indian and Japanese scientists (Rao 1979; Kirti and Rao 1982a, b). Four Ph.D. theses at the University of Birmingham (Pearce 1975; Niakan 1980; Hasan 1989; Al-Ani 1991) as well as research carried out at INRA in the 1990s (Daunay et al. 1998) achieved large-scale interspecific experiments. The rest of the information is scattered among many publications from the 1960s to now. Results were compiled and updated several times (Hasan 1989; Daunay et al. 1991; Collonnier et al. 2001a; Kashyap et al. 2003; Daunay 2008; Daunay and Hazra 2012).

We provide here the next synthesis, based on a stepwise analysis of the literature. First, we compiled information from references which specify the species used as female or male in the crosses (Al-Ani 1991; Ano et al. 1989, 1991; Ano 1990; Behera and Singh 2002; Bletsos et al. 1998; Bletsos et al. 2004; Bukenya and Carasco 1995; Callano et al. 2015; Cao et al. 2009; Daunay et al. 1998; Garcia-Fortea et al. 2019; Gowda et al. 1990; Isshiki and Kawajiri 2002; Khan and Isshiki 2008, 2009, 2010, 2011; Khan et al. 2017; Kirti and Rao 1980, 1981, 1982a, b, 1983; Kouassi et al. 2016; Kumchai et al. 2013; Lester and Hasan 1991; Lester and Kang 1998; Lester and Niakan 1986; Liu et al. 2015; Mc Cammon and Honma 1983; Olet and Bukenya-Ziraba 2001; Omidiji 1979, 1983, 1982; Oyelana and Ogunwenmo 2009; Oyelana and Ugborogho 2008; Oyelana et al. 2009; Plazas et al. 2016; Prabhu et al. 2009; Prohens et al. 2012; Rajasekaran 1971; Rao and Rao 1984; Rattan et al. 2015; Robinson et al. 2001; Schaff et al. 1982; Sharma et al. 1980; Zhou et al. 2018). The next step aimed at simplifying the information by keeping only the best result obtained for a given cross, whatever the authors or the cross direction. This simplified file was then (1) merged together with the similarly simplified data of Daunay et al. (1991), and (2) sorted in order to keep the best result obtained for each interspecific cross and to eliminate duplicated crosses.

On the whole, 67 spiny species have been used so far in interspecific crosses, including 51 African and Asian species, nine Australian and seven American. When compared to the over 500 spiny species inventoried presently (Chap. 10), it is clear that the knowledge about crossability between spiny solanums is a research field barely investigated, which deserves strong efforts in the future, in particular for crosses involving eggplants and their African and Asian closest relatives (see 11.4.2 and 11.4.3).

Surveying interspecific crossability in spiny solanums is challenging for many reasons, in particular because of the large number of species and crosses involved, of frequent inappropriate use of nomenclature and of occasional species misidentification. Further, a wide range of crossability criteria is found in the literature, given that the expression of pre- or post-zygotic barriers induces a diversity of effects. Lastly, results obtained by different authors for a given interspecific cross are often conflicting, because of the influence of cross direction (partner used as female or male), genotype of parental accessions, as well as environmental conditions. Hence, before entering into a summary of the interspecific crosses achieved so far, we first review the prezygotic and post-zygotic barriers that contribute to the complexity of the results published. We will also emphasise the interest of cytogenetic studies (1) for understanding F1 fertility troubles, together with (2) assessing genetic relationships between the parental species. We then provide examples illustrating the heterogeneity of the information found in the literature, before summarising the best results obtained for the over 200 interspecific crosses attempted so far and structured into four types of crosses:

1. Crosses between cultivated eggplants (*Solanum aethiopicum*, *S. macrocarpon*, *S. melongena*);
2. Crosses between cultivated eggplants and their wild progenitors *S. anguivi* Lam.,

[2]Transfer is possible either between cultivated eggplants or from wild species to cultivated eggplant, as well as from wild to wild when relevant.

S. dasyphyllum Schumach. & Thonn. and *S. insanum*, respectively, as well as crosses between these wild progenitors;
3. Crosses between cultivated eggplants and (non-progenitor) wild species;
4. Crosses between wild species.

Phenotypes of interspecific hybrids will be discussed in relation to trait heredity patterns. We will continue by reviewing the occasional use of artificial tetraploidisation for restoring male fertility of interspecific hybrids. Next, a special section is dedicated to the cytoplasmic male sterilities obtained by crossing *Solanum melongena* with several wild species.

Given the wealth of information we provide, we skipped presenting the control data obtained on the parental species, in particular for pollen stainability, given this one is generally above 80% throughout all publications reviewed. Apart some exceptions for which we provide accurate figures, hybrid fertility has been categorised on the basis of pollen stainability values as virtual sterility (<10% pollen stainability), partial fertility (10–50%) and fertility (>50%). The relationships between pollen stainability, viability and fertility are a subject of debate, but as all publications use pollen stainability as a measure of viability or fertility, we kept this criterion. Some publications mention also pollen *in vitro* germination as a complementary measurement of pollen fertility; this criterion yields generally smaller values than stainability.

By convention, any interspecific cross is written in the following text as "female x male" when cross direction is known and "partner 1 and partner 2" when it is not specified. We only partially rationalised species nomenclature, given its complexity in the literature, in order to keep close to the names used in the literature together with the accepted names. Hence, we provide the accepted species name together with the name used by the authors (in parentheses), when their correspondence was easy to establish:

S. campylacanthum Hochst. ex A.Rich. (*S. incanum* group A, group B, *S. panduriforme* Drège ex Dunal, *S. delagoense* Dunal);
S. forskalii Dunal (*S. albicaule* Kotschy ex Dunal);
S. incanum (*S. incanum* group C);
S. insanum (*S. melongena* group E, group F);
S. lichtensteinii Willd. (*S. incanum* group D);
S. multiflorum Roth (*S. indicum* L. var. *multiflorum* (Roth) C.B. Clarke;
S. viarum Dunal (*S. khasianum* C.B.Clarke);
S. violaceum Ortega (*S. indicum* L., *S. kurzii* Brace ex Prain, *S. sanitwongsei* Craib);
S. virginianum L. (*S. surattense* Burm.f., *S. xanthocarpum* Willd. ex Walp.[3]).

However, in several cases, the transposition of species names used in the publications to the now accepted names according to recent nomenclature changes could have blurred or mixed up our discussion of interspecific cross results. That is the reason why we decided to keep the species names used in the literature for the following cases:

S. capense L. and *S. dinteri* Bitter (now both under the accepted name *S. capense*);
S. rigescens Jacq., *S. rigescentoides* Hutch., *S. giftbergense* Dunal (now all under the name *S. humile*);
S. *tomentosum* L. and *S. coccineum* Jacq. (now under the name *S. tomentosum*);
S. sessilistellatum Bitter (now under the name *S. nigriviolaceum* Bitter).

11.3.1 Prezygotic and Post-zygotic Barriers

Results of interspecific crosses between *Solanum* species depend on pre- or post-zygotic barriers, the expression of which is assigned to the relationships (genetic or phylogenetic) between parental partners. Prezygotic barriers include absence of pollen germination on the stigma, abnormal or insufficient pollen tube growth

[3]The name *S. xanthocarpum* is extremely tricky because, depending on the author(s) names associated to it, it matches different accepted species names. In this very case that is *S. xanthocarpon* Schrad. & Wendl. that matches *S. virginianum* (Daunay et al. 1991).

through the style[4] and as a result absence of fertilisation of polar nuclei (future endosperm) and egg cell (future zygote) by the pollen nuclei. Flowers and fruits' drops and/or parthenocarpic fruits[5] are observed in such cases. Post-zygotic barriers are expressed after fertilisation occurred, and they involve unbalanced collaboration between the parental genomes in the fertilised cells, i.e. the endosperm[6] and/or the zygote. Their expression is visible along different development stages of the F1 embryo, plantlet or adult plant. The genetic imbalance between parental genomes is suggested to explain dysfunction of endosperm growth and of endosperm–embryo metabolic relationships, with consecutive embryo starvation and death, or endosperm autolyse and embryo digestion at an early stage (Lester and Kang 1998). In interspecific crosses between *Solanum arcanum* Peralta, *S. chilense* (Dunal) Reiche and *S. peruvianum* L. (wild tomatoes), endosperm–embryo interactions have been recently investigated at intimate levels (endosperm early cellular stages and maternal and paternal genes expression) for unravelling the genetic parental conflicts at the origin of embryo growth stop and degeneration, resulting in hybrid seed failure (Roth et al. 2018a, b, c). Dysfunction between parental genomes ends up with parthenocarpic fruits, or fruit set with aborted seeds or variable proportion of abnormal seeds. According to Lester and Kang (1998) seed abnormality rate, when used carefully, is a good and easy measure of this early post-zygotic reproductive barrier between species. When this barrier is overcome artificially via careful sowing of the normal seeds or via in vitro embryo rescue (Kharkongar et al. 2013; Sharma et al. 1996), genetic imbalance affecting directly the zygote can lead to seedlings or plantlet death, abnormal, weak interspecific hybrid plants and also rooting difficulties.[7] When the two parental genomes collaborate relatively correctly, the hybrid plants are vigourous. However, later dysfunctional genetic control of the reproductive process can induce hybrid fertility troubles, frequently observed (next section). This late post-zygotic barrier, that in Nature protects species from gene exchange, is sometimes described as "hybrid breakdown". The accumulation during lineage divergence of loci interacting negatively and responsible for interspecific hybrids sterility has been theorised on the basis of tomato introgression lines phenotyped for pollen and seed sterility (Moyle and Nakazato 2010).

Another event reported (Rao and Rao 1984) is the occurrence of maternal seeds in a variable proportion, up to 100%, in the fruits set up after an interspecific pollination (examples are provided in Table 11.1). It seems that the foreign pollen induces the development of unfertilised maternal ovules into seeds, instead of, or conjointly with, the fertilisation of these ovules and the development of seeds containing an interspecific embryo. The hypothesis of an apomictic behaviour of the maternal parent was suggested by Rao and Rao (1984). The unexpected and occasional harvest of maternal seeds issued from several interspecific pollinations has also been observed by Daunay et al. (unpubl.).

If species identity is a major factor of the success or failure of any interspecific cross, several authors point out also the influence of parental genotypes (Bletsos et al. 2004; Cao et al. 2009; Daunay 2008; Daunay and Hazra 2012; Devi et al. 2015; Gowda et al. 1990; Kirti and Rao 1982a, b; Lester and Niakan 1986; Omidiji 1979; Plazas et al. 2016; Rajasekaran 1970; Rao 1979; Rao and Rao 1984; Rattan et al. 2015; Schaff et al. 1982; Zhou et al. 2018). The impact of parental genotypes has also been observed in genus *Datura* and was interpreted as an evidence of the influence of genes or gene complexes.

[4]In some cases, mismatch between constitutive pollen tube length and stigma length explains mechanically the incapability of the pollen of one species to reach the ovules of another species.

[5]Parthenocarpic fruits can be the response of the ovary to hormones released through the stimulus of pollination.

[6]Endosperm is a triploid tissue issued from the fertilization of two maternal and one paternal nuclei. Hence maternal and paternal genetic dosages differ (2 vs. 1).

[7]Both these last troubles can be solved either with hormonal treatment of the hybrid plantlets in vitro (e.g. IAA, gibberellic acid) or by their grafting onto roots of one of their parents.

Table 11.1 Examples of interspecific crosses for which maternal diploids seeds were obtained in various proportions with hybrids seeds (Rao and Rao 1984)

Female	Male	Direct result of the cross (seeds obtained)	F1 traits
S. torvum	*S. violaceum (S. indicum)*	100% maternal diploids (no hybrid)	n.d.
S. trilobatum	*S. melongena*	100% maternal diploids (no hybrid)	n.d.
S. trilobatum	*S. virginianum (S. surattense)*	F1 + 90% maternal diploids	F1 weak, 3% occurrence of bivalents at meiosis, virtually sterile (<15% pollen fertility)
S. virginianum (S. surattense)	*S. melongena*	F1 + 1% maternal diploid	F1 vigourous, 50% occurrence of bivalents at meiosis, virtually sterile (<15% pollen fertility)
S. virginianum (S. surattense)	*S. multiflorum (S. indicum var. multiflorum)*	F1 + 30% maternal diploids	F1 weak, 56% occurrence of bivalents at meiosis, virtually sterile (<15% pollen fertility)
S. virginianum (S. surattense)	*S. torvum*	100% maternal diploid (no hybrid)	n.d.
S. virginianum (S. surattense)	*S. trilobatum*	F1 + 17% maternal diploids	F1 medium vigour, 21% occurrence of bivalents at meiosis, virtually sterile (<15% pollen fertility)

Species names into brackets are those used in the publication

Those genetic factors are distributed throughout the genome and act as a barrier against successful hybridisation, possibly in a complementary way (Rao 1979). Environmental conditions also affect the results of interspecific crosses and, together with the genotypes, are probably at the origin of the heterogeneous results obtained by different authors for a same interspecific cross (for instance with regards to fruit set, hybrid meiosis features or hybrid fertility). Hence in the present state of the art, it is safer not to conclude definitively about the failure of any apparently recalcitrant crosses. For the reasons detailed above and because of the potential continuous improvement in the use of in vitro embryo rescue, tetraploidisation, somatic hybridisation or bridge species, interspecific cross results should be considered as provisional.

11.3.2 Cytogenetic Observations of Late Post-zygotic Barriers

Chromosomes structural repatterning having occurred during the evolutionary process of the species (interchanges, interstitial breakpoints, heteromorphy) maintained the individuality of each taxa (Kirti and Rao 1982b) and is considered as a major factor causing gametic lethality of interspecific hybrids. Hence, chromosome behaviour and shapes during diakinesis (end of prophase I) and metaphase I at the time of F1 pollen mother cell (PMC) meiosis provide information about homologies or homeologies[8] between parental chromosomes (Kirti and Rao 1980, 1981, 1982a, b, 1983). As chromosome behaviour differs from one PMC to another and depends also on the meiosis step (diakinesis or metaphase I), cytological observations must be carefully done on several PMC of a given hybrid

[8]In any given species, chromosomes of each pair share a same genetic structure (homology), which allows their close pairing and the formation of bivalents during diakinesis and metaphase I of meiosis. The word "homeology" was coined for designating, for a given pair, the partial similarity between chromosomes originating from different parental species. When homeology between parental chromosomes is sufficient, the meiosis of an interspecific hybrid is possible, but because chromosomes similarity it incomplete, various abnormalities occur at various frequencies during the course of the meiotic divisions.

in order to calculate a reliable estimation of the frequencies of univalent, bivalent and other multivalent occurrence at each meiosis stage. The more univalents, the less homeology between the chromosomes pairs of both parents. The cross between *Solanum trilobatum* L. and *S. virginianum* illustrates a case of poor homeology of their chromosomes, with a frequency of bivalents in their F1 varying from 3% to 21%, depending on the cross direction (Table 11.2). Conversely, occurrence of bivalents in hybrids indicates that the concerned chromosome pairs retained sufficient ancestral similarities to allow their pairing. The closer to 12 the number of bivalents, the better the homeology between the parental chromosomes. High chromosome homeology is found between *S. melongena* and *S. violaceum*, the reciprocal hybrids of which both display 99% of bivalents during their meiosis (Table 11.2). Hence, frequency of bivalents, or more globally regular or irregular meiosis, depends clearly on cross partners. Cross direction effect on F1 meiosis is less clear, given there are some differences between reciprocal hybrids (e.g. for the F1 *S. multiflorum* and *S. virginianum*, with 43 and 56% bivalents) or no differences (e.g. F1 *S. aethiopicum* and *S. macrocarpon*, both with irregular meiosis) (Table 11.2). Meiotic behaviour of hybrids *S. aethiopicum* Aculeatum group (*S. integrifolium* Poir.) X *S. melongena* and hybrids *S. aethiopicum* Aculeatum group x *S. insanum* (*S. melongena* var. *insanum*) was compared (Kirti and Rao 1982b). The high frequency of bivalents in both hybrids led the authors to conclude about homeologies between the three species. Because of differences between both hybrids for types and frequency of chromosomes associations, they also suggested differences "to some extent" between *S. melongena* and *S. insanum.*

Pollen stainability is given in most publications as a criterion of interspecific hybrid fertility, and following Daunay et al. (1991), we will reduce hybrid fertility into three classes: (1) F1 virtually sterile with less than 10% pollen stainability, (2) F1 partially fertile (10-50% pollen stainability) and (3) F1 fertile (>50% pollen stainability). On this basis, we state that irregular meiosis can end up either with virtually sterile (e.g. cross *S. aethiopicum* and *S. multiflorum*) or partially fertile hybrids (e.g. *S. aethiopicum* and *S. macrocarpon*). This means that at least some viable microspores can be produced from abnormal meiosis. On the other hand, a regular or almost regular meiosis, with high bivalents occurrence frequency followed by regular chromosome separation and microspore formation, can end up with fertile or only partially fertile hybrids (e.g. crosses between *S. melongena*and *S. violaceum* and *S. melongena* and *S. viarum*), or even with virtually sterile ones (*S. melongena* and *S. aethiopicum*). In the two latter cases, post-meiotic degenerative events affecting tetrads or maturing microspores probably occur. In cases of highly sterile F1 pollens, the late expression of the reproductive barrier was attributed either to cryptic chromosomal structural differences or to recombination and segregational events of insufficiently homeologous chromosomes leading to unbalanced gametes (Kirti and Rao 1980, 1982a, b, 1983).

Lastly, one notices that progenies were obtained from interspecific F1, regardless of pollen stainability (Table 11.2), including very poor one as illustrated by the striking case of the virtually sterile hybrids (*S. multiflorum* x *S. aethiopicum*), (*S. virginianum* x *S. trilobatum*) and (*S. virginianum* x *S. melongena*).

Apart from chromosome global pairing at diakinesis and metaphase I, careful cytological observations may reveal abnormal shapes of bivalents (e.g. rods, rings) and of tetravalents (e.g. chains, Y, fish, ring or double-ring types), which are also evidence of multiple homeologies between parental chromosomes and of structural re-organisation/repatterning. For instance, fish-type and double-ring configurations suggest interstitial translocation breakpoints.

Comparative chiasma (crossing over) frequencies per bivalent between a hybrid and its parental species is another indicator of the level of homeology between the chromosomes: the closer the chiasma frequency of the hybrid to that of its parental species, the more homeologous their chromosomes; and the higher the recombination potential between the parental genomes, the more closely related the two parental species.

Table 11.2 Meiosis and pollen stainability of interspecific hybrids

Female	Male	Reciprocal cross	F1 meiosis and pollen stainability	Progenies obtained	Source
S. aethiopicum Aculeatum group (*S. integrifolium*)	*S. melongena*	R1	Normal meiosis, F1 virtually sterile	n.d.	Callano et al. (2015)
S. melongena	*S. aethiopicum* Aculeatum group (*S. integrifolium*)	R1	Normal meiosis, F1 virtually sterile	n.d.	Callano et al. (2015)
S. aethiopicum Aculeatum group (*S. integrifolium*)	*S. melongena*	R1	High occurrence of bivalents at meiosis, F1 virtually sterile	n.d.	Kirti and Rao (1982b)
S. aethiopicum Aculeatum group *S.* (*integrifolium*)	S. *multiflorum* (*S. indicum* var. *multiflora* Wight)	R2	69% occurrence of bivalents at meiosis, F1 virtually sterile	n.d.	Kirti and Rao (1980)
S. *multiflorum* (*S. indicum* var. *multiflorum*)	*S. aethiopicum* Aculeatum group (*S. integrifolium*)	R2	76% occurrence of bivalents at meiosis, F1 virtually sterile	F2	Kirti and Rao (1980, 1983)
S. aethiopicum Aculeatum group (*integrifolium*)	*S. violaceum* (*S. indicum*)	R3	Regular meiosis, F1 virtually sterile	n.d.	Kirti and Rao (1982a)
S. violaceum (*S. indicum*)	*S. aethiopicum* Aculeatum group (*S. integrifolium*)	R3	Regular meiosis, F1 virtually sterile	n.d.	Kirti and Rao (1982a)
S. aethiopicum Gilo	*S. macrocarpon*	R4	Irregular meiosis, F1 partially fertile (34% pollen stainability)	n.d.	Oyelana and Ogunwenmo (2009)
S. macrocarpon	*S. aethiopicum* Gilo	R4	Irregular meiosis, F1 partially fertile (21% pollen stainability)	n.d.	Oyelana and Ogunwenmo (2009)
S. melongena	*S. violaceum* (*S. indicum*)	R5	99% occurrence of bivalents at meiosis, F1 fertile (92% pollen fertility)	n.d.	Rao and Rao (1984)
S. violaceum (*S. indicum*)	*S. melongena*	R5	99% occurrence of bivalents at meiosis, F1 fertile (95% pollen fertility)	n.d.	Rao and Rao (1984)
S. violaceum	*S. melongena*	R5	Imperfect meiosis (some univalents), F1 partially fertile (31% stainable pollen)	BC1 to BC4	Ishhiki and Kawajiri (2010)
S. *multiflorum* (*S. indicum* var. *multiflorum*)	*S. virginianum* (*S. surattense*)	R6	43% occurrence of bivalents at meiosis	n.d.	Rao and Rao (1984)

(continued)

Table 11.2 (continued)

Female	Male	Reciprocal cross	F1 meiosis and pollen stainability	Progenies obtained	Source
S. virginianum (*S. surattense*)	S. *multiflorum* (*S. indicum* var. *multiflorum*)	R6	56% occurrence of bivalents at meiosis, F1 virtually sterile	n.d.	Rao and Rao (1984)
S. trilobatum	*S. virginianum* (*S. surattense*)	R7	3% occurrence of bivalents at meiosis, F1 virtually sterile	n.d.	Rao and Rao (1984)
S. virginianum (*S. surattense*)	*S. trilobatum*	R7	21% occurrence of bivalents at meiosis, F1 virtually sterile	F1 "derivatives"	Rao and Rao (1984)
S. aethiopicum Aculeatum group (*S. integrifolium*)	*S. insanum* (*S. melongena* var. *insanum*)		High occurrence of bivalents at meiosis, F1 virtually sterile	n.d.	Kirti and Rao (1982b)
S. aethiopicum Aculeatum group (*S. integrifolium*)	*S. virginianum* (*S. surattense*)		Regular meiosis, F1 virtually sterile	n.d.	Kirti and Rao (1982a)
S. melongena	*S. viarum* (*S. khasianum*)		Regular meiosis, F1 fertile (62% stainable pollen)	F2	Sharma et al. (1980)
S. melongena ($2n = 24$)	*S. scabrum* ($2n = 48$)		Regular meiosis but few univalents, F1 partially fertile (38% pollen stainability), dropping of many flowers buds, seedless fruits	n.d.	Oyelana et al. (2009)
S. trilobatum	S. *multiflorum* (*S. indicum* var. *multiflorum*)		46% occurrence of bivalents at meiosis, F1 virtually sterile	n.d.	Rao and Rao (1984)
S. violaceum (doubt about species identity)	*S. torvum*		Fully abnormal meiosis, dropping off of immature flowers, F1 sterile	n.d.	Kirti and Rao (1981))
S. virginianum (*S. surattense*)	*S. melongena*		50% occurrence of bivalents at meiosis, F1 virtually sterile	F1 "derivatives"	Rao and Rao (1984)
S. virginianum (*S. xanthocarpum*)	*S. melongena*		Normal meiosis except rare occurrence of few univalents, F1 virtually sterile	n.d.	Rajasekaran (1971)

Reciprocal hybrids, when existing, are gathered in successive lines, and identified in column "reciprocal cross". When known, the obtaining of progenies from the F1 is indicated. Hybrids are recorded as "virtually sterile" when their pollen stainability is less than 10%. Note that some crosses have been realised by different authors, with similar or different results

Differences in chiasma frequencies between reciprocal hybrids indicate cytoplasmic influence on meiotic behaviour; this is the case for the cross between *S. aethiopicum* (*S. integrifolium*) and *S. multiflorum* (*S. indicum* var. *multiflorum*), with 1.23–1.27 average chiasma frequency per bivalent when *S. aethiopicum* is the female parent and 1.31–1.34 when it is the male parent (Kirti and Rao 1980). The controls, i.e. the parents, displayed a chiasma frequency of 1.59–1.63.

On the whole, cytogenetic observations reveal the expression of late post-zygotic barriers that

are expressed at the time of, or after, F1 flower meiosis. However, the border between impossibility and possibility to go through these late barriers is labile, as exemplified by cases for which progenies are sometimes obtained from virtually sterile hybrids producing a high percentage of sterile pollen (Garcia-Fortea et al. 2019; Kirti and Rao 1980, 1983; Rao and Rao 1984).

11.3.3 Variation of Hybridisation Results

Same species combinations have been used by a number of authors, with either consistent results (e.g. crosses between *S. melongena* and *S. incanum*), or with inconsistent results ranging from cross failure to obtaining fertile hybrids (e.g. crosses between *S. melongena* and *S. violaceum*; cf. Table 11.3). This could point out that the influence of different parental genotypes and environmental conditions on a crossing result varies with regard to species partnership. Table 11.3 illustrates also the variation of in depth investigation from one author to another; some stopped with the observation of F0 → F1 seed germination, while others went as far as obtaining advanced progenies from the F1.

11.4 Overview of the Best Results Obtained When Crossing Spiny Solanums

For the sake of clarity, as over 200 species combinations have been used in interspecific crosses attempted so far, we decided to split the results into the four crossing categories listed in Sect. 11.3.

The statistical overview of the best results obtained within these four categories of crosses is summarised in Table 11.4. *Solanum melongena* is by far the cultivated eggplant for which the number of interspecific crosses attempted is the highest (61 crosses, vs. 16 and 3 for *S. aethiopicum* and *S. macrocarpon*, respectively). Most of the crosses (116) were attempted between wild species. The best results obtained are distributed along a stepwise scale, from fertile hybrids to no fruit set or setting of parthenocarpic fruits on the maternal parent at the time of the cross. Globally, few publications went as far as attempting to obtain progenies from interspecific hybrids; hence, the data presented in Table 11.4 cannot be used to predict what could be achieved if attempted.

11.4.1 Crosses Between Cultivated Eggplants

Solanum melongena, *S. aethiopicum* and *S. macrocarpon* have been crossed in all reciprocal combinations (Table 11.5). The hybrids between *S. aethiopicum* and *S. macrocarpon* as well as those between *S. aethiopicum* and *S. melongena* are frequently reported as vigourous, whereas those between *S. macrocarpon* and *S. melongena* have generally a poor vigour. For this latter species combination, the vigour depends on the parental genotypes (Schaff et al. 1982), regardless of the direction of the cross. Although results differ between authors, all species combinations have produced at best partially fertile of fertile hybrids. In all cases, progenies were obtained from the hybrids, although in the case of *S. aethiopicum* and *S. macrocarpon*, observations stopped at the seed set of one of the reciprocal hybrids. Hence, despite some sterility troubles occurring at the level of F1 or of later progenies, the three cultivated eggplants are usable in breeding as sources of traits for each other.

11.4.1.1 *Solanum aethiopicum* and *S. macrocarpon*

Partially fertile (10% < pollen stainability < 50%) or virtually sterile hybrids (pollen stainability <10%) with meiotic abnormalities were obtained from this cross (Table 11.5). In the virtually sterile hybrid obtained with *Solanum aethiopicum* (probably Kumba group) used as the female parent (Omidiji 1983), twelve bivalents were formed in 78% of the F1 pollen mother cells (PMC); however, for other PMC, bivalents were associated to low proportion of

Table 11.3 Variation of interspecific crossability results among publications illustrated for the crosses between (A) *S. melongena* and *S. incanum* and (B) between *S. melongena* and *S. violaceum*

Female	Male	Direct result of the cross	F1 pollen stainability meiosis	F1 seed set and/or progenies obtained	Source
A					
S. melongena	*S. incanum*	14–46% fruit set, 79–88% normal seeds	F1 fertile (>60% pollen stainability)	n.d.	Lester and Kang (1998)
S. melongena	*S. incanum*	18% fruit set, 60% germination	n.d.	n.d.	Plazas et al. (2016)
S. melongena	*S. incanum*	F1 obtained	F1 fertile (61% stainability)	BC1 progenies obtained whatever BC direction	Kouassi et al. (2016)
S. melongena	*S. incanum*	F1 obtained	n.d.	Advanced progenies obtained	Robinson et al. (2001)
S. melongena (group H)	*S. incanum* (group C)	14% fruit set, 88% normal seeds, 2% germination	F1 fertile (65% pollen stainability)	n.d.	Lester and Hasan (1991)
S. melongena (group G)	*S. incanum* (group C)	46% fruit set, 79% normal seeds, 73% germination	F1 partially fertile to fertile (53% pollen stainability)	n.d.	Lester and Hasan (1991)
S. incanum	*S. melongena*	23–26% fruit set, 1–11% normal seeds	F1 fertile ($\geq$60% pollen stainability)	n.d.	Lester and Kang (1998)
S. incanum	*S. melongena*	25% fruit set, 77% germination	n.d.	n.d.	Plazas et al. (2016)
S. incanum (group C)	*S. melongena* (group G)	26% fruit set, 11% normal seeds, 55% germination	F1 fertile (67% pollen stainability)	n.d.	Lester and Hasan (1991)
S. incanum (group C)	*S. melongena* (group H)	23% fruit set, 1% normal seeds, no germination	n.d.	n.d.	Lester and Hasan (1991)
B					
S. melongena	*S. violaceum*	5% fruit set, 25% germination	n.d.	n.d.	Plazas et al. (2016)
S. melongena	*S. violaceum*	No fruit set,-	n.d.	n.d.	Al Ani (1991)
S. melongena	*S. violaceum* (*S. indicum*)	F1 vigourous	F1 fertile (92% pollen fertility)	n.d.	Rao and Rao (1984)
S. melongena	*S. violaceum* (*S. kurzii*)	Viable plants	F1 partially fertile	56–75% normal seeds	Daunay et al. (1998), Daunay, unpubl.

(continued)

Table 11.3 (continued)

Female	Male	Direct result of the cross	F1 pollen stainability meiosis	F1 seed set and/or progenies obtained	Source
S. melongena	*S. violaceum* (*S. indicum*)	F1 obtained	n.d.	Viable seeds	Behera and Singh (2002)
S. violaceum	*S. melongena*	65% fruit set, 87% normal seeds	F1 partially fertile	n.d.	Al Ani (1991)
S. violaceum	*S. melongena*	F1 obtained	F1 partially fertile (31% stainable pollen)	BC1-4 progenies obtained	Ishhiki and Kawajiri (2010)
S. violaceum	*S. melongena*	No fruit set	n.d.	n.d.	Plazas et al. (2016)
S. violaceum (*S. kurzii*)	*S. melongena*	F1 obtained	F1 partially fertile (30% pollen stainability, but 1% germination in vitro)	BC1-BC3 populations obtained	Khan and Isshiki (2009)
S. violaceum (*S. kurzii*)	*S. melongena*	Viable plants	F1 partially fertile	23–83% normal seeds	Daunay et al. (1998), Daunay, unpubl.
S. violaceum (*S. indicum*)	*S. melongena*	Death of F1 seedlings	n.d.	n.d.	Behera and Singh (2002)
S. violaceum (*S. indicum*)	*S. melongena*	F1 vigourous	F1 fertile (95% pollen fertility)	n.d.	Rao and Rao (1984)

Species names into brackets are those used in the publications

univalents, trivalents and tetravalents. Omidiji concluded that the chromosomes of both parental species were sufficiently homeologous for permitting pairing in most PMC, despite cryptic differences (translocations, inversions). Despite metaphase I and later stage meiosis irregularities, the low pollen fertility due to unbalanced gametes did not hamper the hybrid undersized fruits to contain some seeds. In reciprocal hybrids obtained from the cross between *S. aethiopicum* Gilo group and *S. macrocarpon* (Oyelana and Ogunwenmo 2009) and displaying partial fertility (21 to 34% pollen stainability), meiotic irregularity was also observed (about 50% bivalents, trivalents, tetravalents, clumps and laggards). Interestingly Omidiji (1983) noticed meiotic irregularities in *S. macrocarpon* (not mentioned by Oyelana and Ogunwenmo 2009) and questioned a possible hybrid origin of this species.

11.4.1.2 *Solanum aethiopicum* and *S. melongena*

Depending on the crosses, hybrids virtually sterile, partially fertile or fertile are described in the literature (Table 11.5). Meiosis of virtually sterile reciprocal F1 is reported as normal (Callano et al. 2015; Kirti and Rao 1982b). Persisting sterility troubles in first backcross (BC) generations are mentioned for a virtually sterile F1 obtained with *Solanum aethiopicum* Aculeatum group used as female (Ano 1990; Ano et al. 1989, 1991). In BC generations obtained with a similar hybrid and *S. melongena* used as male recurrent parent, segregation for cytoplasmic male sterility was detected from BC1 onwards (Khan and Isshiki 2010), whereas the male fertile plants still suffered fertility troubles even in BC4 (maximum of 50% stainable pollen). A reciprocal hybrid obtained with *S. melongena* used as female and *S. aethiopicum* Kumba group

Table 11.4 Overview of the number of interspecific crosses realised so far, distributed across four cross categories (best results obtained)

Total number of interspecific crosses (number of partner species)	Cultivated × cultivated			Cultivated × progenitors			Cultivated × other wild			Wild × wild
	mel	*aet*	*mac*	*mel*	*aet*	*mac*	*mel*	*aet*	*mac*	
Realised	2	2	2	3	3	2	61	16	3	116
Fertile hybrids were obtained				1	1	1	6	0	1	12
Partially fertile hybrids were obtained	2	2	2	2	1	1	19	2	1	13
Virtually sterile hybrids were obtained					1		10	5	1	12
For which progenies beyond F1 were obtained	2	1	1	3			9	1		1
F0 → F1 seeds (3–100% normal) were obtained							1	3		22
F0 → F1 seeds (0% normal) were obtained							2	5	1	21
F0 → F1 seeds (without detail) were obtained							2			
Hybrids (embryo, plantlet) were not viable							5			3
Fruit set (without detail)							1			2
Cross failed (no fruit set or parthenocarpic fruit)							15	1	1	31

The eggplant species are abbreviated to the first three letters of their specific epithet

Table 11.5 Overview of the results obtained from crosses between cultivated eggplants

Female	Male	*aet* cultigroup (if known)	Direct result of the cross	F1 pollen stainability or viability or meiosis	F1 fruit and/or seed set and/or progeny	Source
aet	*mac*	n.d.	F1 vigourous	F1 partially fertile (17% stainability)	n.d.	Omidiji (1979)
aet	*mac*	Gilo	F1 obtained	Irregular meiosis, F1 partially fertile (34% pollen stainability)	n.d.	Oyelana and Ogunwenmo (2009)
aet	*mac*	Kumba?	F1 vigourous	F1 virtually sterile (9% stainable pollen)	Seed set	Omidiji (1983)
mac	*aet*	Gilo	F1 obtained	Irregular meiosis, F1 partially fertile (21% pollen stainability)	n.d.	Oyelana and Ogunwenmo (2009)
aet	*mel*	n.d.	F1 vigourous	F1 partially fertile (13% stainable pollen)	n.d.	Omidiji (1979)
aet	*mel*	Aculeatum	F1 obtained	F1 virtually sterile	Sterility troubles in first BC progenies	Ano et al. (1989, 1990, 1991)
aet	*mel*	Aculeatum (*S. integrifolium*)	Commercial F1 'Assist'	F1 virtually sterile (<10% pollen stainability)	BC progenies obtained, segregating for male sterility	Khan and Isshiki (2010)
aet	*mel*	Aculeatum (*S. integrifolium*)	F1 obtained	Normal meiosis, F1 virtually sterile	n.d.	Callano et al. (2015)
aet	*mel*	Aculeatum (*S. integrifolium*)	F1 vigourous	High occurrence of bivalents at meiosis, but F1 virtually sterile	n.d.	Kirti and Rao (1982b)
aet	*mel*	Gilo	F1 obtained	F1 virtually sterile	F2 and BC progenies obtained	Ano, unpubl.
mel	*aet*	n.d.	F1 obtained	F1 fertile (57% pollen stainability)	Seeds in F1 fruits	Oyelana and Ugborogho (2008)
mel	*aet*	Aculeatum (*S. integrifolium*)	F1 obtained	Normal meiosis, F1 virtually sterile	n.d.	Callano et al. (2015)
mel	*aet*	Aculeatum (*S. integrifolium*)	No fruit set	n.d.	n.d.	Kirti and Rao (1982b)
mel	*aet*	Gilo	F1 obtained	>85% sterile pollen	Parthenocarpic fruits	Behera and Singh (2002)
mel	*aet*	Gilo	F1 obtained	n.d.	Advanced progenies obtained	Robinson et al. (2001)
mel	*aet*	Kumba	F1 vigourous	F1 virtually sterile (0–2% pollen stainability)	Seedless spontaneous fruits, BC progeny obtained	Prohens et al. (2012)
mac	*mel*		20–35% of fruits with seeds, F1 weak	F1 partially fertile (10–21% stainability)	No fruits or seedless fruits after selfing or BC	Bletsos et al. (2004)

(continued)

Table 11.5 (continued)

Female	Male	*aet* cultigroup (if known)	Direct result of the cross	F1 pollen stainability or viability or meiosis	F1 fruit and/or seed set and/or progeny	Source
mac	*mel*		21% fruit set, F1 of variable vigour	F1 virtually sterile (1–9% pollen stainability)	F2 and BC1 obtained	Schaff et al. (1982)
mac	*mel*		F1 obtained	F1 fertile (52% pollen stainability)	F2, F3 segregating progenies obtained	Oyelana and Ugborogho (2008)
mac	*mel*		F1 of poor vigour	F1 partially fertile (30% stainability)	Parthenocarpic fruits	Gowda et al. (1990)
mel	*mac*		4% fruit set, F1 of variable vigour	F1 partially fertile (10–15% pollen stainability)	F2 and BC1 obtained	Schaff et al. (1982)
mel	*mac*		8–30% of fruits with seeds, F1 weak	F1 partially fertile (5–16% stainability)	No fruits or seedless fruits after selfing or BC	Bletsos et al. (2004)
mel	*mac*		F1 of poor vigour	F1 partially fertile (40% stainability)	Parthenocarpic fruits	Gowda et al. (1990)
mel	*mac*		F1 vigourous	F1 partially fertile (49% pollen stainability)	F2, F3 segregating progenies obtained	Oyelana and Ugborogho (2008)

The eggplant species are abbreviated to the first three letters of their specific epithet

(Prohens et al. 2012) as male, also poorly fertile (0–2% pollen stainability; 28% fruit set) yielded also BC progenies (with each parental species) with limited (but improved) pollen stainability (1–62%) and fruit set (53%).

11.4.1.3 *Solanum macrocarpon* and *S. melongena*

F1 meiosis revealed regular chromosome pairing in most pollen mother cells (PMC) with occasional multivalents and univalents in some PMC (Schaff et al. 1982; Wanjari 1976). Hybrid pollen stainability varied from 5 to 21%, depending on the cross direction and parental accessions (Bletsos et al. 2004); it was observed that pollen stainability was better when *Solanum melongena* was the maternal parent: 10–15% versus 1–9% for *S. macrocarpon* as the maternal parent (Schaff et al. 1982), but this difference seems arguable. F2, F3 and BC progenies were obtained from reciprocal hybrids, with better pollen stainability than the hybrid, although still lower than that of the parental species (Oyelana and Ugborogho 2008; Schaff et al. 1982).

11.4.2 Crosses Between Cultivated Eggplants and Their Wild Progenitors

Each cultivated eggplant species is fully interfertile with its own wild progenitor, i.e. *S. aethiopicum* with *S. anguivi*, *S. macrocarpon* with *S. dasyphyllum* and *S. melongena* with *S. insanum* (Table 11.6A). This is the case regardless of the direction of the cross, i.e. cultivated species used as female or as male (data not shown).

Crosses between each cultivated eggplant and the wild progenitors of the two other cultivated species were also investigated (Table 11.6B). Data are insufficient to look for a possible difference between reciprocal crosses. A rough

Table 11.6 Best results obtained for crosses involving the three cultivated eggplants

Partner 1	Partner 2	Best result simplified	Detailed source
A			
S. aethiopicum	*S. anguivi*	F1 fertile (95% pollen stainability), vigourous	Niakan (1980), Lester and Niakan (1986)
S. macrocarpon	*S. dasyphyllum*	F1 fertile (92–100% pollen stainability), normal seeds produced	Omidiji (1979), Bukenya and Carasco (1995, 1999)
S. melongena	*S. insanum*	F1 fertile (62–98% pollen stainability), progenies obtained	Lester and Hasan (1991), Kouassi et al. (2016), Plazas et al. (2016)
B			
S. aethiopicum	*S. dasyphyllum*	F1 partially fertile, vigourous	Omidiji (1979), Niakan (1980)
S. aethiopicum	*S. insanum*	F1 virtually sterile	Kirti and Rao (1982b)
S. macrocarpon	*S. anguivi*	F1 partially fertile (pollen stainability <15%)	Omidiji (1982) quoted by Lester and Niakan (1986)
S. macrocarpon	*S. insanum*	–	–
S. melongena	*S. anguivi*	F1 partially fertile, progenies obtained	Al-Ani (1991), Kouassi et al. (2016), Plazas et al. (2016)
S. melongena	*S. dasyphyllum*	F1 partially fertile, progenies obtained	Daunay et al. (1998), Kouassi et al. (2016), Plazas et al. (2016)

(A) when crossed with their respective wild progenitor and (B) when crossed with the wild progenitors of the other cultivated species

comparison of crossability results between partnerships "cultivated$_i$ − cultivated$_j$" (Table 11.5) and "cultivated$_i$-wild progenitor$_j$" is possible. The results of such comparisons seem consistent for the crosses involving:

- *S. aethiopicum* crossed with *S. macrocarpon* or *S. dasyphyllum* (F1 partially fertile);
- *S. aethiopicum* crossed with *S. melongena* (F1 partially fertile) or *S. insanum* (F1 virtually sterile);
- *S. melongena* crossed with *S. aethiopicum* or *S. anguivi* (F1 partially fertile);
- *S. melongena* crossed with *S. macrocarpon* or and *S. dasyphyllum* (F1 partially fertile);
- *S. macrocarpon* crossed with *S. aethiopicum* or *S. anguivi* (F1 partially fertile);
- Incomplete data hamper the comparison between *S. macrocarpon* crossed with *S. melongena* (F1 partially fertile) or *S. insanum* (no data).

11.4.3 Crosses Between Cultivated Eggplants and (Non-progenitor) Wild Species

11.4.3.1 Reciprocal Crosses

Many crosses have been attempted by using the parental partners as female and as male parent. We compare the best results obtained so far for reciprocal crosses in the case of three species partnerships for *Solanum aethiopicum*, one for *S. macrocarpon* and 52 for *S. melongena* (see Table 11.7). This table once more illustrates the heterogeneous information available in the literature, as well as the extreme diversity of cases obtained throughout the crosses. Here, we will only discuss the diversity of results obtained in crosses involving *S. melongena*, since they are numerous enough to provide a general overview. Hybrids virtually sterile, partially fertile or fertile are obtained whether *S. melongena* is used as

Table 11.7 Overview of the best results obtained for reciprocal crosses between cultivated eggplant and wild species (wild progenitors of cultivated eggplants excluded)

Cultivated species	Wild species	Best cross result (cultivated = female parent)	Best cross result (wild = female parent)
S. aethiopicum	*S. multiflorum*	F1 vigourous, virtually sterile, 69% occurrence of bivalents at meiosis	F1 vigourous, virtually sterile (76% occurrence of bivalents at meiosis), seed set, 100% germination, F2 progeny obtained
S. aethiopicum	*S. violaceum*	F1 virtually sterile	F1 partially fertile
S. aethiopicum	*S. virginianum*	F1 virtually sterile	No success
S. macrocarpon	*S. linnaeanum*	0–80% fruit set, abortive seeds	25–50% fruit set, abortive seeds
S. melongena	*S. aculeastrum*	Parthenocarpic fruits	No fruit set
S. melongena	*S. aculeatissimum*	Tetraploidised F1 partially fertile (25% pollen stainability)	No fruit set
S. melongena	*S. beaugleholei*	Parthenocarpic fruits	No fruit set
S. melongena	*S. burchellii*	F1 partially fertile, fruit set, 20% normal seeds	F1 obtained
S. melongena	*S. campylacanthum*	F1 fertile	No fruit set
S. melongena	*S. capense*	F1 virtually sterile, 67% normal seeds	F1 obtained
S. melongena	*S. catombelense*	F1 partially fertile, 10–33% normal seeds	F1 partially fertile, 42–57% normal seeds
S. melongena	*S. cerasiferum*	F1 partially fertile, 95–97% normal seeds	F1 partially fertile, 96–99% normal seeds
S. melongena	*S. chippendalei*	No viable plantlets	No fruit set
S. melongena	*S. clarkiae*	No viable embryos	No fruit set
S. melongena	*S. coagulans*	F1 virtually sterile, 66% normal seeds	Parthenocarpic fruits
S. melongena	*S. coccineum*	F1 virtually sterile, 51–69% normal seeds	F1 partially fertile, 77–98% normal seeds
S. melongena	*S. cyaneopurpureum*	F1 partially fertile	F1 obtained
S. melongena	*S. dennekense*	No fruit set	No fruit set
S. melongena	*S. dinteri*	F1 partially fertile, 58–74% normal seeds	F1 partially fertile, 69–75% normal seeds
S. melongena	*S. dioicum*	Parthenocarpic fruits	No fruit set
S. melongena	*S. diversiflorum*	Parthenocarpic fruits	No fruit set
S. melongena	*S. elaeagnifolium*	F1 virtually sterile—BC1 and BC2 progenies obtained (*S. melongena* used as male)	No fruit set
S. melongena	*S. forskalii*	F1 virtually sterile, no fruit set	No viable embryo
S. melongena	*S. giganteum*	Parthenocarpic fruits	No fruit set
S. melongena	*S. goetzii*	Parthenocarpic fruits	F1 virtually sterile
S. melongena	*S. hastifolium*	F1 partially fertile, 18–23% normal seeds	No fruit set

(continued)

Table 11.7 (continued)

Cultivated species	Wild species	Best cross result (cultivated = female parent)	Best cross result (wild = female parent)
S. melongena	*S. heinianum*	No fruit set	No fruit set
S. melongena	*S. incanum*	F1 fertile—advanced progenies obtained	F1 fertile
S. melongena	*S. lichtensteinii*	F1 fertile—BC1 obtained (S. melongena used as male)	18% fruit set
S. melongena	*S. lidii*	3% fruit set, presence of seeds	F1 partially fertile, 77–86% normal seeds
S. melongena	*S. linnaeanum*	9% fruit set, 0% germination	F1 obtained with "good fertility"—BC1 obtained with F1 used as female
S. melongena	*S. mahoriensis*	No viable embryos	No fruit set
S. melongena	*S. melanospermum*	F1 partially fertile, no fruit set	No fruit set
S. melongena	*S. multiflorum*	Parthenocarpic fruits	Parthenocarpic fruits
S. melongena	*S. myoxotrichum*	Parthenocarpic fruits	No fruit set
S. melongena	*S. phlomoides*	No viable embryos	No fruit set
S. melongena	*S. pyracanthos*	33% fruit set, 0% normal seeds	5% fruit set, 8% germination
S. melongena	*S. richardii*	F1 partially fertile, 0–12% normal seeds	No fruit set
S. melongena	*S. rigescens*	F1 partially fertile, 9–63% normal seeds	Parthenocarpic fruits
S. melongena	*S. rigescentoides*	F1 partially fertile, 41–84% normal seeds	F1 partially fertile, 42% normal seeds
S. melongena	*S. rubetorum*	Abnormal adult plants	No viable plantlets
S. melongena	*S. scabrum* ($2n = 48$)	F1 ($2n = 72$), partially fertile, almost regular meiosis (few univalents), dropping of flowers buds, seedless fruits	No fruit set
S. melongena	*S. schimperianum*	Parthenocarpic fruits	No fruit set
S. melongena	*S. sessilistellatum*	F1 partially fertile, no fruit set	F1 partially fertile, 74% normal seeds
S. melongena	*S. sisymbriifolium*	Presence of embryos in ovules, but no germination	11% fruit set, parthenocarpic fruit
S. melongena	*S. supinum*	Parthenocarpic fruits	F1 partially fertile, 41–49% normal seeds
S. melongena	*S. toliaraea*	Parthenocarpic fruits	No fruit set
S. melongena	*S. tomentosum*	F1 partially fertile—BC1 obtained (S. melongena used as male)	F1 virtually sterile, 52–63% normal seeds
S. melongena	*S. torvum*	F1 virtually sterile or fertile—BC1 (S. melongena used as female)	No fruit set
S. melongena	*S. trilobatum*	Parthenocarpic fruits	Fruit set, 100% maternal diploids (no hybrid)
S. melongena	*S. tudununggae*	Parthenocarpic fruits	No fruit set
S. melongena	*S. vespertilio*	10% fruit set, presence of seeds	No fruit set

(continued)

Table 11.7 (continued)

Cultivated species	Wild species	Best cross result (cultivated = female parent)	Best cross result (wild = female parent)
S. melongena	*S. viarum*	F1 fertile—F2 and advanced BC progenies obtained	No fruit set
S. melongena	*S. violaceum*	F1 vigourous, fertile, 99% occurrence of bivalents at meiosis, viable seeds	F1 vigourous, fertile, 99% occurrence of bivalents at meiosis—BC1 to BC4 obtained
S. melongena	*S. virginianum*	No fruit set	F1 vigourous, virtually sterile, 50% occurrence of bivalents at meiosis—BC1 to BC4 obtained
S. melongena	*S. zanzibarense*	Parthenocarpic fruits	F1 virtually sterile, no fruit set

female (for six crosses, thirteen and five, respectively) or male parent (four, eight and two, respectively). Hybrid fertility level does not seem to be related to the phylogenetic proximity between *S. melongena* and the wild species involved. In a number of cases, crosses yielded fertile or partially fertile hybrids regardless of the cross direction, e.g. those involving *S. melongena* on one hand and *S. catombelense* Peyr., *S. cerasiferum* Dunal, *S. dinteri*, *S. incanum*, *S. rigescentoides*, *S. sessilistellatum* and *S. violaceum* on the other hand. Several reciprocal crosses produced fertile or partially fertile hybrids for one cross direction only. This is the case for *S. melongena* used as female and pollinated with *S. campylacanthum*, *S. hastifolium* Hochst. ex Dunal, *S. lichtensteinii*, *S. melanospermum* F. Muell., *S. rigescens* Dunal, *S. viarum* as well as with the nightshade *S. scabrum* Mill. This is also the case for *S. lidii* Sunding, *S. linnaeanum*, Hepper & P.-M.L. Jaeger, S. supinum Dunal (and possibly *S. capense* and *S. cyaneopureum* De Wild.[9]) when used as female and pollinated with *S. melongena*.

One observes also that there are as many as five different types of crossing results (Table 11.8). Fertile (1st type), partially fertile (2nd), virtually sterile (3rd) or unviable interspecific hybrids (4th) together with cross failure (5th type) are obtained for crosses whether *S. melongena* is used as female or as male parent. On the basis of the available set of reciprocal crosses involving *S. melongena* and wild species (Table 11.7), it seems that there is no relationship between reciprocal results; indeed, almost every type of result obtained with *S. melongena* used as female matches with the ones retrieved when *S. melongena* is used as male and conversely (Table 11.8). Last but not least, progenies can be obtained from any given fertility level (fertile, partially fertile or virtually sterile) of the interspecific hybrids (Table 11.7).

11.4.3.2 Global Results for All Types of Crosses

In a number of publications, results are provided without specification of cross direction, or only with a mention of a single cross direction. Therefore, such crosses' results are excluded from Table 11.7, which gathers only the reciprocal crosses. In order to provide a global overview of the interspecific crosses results (out of the wild progenitors of cultivated eggplants, which are detailed in Sect. 11.4.2), we have gathered the best results obtained from such "one way" crosses as well as "unknown direction" crosses together with the best results obtained from "reciprocal crosses"; we then selected the "top one" results. The global synthesis involving *Solanum aethiopicum* and *S. macrocarpon* is provided in Table 11.9 and for *S. melongena* in Table 11.10.

To date, no fertile hybrids have been obtained when crossing *Solanum aethiopicum* with any of the 16 wild species tested; however, partially

[9]The fertility of the hybrids *S. capense* × *S. melongena* and *S. cyaneopurpureum* × *S. melongena* being not indicated (Table 11.7) we hypothesize here that they are fertile or partially fertile.

Table 11.8 Diversity of results obtained from reciprocal results between *Solanum melongena* and wild species

S. melongena female	*S. melongena* male
Fertile hybrids obtained	Fertile hybrids obtained
Fertile hybrids obtained	No fruit set
Partially fertile hybrids obtained	Partially fertile hybrids obtained
Partially fertile hybrids obtained	Virtually sterile hybrids obtained
Partially fertile hybrids obtained	No fruit set
Virtually sterile hybrids obtained	Partially fertile hybrids obtained
Virtually sterile hybrids obtained	Hybrids (embryo, plantlet) not viable
Virtually sterile hybrids obtained	No fruit set
Hybrids (embryo, plantlet) not viable	No fruit set
Cross failure (no fruit set or parthenocarpic fruit)	Partially fertile hybrids obtained
Cross failure (no fruit set or parthenocarpic fruit)	Virtually sterile hybrids obtained
Cross failure (no fruit set or parthenocarpic fruit)	Cross failure (no fruit set or parthenocarpic fruit)

This Table derives from Table 11.7

Table 11.9 Global overview of the best results obtained when crossing *S. aethiopicum* and *S. macrocarpon* with wild *Solanum* species (crosses with the wild progenitors of cultivated eggplants, not here, are detailed in Sect. 11.4.2)

Best result simplified	Partner 1	Partner 2	Source
F1 partially fertile, vigourous	*S. aethiopicum*	***S. incanum***	Daunay et al. (1991)
F1 partially fertile, vigourous	*S. aethiopicum*	*S. violaceum*	Literature compilation
F1 virtually sterile, vigourous	*S. aethiopicum*	***S. cinereum***	Daunay et al. (1991)
F1 virtually sterile, vigourous	*S. aethiopicum*	***S. marginatum***	Daunay et al. (1991)
F1 virtually sterile, vigourous, progenies obtained	*S. aethiopicum*	*S. multiflorum*	Literature compilation
F1 virtually sterile, vigourous	*S. aethiopicum*	***S. tomentosum***	Daunay et al. (1991)
F1 virtually sterile, vigourous	*S. aethiopicum*	*S. virginianum*	Daunay et al. (1991)
F0 → F1 seeds (50–100% normal)	*S. aethiopicum*	***S. sisymbriifolium***	Daunay et al. (1991)
F0 → F1 seeds (50–100% normal)	*S. aethiopicum*	***S. viarum***	Daunay et al. (1991)
F0 → F1 seeds (5–50% normal)	*S. aethiopicum*	***S. capsicoides***	Daunay et al. (1991)
F0 → F1 seeds (abnormal)	*S. aethiopicum*	***S. campanulatum***	Daunay et al. (1991)
F0 → F1 seeds (abnormal)	*S. aethiopicum*	***S. capense***	Daunay et al. (1991)
F0 → F1 seeds (abnormal)	*S. aethiopicum*	***S. linnaeanum***	Daunay et al. (1991)
F0 → F1 seeds (abnormal)	*S. aethiopicum*	***S. pyracanthos***	Daunay et al. (1991)
F0 → F1 seeds (abnormal)	*S. aethiopicum*	***S. torvum***	Daunay et al. (1991)
Parthenocarpic fruits	*S. aethiopicum*	***S. rubetorum***	Daunay et al. (1991)
F1 obtained	*S. macrocarpon*	***S. incanum***	Literature compilation
F0 → F1 seeds (abnormal)	*S. macrocarpon*	*S. linnaeanum*	Literature compilation
No fruit set	*S. macrocarpon*	***S. violaceum***	Daunay et al. (1991)

Data are ranked by results and wild species names. In bold, species absent from Table 11.7

Table 11.10 Global overview of the best results obtained when crossing *S. melongena* with wild *Solanum* species (crosses with the wild progenitors of cultivated eggplants, not here, are detailed in Sect. 11.4.2). Data are ranked by results and wild species names

Best result simplified	Partner 2	Source
F1 fertile	*S. campylacanthum*	Literature compilation
F1 fertile, vigourous, progenies obtained	*S. incanum*	Literature compilation
F1 fertile, progenies obtained	*S. lichtensteinii*	Literature compilation
F1 fertile, progenies obtained	*S. linnaeanum*	Literature compilation
F1 fertile, vigourous, progenies obtained	*S. viarum*	Literature compilation
F1 fertile, vigourous, progenies obtained	*S. violaceum*	Literature compilation
tetraploidised F1 partially fertile	*S. aculeatissimum*	Literature compilation
F1 partially fertile	*S. burchellii*	Literature compilation
F1 partially fertile	*S. catombelense*	Literature compilation
F1 partially fertile	*S. cerasiferum*	Literature compilation
F1 partially fertile	*S. coccineum*	Literature compilation
F1 partially fertile	*S. cyaneopurpureum*	Literature compilation
F1 partially fertile	*S. dinteri*	Literature compilation
F1 partially fertile	*S. hastifolium*	Literature compilation
F1 partially fertile	*S. lidii*	Literature compilation
F1 partially fertile	*S. melanospermum*	Literature compilation
F1 partially fertile	*S. richardii*	Literature compilation
F1 partially fertile	*S. rigescens*	Literature compilation
F1 partially fertile	*S. rigescentoides*	Literature compilation
F1 partially fertile	*S. rubetorum*	Daunay et al. (1991)
F1 partially fertile, dropping of flower buds, seedless fruits	*S. scabrum* ($2n = 48$)	Literature compilation
F1 partially fertile	*S. sessilistellatum*	Literature compilation
F1 partially fertile	*S. supinum*	Literature compilation
F1 partially fertile, vigourous, progenies obtained	*S. tomentosum*	Literature compilation
F1 partially fertile, vigourous, progenies obtained	*S. virginianum*	Daunay et al. (1991)
F1 virtually sterile	*S. capense*	Literature compilation
F1 virtually sterile, vigourous	***S. cinereum***	Daunay et al. (1991)
F1 virtually sterile	*S. coagulans*	Literature compilation
F1 virtually sterile, progenies obtained	*S. elaeagnifolium*	Literature compilation
F1 virtually sterile	*S. forskalii*	Literature compilation
F1 virtually sterile	*S. goetzii*	Literature compilation
F1 virtually sterile, vigourous	***S. hispidum***	Daunay et al. (1991)
F1 virtually sterile, vigourous	***S. marginatum***	Daunay et al. (1991)
F1 virtually sterile (or fertile), progenies obtained	*S. torvum*	Literature compilation
F1 virtually sterile	*S. zanzibarense*	Literature compilation
F0 → F1 seeds (5–50% normal)	***S. capsicoides***	Daunay et al. (1991)
F0 → F1 seeds	*S. pyracanthos*	Literature compilation
F0 → F1 seeds	*S. vespertilio*	Literature compilation

(continued)

Table 11.10 (continued)

Best result simplified	Partner 2	Source
F0 → F1 seeds (abnormal)	***S. campanulatum***	Daunay et al. (1991)
F0 → F1 seeds (abnormal)	***S. mammosum***	Daunay et al. (1991)
No viable F1	*S. chippendalei*	Literature compilation
No viable F1	*S. clarkiae*	Literature compilation
No viable F1	*S. mahoriensis*	Literature compilation
No viable F1	*S. phlomoides*	Literature compilation
No viable F1	*S. sisymbriifolium*	Daunay et al. (1991)
Fruit set (no detail)	*S. trilobatum*	Literature compilation
Parthenocarpic fruits	*S. aculeastrum*	Literature compilation
Parthenocarpic fruits	*S. beaugleholei*	Literature compilation
Parthenocarpic fruits	*S. dioicum*	Literature compilation
Parthenocarpic fruits	*S. diversiflorum*	Literature compilation
Parthenocarpic fruits	*S. giganteum*	Literature compilation
Parthenocarpic fruits	*S. multiflorum*	Literature compilation
Parthenocarpic fruits	*S. myoxotrichum*	Literature compilation
Parthenocarpic fruits	*S. schimperianum*	Literature compilation
Parthenocarpic fruits	*S. toliaraea*	Literature compilation
Parthenocarpic fruits	*S. tudununggae*	Literature compilation
No fruit set	***S. bonariense***	Literature compilation
No fruit set	*S. dennekense*	Literature compilation
No fruit set	***S. giftbergense***	Literature compilation
No fruit set	*S. heinianum*	Literature compilation
No fruit set	***S. platacanthum***	Literature compilation

In bold, species absent from Table 11.7

fertile hybrids were obtained with *S. incanum* and *S. violaceum*. Progenies were obtained from only one of the virtually sterile hybrids (*S. multiflorum*). It is worthwhile to retry some of the crosses since they produced a proportion of normal seeds and could perhaps give rise to hybrids. Only one out of the three interspecific crosses attempted so far with *S. macrocarpon* has yielded a hybrid, the fertility of which is however not known (Robinson et al. 2001).

Interspecific crosses involving *Solanum melongena* are much more numerous (61) than those involving *S. aethiopicum* (16) and *S. macrocarpon* (3). Over half of the crosses yielded hybrids of variable fertility (from fertile to virtually sterile) and from which nine progenies were obtained so far (Table 11.10).

The species yielding fertile or partially fertile hybrids belong either the Melongena clade (*Solanum campylacanthum*, *S. cerasiferum*, *S. incanum*, *S. linnaeanum* and *S. lichtensteinii*), to the poorly resolved Old World Anguivi grade (*S. burchellii*, *S. catombelense*, *S. coccineum*, *S. cyaneopurpurem*, *S. dinteri*, *S. hastifolium*, *S. lidii*, *S. rigescens*, *S. rigescentoides*, *S. rubetorum* Dunal,, *S. sessilistellatum* (=*S. nigriviolaceum*), *S. supinum*, *S. tomentosum* and *S. violaceum*), to other Old World clades (*S. melanospermum*, *S. virginianum*) as well as to New World clades (*S. aculeatissimum* Jacq., *S. viarum*) (Vorontsova et al. 2013; Aubriot et al. 2018). For the hybrid between *S. melongena* and *S. aculeatissimum*, information is given only for its tetraploidized form. Unexpectedly, one tetraploid species of

subgenus *Solanum*, *S. scabrum*, is one of the species yielding partially fertile hybrids when crossed with *S. melongena*. The species yielding virtually sterile hybrids, or no hybrids at all, display a similar phylogenetic diversity, as those yielding fertile or partially fertile hybrids.

Interestingly, when crossed with *Solanum melongena*, some species belonging to the New World clade (Stern et al. 2011) yield hybrids. That is the case of *S. viarum* which produces a fertile hybrid (Sharma et al. 1980), as well as *S. elaeagnifolium* (Garcia-Fortea et al. 2019) and *S. hispidum* Pers. (= *S. asperolanatum* Ruiz & Pav.; Daunay et al. 1991) which produce virtually sterile hybrids. The case of *S. aculeatissimum* is unclear since the fertility of the diploid hybrid is not indicated (Zhou et al. 2018). That is also the case for the fertile hybrid between *S. melongena* (female) and *S. torvum* (Cao et al. 2009) although all other authors having worked on this hybrid report its high sterility (Bletsos et al. 1998, 2004; Daunay unpub.; Mc Cammon and Honma 1983; Plazas et al. 2016; Robinson et al. 2001).

On the whole, this survey of the crossability results between cultivated eggplants and wild relatives indicates that a lot of work has still to be carried out in the future for completing and rationalising the current knowledge, both by extending the range of wild species available (African, Asian and Australian species) and by homogenising of the types of criteria to record. The possibility of obtaining progenies from interspecific hybrids has to be investigated as a priority, because this is the criterion that at the end is essential to breeders for the transfer of wild traits into cultivated germplasm. The apparent loose link between interspecific crosses results and phylogenetic relatedness of the partner species is a questioning matter that constitutes a promising research field for further comparative studies.

11.4.4 Crosses Between Wild Species

One hundred sixteen crosses involving 33 wild species have been attempted between wild *Solanum* species, out of which 26 crosses were reciprocals. Reciprocal and fertile or partially fertile hybrids were obtained only from the crosses involving *S. coccineum* on one hand and *S. capense* or *S. violaceum* on the other hand (Table 11.11). One cross direction and fertile or partially fertile hybrids were obtained from eight other crosses, involving mostly species of the former Oliganthes section, now included in the Anguivi grade (i.e. *S. anguivi*, *S. capense*, *S. coccineum*, *S. rubetorum*, *S. violaceum*) and some species of the Melongena clade (*S. campylacanthum* crossed with *S. cerasiferum* and *S. incanum*). One partially fertile hybrid was unexpectedly obtained when crossing *S. violaceum* (female) with *S. virginianum*, two species that are partly in sympatry[10] but also rather distantly related (Chap. 10).

The global overview of the best results obtained when crossing wild × wild, and that for any cross direction, is provided in Tables 11.12 and 11.13. The global picture is that roughly half (62) of the crosses were "successful" (Table 11.12) and half (54) failed (Table 11.13). Among the species combinations yielding fertile hybrids, one notices members of the Melongena clade that are closely related to each other, namely *S. campylacanthum–S. cerasiferum*,[11] *S. incanum–S. campylacanthum*, *S. incanum–S. insanum* and *S. incanum–S. lichtensteinii*. As already mentioned when discussing the reciprocal crosses, members of the former Oliganthes section are also often cross compatible. Detailing the cross failures (Table 11.13) is of limited use given many crosses have been attempted by only one author or with few parental accessions. Some failures are questionable, in particular for crosses between phylogenetically close species of the Melongena clade (Chap. 10), such as *S. campylacanthum* and *S. insanum*, *S. campylacanthum* and *S. lichtensteinii* and *S. incanum* and *S. linnaeanum*.

A few New World species, *Solanum sisymbriifolium, S. torvum and S. viarum,* have been

[10]Both are found in the same geographical and ecological areas.

[11]Because of this interfertility, Olet and Bukenya-Ziraba (2001) suggested *S. campylacanthum* and *S. cerasiferum* belong to the same biological species.

Table 11.11 Overview of the best result obtained from reciprocal crosses between wild species (wild progenitors of cultivated eggplants included)

Female	Male	Best result of the cross	Best result of the reciprocal cross
S. anguivi	*S. capense*	25% fruit set, 1% normal seeds	F1 partially fertile
S. anguivi	*S. coccineum*	30% fruit, 0% normal seeds	F1 fertile
S. anguivi	*S. rubetorum*	No fruit set	F1 partially fertile
S. anguivi	*S. violaceum*	Hybrid death	F1 partially fertile (16% pollen stainability)
S. campylacanthum	*S. cerasiferum*	F1 fertile	No fruit or not seeds
S. campylacanthum	*S. incanum*	No fruit set	F1 fertile
S. capense	*S. coccineum*	F1 partially fertile	F1 fertile
S. capense	*S. pyracanthos*	10% fruit set, 0% normal seeds	No fruit set
S. capense	*S. rubetorum*	0–5% fruit set, 0% normal seeds	16% fruit set
S. capense	*S. violaceum*	F1 virtually sterile	F1 virtually sterile
S. coccineum	*S. pyracanthos*	40% fruit set, 0% normal seeds	No fruit set
S. coccineum	*S. rubetorum*	50–60% fruit set, 40–60% normal seeds	F1 fertile
S. coccineum	*S. violaceum*	F1 partially fertile	F1 partially fertile
S. dasyphyllum	*S. linnaeanum*	25–80% fruit set, abortive seeds	25–50% fruit set, abortive seeds
S. multiflorum	*S. torvum*	Parthenocarpic fruits	No fruit set
S. multiflorum	*S. trilobatum*	Parthenocarpic fruits	F1 vigourous, virtually sterile, 46% bivalents at meiosis
S. multiflorum	*S. violaceum*	Parthenocarpic fruits	Death of F1
S. multiflorum	*S. virginianum*	F1 obtained, 43% occurrence of bivalents at meiosis	F1 weak + 30% maternal diploids—F1 virtually sterile, 56% occurrence of bivalents at meiosis
S. pyracanthos	*S. rubetorum*	No fruit set	7% fruit set, 0% normal seeds
S. rubetorum	*S. violaceum*	7% fruit set, 0% normal seeds	35–50% fruit set, 43–59% normal seeds
S. torvum	*S. trilobatum*	No fruit set	Parthenocarpic fruits
S. torvum	*S. violaceum*	Fruit set, 100% maternal diploids (no hybrid)	Death of F1 seedlings, or F1 weak, fully abnormal meiosis, dropping off of immature flowers, hybrid 100% sterile
S. torvum	*S. virginianum*	No fruit set	Fruit set, 100% maternal diploid (no hybrid)
S. trilobatum	*S. violaceum*	Parthenocarpic fruits	No fruit set
S. trilobatum	*S. virginianum*	90% maternal diploids, F1 weak and virtually sterile, 3% occurrence of bivalents at meiosis	17% maternal diploid, F1 of medium vigour and virtually sterile, 21% occurrence of bivalents at meiosis, progenies obtained
S. violaceum	*S. virginianum*	F1 partially fertile	Death of F1 seedlings

Table 11.12 Global overview of the best and most successful results obtained when crossing wild *Solanum* species (wild progenitors of cultivated eggplants included). Data are ranked by results and wild species names (partner 1 first, and then partner 2)

Best result simplified	Partner 1	Partner 2	Source
F1 fertile	*S. anguivi*	*S. coccineum*	Compilation
F1 fertile	***S. anguivi***	***S. platacanthum***	Compilation
F1 fertile	*S. campylacanthum*	*S. cerasiferum*	Compilation
F1 fertile	*S. capense*	*S. coccineum*	Compilation
F1 fertile	***S. capense***	***S. tomentosum***	Compilation
F1 fertile	***S. coccineum***	***S. giftbergense***	Compilation
F1 fertile	***S. coccineum***	***S. rigescens***	Compilation
F1 fertile	*S. coccineum*	*S. rubetorum*	Compilation
F1 fertile	***S. coccineum***	***S. tomentosum***	Compilation
F1 fertile	*S. incanum*	*S. campylacanthum*	Compilation
F1 fertile	***S. incanum***	***S. insanum***	Compilation
F1 fertile	***S. incanum***	***S. lichtensteinii***	Compilation
F1 partially fertile	*S. anguivi*	*S. capense*	Compilation
F1 partially fertile, vigourous	*S. anguivi*	*S. rubetorum*	Compilation
F1 partially fertile, vigourous	*S. anguivi*	*S. violaceum*	Daunay et al. (1991)
F1 partially fertile	***S. capense***	***S. supinum***	Compilation
F1 partially fertile	***S. coccineum***	***S. cinereum***	Compilation
F1 partially fertile	*S. coccineum*	*S. violaceum*	Compilation
F1 partially fertile, vigourous	***S. incanum***	***S. pubescens***	Daunay et al. (1991)
F1 partially fertile	***S. violaceum***	***S. giftbergense***	Compilation
F1 partially fertile, vigourous	***S. violaceum***	***S. pubescens***	Daunay et al. (1991)
F1 partially fertile	***S. violaceum***	***S. rigescens***	Compilation
F1 partially fertile, vigourous	***S. violaceum***	***S. tomentosum***	Daunay et al. (1991)
F1 partially fertile	*S. violaceum*	*S. virginianum*	Compilation
F1 partially fertile	***S. violaceum***	***S. zanzibarense***	Compilation
F1 weak	***S. anguivi***	***S. cinereum***	Daunay et al. (1991)
F1 virtually sterile, vigourous	***S. anguivi***	***S. incanum***	Daunay et al. (1991)
F1 vigourous, but no flowers	***S. anguivi***	***S. linnaeanum***	Daunay et al. (1991)
F1 virtually sterile	*S. capense*	*S. violaceum*	Compilation
F1 virtually sterile	***S. coccineum***	***S. zanzibarense***	Compilation
F1 virtually sterile, vigourous	***S. incanum***	***S. virginianum***	Daunay et al. (1991)
F1 virtually sterile, vigourous	*S. multiflorum*	*S. trilobatum*	Compilation

(continued)

Table 11.12 (continued)

Best result simplified	Partner 1	Partner 2	Source
F1 virtually sterile, weak	*S. multiflorum*	*S. virginianum*	Compilation
F1 virtually sterile, medium vigour, progenies obtained	*S. trilobatum*	*S. virginianum*	Compilation
F1 virtually sterile, vigourous	***S. violaceum***	***S. marginatum***	Daunay et al. (1991)
F1 virtually sterile, vigourous	*S. violaceum*	*S. torvum*	Daunay et al. (1991)
F1 virtually sterile, vigourous	*S. violaceum*	*S. trilobatum*	Daunay et al. (1991)
No viable F1	***S. anguivi***	***S. marginatum***	Daunay et al. (1991)
No viable F1	***S. violaceum***	***S. linnaeanum***	Daunay et al. (1991)
No viable F1	*S. violaceum*	*S. multiflorum*	Compilation
F0 → F1 seeds (100% normal)	***S. capense***	***S. burchelli***	Compilation
F0 → F1 seeds (100% normal)	***S. rubetorum***	***S. cyaneopurpureum***	Compilation
F0 → F1 seeds (50–100% normal)	***S. incanum***	***S. cinereum***	Daunay et al. (1991)
F0 → F1 seeds (50–100% normal)	***S. incanum***	***S. dasyphyllum***	Daunay et al. (1991)
F0 → F1 seeds (50–100% normal)	***S. incanum***	***S. marginatum***	Daunay et al. (1991)
F0 → F1 seeds (50–100% normal)	***S. incanum***	***S. tomentosum***	Daunay et al. (1991)
F0 → F1 seeds (50–100% normal)	***S. rubetorum***	***S. platacanthum***	Compilation
F0 → F1 seeds (50–100% normal)	***S. violaceum***	***S. platacanthum***	Compilation
F0 → F1 seeds (50–100% normal)	*S. violaceum*	*S. rubetorum*	Compilation
F0 → F1 seeds (5–50% normal)	***S. capense***	***S. campylacanthum***	Compilation
F0 → F1 seeds (5–50% normal)	***S. capense***	***S. cyaneopurpureum***	Compilation
F0 → F1 seeds (5–50% normal)	***S. capense***	***S. platacanthum***	Compilation
F0 → F1 seeds (5–50% normal)	***S. capense***	***S. virginianum***	Compilation
F0 → F1 seeds (5–50% normal)	***S. coccineum***	***S. burchelli***	Compilation
F0 → F1 seeds (5–50% normal)	***S. coccineum***	***S. campylacanthum***	Compilation
F0 → F1 seeds (5–50% normal)	***S. coccineum***	***S. cyaneopurpureum***	Compilation
F0 → F1 seeds (5–50% normal)	***S. coccineum***	***S. platacanthum***	Compilation
F0 → F1 seeds (5–50% normal)	***S. violaceum***	***S. burchelli***	Compilation
F0 → F1 seeds (5–50% normal)	***S. violaceum***	***S. cyaneopurpureum***	Compilation
F0 → F1 seeds (<5% normal)	***S. capense***	***S. rigescens***	Compilation
F0 → F1 seeds (<5% normal)	***S. rubetorum***	***S. campylacanthum***	Compilation
F0 → F1 seeds (<5% normal)	***S. violaceum***	***S. campylacanthum***	Compilation

In bold, partner' species absent from Table 11.11

Table 11.13 Global overview of cross failures when crossing wild *Solanum* species to each other (wild progenitors of cultivated eggplants included). Data are ranked by results and wild species names (partner 1 first, and then partner 2)

Best result simplified	Partner 1	Partner 2	Source
F0 → F1 seeds (0% normal)	***S. anguivi***	***S. campanulatum***	Daunay et al. (1991)
F0 → F1 seeds (0% normal)	***S. anguivi***	***S. capsicoides***	Daunay et al. (1991)
F0 → F1 seeds (0% normal)	***S. anguivi***	***S. sisymbriifolium***	Daunay et al. (1991)
F0 → F1 seeds (0% normal)	***S. anguivi***	***S. tomentosum***	Daunay et al. (1991)
F0 → F1 seeds (0% normal)	***S. anguivi***	***S. torvum***	Daunay et al. (1991)
F0 → F1 seeds (0% normal)	***S. anguivi***	***S. viarum***	Daunay et al. (1991)
F0 → F1 seeds (0% normal)	***S. capense***	***S. cinereum***	Compilation
F0 → F1 seeds (0% normal)	*S. capense*	*S. pyracanthos*	Compilation
F0 → F1 seeds (0% normal)	*S. capense*	*S. rubetorum*	Compilation
F0 → F1 seeds (0% normal)	***S. coccineum***	***S. giganteum***	Compilation
F0 → F1 seeds (0% normal)	*S. coccineum*	*S. pyracanthos*	Compilation
F0 → F1 seeds (0% normal)	***S. coccineum***	***S. supinum***	Compilation
F0 → F1 seeds (0% normal)	*S. dasyphyllum*	*S. linnaeanum*	Compilation
F0 → F1 seeds (0% normal)	***S. incanum***	***S. campanulatum***	Daunay et al. (1991)
F0 → F1 seeds (0% normal)	***S. incanum***	***S. violaceum***	Daunay et al. (1991)
F0 → F1 seeds (0% normal)	*S. pyracanthos*	*S. rubetorum*	Compilation
F0 → F1 seeds (0% normal)	***S. violaceum***	***S. cinereum***	Compilation
F0 → F1 seeds (0% normal)	***S. violaceum***	***S. giganteum***	Compilation
F0 → F1 seeds (0% normal)	***S. violaceum***	***S. pyracanthos***	Compilation
F0 → F1 seeds (0% normal)	***S. violaceum***	***S. sisymbriifolium***	Daunay et al. (1991)
F0 → F1 seeds (0% normal)	***S. violaceum***	***S. viarum***	Daunay et al. (1991)
Fruit set (no detail)	***S. anguivi***	***S. giganteum***	Compilation
Fruit set (no detail)	*S. torvum*	*S. virginianum*	Compilation
No fruit set	***S. anguivi***	***S. giftbergense***	Compilation
No fruit set	***S. anguivi***	***S. pyracanthos***	Compilation
No fruit set	***S. anguivi***	***S. rigescens***	Compilation
No fruit set	***S. anguivi***	***S. supinum***	Compilation
No fruit set	***S. anguivi***	***S. virginianum***	Daunay et al. (1991)
No fruit set	***S. anguivi***	***S. zanzibarense***	Compilation
No fruit set	***S. campylacanthum***	***S. insanum***	Compilation
No fruit set	***S. campylacanthum***	***S. lichtensteinii***	Compilation
No fruit set	***S. capense***	***S. giftbergense***	Compilation
No fruit set	***S. capense***	***S. giganteum***	Compilation
No fruit set	***S. coccineum***	***S. virginianum***	Compilation
No fruit set	***S. incanum***	***S. capsicoides***	Daunay et al. (1991)
No fruit set	***S. incanum***	***S. linnaeanum***	Daunay et al. (1991)
No fruit set	***S. incanum***	***S. pyracanthos***	Daunay et al. (1991)
No fruit set	***S. incanum***	***S. torvum***	Daunay et al. (1991)

(continued)

Table 11.13 (continued)

Best result simplified	Partner 1	Partner 2	Source
No fruit set	***S. incanum***	***S. viarum***	Daunay et al. (1991)
No fruit set	***S. pyracanthos***	***S. giftbergense***	Compilation
No fruit set	***S. pyracanthos***	***S. platacanthum***	Compilation
No fruit set	***S. pyracanthos***	***S. tomentosum***	Compilation
No fruit set	***S. pyracanthos***	***S. virginianum***	Compilation
No fruit set	***S. pyracanthos***	***S. zanzibarense***	Compilation
No fruit set	***S. rubetorum***	***S. giftbergense***	Compilation
No fruit set	***S. rubetorum***	***S. supinum***	Compilation
No fruit set	***S. rubetorum***	***S. tomentosum***	Compilation
No fruit set	***S. rubetorum***	***S. virginianum***	Compilation
No fruit set	***S. violaceum***	***S. supinum***	Compilation
Parthenocarpic fruits	***S. incanum***	***S. sisymbriifolium***	Daunay et al. (1991)
Parthenocarpic fruits	*S. multiflorum*	*S. torvum*	Compilation
Parthenocarpic fruits	*S. torvum*	*S. trilobatum*	Compilation
Parthenocarpic fruits	***S. violaceum***	***S. campanulatum***	Daunay et al. (1991)
Parthenocarpic fruits	***S. violaceum***	***S. capsicoides***	Daunay et al. (1991)

In bold, partner' species absent from Table 11.11

crossed so far with Old World ones (Tables 11.11, 11.12 and 11.13). *Solanum sisymbriifolium* was crossed with *S. anguivi* and *S. violaceum* (Niakan 1980), as well as with *S. incanum* (Pearce 1975; Rao 1979). *Solanum torvum* was also crossed with *S. anguivi* (Niakan 1980), *S. violaceum* (Kirti and Rao 1981; Niakan 1980) and *S. incanum* (Pearce 1975). *Solanum torvum* was further crossed with *S. multiflorum*, *S. trilobatum* and *S. virginianum* (Rao and Rao 1984). *Solanum viarum* was crossed with *S. anguivi* and *S. violaceum* (Niakan 1980) as well as with *S. incanum* (Pearce 1975). All these crosses failed except for the cross between *S. torvum* and *S. violaceum* which yielded a virtually sterile hybrid (Table 11.12), as did the cross between *S. torvum* and *S. melongena* (Table 11.10).

11.5 Is Interspecific Crossability Predictable?

The genepool concept (Harlan and de Wet 1971) was set up for hierarchising the species related to a crop, on the basis of their crossability potential with the crop. Genepools (GP) were conceptualised as GP1 (biological species[12] including wild, weedy and cultivated forms of the crop, all interfertile), GP2 (species that are crossable with GP1 however with some difficulty and hybrids more or less fertile) and GP3 (species that are not crossable with GP1, forming abnormal, lethal or sterile hybrids, or hybrids that request radical techniques for getting success).

Applied to *Solanum melongena* (Hasan 1989), GP1 was first defined with *S. insanum* (*S. melongena* groups E and F *sensu* Lester) and *S. melongena* (groups G and H) on the basis of (1) their complete intercrossability (F1 plants with >80% pollen stainability), and (2) of the fact that, at that time, they were belonging to a same biological species. Hasan placed *S. incanum* (group C) and *S. lichtensteinii* (*S. incanum* group D) in GP2; together with *S. campylacanthum* (*S. incanum* groups A and B). In later research (Plazas et al. 2016) *S. insanum*, *S. melongena* and *S. incanum* were all (arguably) included in GP1. *Solanum*

[12]The biological species concept is based on successful interbreeding between the members of a given (biological) species, and their reproductive isolation from other species.

lichtensteinii and *S. campylacanthum* were included in GP2, together with *S. linnaeanum*, several species of the Anguivi grade (including the cultivated *S. aethiopicum* and *S. macrocarpon* and their wild progenitors) as well as species of the Madagascar clade (*S. pyracanthos* Lam.). Other Old World species, as well as New World species including *S. sisymbriifolium*, *S. torvum* and *S. elaeagnifolium*, were gathered into GP3. These examples illustrate the fluidity in the application of GP definitions for spiny solanums. Also, the global overview of the interspecific results involving *S. melongena* (see above) shows the limited practical value of the genepool system applied to spiny solanums. The example of *S. melongena* (Table 11.10) indicates that viable hybrids of various pollen fertilities were obtained when crossed with wild species of any given GP and that progenies can be obtained even from hybrids obtained with GP3 wild species.

Phylogenetic relationships between spiny solanums do not seem to be entirely helpful for predicting interspecific crossability. Indeed, closely related species can yield fertile or partially fertile hybrids when crossed to each other (e.g. *S. melongena* with other species of the Melongena clade), but species that are far more distant can also yield such hybrids (e.g. *S. melongena* with the New World *S. viarum* or the Australian *S. melanospermum*). Conversely species distantly related to *S. melongena* can yield hybrids from which progenies were obtained (e.g. *S. elaeagnifolium* and *S. torvum*). The ultimate inconsistency is illustrated by the successful cross between two species that are phylogenetically very distant, the tetraploid *S. scabrum* of subgenus *Solanum* (Chap. 10) and the diploid eggplant, *S. melongena*. Indeed, the cross *S. melongena* ($2n = 24$) *S. scabrum* ($2n = 48$) yielded a few hexaploid F1 plants, partially fertile. The authors related the unusual ploidy level to the endo-duplication of the triploid zygote (Oyelana et al. 2009). Despite partial pollen stainability (38%), the hybrids produced only parthenocarpic fruits.[13]

[13]Interestingly, mature fruit colour of the hybrid between *S. melongena* (yellow) and *S. scabrum* (purple-black) was red (Oyelana et al. 2009).

Knowledge on crossability combinations between cultivated eggplants and wild species and between wild species is by far very incomplete; this reflects (1) the very rich species diversity in spiny solanums, (2) and the still incomplete knowledge on phylogenetic relationships among Old World spiny solanums. However, the current state of the art and the apparent loose consistency between crossability and phylogenetic relationships seem to indicate that predicting crossability between species is illusory. This has implications on research fields that investigate (1) the biological meaning of current phylogenetic hypotheses and traditional species concept, (2) the range and nature of species chromosomal (and genomic) differentiation making interspecific crosses possible or not, and (3) the identity of the genetic factors that can rock an interspecific cross from impossible with some parents to possible with others.

11.6 Overcoming Interspecific Hybrid Sterility via Tetraploidisation

Several cases of F1 hybrid fertility restauration thanks to chromosome doubling are reported in the literature. Amphidiploids ($4x$) issued from colchicine treatment of reciprocal hybrids between *Solanum melongena and S. aethiopicum* Aculeatum group (*S. integrifolium*) displayed a clear increase of pollen stainability (70–72%), when compared to their diploid counterpart (9–12%); they yielded seeded fruits (86–91% normal seeds), whereas the diploids did not set fruits or set parthenocarpic ones (Ali et al. 1992). Bivalents and quadrivalents were observed at metaphase I in meiosis of a $4x$ F1 (*S. aethiopicum* Aculeatum group [*S. integrifolium*] × *S. melongena*), which indicates high homeology of the genomes (Isshiki et al. 2000).

F1 (*Solanum melongena* × *S. aethiopicum* Gilo group) pollen stainability was improved from 7% (diploid hybrid) up to 67% (tetraploid version) (Isshiki and Taura 2003). The reciprocal hybrid F1 (*S. aethiopicum* Gilo group x *Solanum melongena*) whether $2x$ or $4x$ did not produce

pollen at all. Fruit set was obtained on the reciprocal 4*x* via selfing or intercross, whereas the diploids did not set fruits. In addition to the interest of chromosome doubling for restoring the fertility of this interspecific hybrid, Isshiki and Taura (2003) demonstrated also that there was a correlation between pollen sterility and cytoplasm donor, but no correlation between ability to set seed and cytoplasm. Contradictory findings on pollen fertility obtained by other authors suggest the existence of intraspecific variations of the cytoplasm between *S. aethiopicum* cultigroups or accessions, in line with mitochondrial DNA variations previously revealed by RFLPs (Isshiki et al. 2003).

In the case of crosses between *Solanum melongena* and *S. macrocarpon*, partial restauration of F1 pollen stainability was achieved by chromosome doubling induced by colchicine treatment (Khan et al. 2013a). The tetraploid hybrids displayed 40% pollen stainability versus 0.9% for its diploid counterpart. Whereas the diploid hybrid did not set fruits, F2 seeds were obtained by selfing the tetraploid F1 and BC1 seeds by backcrossing the tetraploid F1 with the diploid *S. macrocarpon* (ploidy level of this BC1 progeny was not specified).

Another example is provided by the tetraploidised F1 (*Solanum virginianum* [*S. xanthocarpum*] × *S. melongena*) that produced 78% stainable pollen and its progeny was fertile; on the contrary the diploid (2*x*) hybrid was highly sterile with 1% stainable pollen (Rajasekaran 1971).

The F1 (*S. violaceum* [*S. indicum*] × *S. melongena*), 2*n* = 2*x*, was partially fertile with 49% stainable pollen; after colchicine treatment, its amphidiploid (2*n* = 4*x* = 48) was fully fertile (92% stainable pollen) and produced seeds and further fertile progenies (Rajasekaran 1970). The 4*x* plants were slow in growth, but did not show any gigantism, usually observed in polyploids. Meiosis was normal in the diploid (12 bivalents). The meiosis of tetraploid plants diakinesis and metaphase I yielded more bivalents and tetravalent than univalents and trivalents, but the subsequent stages were mostly normal. Based on chromosome pairing in the F1 and its derived amphidiploid, this latter was classified as a segmental allopolyploid.

The F1 (*S. melongena* × *S. aculeatissimum*) hybrid, obtained via embryo rescue (Zhou et al. 2018) was immediately treated with colchicine. The meiotic configuration of the resulting amphidiploid mostly consisted in bivalents, although multivalents were also observed but in low frequency. Lagging chromosomes were observed in later meiosis divisions, and the resulting pollen had 25% stainability.

F1 (*S. melongena* × *S. torvum*) has also been tetraploidised with colchicine (Daunay 1987–1988; Cürük and Dayan 2018). Both authors report virtual sterility (pollen stainability <5%) of the hybrids, although Cürük and Dayan (2018) describe two plants (out of 77 obtained) that yielded 8–11% pollen stainability. The tetraploid hybrids displayed improved pollen stainability, although still mediocre (10–15% in Daunay (1987–1987) and less than 3% in Cürük and Dayan (2018)).

These various examples show the interest of doubling the chromosome set for overcoming some F1 hybrid sterility barriers. However, information about the inevitable return, sooner or later, to diploid level is scarcely mentioned by authors. Isshiki and Taura (2003) on the basis of successful production of dihaploids by anther culture of somatic amphidiploids *S. aethiopicum* Gilo group × *S. melongena* (Rizza et al. 2002) suggested that anther culture could constitute a promising technique to move tetraploid progenies to the diploid level.

11.7 Disharmonic Interaction Between Wild Cytoplasms and Eggplant Nucleus: An Opportunity for Breeders

Male sterility has an interest for breeding, because it facilitates the production of commercial F1 seeds, given no emasculation of the maternal parent is needed. Cytoplasmic male sterility (CMS) has been found in several interspecific crosses between *Solanum* species used as females and *Solanum melongena*. It is explained

by an incompatibility between the *Solanum* cytoplasm and *S. melongena* nuclear genome. It is a maternally inherited trait that is characterised by a failure to produce or to release functional pollen. In order to be workable for breeding, its expression must be stable regardless of the environmental conditions and must be associated to normal seed set. Cytoplasmic male sterilities of several phenotypes have been obtained from several interspecific crosses involving wild species and *S. melongena*. They result from unbalanced interactions between wild cytoplasm factor (s), of mitochondrial origin in most cases, and eggplant nuclear factor(s). We detail here two CMS systems. The anther indehiscent type was obtained with cytoplasms of *S. violaceum* (*S. kurzii*) and *S. virginianum*, for which anthers contain normal pollen but do not release it because their terminal pores do not open. The second system is the pollen non-formation type, obtained with cytoplasms of *S. aethiopicum* Aculeatum group, "*S. grandifolium*"[14] and *S. anguivi* for which the anthers are completely devoid of pollen. Both systems have been summarised (Khan and Isshiki 2016). Other CMS types (Fang et al. 1985; Khan and Isshiki 2008), the petaloid and vestigial anther types, were obtained from a cross between *S. aethiopicum* Gilo group × *S. melongena*.

11.7.1 Indehiscent Anthers—Non-release Type

The cross between *Solanum violaceum* (female) and *S. melongena* yielded a hybrid with 31% pollen stainability (Isshiki and Kawajiri 2002). When backcrossing it (as female) with *S. melongena* as recurrent parent, the BC1 and BC2 segregated for anther indehiscence. This trait was fixed in BC3 and BC4, which possessed *S. violaceum* mitochondrial (mt) and chloroplast (cp) DNAs. All BCs displayed low pollen stainability (0–70%), despite an almost normal meiosis in the advanced BC4 (average chromosome association was 11.6 bivalents + 0.8 univalents, up to 12 bivalents). Similarly, the hybrid between a prickleless form of *S. violaceum* (*S. kurzii*) and *S. melongena* yielded a hybrid with 30% pollen stainability and only 1% in vitro germination (Khan and Isshiki 2009). Segregation for releasing/not releasing the pollen appeared in the BC1 generation, which produced pollen grains regardless of the pollen release ability of the plants. The "not releasing pollen" trait was transmitted to the next BC2 progeny and was fixed without exception in BC3. "Releasing pollen" BC1 and BC2 plants yielded BC2 and BC3 segregating progenies, progressively nearing 100% "not releasing" plants. Average pollen stainability (63–68%) and in vitro germination ability (8–24%) of the BC progenies remained relatively low. Because meiosis of BC3 was normal (complete bivalents at metaphase I), this low pollen quality was attributed to the wild cytoplasm. All BC progenies, regardless of their pollen release type, had the cytoplasm of the wild parent (mtDNA and cpDNA). Fruit set and seed set (after pollination with the recurrent *S. melongena* parent) increased gradually with successive BC generations, thus indicating the absence of negative effect of the *S. kurzii* cytoplasm on this trait. This CMS was stable over seasonal climatic changes, but no restorer genes were identified. This is not a problem given that the male sterile plants produce some viable pollen; hence, their maintenance by selfing is potentially feasible.

The hybrid *Solanum virginianum* × *S. melongena* is virtually sterile with 5% stainable pollen (Khan and Isshiki 2008). Backcrossed with *S. melongena* (male parent), all plants of BC1 to BC4 generations displayed indehiscent anthers, although the parents and the F1 had dehiscent ones. The expression of this sterility was shown to be stable over four months, despite warm temperatures varying from 26 to 38 °C. Mitochondrial genomes of F1 and BCs were inherited from *S. virginianum* (maternal inheritance), while their chloroplast genomes

[14]This name is a synonym of the accepted name *S. sessile*, an American species of the Geminata Clade. However the species designated under this name in the publications on male sterility is probably another taxon.

originated from recombination of parental cpDNAs (biparental inheritance). Average chromosome pairing of the F1 at metaphase I was 11.7 bivalents and 0.6 univalents. Despite this ratio reaching 12 bivalents for some plants in the BC generations, microspores degenerated post-meiosis and BC progenies displayed partially stainable pollen, with a tendency to decrease in later generation BCs (67% in BC1, down to 37% in BC4). This research pointed out, for the first time, the presence of recombined cpDNA in progenies of sexual crosses among non-tuberous solanums. If confirmed, this finding would impact the interpretation of phylogenetic trees based on chloroplast markers only, these latter being hypothesised to only reflect maternal inheritance.

Male sterile lines having one or the other of the above-mentioned cytoplasms, *S. violaceum* (*S. kurzii*) and *S. virginianum*, were compared in two studies (Hasnunnahar et al. 2012; Khan et al. 2015). For all of these lines, pollen stainability evaluated with acetocarmine was lower (50–75%) than eggplant control (90–100%) in the first publication. Pollen stainability was even lower for the second study, with 49–56% for lines with *Solanum violaceum* cytoplasm and 42% for lines with *S. virginianum* cytoplasm, whereas *in vitro* pollen germination dropped down to 25% (*S. violaceum* cytoplasm) and 14% (*S. virginianum*). Quantitatively, male sterile lines produced as much pollen grains per anther as the *S. melongena* control, with the exception of those with the *S. virginianum* cytoplasm that significantly produced less pollen grains (Khan et al. 2015). Fruit set of the lines after manual selfing was correct but variable (53% for lines with *S. virginianum* cytoplasm, 75–91% for lines with *S. violaceum*); it was improved (up to 71% and 87–100%, respectively) when the male sterile lines were backcrossed with *S. melongena* (Hasnunnahar et al. 2012). The average number of seeds per fruit was less than the selfed S. melongena control (784 seeds) for the selfed male sterile lines (362–518 seeds), but similar to it (767–834 seeds) when the lines were backcrossed with *S. melongena* (Hasnunnahar et al. 2012). The mediocre pollen stainability of the male sterile lines, evaluated with a starch staining solution (Lugol's), indicated that at the time of pollen maturation their carbohydrate metabolism was abnormal with incomplete starch degradation (Hasnunnahar et al. 2012; Khan et al. 2015). Pollen degeneration in indehiscent CMS lines having *S. violaceum* or *S. virginianum* cytoplasms occurs along all stages of pollen development, from unicellular microspores released by the tetrads (29–36%), early bicellular pollen (6–12%) to late bicellular pollen (9–10%).

Given pollen quality of these CMS sources is low and hampers their maintenance by hand selfing and given no restorer genes were identified so far, their use in breeding remains hypothetical.

11.7.2 No Formation of Pollen Grains

The absence of pollen production in the anthers was found in progenies issued from a hybrid between "*Solanum grandifolium*" (possibly a misidentified germplasm of *S. aethiopicum* Aculeatum group) and *S. melongena* (Saito et al. 2009). Genetic study with sterile and fertile progenies led the authors to identify this sterility as a cytoplasmic male sterility (CMS), restorable thanks to a single (Saito et al. 2009) or two (Khan et al. 2013b) dominant gene(s) *Rf*. This CMS is stable over a range of environments.

A similar expression of male sterility was found in the BC1 progeny issued from the F1 (*Solanum aethiopicum* Aculeatum group [female] × *S. melongena*) (Khan and Isshiki 2010). This hybrid (10% pollen stainability) when backcrossed as female with *S. melongena* produced BC1 plants segregating for male sterility; the male sterile BC1 did not produce pollen. BC2 to BC4 progenies obtained from male sterile plants were fixed for this trait, whereas they still segregated for male sterility and male fertility when obtained from fertile mother plants. Pollen stainability of male fertile BCs remained low (<60%). Genetic analysis showed that the sterility had a cytoplasmic origin and that two

independent and dominant genes (*Rf*) controlled the fertility restoration of this CMS. Whether the BC4 plants were male sterile or male fertile, they displayed the cytoplasm of the wild parent (mt and cpDNA).

Segregation for the absence versus presence of pollen grains within the stamens was observed directly on the F1 (*Solanum anguivi* × *S. melongena*) plants (Khan and Isshiki 2011). BC1 progenies obtained from the male sterile F1 plants were all male sterile, whereas the BCs obtained from fertile F1 plants continued to segregate down to BC5. Pollen stainability of the male fertile F1 was 17% and remained low in the BCs (43–56%), although meiosis observed in some BC5 plants was normal (with the exception of rare cases of few univalents). No meiosis at all was detected in the male sterile BC5 plants. All BC progenies possessed *S. anguivi* cytoplasm. Genetic analysis identified two independent and dominant restorer genes, originating from *S. anguivi*, each controlling pollen formation in the presence of *S. anguivi* cytoplasm. Fruit set and seed germination of BC5 were as good as for the *S. melongena* recurrent parent, although the number of seeds per fruit was lower. The expression of this male sterility being stable, it looks promising for use in breeding.

As we have seen, CMS originating from "*S. grandifolium*", *S. aethiopicum* Aculeatum group and *S. anguivi* segregate along the successive backcrossing (or selfing) of male fertile plants, given that the restoration of male fertility is under control of either the one or the other or both dominant restorer *Rf* genes identified in this set of material. In order to speed up the fixation of restorer lines homozygous for the one, the other of both *Rf* genes, Khan et al. (2013b) experimented anther culture of male fertile plants for producing haploids. They obtained few haploids from two ("*S. grandifolium*" and *S. anguivi*) out of the tree cytoplasms tested, thus demonstrating that this technique was workable for fixing eggplant material carrying a wild cytoplasm. Applied to male fertile plants segregating for male sterility, this technique looks promising to produce rapidly homozygous male fertile restorer lines together with male sterile lines. This work opens the path for the use of this CMS in the production of eggplant commercial F1 hybrids.

11.7.3 Towards Genetic Comparisons Between the Two CMS Types

In a wide cross combination experiment, male sterile plants of each cytoplasmic origin were pollinated with male fertile line of their own CMS system and of the other cytoplasms (Khan et al. 2014). The segregation patterns revealed again the occurrence of two independent and dominant restorer genes operating in each CMS system, each *Rf* gene being able to restore fertility in its own CMS system and also in the other CMS, with similar recovery actions in terms of male and female functionality and seed production. The authors concluded that this similarity was indicative of the close relationships between "*S. grandifolium"*, *S. aethiopicum* and *S. anguivi*. All restorer genes were found to be of wild origin. A single reliable SCAR marker ($SCAB10_{1900}$), linked to *Rf* genes, was set up and provides the first facility for early and efficient selection in any marker-assisted CMS breeding programme. This marker will facilitate the exploration of CMS and corresponding *Rf* genes within wild *Solanum* germplasm, although the authors mention the need for the future to develop further markers more tightly linked than $SCAB10_{1900}$ to Rf genes. The molecular basis of both cytoplasmic male sterilities has been unravelled at the level of mitochondrial genes (Yoshimi et al. 2013).

11.8 Genetic Information Drawn from Interspecific Hybrid Phenotypes

Interspecific hybrids display variable redistributions of parental morphological traits depending on the qualitative or quantitative expression of the traits and on the underlying genetic effects controlling their expression (recessiveness, dominance, additivity, epistasis, etc.). Heterosis

for plant vigour, mentioned for a number of interspecific crosses (see Tables 11.3, 11.5, 11.9, 11.10 and 11.12), is observed in hybrids, regardless of pollen fertility. Hence, it seems that the dysfunctioning between parental genomes, expressed at the level of reproductive functions, does not affect development events, as this is exemplified by virtually sterile hybrids that are however vigourous.

11.8.1 Hybrids Between Cultivated Eggplants

11.8.1.1 *Solanum aethiopicum* and *S. macrocarpon*

The hybrid obtained with *Solanum aethiopicum* used as female parent expressed heterosis for plant height and displayed intermediate features between those of the parents for traits such as leaf blade size (Omidiji 1983). The many branched phenotype of the hybrid indicated that this trait is dominant over the less branched one (type of *S. macrocarpon*). Unexpected prickliness and hairiness absent from both parents were observed in the hybrids issued from this cross (Omidiji 1979, 1983), but the occurrence of this phenotype depends on the parental accessions used (Oyelana and Ogunwenmo 2009). Prickliness was also observed in another hybrid between *S. aethiopicum* Kumba group and *S. macrocarpon* (cross direction not specified) as well as unexpected many flowered inflorescences despite the parents having few flowers (Lester 1986). It was hypothesised that the resurgence of these wild or atavic traits (prickliness, hairiness and many flowered inflorescences) in the hybrid was due to loss mutations in the parents and gene complementation in the hybrid.

Also, plants unexpectedly resembling *S. macrocarpon* were found in the F2 progeny issued from a cross between *S. aethiopicum* Kumba group (hairless and prickleless) and *S dasyphyllum*, the wild progenitor of *S. macrocarpon* (hairy and prickly) (Omidiji 1986).

11.8.1.2 *Solanum aethiopicum* and *S. melongena*

The hybrid *Solanum melongena* × *S. aethiopicum* Aculeatum group (*S. integrifolium*) displayed pink flowers and purple fruits (before physiological maturity) as did its *S. melongena* parent, small fruits as did *S. aethiopicum* and intermediate plant vigour, leaf and flower sizes (Oyelana and Ugborogho 2008). The single flower observed by these authors (both parents had few or several flowers per inflorescence) is a unique finding since other hybrids, obtained with other *S. melongena* accessions × *S. aethiopicum* Kumba group, displayed more flowers than both their parents (Prohens et al. 2012). These hybrid plants were also much taller than each of their parent, but were intermediate for leaf size and flower diameter. They displayed *S. melongena* traits for anthocyanins on plant parts and *S. aethiopicum* fruit shape ratio and low fruit phenolic content. They had much smaller fruits than each parental species. Reversion to the wild state was observed for hybrids between *S. melongena* and *S. aethiopicum* Kumba group, which displayed prickly leaves although neither of their parents had prickles (Prohens et al. 2012).

11.8.1.3 *Solanum macrocarpon* and *S. melongena*

Regardless of the cross direction, hybrids display variable vigour (plant height and number of branches) from very weak to vigourous, depending on the publications (Schaff et al. 1982; Gowda et al. 1990; Bletsos et al. 2004; Oyelana and Ugborogho 2008) or on the parental accessions that were used (Schaff et al. 1982). These hybrids displayed several traits similar to those of *Solanum macrocarpon* (high number of flowers per inflorescence, accrescent calyx, small round fruits, yellow mature fruit), of *S. melongena* (presence of prickles on calyx, presence of hairs on leaves, purple fruit), or intermediate between those of both parents (plant height, growth habit, leaf size, petiole length) (Bletsos et al. 2004; Oyelana and Ugborogho 2008;

Schaff et al. 1982). Interestingly the hybrids obtained by Schaff et al. (1982) and Bletsos et al. (2004) displayed prickles on their leaves midribs that were absent from both parents. Unexpected prickliness was also observed on other hybrids issued from crosses between other parental accessions (Omidiji 1979). Hence, reversion to the wild prickliness, previously mentioned for hybrids between *S. aethiopicum* and *S. macrocarpon*, *S. aethiopicum* and *S. melongena*, is also observed for hybrids between *S. macrocarpon* and *S. melongena.*

11.8.2 Hybrids Between Cultivated Eggplants and Wild Species

Generally, reciprocal hybrids display identical phenotypes (Kirti and Rao 1982a), although slight differences are sometimes mentioned, such as in the case of the hybrid *S. aethiopicum* Aculeatum group (*S. integrifolium*) × *S. multiflorum* (*S. indicum* var. *multiflorum*) which attained a greater height than its reciprocal (Kirti and Rao 1980). When the crosses involve cultivated eggplants and wild species, the hybrid general outline is closer to that of their wild parent than to their cultivated one (Bletsos et al. 1998; Kaushik et al. 2016); Daunay et al. unpub. results). This tendency is explainable by the overall dominance of wild traits over domesticated ones (Lester 1989). However, depending on the quantitatively inherited traits, the phenotype of the hybrid moves closer to one or the other parent and sometimes exceeds them (in the case of transgression).

Although the concept of heterosis is usually used and interpreted only in terms of superiority of the hybrid compared to its parents, it was used as a tool for comparing phenotypes of interspecific hybrids issued from crosses between *Solanum melongena* and seven wild species,[15] to those of their parents (Kaushik et al. 2016). Indeed, calculation of heterosis (H) yields values which position the hybrid phenotype by the comparison with its parents. When calculated on the basis of the deviation between the hybrid and its mid parents values for a given trait,[16] H ranges theoretically from zero (hybrid equals parents average) to +100% (hybrid equals its parent displaying the highest value) or −100% (hybrid equals its parent displaying the lowest value). Positive values intermediate between 0 and 100 mean that the hybrid displays intermediate features that are skewed towards the parent with the highest value, and conversely when negative, values indicate that the hybrid displays intermediate features that are skewed towards the parent with the lowest values. H values over 100% (case of transgressive traits) indicate that the hybrid phenotype is beyond the parent with the highest value (if H is positive) or beyond the parent with the lowest value (if H is negative). Kaushik et al. (2016) showed that, depending on trait types (plant height, stem diameter, leaf size, number of flowers per inflorescence, number of petals, calyx prickliness) and species cross combinations, heterosis displayed variation ranging from −100% up to +91%. For example, for plant height H varied from 2.3% for F1 (*S. linnaeanum* × *S. melongena*) to +91% for F1 (*S. melongena* × *S. dasyphyllum*). For fruit calyx prickles H varied much more, from −100% for reciprocal F1 between *S. melongena* and *S. anguivi*, to +80% for F1 (*S. melongena* x *S. dasyphyllum*). Heterosis for the number of petals ranged much less, from −4.8% to +1.9% for the six interspecific hybrids studied. Fruit weight and leaf prickliness behaved differently from the above-mentioned traits. Fruit weight displayed only negative H values, ranging from −6 to −99%, meaning all hybrid combinations bore fruits of a size skewed towards their wild parent. On another extreme, heterosis for leaf prickliness displayed only positive values, some shooting very high for hybrids between *S. melongena* on one hand and *S. incanum* (H = 733%) or

[15]Namely *S. anguivi*, *S. dasyphyllum*, *S. incanum*, *S. insanum*, *S. lichtensteinii*, *S. linnaeanum* and *S. tomentosum*.

[16]Given P1 is the value of parent 1, P2 the value of parent 2, F1 the value of the F1 (P1 × P2), Heterosis H is calculated as $H = 100 * ((F1 - (P1 + P2)/2)/(P1 - P2)/2)$.

S. tomentosum (H = 800%) on the other hand. This means that these hybrids were up to seven or eight times pricklier than their prickliest parent.

Partly consistent as well as complementary results about trait heredity pattern were obtained with an F1 (*Solanum melongena* × *S. incanum*) (Prohens et al. 2013). This hybrid expressed higher values than its parents, in particular for plant height, leaf length and lobing, prickliness, as well as for fruit browning after being cut. The presence of prickles and of anthocyanins on vegetative parts and fruit epidermis was dominantly expressed (over their absence) in the hybrid. F1 small fruits size was skewed towards the wild parent, which is in favour of dominance of small fruit size over large one. However, it is hazardous to assess the inheritance mode of this trait on the sole basis of interspecific hybrid phenotypes; indeed, the frequently observed absence or reduced number of seeds within the F1 fruits can partly explain the reduction of their sizes. For all the other traits, the hybrid was intermediate between the two parents (incomplete dominance).

Reversion to the wild prickly state was observed in hybrids generated by crosses between an accession of *Solanum melongena* without prickles and two non- or poorly prickly accessions of *S. insanum* and *S. tomentosum* (Plazas et al. 2016).

11.8.3 Hybrids Between Wild Species

The phenotype of interspecific hybrids obtained from the cross between wild species is also informative for accessing trait heredity. Several traits were identified as dominantly expressed (Kirti and Rao 1981; Rao and Rao 1984) such as erect habit over pendant habit, long branches over short branches, hairy brittle leaves over soft textured ones, lengthy many (6–10) flowered inflorescences over short and less (1–3) flowered ones, red or orange mature fruit over yellow ones (Rao and Rao 1984), lobed ovaries over globular ones (Kirti and Rao 1980) and racemose over umbellate inflorescence type (Oyelana et al. 2009). The hybrids express features intermediate to those of their parents for quantitative traits such as dimensions of various plant parts (petioles, leaves, flowers, fruit (e.g. in Oyelana et al., 2009).

11.9 Somatic Interspecific Hybrids

From the 1980s onwards, fusion between protoplasts via polyethylene glycol (PEG) exposure or electrofusion, allied to plant regeneration techniques, allowed for the production of a set of interspecific somatic hybrids (symmetric fusion) or of cybrids (asymmetric fusion) between *Solanum* species (eggplant, potato, tomato, spiny solanums and black nightshade), as well as of some intergeneric hybrids (*Solanum melongena* + *Nicotiana* spp.). Somatic hybridisation was investigated as (1) an alternative route to the sexual crosses for transferring traits of interest (mostly disease resistances) from one species to another, and (2) a method to increase cytoplasmic and nuclear genetic diversity (Sihachakr et al. 1994). Results of hybridisations involving *S. melongena* were reviewed twice (Collonnier et al. 2001a; Kashyap et al. 2003).

11.9.1 *Solanum Melongena* + New World Spiny Solanums

Three wild species have been used so far, *Solanum sisymbriifolium*, *S. torvum* and *S. viarum*.

Solanum melongena + *S. sisymbriifolium*

The first somatic hybrids were obtained by PEG fusion of protoplasts of eggplant (*Solanum melongena*) and *S. sisymbriifolium* (Gleddie et al. 1986). They were aneuploid (but close to the 48 expected chromosomes), and plants were smaller than their parents and produced abnormal flowers and pollenless anthers. They segregated for flower colour (purple like the eggplant or white like the wild species) and leaf shape, pubescence and prickliness, but on the whole leaf morphology was closer to that of the eggplant than to *S. sisymbriifolium*. The hybrids had both the stellate trichomes of eggplant and the glandular ones of *S. sisymbriifolium*; those having the highest

proportion of glandular trichomes displayed resistance and antibiosis to the mite *Tetranychus cinnabarinus* comparable to that of *S. sisymbriifolium* (Gleddie et al. 1985). When inoculated with the root knot nematode *Meloidogyne incognita,* the hybrids developed a few galls, but the nematodes did not reproduce as was observed for *S. sisymbriifolium* (Gleddie et al. 1985). These observations indicate that trait inheritance in aneuploid hybrids is both conventional and not conventional, depending on the hybrids and on the traits. Later hybrids obtained by electrofusion were tetraploid ($2n = 4x = 48$) and homogeneous. Their phenotype was intermediate between those of their parents (Collonnier et al. 2003b). Although their pollen stainability ranged from 20 to 30%, they produced fruits with empty seeds. Interestingly, the hybrids inoculated with *Verticillium* wilt (filtrate of culture medium) and *Ralstonia solanacearum* (two isolates) displayed resistance levels intermediate between those of the resistant parent, *S. sisymbriifolium*, and the sensitive one, the eggplant. All hybrids possessed the wild parent chloroplasts (Collonnier et al. 2003b; Gleddie et al. 1986).

Solanum melongena + *S. torvum*

Solanum torvum was also used for attempting to transfer its pest and disease resistances to eggplant (*S. melongena*) by the somatic route. The first hybrids, obtained with PEG technique, ranged from possessing 46–48 chromosomes and displayed 5–70% pollen stainability (Guri and Sink 1988a, c). Prickles were present on all but one hybrid, but their colour (purple) differed from the colour of those of *S. torvum* (green). The long sepals resembled those of eggplant, but petals' colour was a deeper purple. The hybrids exhibited intermediate morphological characteristics for plant stature, leaf and flower size and shape. Some hybrids had eggplant cpDNA and some had both eggplant and *S. torvum* cpDNA. The structure of mtDNA was the result of rearrangements between the mtDNA of the parents. Natural infestation with spider mites was strong on eggplant, weak on the wild species and intermediate on the hybrids. When inoculated with *Verticillium* extracts, hybrid cuttings displayed the resistance of their wild parent. Other authors observed that 15% of somatic hybrids issued from electrofusion had a chromosome number approaching (35 to 46) or reaching tetraploid (48) status (Sihachakr et al. 1989). Leaf shape and flowers number per inflorescence were intermediate to those of the parents, whereas the hybrids expressed the wild parent traits for anthocyanins presence, prickle location and eggplant traits for calyx length and plant height. Interestingly, hairiness was transgressive, with the hybrids displaying a greater hairs density and length. The plants with less chromosomes exhibited a greater morphological variation than those close to $4x = 48$.

Another set of somatic hybrids, all tetraploid, acquired the chloroplast from either one parent or the other one; they were vigourous, relatively homogeneous and morphologically intermediate between the parents and displayed 2–20% pollen stainability (Collonnier et al. 2003a). No translocations or recombinations between parental chromosomes could be observed by genomic in situ hybridisation (GISH). Similar to *S. torvum*, the majority of the hybrids were resistant to *Verticillium* and bacterial wilt.

Asymmetric hybridisation obtained after irradiation of *S. torvum* protoplasts (in order to fragment their nuclear DNA) followed by chemical or electrical fusion with normal eggplant protoplasts yielded a wealth of plants, 15% of which were tetraploid, the rest being diploid (Jarl et al. 1999). The majority of the regenerated plants were morphologically similar to eggplant. The tetraploid plants could be distinguished from the diploids by their broad dark green leaves, short internodes, vigourous growth and a slight decrease of pollen stainability. Agronomic and *Verticillium* tests, performed on hundreds of regenerated plants, identified one highly resistant $4x$ plant, looking like eggplant with normal fruit and seed set. This plant displayed an *Eco*RV DNA restriction pattern similar to that of eggplant, except for few bands similar to *S. torvum*. Although this research did not explain the tetraploid status of this plant, it was the first to indicate that the transfer of a limited amount of DNA

of the donor wild parent was possible while keeping eggplant morphological, fertility and agronomical traits.

Solanum melongena + *S. viarum*

Somatic hybrids issued from *S. melongena* and *S. viarum* (*S. khasianum*) protoplast electrofusion represented 40–50% of the regenerated plants and had a chromosome number ranging from 46 to 48 (Sihachakr et al. 1988). Plants were less vigourous than their parents and relatively homogeneous. Depending on the traits, hybrid phenotype (1) was intermediate (e.g. leaf shape and base blade), (2) expressed dominant traits originating from *S. viarum* (e.g. anthocyanin presence) or from *S. melongena* (stem and petiole thickness and shortness), or (3) was transgressive (e.g. higher number of flowers per inflorescence than each of the parents, distribution of prickles over a larger range of plant parts). Pollen stainability ranged 10–15% (it was >98% for the parents), and no fruit set was observed. Sexual hybridisation was more successful (Table 11.10), by yielding a hybrid with c. 62% pollen stainability (Sharma et al. 1980).

Somatic fusion between eggplant and New World species: uncertain potential for breeding

Regardless of the wild species used, flowering of the somatic hybrids was precocious (Gleddie et al. 1986; Sihachakr et al. 1988, 1989). Ultimately, somatic hybridisation between *S. melongena* and three New World species is as much hopeful as it is hopeless for introgressing wild resistance traits into *Solanum melongena*. It is proven that their disease resistances can be transferred into interspecific somatic hybrids, but the improved pollen stainability of these hybrids, when compared to that of their sexual counterparts, is not sufficient for ensuring their reproductive fertility (seed set). Hence, no progenies usable in a breeding programme have been obtained so far. Further, the return of tetraploid somatic hybrids to the diploid status is a supplementary difficulty.

11.9.2 *Solanum Melongena* + Old World Spiny Solanums

Solanum melongena + *S. marginatum*

With the aim of transferring the arborescent and perennial characters of *Solanum marginatum* L.f. into *S. melongena*, protoplasts of both species were electrofused and somatic hybrids regenerated (Borgato et al. 2007). These hybrids were tetraploid, vigourous and homogeneous, and plants displayed morphological features intermediate to those of the parents, including flower colour (purple-edged petals with central white sector, whereas eggplant has purple flowers and *S. viarum* has white ones). These plants, grown over three years, displayed the arborescent habit of their wild parent, together with its secondary wood tissues. Cytological observations of the hybrids showed a high frequency of bivalents, together with a low frequency of abnormalities (multivalents, univalents, heteromorphic bivalents and lagging chromosomes). Despite this imperfect homeologous pairing during meiosis division I, the somatic hybrids unexpectedly produced pollen of 85% stainability, a much better score than the virtual sterility obtained with sexual hybrids (Table 11.10); hybrids also set fruits and seeds. The germination of the seeds yielded S1 generation plants that were also arborescent, fertile and similar to the former generation S0 for flower and fruit morphology. Segregation for other traits is not mentioned by the authors; hence, recombination events between the parental chromosomes deserve to be clarified in future.

Solanum melongena + *S. violaceum*

In order to transfer to eggplant the bacterial wilt (*Ralstonia solanacearum*) resistance of *S. violaceum* (*S. sanitwongsei* in the publication), protoplasts of both species were electrofused and screened on a medium containing bacterial toxins. Plants regenerated from the surviving cells were further screened in contaminated soil and a

single one survived (Asao et al. 1994). This plant was tetraploid and expressed intermediate traits (e.g. leaf shape, flower size, colour and diameter, stem anthocyanins), or traits of the cultivated parent (immature fruit black colour[17]), or of the wild parent (mature fruit orange colour,[18] numerous flowers per inflorescence).Transgressive traits were not observed. Pollen stainability was 82%, i.e. comparable to the score reached by the sexual hybrid (Table 11.10), and hybrids set seeded fruits and the S1 progeny was also tetraploid and fertile. S0 plants as well as S1 progeny were as resistant to bacterial wilt as *S. violaceum*.

Solanum melongena + *S. aethiopicum*

This interspecific fusion aimed at transferring *S. aethiopicum* disease resistances to *S. melongena*. Iodoacetamide-treated eggplant protoplasts, fused (by dextran method) with *S. aethiopicum* Aculeatum group (*S. integrifolium* in the publication) protoplasts, gave rise to vigourous hybrids displaying characters intermediate to those of the parents for flower size and colour, fruit shape and trichome density on the petiole (Kameya et al. 1990). Hybrids were tetraploid ($2n = 48$) except one which was diploid ($2n = 24$) and sterile. Progenies issued from selfing of one of the tetraploid plants and tested with *Ralstonia solanacearum* segregated for resistance; some plants expressed transgression for resistance (higher level than for *S. aethiopicum*). Other hybrids obtained by electrofusion of the same species displayed also heterosis for plant vigour as a whole: plant height, leaves and stem size (Daunay et al. 1993). All but three plants were intermediate between the parents for morphological traits,[19] with the exception of prickliness and anthocyanin presence which were similar to *S. aethiopicum* and dominantly inherited. Most hybrids were tetraploid, and some were hexaploid or mixoploid. Some of the hybrids displayed cpDNA of *S. melongena* and the others cpDNA of *S. aethiopicum*. The hybrids segregated for pollen stainability (30–85%[20]) and fruit production (from 3 to >9 kg per plant). The authors noticed that good fertility was mostly associated to tetraploidy and the capture of eggplant chloroplasts. Hybrids obtained again with *S. aethiopicum* Aculeatum group, as well as with Gilo group (Collonnier et al. 2001b), provided results globally similar to those of Daunay et al. (1993). Tested with *Ralstonia solanacearum*, most hybrids were as resistant as their *S. aethiopicum* parents, a few of them being transgressive towards a better resistance (Collonnier et al. 2001b). In vitro anther culture was successful (Rizza et al. 2002; Rotino et al. 2005) in yielding dihaploids ($2n = 2x = 24$) from the $2n = 4x = 48$ somatic hybrids previously obtained by (Collonnier et al. 2001b). The segregation of the dihaploids for flower and fruit traits confirmed that genetic recombination between *S. melongena* and *S. aethiopicum* genomes had occurred at the time of the meiosis of the tetraploid somatic hybrids. Return to diploidy was associated to a strong drop of pollen stainability, ranging 8–16% on average for the dihaploids, whereas their tetraploid parents ranged 54–71% (Rotino et al. 2005). Most dihaploids produced parthenocarpic fruits, and the rest of them produced no fruits at all (Rizza et al. 2002). The resistance of *S. aethiopicum* Gilo and Aculeatum groups to *Fusarium* wilt was transferred to the dihaploids, which segregated for this trait (Rizza et al. 2002; Rotino et al. 2005). A further biotechnological feat was achieved by producing, with the same anther culture technique, dihaploids from a double somatic hybrid obtained by sexual cross between two simple somatic hybrids (eggplant + *S. aethiopicum* Aculeatum group) and (eggplant + *S. aethiopicum* Gilo group) (Rotino et al. 2005). These dihaploids also segregated for *Fusarium* wilt resistance. Via backcrosses, the resistance of the best dihaploids was further introgressed into *S. melongena* and integrated into a breeding

[17]Presence of anthocyanins, which confers purple or black fruit colour, is dominant over their absence.

[18]Orange (*S. violaceum*) is dominant over yellow (*S. melongena*) mature fruit colour.

[19]Mature fruits turned orange, an intermediate state between yellow (eggplant) and red (S. aethiopicum).

[20]The sexual hybrid also phenotyped in Daunay et al. (1993) displayed 10–30% pollen stainability, very poor fruit set and parthenocarpic fruits.

programme (Rotino et al. 2005). The extent of genetic recombination between the genomes of *S. melongena* and *S. aethiopicum* Gilo group was analysed on a population of dihaploids obtained by Rizza et al. (2002), with 280 ISSR markers (71 genotypes) and 3 isozyme systems (70 genotypes) (Toppino et al. 2008a). Disomic and tetrasomic inheritance patterns were identified for ISSR markers. Distorted segregations patterns, not fitting disomic or tetrasomic patterns, were observed for isozymes. These careful analyses confirmed that genes were exchanged between the parental genomes at the time of the meiosis of the somatic hybrid mother plants.

Somatic fusion between eggplant and Old World species: potentials for eggplant breeding

On the whole, somatic hybrids obtained so far between *S. melongena* and three Old World spiny solanums produce a pollen which stainability is equivalent to that of their sexual counterparts (*S. violaceum*, see Table 11.10 and *S. aethiopicum*, see Table 11.5) or a pollen of much better fertility (*S. marginatum,* see Table 11.10); these hybrids also produce seeded fruits. The transfer of disease resistance was proved successful in the somatic hybrids, as well as in their progenies issued from selfing (*S. violaceum*) or dihaploidisation (*S. aethiopicum*). These results, together with segregation events for resistance and morphological traits, as well as genetic analysis with markers (ISSR, isozymes), indicate that recombination between parental genomes occurs at the time of the meiosis of the somatic hybrids. Interestingly for breeders, transgressions towards disease resistance levels that are higher than that of the resistant parent were observed. Importantly, return to diploid status via anther culture and dihaploid production was proved feasible in the case of somatic hybrids obtained with *S. aethiopicum*; this remains to be demonstrated for the hybrids obtained with other species. In the case of *S. aethiopicum*, the ploidy status conversion from $2n = 48$ to $2n = 24$ was associated with an important decrease of pollen fertility. On the whole, the results obtained so far indicate that somatic hybridisation might be complementary to sexual hybridisation, in the specific cases of (1) transgressive resistance, (2) low fertility of sexual hybrids, and (3) if the change of cpDNA and/or mtDNA brings a capital gain over sexual hybrids carrying their maternal cytoplasmic DNA, the agronomic interest of which remains to be demonstrated.

11.9.3 Other Somatic Hybridisations Involving Spiny Solanums

Solanum aethiopicum (Aculeatum group) + *S. violaceum*

This somatic hybridisation aimed at transferring bacterial wilt tolerance of *Solanum violaceum* to *S. aethiopicum* Aculeatum group (*S. integrifolium*) (Tamura et al. 2002). Despite the low success rate (1.5%) of the electrofusion and plant regeneration, one amphidiploid ($2n = 48$) hybrid plant grew well. After inoculation, inhibition of bacterial multiplication in the roots and of its spread to plant upperparts was observed in this hybrid as well as in S. *violaceum*. The hybrid displayed *S. aethiopicum* anthocyanins pigmentation of stems, prickles and veins, but the general habit and leaf shape of *S. violaceum*, as well as intermediate flower colour (pale mauve). It bore many small fruits, containing seeds larger than those of each parent and with a germination rate >90%. Another electrofusion experiment (Iwamoto et al. 2007) was carried out with iodoacetamide-treated protoplasts of *S. violaceum* (*S. sanitwongsei*, *S. kurzii* in the publication) and UV-irradiated protoplasts of *S. aethiopicum* Aculeatum group (*S. integrifolium*). The putative hybrids, regenerated from some 1000 calli, were classified into three groups, according to their chromosome set and phenotype. One group included amphidiploids ($2n = 4x = 48$), displaying homogeneous and intermediate morphological features (leaf size, flower colour, fruits shape size and colour). These plants displayed 79% averaged pollen stainability, set fruits and seeds and expressed heterosis for plant vigour and seed size. The two other groups included asymmetric and mostly hexaploid hybrids ($2n = 6x = 72$), one group

with 1-2 *S. aethiopicum-S. violaceum* parental chromosome dosage and the other group with 2-1 dosage.

Somatic hybridisation between *S. aethiopicum* Aculeatum group and *S. violaceum* yielded fertile tetraploid material, whereas sexual hybridisation yielded at best, when *S. violaceum* is used as female parent (see Table 11.7) a partially fertile diploid hybrid (Lester and Niakan 1986). Given the incompleteness of the available data (possibility to return to the diploid state for the somatic hybrid and obtaining progenies from the sexual hybrid), there is again no clear advantage of somatic hybridisation over sexual hybridisation.

Solanum viarum + *S. aculeatissimum*

Tetraploid somatic hybrid was regenerated at a rate of 45% from electrofusion of *S. viarum* (*S. khasianum* in publication) and *S. aculeatissimum* protoplasts (Stattmann et al. 1994). Grown in greenhouse, the hybrids were relatively homogeneous, of intermediate phenotype for some traits such as prickliness and leaf shape. They expressed heterosis for plant vigour, leaf and flower size. Flowers were normal, with pollen stainability over 87%, and set fruits with seeds that were germinated. Hence, the somatic hybrids between these two species of the Acanthophora clade are fully fertile.

Solanum torvum + *S. tuberosum* (potato)

In order to transfer *S. torvum* resistance to *Verticillium dahliae* to potato, electrofusion of protoplasts of both species was processed (Jadari et al. 1992). Out of hundreds of calli, four tetraploid hybrids were regenerated. They were vegetatively propagated, in order to be phenotyped in vitro and in greenhouse. Rooting troubles, observed in greenhouse only, were overcome by grafting on parental roots. The plants exhibited intermediate morphology, leaf shape and anthocyanin pigmentation, but their flowers aborted precociously. *In vitro* inoculation with *Verticillium* filtrate demonstrated that the hybrids were as resistant as *S. torvum*.

11.9.4 *Solanum Melongena* + Distantly Related Solanaceae Crops

A number of somatic hybrids have been regenerated from the fusion of *Solanum melongena* protoplasts with *Solanum* species of subgenus *Solanum* (*S. nigrum*) and Potatoe (tomato, potato), as well as with other genera (*Nicotiana*).

Solanum melongena + *S. lycopersicum* (tomato)

Asymmetric somatic plants were obtained by fusion of gamma irradiated protoplasts of a sexual interspecific tomato hybrid (*S. lycopersicum* x *S. pennellii* Correll), together with eggplant protoplasts (Liu et al. 1995). The four plants obtained had abnormal chromosome numbers (42, 45, 57, 60) and were all sterile (flowers drop after self-pollination). Only two of them survived after a few months; they exhibited a branching pattern resembling eggplant and compound leaves as their tomato parent. Other putative asymmetric hybrids obtained with the same partners were close to the expected tetraploidy ($2n$?=?48) and displayed eggplant morphology (Samoylov and Sink 1996).

Solanum melongena + *S. tuberosum* (potato)

In order to transfer eggplant bacterial wilt resistance (accession cv.508.3) into a diploid potato (*Solanum tuberosum* L.), protoplasts of both species were symmetrically fused in a helix fusion chamber (Yu et al. 2013). The hybrids exhibited various ploidy levels (4*x*, 6*x*, aneuploidy) with three types of nuclear genomes, potato cpDNA, as well as different phenotypes segregating for parental traits (stem colour), or displaying intermediate features (leaf shape) or trait states similar to or different from their parents (internode length, plant vigour, foliage colour). Screening tests carried out *in vitro* as well as with potted plants, with the agent of bacterial wilt, revealed segregation of the hybrids for

resistance, the best ones having levels of resistance similar to their eggplant-resistant parent. Other hybrids obtained with other parental accessions were obtained via the asymmetric fusion between UV-treated eggplant protoplasts and potato protoplasts (Liu et al. 2016). The potato genome of these hybrids had integrated one to eight eggplant chromosome fragments, in a non-selective manner.[21] This result demonstrates that breeding potato for resistance to bacterial wilt issued from *S. melongena* is possible. Some hybrids produced tubers, shaped or not as their potato parent and developed no flowers, abnormal or normal flowers, but none produced pollen. However, as the potato parent unexpectedly did not produce pollen either, the hybrid fertility remains unknown. The authors were very confident in the feasibility of introgressing eggplant bacterial wilt resistance into potato via asymmetric protoplast fusion.

Solanum melongena + *Nicotiana* sp.

Hybrid plants were obtained by the fusion (dextran method) of protoplasts of a triple tobacco mutant set-up for *in vitro* selection of the regenerants, together with a "wild type *Solanum melongena*", but details about these hybrids were not given (Toki et al. 1990).

Somatic fusion between eggplant and distantly related Solanaceae crops: a field of research insufficiently investigated

The potential of plant breeding using protoplast fusion techniques between distantly related species is far from being sufficiently investigated. The few results obtained so far indicate that transfer of traits is possible, but they also point out recurrent sterility troubles. Asymmetric fusion techniques that allow the transfer of pieces of the donor genome into the recipient species seem to be promising. The transfer of eggplant bacterial wilt resistance into potato seems to be the most promising application of this research domain.

11.10 Conclusions

11.10.1 Germplasm Characterisation

Efficiency of *Solanum melongena* breeding is on the way to be upgraded thanks to various DNA and RNA technologies (markers, QTLs mapping, sequencing, genes expression, etc.). However, the main challenge of future breeding of this species as well as of the two African eggplants is based on the genetic and phenotypic characterisation of their cultivated germplasm and of the wild relatives, since all this material is entangled in a complex network of relationships (c.f. Chap. 10 and Sect. 11.4). The characterisation carried out so far (Sect. 11.2) was limited by the difficulty of germplasm holders and breeders to outline the species content of eggplants and relatives germplasm, and to access it. Therefore, the phenotypic and genetic potential of subgenus *Leptostemonum* diversity, far from being unravelled yet, constitutes a promising field of research in many aspects all the more because most traits of interest are common to *S. melongena*, *S. aethiopicum* and *S. macrocarpon*. The breeding of each of these cultivated species will be boosted by the use of an enlarged diversity.

A second challenge relates to the phenotyping methods. Methodologies with improved accuracy that would allow for a better dissection of traits of interest must be set up. Until now phenotyping has been often coarsely carried out; this is the case for graft affinity between rootstock and scion assessed on few genotypes and few criteria (plant survival, growth, earliness, yield and fruit quality) or for resistance to pests, mostly assessed by field observations (degree of infestation). Such traits, based on partner's interactions, deserve to be more closely looked at from both partner's sides, at the intimate level of their interaction. For instance, for graft affinity nearly

[21] *Solanum melongena* + *S. nigrum* PEG fusion between protoplast of *Solanum nigrum* and iodoacetate-inactivated eggplant protoplasts aimed at transferring atrazine (herbicide) resistance carried out by the chloroplasts of the wild partner into eggplant (Guri and Sink 1988b). The regenerated plants displayed *S. nigrum* cpDNA pattern and were resistant to atrazine *in vitro*. The single plant phenotyped resembled *S. nigrum* had white flowers (although the purple colour of eggplant flower is usually dominant) and sterile (no stainable pollen grains).This means that any part of eggplant chromosomes can be integrated.

nothing is known so far in terms of histological and biochemical interactions between scion and rootstock, although graft affinity is located at the level of the graft union. Another relevant example concerns the interactions between plants and insects. The influence of plant genotype on insect biotic criteria (e.g. adult longevity, female fecundity, larvae mortality) allows for an accurate identification of possible antibiotic actions of some genotypes towards the insect. Identification of such new and accurate plant traits, unfavourable to the targeted insect, would provide breeders with powerful breeding criteria that should boost forward efficiency of breeding for resistance to insects.

The third promising aspect of future characterisation concerns the traits to be phenotyped. Evaluation for traits currently much sought-after, such as resistance to major pests (root knot nematodes, mites, and most damaging insects such as the fruit and shoot borer and the leaf hopper) as well as pathogens (in particular soil-borne vascular diseases), is a priority. This should allow the discovery of resistances so far unavailable (e.g. resistance to *Verticillium* wilt and to root knot nematodes within cultivated eggplant germplasm) or impossible to handle because of interspecific cross barriers (resistances to several soil-borne pests and diseases of *Solanum torvum*). The evaluation of an enlarged germplasm resource should also lead to the identification of different resistance types and genetic systems controlling different strains of a given pathogen, of the utmost breeding interest. An outstanding example is that of *S. melongena* and the very damaging *Ralstonia solanacearum* species complex (RSSC) in tropical conditions. Several local *S. melongena* accessions have been identified as being resistant in their country of origin, but these resistances are rarely effective in other places, likely because the bacterial strains are different. Indeed, strong interactions characterise this host–pathogen couple (Lebeau et al. 2011). Hence, in such a case, a breeder's utmost dream is to build an "universal resistance", efficient towards any bacterial strain in any country where the crop has economic importance. When complementary genetic systems (genes and QTLs), originating from different sources and controlling resistance to different strains, are available in the germplasm (and have been characterised), it is theoretically possible to build up, by genetic recombination between the sources, resistance that controls a range of strains wider than the range controlled by each source individually. Such a strategy, involving geneticists and bacteriologists, is ongoing (Salgon et al. 2017, 2018). For other diseases affecting eggplants, if breeders one day face such a case of strong host–pathogen interactions,[22] they will have to turn to the natural genetic diversity for resistance.

New traits must attract attention of breeders in the near future, such as those directly related to the adaptation to abiotic constraints (e.g. drought). They deserve a special attention, in particular root system structural (e.g. hierarchical ranks between roots, vigour components) and dynamic characteristics (e.g. emission of adventitious roots along plant development steps). Another "new" trait, poorly investigated so far within the germplasm of eggplants and relatives, concerns the alkaloids produced by most of *Solanum* species. These substances are involved in the bitter taste of the fruits and are toxic at high concentrations. Identifying the chemical diversity of the alkaloids synthetised by *Leptostemonum* species, quantifying their presence (in particular in the wild germplasm) and unravelling the genetic controls of their biosynthetic pathway are important. Indeed, there is a non-negligible risk of transfer of alkaloids from wild to cultivated eggplants, either by their grafting on wild rootstocks, or by interspecific crosses. Attention should also be turned to a possible resurgence, by genetic complementation, of this wild (atavic) trait when crossing cultivated forms, although this has not been proved yet for alkaloids (Sect. 11.8.1).

[22]It is possible for instance, that when looked at more closely in the future, eggplants resistance to *Fusarium oxysp*. f. sp. *melongenae* will reveal interactions with the fungus diversity, as it is the case for tomato (different genitors control different races of *Fusarium oxysp.* f. sp. *lycopersici*).

Given the expected increasing pressures of abiotic and biotic stresses in a near future, in particular because of the oncoming climatic changes, characterisation of cultivated and wild germplasm is of particular importance for future breeding of eggplants. Genetic and genomic techniques, taking advantage of the syntenic features among solanaceous crops, are complementary tools to phenotyping largely sampled intra- and interspecific germplasm, given they offer another path for mining genes controlling traits of interest and for discovering allelic diversity.

11.10.2 Sexual Crossability

Knowledge on the potential of crossability between species is extremely important for breeders; it gives the information on the basic requirements for transferring traits of interest from one species to another. Also, new traits of interest can arise from interspecific hybridisation, in particular cytoplasmic male sterilities that are of the utmost interest for the production of hybrid seeds (see 11.7). Cultivated eggplant species can be hybridised experimentally to each other and give rise, with some difficulties, to interspecific progenies (see 11.4.1). Although gene transfer from one eggplant species to another is possible, it has been so far barely practised by breeders, since only resistance genes (*Fusarium* wilt and bacterial wilt) originating from *Solanum aethiopicum* have been transferred to *S. melongena* (11.4.1.2 and 11.9.2). Gene transfer from wild species to cultivated eggplants was not carried out for long because the most interesting species carrying breeding strategic traits such as resistance to several soil-borne pests and diseases did not yield hybrids (*S. sisymbriifolium*) or yielded only virtually sterile ones (*S. torvum*) when crossed with *S. melongena* (Table 11.7). The transfer of other wild traits is ongoing, with in particular the transfer of *S. elaeagnifolium* and *S. incanum* drought resistance to *S. melongena* (see 11.2.3). As for *S. aethiopicum* and *S. macrocarpon*, the breeding efforts have been much less consistent than for *S. melongena*, and until now, there has been no attempt of introgressing them with wild traits of interest.

Although a rather high number of *Solanum* species (67) have been used in interspecific crossability studies (see 11.3), this number is low when compared to the size of *Leptostemonum* subgenus (over 500 species, see Chap. 10) and hence it is clear that crossability attempts will still keep scientists busy in the future. The apparent inconsistency between interspecific crossability results and phylogenetic relationships of the parental species (see 11.5) suggests that predicting crossability between species is for the present time illusory. It indicates also that interspecific crossability between species provides another insight at species relationships, complementary to phylogenetics and other criteria such as phenotype, genetic distance, geographical and ecological distribution (Chap. 10). Indeed, interspecific zygote formation and growth within the seed, and later hybrid growth provide information about the ability of parental genomes to collaborate and ensure or not a normal plant development. Meiosis patterns at diakinesis and metaphase I of interspecific hybrids provide precious information on parental chromosomal interactions, and hence on their chromosomes homologies, homeologies and/or rearrangements. Full sequences of chromosomes of an increasing number of *Solanum* species will provide a way complementary to cytogenetics for assessing chromosomal and genetic rearrangements between species.

So far crossability studies have been most often "roughly" carried out for two main reasons. First, only a small proportion of the publications went as far as attempting to obtain progenies from the hybrids, although for an eggplant breeder, this is the ultimate criterion to assess the success (or failure) of a given interspecific cross. Second, crossability has been assessed by nearly as many criteria combinations as the number of publications (11.3). This situation can be explained by the fact that results of any interspecific cross depend on many factors, in particular (1) prezygotic and post-zygotic barriers, (2) cross direction (which species is the female or male), (3) genotypes of the parental species, and

(4) environmental conditions. As a result of such combinatory conditions, interspecific crosses yield a great variety of results, from no fruit set on the maternal parent to fully fertile hybrids at the extremes of the possible range of responses. Measurements for assessing cross success or failure are consequently also diverse and range from percentages of fruit set, seed set of the maternal parent, F0–>F1 seed normality and germination rate, F1 characteristics (lethality at embryo or plantlet stages, abnormal features, weakness), F1 male meiosis and pollen stainability or germinability, up to F1 fruit set and seed set. Results of any interspecific cross can also change when various techniques are implemented, such as embryo rescue, hormonal treatment or grafting for boosting weak hybrids, artificial chromosomes doubling and other biotechnologies such as somatic hybridisation. As a consequence, results in the literature are extremely heterogeneous and it is rather difficult to unambiguously characterise a "successful cross". Also, the use of interspecific F1 pollen fertility as a criterion is questioning for at least two reasons. First there is no strict link between meiosis regularity or irregularity and pollen stainability (11.3.2). For this reason, a statistical approach of PMC meiotic behaviour (in the cases where abnormal meiosis yields some proportion of stainable pollen grains) is necessary, together with the identification of additional post-meiotic factors (for the cases where a regular or almost regular meiosis ends up with a rather poor pollen stainability). Second, the ability of an interspecific hybrid to produce F2 or BC progenies is not clearly related to its (male) fertility, since hybrids virtually sterile (e.g. *Solanum melongena* × *S. elaeagnifolium*), partially fertile (e.g. *S. melongena* × *S. tomentosum*) and fertile (*S. violaceum* × *S. melongena*) can yield such progenies. Definitely, anything seems possible when crossing spiny solanums!

When interspecific crosses fully fail or fail in producing interspecific progenies beyond the F1 crucial step, breeders can nonetheless valorise the wild material. This is the case when the species of interest (1) carries resistances to soil-borne pests and pathogens, (2) displays a vigourous growth in unfavourable conditions (water excess or shortage, drought, cold, salinity) or (3) boosts plant vigor, qualitative and/or qualitative yield. The wild species of interest or the interspecific hybrid itself can then be used as eggplant(s) rootstock, provided it has a good graft affinity with the cultivated eggplant used as scion. Grafting is a technique commonly used for *S. melongena*, and it is workable for the African eggplants. Hence, breeding innovative rootstocks has agronomic and economic interests.

All this means that for the future, much research is still necessary in the field of interspecific crosses between *Leptostemonum* species and although crossability and phylogenetic relatedness are not clearly associated, it is probably more secure to begin with the closest relatives of eggplants (species belonging to Melongena clade and Anguivi grade). Internationally collaborative initiatives are needed in order to guaranty full coverage of the crosses, use of shared success criteria and clarification of several pending questions.

11.10.3 Somatic Crossability

Somatic hybridisation experiments between spiny solanums and other Solanaceae had its peak in the 1980s–1990s, and its agronomic motivation was mostly the transfer of disease resistances. The techniques for regenerating amphidiploids or asymmetric hybrids are functional. Although morphological features of the polyploids, aneuploids or introgressed somatic hybrids display both expected and unexpected heredity patterns, their expression of disease resistance levels similar to those of their donor parent is a constant throughout the examples reviewed here. The general trend is that somatic hybridisation yields fertile hybrids when partner species share close phylogenetic relationships and yields sterile hybrids when the sexual cross is either impossible or yields sterile material. However, there are some exceptions for which somatic hybridisation is superior to sexual hybridisation (e.g. *Solanum melongena* + *S. marginatum*; *S. aethiopicum* + *S. violaceum*). In

these cases somatic hybrids display better pollen stainability than their sexual counterparts. Somatic hybrid sterility might be compatible with breeding of a vegetatively propagated crop such as potato, since flower fertility is not indispensable. But genetic recombination between parental genomes and fertility of the progenies is indispensable for breeding sexually reproduced crops, such as *S. melongena*. In such cases, the next obstacle is the return to the diploid status. This was proved feasible thanks to dihaploids production via anther culture on the single example of somatic hybrids between *S. melongena* and *S. aethiopicum*. However, return to diploidy came with a strong reduction in pollen fertility. On the whole, *S. melongena-S. aethiopicum* progenies were obtained and used in breeding from the hybrids, regardless of their sexual or somatic origin. It would be interesting to know if genetic recombination was different between both kinds of hybrids, because this could be a reason for choosing the best "recombining" technique. With the exception of these somatic hybrids, return to diploidy is neither questioned not solved for all other somatic hybrids involving other species combinations.

11.10.4 Hybrid Phenotypes and Genetics of Morphological Traits

Mendelian and quantitative genetics of traits of interest to breeders are not developed in this chapter because they are beyond its scope. Nonetheless some trait heredity patterns are presented, given that the literature offers information on some interspecific hybrid phenotypes. When differences exist between parents (e.g. prickly vs non prickly, resistant to a given pathogen vs sensitive, etc.), F1 hybrid phenotypes (Sect. 11.8) allow us to determine whether a given trait is dominant, incompletely dominant or recessive. Heterosis, or hybrid vigour, is frequently observed for some traits such as plant height and leaves sizes, whereas resurgence of a few atavic (wild) traits (prickliness in particular) occurs in crosses between cultivated eggplants (c.f. 11.8). However, the interspecific F1 phenotype is sometimes biased, such as in the case of fruit size: this trait depends not only on fruit size genes but also on the presence of seeds. As interspecific hybrids frequently display fertility troubles, F1 fruit size must be interpreted with caution. F2 or backcross generations issued from F1 theoretically provide further information on the genetic control of the segregating traits, but in the case of interspecific hybrids progenies, this information is absent because of the sterility of the hybrids or biased because of distorted segregations. Phenotypes of symmetrical or asymmetrical somatic hybrids are even more difficult to interpret in terms of traits genetics, because of the tetraploid or aneuploid status of such hybrids together with cytoplasmic changes.

Along the successive parts of this chapter, we hope to have convinced our readers that examining the diversity and intercrossability of eggplants and relatives is of key importance for future research programmes.

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Domestication of Eggplants: A Phenotypic and Genomic Insight

12

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Abstract

Agriculture, and in particular systematic and repeated cultivation of plants, is one of the main characteristics of post-Neolithic sedentary human societies. Deciphering the domestication pathways that have allowed for extensive cultivation of crops is of great scientific importance: first, because it can reveal the patterns and processes of human-induced selection and contribute to the knowledge of the genetic basis of adaptive traits, and second, because identifying the times and locations of domestication is crucial to the understanding of our own evolutionary history, in particular for the last ca. 12,000 years. Finally, the identification of genes involved in domestication could offer potential for future crop improvement. In some instances, knowledge from one crop can be transferred to another to reveal broad patterns, as well as the extent to which parallel evolution has given rise to the crops we rely on today. There have been a number of studies into eggplant domestication, but clarifying the routes and even the number of domestications has until today been limited. This is due to (1) partial knowledge on the identity of eggplant wild relatives, (2) sparse sampling (both in terms of species/accessions and types of data), and (3) inadequacy of the statistical tools used for phylogenetic/demographic inferences. However, the most recent analyses of *Solanum melongena* point to a single domestication and significant crop-wild-weedy gene flow, which likely hampered earlier phylogenetic attempts. Here, we provide an overview of the current understanding of the domestication frameworks for the three eggplants, *Solanum melongena*, *S. aethiopicum* and *S. macrocarpon*. First, we detail the phenotypical traits of the crops and of their wild progenitors. Then, we detail the historical hypotheses on domestication of eggplants and, when possible, we re-evaluate them in the light of the genomic data generated within the last couple of years.

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12.1 Domestication: An Overview

For millennia, Palaeolithic hunter-gatherers engaged in various types of early cultivation and low-level farming to enhance or supplement

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Table 12.1 Examples of traits affected by the transition between wild *S. anguivi* and cultivated *S. aethiopicum* cultigroups

Trait	Wild (*S. anguivi*)	Cultivated (*S. aethiopicum*)
Vegetative traits		
Longevity	Over 2 years	1/2 year
Plant habit	Shrub	Herb
Stem prickliness	Prickly	Non-prickly
Pubescence	Hairy	Glabrous
Leaf size (young plant)	Large	Small
Leaf lobing (young plant)	Lobed	Entire
Leaf tip angle (°)	60	90
Flower traits		
First inflorescence height (cm)	100	20
Number of flowers per inflorescence	Over 10	Single
Peduncle and rachis	Long	Short
Pedicel thickness	Thin	Thick
Corolla diameter	Small	Large
Perianth lobe number	5	6 to over 10
Fruit traits		
Number of fruits per inflorescence	Over 6	Single
Pedicel length	Long	Short
Pedicel thickness	Thin	Thick
Position	Erect	Pendant
Detachment from calyx	Easy	Hard
Colour (immature)	Green	White, violet
Stripes (immature)	None	Striped
Colour (mature)	Red	Orange
Diameter	1 cm	10 cm or more
Length/breadth ratio	As long as broad	Longer than broad, or vice versa
Groove number	None	Many
Groove depth	None	Deep
Locules number	2	Over 10
Inner locules number	None	Some
Placentas	Simple	Proliferate
Septa (divisions between tissues or cavities)	Thin	Thick
Fruit wall thickness	Thin	Thick
Pulp	Juicy	Gelatinous or fleshy
Taste	Bitter	Mild or sweet
Other traits		
Storage life (weeks)	2	20
Seed breadth (mm)	2.5	3.5

their foraging (Barker 2011), but it was not until the end of the Pleistocene (ca. 12,000 years before present [YBP]) that these manipulations of wild species began to develop into what could be recognised as agriculture (Diamond 2002). Agriculture emerged independently in multiple regions between 12,000 and 3000 YBP (Meyer et al. 2012a), and it is likely the reasons this transition was both feasible and beneficial differed significantly between these areas (Larson et al. 2014).

When Vavilov first outlined his theory on the centres of origin of plant domestication in 1926 (Vavilov 1926), he fundamentally changed the intellectual framework for the study of plant domestication and agriculture (Harris 1990). Vavilov's motivation was in the use of plant breeding to improve crop yields in the famine-stricken USSR, theorising that increasing the biodiversity of crop plants would increase food security. During his dozens of collecting trips across the globe, Vavilov realised that crops and their wild relatives were often particularly diverse in relatively small locations, and theorised that this could be the site of origin of the crop. Vavilov identified locations where the centres of diversity overlapped for multiple crops, and suggested there were the centres of origin for agriculture. Although the locations and even reasons for the presence of these centres have been revised over the decades, the work of Vavilov set the stage for this way of thinking.

Plant domestication is a long-term and progressive process driven by an array of selection pressures applied to plants. Agricultural constraints, such as short cultivation duration allied to human choices among natural variations and mutations, progressively modify traits in order to match people's needs (e.g. large palatable fruits providing food) and preferences (attractive fruit

Table 12.2 Examples of traits affected by the transition between wild *S. dasyphyllum* and cultivated *S. macrocarpon*. Data taken from Bukenya and Carasco (1994)

Traits	Wild (*S. dasyphyllum*)	Semi-wild or semi-cultivated	Cultivated (*S. macrocarpon*)
Vegetative traits			
Plant height (cm)	90–122	62–85	40–52
Number of leaf prickles cm^{-2}	3–5	0–3	0 or almost 0
Number of leaf hairs cm^{-2}	30 to over 180	Almost 0–30	0 or almost 0
Leaf length (cm)	22–27	21–31	19–39
Leaf width (cm)	15–20	16–22	11–27
Stem colour	Green	Green or some shades or purple	Green or light purple
Flower traits			
Number of flowers per inflorescence	8–10	7–8	3–6
Corolla colour	Light purple	Light purple	Light purple or white
Corolla diameter (mm)	29–42	24–47	23–54
Fruit traits			
Diameter (cm)	3.1–3.7	3.0–3.7	4.2–7.4
Length/breath ratio	0.9	0.8–0.9	0.7–0.8
Peduncle length (cm)	2.3–2.6	1.9–2.2	1.6–1.7
Calyx length (cm)	2.8–3.1	2.9–3.4	2.8–4.6
Other traits			
Seed width (mm)	2.6–2.8	2.5–2.9	3.2–3.6

Table 12.3 Summary of the range of diversity among 56 accessions of *S. insanum* originating from India, Bangladesh and Sri Lanka (from Karihaloo and Rai 1995), and among 27 accessions of INRA collection originating from India, Sri Lanka, Thailand, Indonesia, Malaysia and Madagascar

Trait	Range of variation (Karihaloo and Rai 1995)	Range of variation (INRA collection)
Vegetative traits		
Plant growth habit	Erect (44%), shrubby (48%), decumbent (7%)	
Plant height		Very low to very high
Number of prickles per leaf	0–26	
Presence of prickles		None (rarely) to all plant parts (slight to very strong)
Hairiness		Weak to strong
Leaf length	4.5–14.9 cm	
Anthocyanins on vegetation		None to strong
Flower traits		
Maximum number of flowers per inflorescence	2–8	1–7
Petal lobing	Shallow (93%), deep (7%)	
Flower colour		White, mauve or violet
Sexuality		Andromonoecious (rarely hermaphrodite)
Fruit traits		
Number of fruits per inflorescence	1–3	1–7
Number of prickles per calyx	0–12.8	
Colour (immature)	Green striped (70%), green striped with anthocyanins (12%), white (9%), pink to purple (9%)	Various shades of green, striped
Fruit length	1.9–4.7 cm	
Fruit breath (cm)	2.0–3.9	1–5
Fruit shape	Globose (66%), ovoid (33%), oblong (1%)	Globose to slightly elongated
Fruit weight (g)	3.5–35	3–40
Taste	Bitter, non-edible	

Percentages of accessions are indicated for some traits

shapes and colours, loss of shattering). Domestication modifies the range of variation of the traits (1) directly affected by the selection pressures (e.g. fruit size), and (2) genetically dragged with them (e.g. seed size within large fruit). When domestication (and diversification) occurs in other areas than the centre of origin, the new environmental conditions apply supplementary selection pressures, which contribute to further crop morphological and physiological change. Major traits affected by domestication of 203 food crops have been categorised into nine classes (Meyer et al. 2012a). As far as eggplants are concerned, these classes are life cycle, vegetation, reproductive strategy, fruits, metabolites and to a lesser extent seeds (Tables 12.1, 12.2 and 12.3).

At the genetic scale, domestication seems generally to be a loss rather than a gain, since most major genes controlling domesticated traits

are under recessive genetic control (Lester 1989). Domestication also has an impact at the whole genome, with changes in the genetic architecture along chromosomes and genome-wide gene expression, as has been shown for example in tomato (Sauvage et al. 2017) and other solanaceous crops, including eggplants (AML Page and MA Chapman, unpublished data).

12.2 The Need to Understand Domestication

If we can identify crop progenitors and other species closely related to modern crops (collectively "crop-wild relatives"; CWRs) we have the ability to breed these taxa and introgress traits of interest from the wild into the crop. Because humans selected only a subset of available genotypes during domestication, for example those with better flavour, harvesting ability, and pest resilience, modern crops have only a subset of the genetic diversity present in their progenitors (Burke et al. 2007). Modern breeding in the last century has often reduced the amount of genetic diversity further, giving rise to highly inbred, uniform crops (Fu 2015). This lack of variation can mean that resilience to a certain pest or pathogen is absent, for example. With the pressures of climate change set to increase over the coming decades, for crops to continue to perform, genetic variation for tolerance to heat and drought and changes in pest and pathogen burden need to be introduced. It could be that the genetic diversity needed has been maintained in the CWRs (Knapp et al. 2013).

12.3 The Pathway(s) to Eggplant Domestication(s)

Genetic techniques have been used to study crops and their wild relatives since the late 1970s, when the first genetic analyses, using seed proteins or isozymes, were carried out (e.g. Decker 1985; Doebley et al. 1984; Lester 1979; McLeod et al. 1983). Since then, the techniques have moved on, with continual updates and improvements based on ease of use, cost, and depth of data. However, to this day the same questions are being asked—where were crops domesticated, how many times, and from which wild species? We begin by describing the three main domesticated eggplants, summing up what is known about the traits and pathways of domestication, where known.

12.3.1 *Solanum aethiopicum* was Domesticated from *S. anguivi*

Historically, scarlet eggplants were split under many botanical names; however, extensive biosystematic studies have proved the interfertility of formerly distinguished taxa and the botanical name was stabilised as *Solanum aethiopicum* L. (Lester 1986; Lester and Niakan 1986). The species it structured into cultigroups, or nodes, on the basis of four usages (Lester 1986; Lester et al. 1986; Lester and Niakan 1986; Lester and Thitai 1989). Three cultigroups are African indigenous leaf and fruit vegetables, mostly grown in West and Central tropical Africa (Gilo, Kumba and Shum), with the fourth primarily an ornamental curiosity. Their biological, agronomical, nutritional characteristics and uses are detailed in "African indigenous vegetables" (Bukenya and Carasco 1999; Lester and Seck 2004; Schippers 2002) and are summarised here:

The Gilo group, previously described under many names (e.g. *Solanum gilo* Raddi, *S. olivare* Paill. & Bois, *S. pierreanum* Paill. & Bois), is cultivated from West to Eastern Central Africa (Daunay et al. 2001b). It is the most commonly cultivated scarlet eggplant (Schippers 2002), for its fruit which display considerable diversity in size (from 1.5 to 12 cm diameter), shape (round or long, grooved or not, flattened or not) and (before ripening) colour (white to dark green with or without stripes; shades of anthocyanins are rarely encountered). At maturity, fruits turn bright red-orange. Plants set single or clustered

fruits (up to ten or more for the small-fruited types). Plants are generally hairy and without prickles.

The Kumba group (*Solanum aethiopicum* L. *sensu* Dunal, and type specimen) is a leaf and/or fruit vegetable, found in semi-arid zones of West and North-Western Africa. Its large fruits (3–20 cm) are generally round, flattened, multi-locular and single, but smaller ones can be clustered. Fruits colour varies as for Gilo group and taste is generally sweeter than Gilo (Schippers 2002). Plants are generally glabrous and without prickles.

The Shum group (*Solanum zuccagnianum* Dunal, *S. aethiopicum* L. *sensu* Bitter) is a leafy (glabrous) vegetable, cultivated in the rainfall parts of most West and Central African countries. The small fruits (12–20 mm) have 2–3 locules, borne singly or in clusters of up to eight fruits, are normally bitter and rarely eaten.

The Aculeatum group (*Solanum integrifolium* Poir.) is common in botanical gardens; it has been distributed commercially as an ornamental curiosity in Europe and elsewhere. It is also found occasionally in gardens in Thailand (Daunay, pers. obs.). The plant is hairy and very prickly; the very bitter, round flattened and many grooved fruits, 3–8 cm diameter, in clusters of half a dozen or more, are generally dark green, sometimes with hints of anthocyanins, and they turn red at maturity. As the Aculeatum group interest is mostly ornamental, the current hypothesis about its origin is that it was produced, intentionally or not, by Europeans selecting progenies from hybrids between *S. aethiopicum* Kumba group and the wild *S. anguivi* (Lester 1986).

Field observations together with a vast crossing programme, detailed morphology and numerical taxonomy, as well as seed proteins electrophoresis have evidenced that *Solanum aethiopicum* has been domesticated from the wild weedy *S. anguivi* (Lester 1986; Lester et al. 1986; Lester and Niakan 1986; Lester and Thitai 1989). *Solanum anguivi* is a polymorphic species, morphologically diverse in terms of general habit, leaf and fruit traits. The very bitter small berries (<1.5 cm), clustered up to 20 on a single inflorescence, have various cooking, condimental, medicinal and ornamental uses (Bukenya and Carasco 1999; Bukenya-Ziraba 2004; Schippers 2002). Bitterness is mostly due to a variety of alkaloids that are widespread in genus *Solanum* (Jayakumar and Murugan 2016).

Recent phylogenetic studies have again evidenced the very close relationship between these two species (Vorontsova et al. 2013; Aubriot et al. 2018). Fully wild and weedy plants can be very prickly on vegetative parts, but the species is most often found in a semi-cultivated state. Both species cohabit in fields or gardens of Africa and Madagascar (Vorontsova and Knapp 2016) where weedy or semi-cultivated *Solanum anguivi* spontaneously occurs and are selectively weeded by humans. Natural fertile hybrids between the two seldom occur spontaneously, despite experimentally being fully cross-compatible (Lester and Niakan 1986). These hybrids (and their progeny) probably suffer from selective disadvantage, given the disruptive selection of man (e.g. selection of large non-bitter fruits, and non-prickly and glabrous plants) and nature (small erected and easy-to-detach fruits held above the foliage, facilitating bird dispersal). As a result, both species remain distinct in Africa.

Human preferences exerted a strong selection pressure at the level of the part of the plant harvested for consumption or reproduction. Unusual mutations could also attract people's attention. Natural selection favours other characters adapted to survival in agricultural systems. And last but not least, the complex floral biology of the wild and cultivated species also acted. Their partial autogamy allows rapid fixation of any chosen phenotype, but partial allogamy, together with the interfertility between wild and cultivated forms, and between cultivated forms, favours hybridisation. Hence, long-lasting selection of cultivated material based on the phenotypes created by all these forces, produced a continuum of great morphological diversity for *Solanum aethiopicum*.

Lester and Niakan (1986) and Lester et al. (1986) have identified characters having

undergone changes during domestication and diversification of the groups. They scored over 30 vegetative, flower and fruit traits and qualified their primitive or advanced (domesticated) states (Table 12.1). This long list of traits outlines the domestication process, which progressively adapts cultivated forms to agricultural constraints (e.g. increased earliness adapted to short cultivation duration) and human preferences, in particular for non-prickly plants, mild tasting, coloured, large and variously shaped fruits, as well as palatable hairless leaves. The domestication selection pressure also indirectly modifies a whole range of other traits dragged together with the directly selected traits, via epistasis and linkage.

Lester et al. (1986) selected 17 traits out of those listed in Table 12.1, and carried out a cladistic analysis to infer potential ancestral features for all these traits. The resulting tree suggested that the Shum group had retained most of the ancestral traits while the Kumba group was suggested to derive from the Gilo group. The cladogram indicated that large fruits (in particular the ones of the Kumba group) developed only once during fruit size evolution. Conversely, changes in several other traits (prickliness, hairiness, leaf size, flower and fruit number, corolla lobe number, fruit pedicel length and fruit length) had occurred several times. Overall, however, based on the wide range of variation of traits within each group (e.g. flower clustering in the Gilo group and fruit size in the Kumba group), the authors concluded that these groups might have undergone reticulate evolution, a phenomenon difficult to represent with a simple bifurcating tree.

In the absence of archaeological information and better knowledge of African tribal migrations, domestication history of scarlet eggplants remains obscure, paving the way to speculations such as those proposed by Lester and Niakan (1986). They suggested that, instead of a single domestication event occurring in one place from a single wild population of the wild *Solanum anguivi*, *S. aethiopicum* would result from several domestication events having occurred many times and in many places in rainforests or woodland savannahs of West and Central Africa.

Inheritance analyses carried out for over 80 vegetative and reproductive characters, on F1 progenies obtained by crossing *Solanum anguivi* with Shum and Kumba, and by crossing Gilo with Shum and Kumba, showed that the hybrids were generally more similar to the less domesticated parent, or else, intermediate, suggesting simple or co-dominant inheritance (Lester and Thitai 1989). Heterosis was noted for plant vigour (height and breath). Results from F2 populations confirm previously reported Mendelian inheritance of dominant genes for the presence of prickles, stellate hairs, anthocyanin pigmentation on petioles; likewise, the long multi-flowered inflorescences of *S. anguivi* were dominant to the short, few flowered inflorescences of Kumba. However, F2 quantitative segregation for these traits indicated a polygenic control, and for some, skewed distributions towards the wild type were observed. This study showed that several wild traits are dominant to their domesticated state; that is, there is a preponderance of recessive genes in scarlet eggplants domesticates.

12.3.2 *Solanum macrocarpon* was Domesticated from *Solanum dasyphyllum*

Domestication of gboma, or the African eggplant (*Solanum macrocarpon*), gave rise to two main types of cultivars: a leafy group and a fruity group (Schippers 2002). The leafy group is used for its small and tender leaves. Its fruits are usually not used because they are hard, bitter, and packed with seeds; at maturity, their surface cracks, suberises and becomes brown. The fruity group usually has very large (up to 750 g) and soft fruits, mainly round and flattened, with a wide and edible calyx. They turn bright yellow at maturity. Their large leaves can be eaten when young. Further details about this crop (agronomy, diseases, nutritional value, etc.), generally considered as a minor crop in Africa, are

available (Bukenya-Ziraba and Bonsu 2004; Schippers 2002).

Solanum dasyphyllum is the wild progenitor of *S. macrocarpon* (Vorontsova et al. 2013; Aubriot et al. 2018). Both are distributed across tropical Africa (Vorontsova and Knapp 2016) and are fully interfertile, as evidenced from experimental crosses (see Chap. 11 and Bukenya and Carasco 1995) and observations in the wild (Lester and Hawkes 2001; N'Gbesso et al. 2016; Schippers 2002). As a result, both taxa are treated as the same biological species. The wild *S. dasyphyllum*, a very prickly and hairy species, is sometimes cultivated for its roots, leaves and bitter fruits that are used as medicine (Bukenya and Carasco 1999). Plants are extremely prickly and hairy, inflorescences are multi-flowered, and the fruits are generally small, clustered and erect.

The effects of domestication on *Solanum macrocarpon*/*S. dasyphyllum* have been much less investigated than for other domesticated-wild eggplant species. The comparison of wild, semi-wild and cultivated forms (Bukenya and Carasco 1994) summarises the effects of domestication on a set of traits (Table 12.2). One notices overall (1) loss of prickles and hairs, (2) reduction of plant height, number of flowers per inflorescence, and peduncle length, (3) increase in diversity for flower/stem colour, and of seed size and (4) general increase in the size and morphological variability of the leaves, calyx, corolla and fruit. Although not recorded by Bukenya and Carasco (1994), other fruit traits are also affected by domestication, such as the switch from erect to pendant fruits, the diversification of immature fruit colour (from only green and striped to various shades of green, white or partly purple) and a reduction in fruit bitterness.

Domestication history of *Solanum macrocarpon* was hypothesised to have followed three steps (Bukenya and Carasco 1994). The first stage could have begun with the gathering of roots, leaves and fruits from wild *S. dasyphyllum* populations for medicinal uses, and consecutive introduction of the species around the settlements. The second step is suggested to involve the use of these plants also for culinary purposes, in the case of hairless and/or non-prickly mutants. The third stage involved planting in gardens. Another hypothesis (Lester et al. 1990) that concerns both *S. macrocarpon* and *S. aethiopicum* is that these crops would have been domesticated throughout extensive areas, since there is no evidence of any centre of origin. This would result from migrations of human tribes in various directions across different agro-ecological habitats, with relief and belts of vegetation as drivers of these migrations. As a result, "today we are left with a confusing mosaic of uncertain patterns" (Lester et al. 1990). However, the authors state that the greatest diversity of cultivars of both species is found today around Ivory Coast in West Africa.

Concerning the domestication of *Solanum macrocarpon*, one can wonder why this species is much less variable than the other two domesticated eggplant species regarding fruit shape, a trait of key economic importance. Indeed, the majority of varieties are oblate or subspherical (Schippers 2002), like the wild type, with only a few varieties that display oblong fruits (e.g. MM 11044 in the INRA collection, originating from Ivory Coast). No long or grooved fruits have been described to date. This specificity is perhaps a trace of a later or less stringent selection pressure than for *S. aethiopicum* and *S. melongena*.

Inheritance of some traits was studied on F1 and F2 progenies deriving from crosses between wild and cultivated forms (Bukenya and Carasco 1995). As expected, the presence of purple colour, prickles and hairs on vegetative parts was shown to be dominant over their absence, as previously shown by Lester and Niakan (1986), and the closest resemblance of F1 and F2 progenies to their wild parent was confirmed for these traits.

12.3.3 *Solanum melongena* was Domesticated from *Solanum insanum*

For eggplant, which is harvested for its fruit, significant evolution of fruit size, shape and taste took place during its domestication (Wang et al.

2008). There has also been in many cases a drastic reduction in the size and density of the prickles that cover the leaves, stems and calyxes of wild eggplants, presumably due to selection for ease of harvesting (Fig. 12.1). Fruit size can vary from a few grams to over one kilo; fruit colour can vary from plain green to green with white and purple together with a diversity of chlorophylls and anthocyanins nuances and distribution patterns; fruit shapes vary from spherical to extremely long and narrow (over 50 cm length); fruit surface can vary from smooth to ribbed. Phytochemical investigations have shown that domestication has also modified fruit composition for phenolic constituents. Content of total phenolic acid conjugates is much higher in *Solanum insanum* fruits than in *S. melongena* (Prohens et al. 2013), and the species differ in the relative quantities of some individual phenolic compounds (Kaushik et al. 2017; Meyer et al. 2015). This change might have been dragged with selection for more palatable fruits, a criterion usually involved in the domestication process (Wang et al. 2008). Fruit bitterness, mostly controlled by alkaloid content, is another trait that was reduced by domestication (Wu et al. 2013), although the range of variation within *S. melongena* germplasm is not quantified yet. Given the consumer's variable organoleptic preferences from one country to another, one can reasonably suppose that there is still variation for this trait, at least among landraces.

This range of variation is much narrower in *Solanum aethiopicum* and even more in *S. macrocarpon*, where fruits remain smaller and much less variable regarding their shape and colour variation. Long fruits barely exist in *S. aethiopicum* and are absent from *S. macrocarpon*, and for both species, fruit anthocyanins are rarely present, and if they are present, their shade is weak and temporary. These differences question whether the three eggplant species reached different stages of domestication, perhaps more recent in the African eggplants, not allowing sufficient time for novel mutations to arise, or whether human selection has favoured other phenotypes.

The taxonomic treatment of the wild ancestors and progenitors of *Solanum melongena* has been the subject of numerous researches, the complexity of which was summarised several times (Aubriot et al. 2018; Daunay and Hazra 2012;

Fig. 12.1 Spiny leaves of the eggplant progenitor *S. insanum* Photograph by Mark Chapman

Khan 1979; Knapp et al. 2013; Mace et al. 1999; Weese and Bohs 2010) and is part of long-lasting controversy among taxonomists about the biological reality of the species concept (see, e.g., De-Queiroz 2007; Knapp 2008; Luckow 1995; Mallet 1995). The closest wild relative to *S. melongena* is *S. insanum*, but the majority of related eggplants are found in Africa and/or the Middle East. A number of similar but varied theories on the origin of cultivated eggplant have been made and are summarised in the next few paragraphs.

We start by describing the informal classification system for eggplant and wild relatives developed by Lester and Hasan (1991); this has often formed the basis of other investigations. These authors gathered a number of taxa into two "species", each represented by four informal groups. The *Solanum incanum* groups (named A–D) were considered the progenitor of the *S. melongena* groups (E–H). Within *S. incanum*, groups A and B are found throughout a large part of Africa in savannah woodlands and grasslands, respectively, with groups C and D derived from these. Group D is found only in Southern African semi-deserts, whereas C has a much larger distribution throughout Central and North-East Africa, extending into the Middle East and as far as Pakistan. *Solanum melongena* group F was considered derived from *S. incanum* C; it is a widespread weedy taxon that was thought to represent the wild progenitor of the cultivated eggplant. Under the scenario of Lester and Hasan (1991), human selection on *S. melongena* F gave rise to primitive cultivars in South-East Asia (group G), which were further dispersed around the region. Further selection gave rise to group H, the advanced cultivars. Group E is morphologically similar to group F, but often with a creeping habit, much more prickles and slightly larger fruit, which Lester and Hasan (1991) and others (e.g. Daunay et al. 2001a) considered to be weedy feral derivatives of the cultivated eggplant.

Many studies have used a range of genetic markers to address aspects of eggplant taxonomy and domestication, yet have often suffered from incomplete sampling of the A to H groups and inconsistent usage of these informal names. For the sake of clarity, we will only restrict our discussion to the first molecular phylogeny of the eggplant direct wild relatives (Weese and Bohs 2010); the reader interested in the previous and subsequent works on the subject is invited to refer to Chap. 10 for a chronological review. Weese and Bohs (2010) sampled all eight groups and other related taxa and showed that at least three of the eight groups held up well (groups A, D and C). Relationships between the groups proposed by Lester and Hasan were not always supported; however, the limited statistical support obtained for the internal nodes of the phylogeny obtained by Weese and Bohs (2010) further complicates the comparison. In particular, resolution between groups E–H was low, but the results were not inconsistent with Lester and Hasan's work; that is, group F was probably the progenitor of eggplant and group E is probably a derivative (Weese and Bohs 2010). The low resolution of the clade containing wild, weedy and crop eggplants can be explained by ongoing gene flow, a genetic bottleneck following split from *S. incanum* and/or multiple domestications (Weese and Bohs 2010).

In an attempt to provide greater resolution, Meyer et al. (2012b) used highly polymorphic AFLP (amplified fragment length polymorphism) markers and a broad sampling of accessions. They concluded at least two origins of domesticated eggplant, with the primitive cultivars (incorrectly named *S. melongena* subsp. *ovigerum*) possibly representing a third domestication. This was based on two genetic groups within cultivated eggplant, roughly corresponding to Indian accessions and South-East Asian accessions. Additionally, the genetic differences between the two wild taxa (groups E and F of Lester and Hasan) were slight and Meyer et al. (2012b) conclude these are the same taxon.

The informal classification system of Lester and Hasan (1991) had the key advantage of having taken in account extensive phenotypic data of plants grown in greenhouses and observed in the wild, in their natural habitats; however, its use was grieved by several difficulties. First, the taxonomy and the nomenclature

of African spiny solanums were at that time significantly fragmentary and unstable. Consequently, the knowledge on the identity of the species that were the closest to the cultivated eggplant was limited. Second, the use of such an informal classification system is problematic because (1) it strongly limits the efforts of biodiversity specialists (taxonomists, phylogeneticists, ecologists, etc.) that all work with formal species (i.e. following the framework of the *International Code of Nomenclature for algae, fungi, and plants*; Turland et al. 2018) and (2) it can lead to considerable confusion on the identity of accessions when the informal designation (viz. the group information) is truncated.

Building on the framework of Lester and Hasan (1991), the genetic data of Weese and Bohs (2010) and Meyer et al. (2012b), and recent breakthroughs on the taxonomy and phylogenetics of African spiny solanums (Aubriot et al. 2018; Vorontsova et al. 2013; Vorontsova and Knapp 2016; see Chap. 10), Knapp et al. (2013) have updated the taxonomy and provided detailed botanical descriptions of the taxa. Formal species delimitations and phylogenetic classification took into account dozens of investigations of wild, weedy and domesticated eggplants which have contributed to the debate, but often only used a subset of the taxa (e.g. Karihaloo et al. 1995; Mace et al. 1999; Sakata and Lester 1997). Their work led to the delimitation of a monophyletic group that include the eggplant and its wild progenitor (*Solanum insanum*), as well as 11 wild African species that are phylogenetically close (Aubriot et al. 2018 and Chap. 10). Of these 11 species, we will mainly focus on the African and Middle East *S. incanum*, closely related to the African *S. campylacanthum*, *S. cerasiferum*, and to the Cape-Verdean *S. rigidum*, i.e. the "Widespread clade" *sensu* Aubriot et al. (2018). This clade is closely related to the aubergine and the Asian *S.*

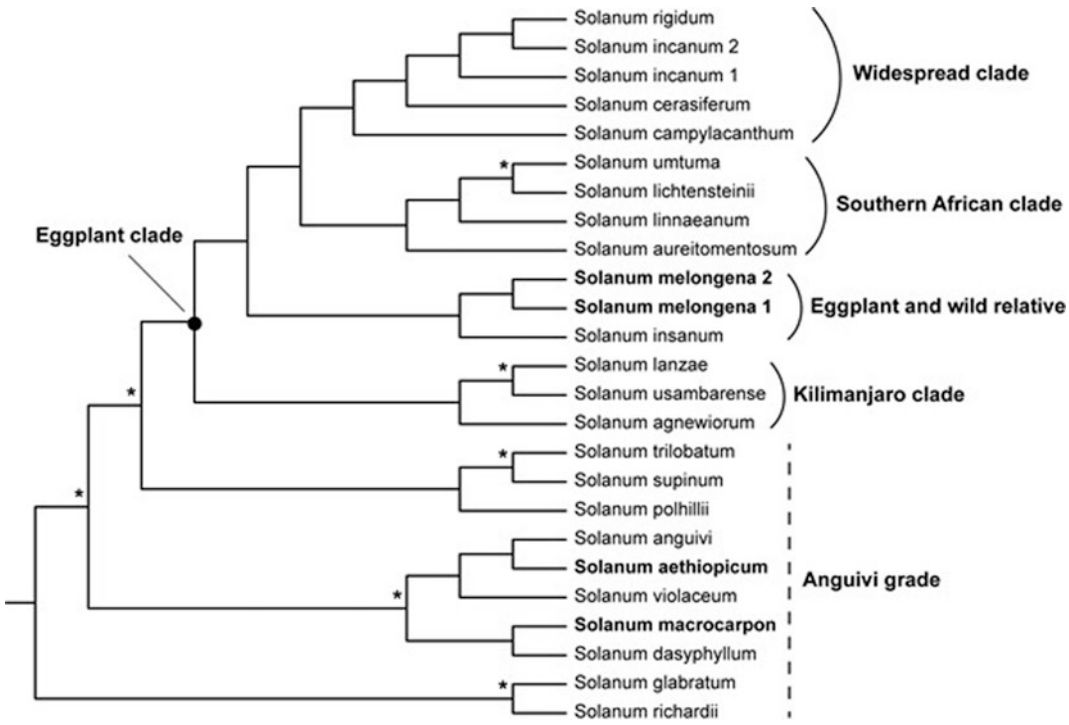

Fig. 12.2 Full plastome phylogeny of the eggplant clade (consensus of 4 BEAST analyses; 159,227 bp matrix). All nodes are well supported except for the nodes designated with *. Names of cultivated species are in bold Modified from Aubriot et al. (2018)

insanum (see also Aubriot et al. 2016; Vorontsova and Knapp 2016; Vorontsova et al. 2013; Fig. 12.2). *Solanum incanum* and *S. insanum* are both used for medicinal purposes (Matu 2008); the former is distributed eastwards as far as Pakistan and the latter westwards as far as Pakistan also, and recent results suggest that the latter is closely related to the former (see below), although this is not borne out in the recently published cpDNA phylogeny (Aubriot et al. 2018).

Solanum insanum has been considered conspecific to *S. melongena* by several authors (Karihaloo and Rai 1995; Lester and Hasan 1991; Lester et al. 1990); however given that the wild and cultivated taxa undergo different selection regimes, their separation as distinct species was proposed (Knapp et al. 2013) and subsequently adopted (e.g. Aubriot et al. 2018; Ranil et al. 2017). At the molecular level, the species are very close (Aubriot et al. 2018; Karihaloo et al. 2002; Mace et al. 1999; Weese and Bohs 2010), and DNA polymorphism is higher in *S. insanum* (Karihaloo et al. 1995; AML Page and MA Chapman, unpublished data). *Solanum insanum* is a morphologically very variable wild or weedy species, widely distributed in fields, disturbed habitats and villages from Pakistan, India and Sri Lanka to the Philippines and Indonesia; it is also found in Madagascar (Deb 1979; Ranil et al. 2017). Comparison between several *S. insanum* accessions of the INRA collection (M-C Daunay, unpublished data) indicates that they are very variable for plant growth habit (from erect to sprawling), prickliness (from zero to very prickly), fruit clustering (from single to several) and fruit size (1–4 cm diameter). All the wildish types have green striped fruits. A more complete survey of the diversity of *S. insanum*, based on 56 accessions (Karihaloo and Rai 1995), demonstrates the range of morphological diversity (Table 12.3).

The historical hypothesis of Lester and Hasan (1991) is that *Solanum insanum* could have developed as a weed in the tropical Asian horticultural situations, derived from *S. incanum*. It would have become progressively adopted as a semi-cultivated taxon, and exposed to progressive selection, first towards primitive types (such as those found in Thailand) and then towards advanced types with large fruits of various shapes and colours (Lester and Hasan 1991).

Vavilov (1935) identified the Indo-Burma region as the area of origin of eggplant. However, he mentioned also the occurrence of small-fruited eggplants in Central and Western China and adjacent areas. Investigations through ancient Chinese literature confirmed China as one centre of domestication and revealed gradual changes towards larger fruit sizes, reduced bitterness and diversification of fruit shapes (Wang et al. 2008). A later synthesis, based on historical, morphological and molecular data, identified several Asian domestication centres, including southern China, India and Malay islands (Meyer et al. 2012b). A comparison of eggplant uses, summing up 77 categories of medicinal attributes mostly specific to each of these three Asian regions (Meyer et al. 2014) reinforced the hypothesis of at least three domestication centres. Comparisons between accessions from different countries, including Sri Lanka and China (Hurtado et al. 2012) and China, India, Indonesia and Indo-China (Cericola et al. 2013), have also shown that the structuring of morphological traits and microsatellite diversity is compatible with the multi-local domestication hypothesis. The partial overlap of these results also suggests diffusion and subsequent gene exchanges between materials originating from the domestication areas. This statement is consistent with human migrations and crops trade in Asia (Meyer et al. 2014).

We (AML Page and MA Chapman, unpublished data) have further examined the amount and partitioning of genetic variation in wild, weedy and domesticated eggplants with broad sampling and using genotyping by sequencing (GBS; Fig. 12.3). Our phylogenetic work backs up the majority of previous work, that is: (1) *Solanum insanum* is the progenitor of cultivated eggplant, (2) *S. incanum* is sister to the group formed by the eggplant and its wild progenitor, and (3) the species previously named *S. ovigerum* appears to correspond to primitive

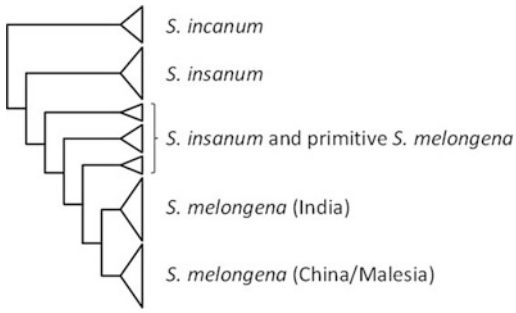

Fig. 12.3 Outline phylogenetic tree of eggplant and its immediate wild relatives based on GBS data (AML Page and MA Chapman, unpublished data)

domesticates. Despite the cultivated accessions being roughly split into an east (China, Malaysia, Indonesia, Thailand) and west (India) gene pool (as found by Meyer et al. 2012b), we found no evidence for multiple domestications of Asian eggplant. Instead, a small number of *S. insanum* accessions appear nested in the domesticated gene pool, suggesting that these are feral escapes, which would be consistent with the earlier hypothesis of Lester and Hasan (1991) and Daunay et al. (2001a, b), that there exist genetically separate wild progenitors and weedy escapes.

We also show extensive contemporary gene flow between wild, weedy and domesticated eggplants, a finding which is backed up by recent field data in India (Davidar et al. 2015; Mutegi et al. 2015). Experimental hybridisations have proved full interfertility between *S. insanum* and *S. melongena* (Lester and Hasan 1991; Plazas et al. 2016). This ongoing gene flow likely complicated earlier analyses of phylogenetic relationships between taxa when small numbers of markers were used. *Solanum insanum* is extremely variable (see above) which may be in part a result of this ongoing gene flow. Gene exchange between wild and cultivated populations is also attested by the various combinations of wild traits (e.g. prickles, deep petal lobing, many flowers per inflorescence, green striped immature fruits, globose shapes) and domesticated ones (e.g. no prickles, shallow petal lobbing, few flowers per inflorescence, immature white and purple fruits, slightly elongated shapes) observable for *S. insanum*. These combinations of wild and domesticated traits allow differentiation between populations of *S. insanum* and even between single individuals.

In India and Thailand, spontaneously occurring eggplants are common in villages' backyards, waste areas and nearby paths and roadsides (M-C Daunay and X Aubriot, pers. observations). Local people distinguish between "wild" and "cultivated" types, although neither are truly wild or cultivated. Their status is closer to semi-domesticates, i.e. spontaneously occurring but submitted to human interference (plants are kept or destroyed). People identify the "wild" type by tasting the fruits, which should be bitter and soft, whereas the "cultivated" type has milder and crispy fruits. Both types share common plant habits (from prostrate to erect, weak or very vigorous), flowers with protruded styles and stigmas, and fruit characteristics (solitary or clustered, small, round, green striped); their prickliness is generally absent or moderate, and their occurrence in the landscape is commonly erratic. A careful observation distinguishes the "wild" from the "cultivated" by their slightly smaller leaves, flowers and fruits, and by the occasional presence of clustered fruits. In the backyards, one also notices escaped hybrid material, presenting domesticated traits, such as white or almost-violet fruits, or oblong ones. All this material cohabits in the villages together with cultivars. And last but not the least, these cultivars, which are bought, sown and planted intentionally in gardens or fields, display a range of variation spreading from "partly wild phenotypes" (small green striped fruits, and non-prickly plants) to advanced phenotypes (large and variously coloured fruits). Asian seed companies' catalogues are very instructive on the diversity of the cultivated material for which F1 hybrids are available in both types. This picture of Tropical Asian eggplants is a good example of the complex relationships existing between wild and cultivated compartments in areas where they coexist.

When looking across the advanced *Solanum melongena* types, one notices also the presence of several wild-like traits in the germplasm. For

instance, roundish green-variegated fruits are common in Asian *S. melongena*, although this fruit colour is typically a wild trait. Similarly, in advanced germplasm, one also finds varieties bearing clustered fruits and some extremely prickly Indian varieties are prized for their special taste. Both of these traits are wild-like traits. These examples, and others, indicate that *S. melongena* domestication does not indicate a clear-cut mono-directional force moving traits from wild states to advanced ones. On the one hand, domestication has retained and bred new traits, in particular elongated and/or large fruits, fruit epidermis glossiness and an additional fruit epidermis pigment (anthocyanins). In addition, fruit colour has also "bloomed" with various pigments combinations (chlorophylls or anthocyanins, or both, or none), pigments intensities (from light to very strong) and hues (from dark green to yellowish green, and all nuances between pink, purple, violet and blackish). Yet on the other hand, *S. melongena* domestication has also retained a number of wild traits, enlarging the range of variation towards important transgressive and opposite states (e.g. from non-prickly to extremely prickly forms).

12.3.4 Ongoing Questions About the Domestication Pathways

The domestication and history of eggplants are beginning to be unravelled, through their botanical, ethnobotanical, morphological and genetical dimensions, but many questions are still pending. For *Solanum macrocarpon* and *S. melongena*, the coexistence in their respective areas of origin with interfertile wild material and more or less advanced varieties leads to a morphological and genetic continuum of diversity, a consequence of millennia of human and natural selection pressures and genetic exchanges. The intermediate material, variously described as semi-wild, semi-domesticated, or pre-domesticated, may be discarded, ignored or sometimes cared for by humans. Are such plants survivors of intermediate steps of domestication process? Does their presence indicate the places where domestication occurred? Do they indicate that the domestication process is not fully complete? Could they help in deciphering the modes and timing of domestication?

For the three eggplants, gene flow between wild and cultivated populations is ongoing (in particular for *Solanum macrocarpon*) but their direction needs clarification. Based on the available clues, one can expect that they work mostly from the cultivated to the wild genepools in the case of *S. melongena*, whereas reciprocal gene flow seems likely for *S. macrocarpon*. Eggplants are therefore material of special interest for comparative domestication processes. The consequences of this are of interest to evolutionary biologists, but have applied consequences too, for example pertaining to the release of GM eggplants.

12.4 Domestication Genetics and Genomics of Eggplant

Domestication is often accompanied by a reduction of the genetic diversity (assessed with molecular markers) within the cultivated taxon, compared to the wild (Smykal et al. 2018). This statement looks at first as paradoxical since domesticated plants are characterised by large phenotypic variation. Available information on eggplants points out indeed such a reduction of genetic diversity; *S. melongena* displays indeed a reduced molecular diversity when compared to *S. insanum* and/or *S. incanum* for cpDNA (Sakata and Lester 1994), RAPDs (Karihaloo et al. 1995; Singh et al. 2006), SSRs (Mutegi et al. 2015; Tümbilen et al. 2011) and genome-wide SNPs (AML Page and MA Chapman, unpublished data). The latter work suggests a reduction of genetic diversity of about 50% during *S. melongena* domestication; however, this is almost completely unknown for the two African eggplants. A better quantification and characterisation of the allelic loss between cultivated and wild taxa is needed, as well as the assessment of possible genetic diversity differences between geographical areas. This latter approach has been

initiated for *S. melongena* (Cericola et al. 2013; Hurtado et al. 2012; Naegele et al. 2014; Vilanova et al. 2012).

Domestication of eggplants has targeted a small number of traits, in particular fruit shape, size, colour and taste, plant prickliness and hairiness (see also above). Seed dormancy, although poorly documented, seems to be another trait having undergone human selection, since this trait is rare in *S. melongena* (Yogeesha et al. 2006), although it is common in wild-related *Solanum* species (Daunay et al. 1999). Most traits affected by domestication are common to the three eggplants, and as far as known, they are most often monogenetically inherited. Genetic mapping has confirmed (for *S. melongena*) the involvement of few loci with major effects controlling these domestication traits (Doganlar et al. 2002); the number and genetic map locations of which, have been refined in more recent studies (Barchi et al. 2012; Frary et al. 2014; Toppino et al. 2016).

Another feature of eggplants domestication is the general dominance of wild traits over cultivated ones. That is the case of prickliness (dominant over the absence of prickles) and hairiness (dominant over the absence of hairs). Interestingly, when these wild traits are present, there is an additional quantitative variation under polygenetic control. Some fruit epidermis colour pattern genes are also dominant (Daunay et al. 2004), for example, the presence and distribution of chlorophylls. Fruit size genetics is more difficult to study; the fruit size of interspecific hybrids is generally close to the wild type, likely through the combined effect of partial dominance of small over large fruits, and the absence or low quantity of seeds due to the frequent sterility of interspecific hybrids resulting in a smaller fruit. Crosses within eggplant germplasm (which do not suffer from sterility) indicate co-dominance of the alleles coding for fruit size and shape (M-C Daunay, pers. observations). Seed dormancy (a wild trait), although poorly documented, is inherited in monogenic and dominant fashion in *S. melongena* (Padmini et al. 2008); however, crosses between *S. aethiopicum* Kumba group cultivars indicate recessive control (Seck and Sow 1994). For many other traits, less clearly affected by domestication, the phenotypes of interspecific hybrids also advocate for a generally dominant inheritance of wild traits.

Dominance of wild traits over cultivated ones, as identified in eggplants, is also observed in other domesticated crops and animals. Together with the observation of a resurgence of atavistic traits when crossing domesticated eggplants, this has led to the suggestion that domestication might be generally accounted for by a loss of genetic function or regulation, instead of by an addition of new variability (Lester 1989; Lester and Daunay 2003). R.N. Lester suggested that human selection of non-functional alleles or gene products would lead to a reduced control of wildlife-adapted metabolism and morphogenesis pathways and that this loss of control could explain the very rapid evolution of crop plants over a mere few millenaries. One can expect that gene sequencing and transcriptomics will bring further insights into this hypothesis, and data from transcriptomics analyses suggests that the majority of differences in gene expression between *S. melongena* and *S. insanum* are conditioned by loss, rather than gain, of expression (AML Page and MA Chapman, unpublished data).

While several genes related to agronomic phenotypes have been cloned in tomato (e.g. Chakrabarti et al. 2013; Cong et al. 2008; Frary et al. 2000; Liu et al. 2002; Xiao et al. 2008), there have been no such successes in eggplant. As mentioned in the Introduction, the worldwide economic importance of eggplant is significantly less than tomato, although locally, especially in the Mediterranean, South and South-East Asia, eggplant makes up a vital portion of the diet. Overall, this has meant that investment in eggplant research, especially the development of a reference genome sequence, has lagged behind other species.

Nevertheless, some progress is being made to identify candidate genes underlying domestication traits. As mentioned in previous chapters, there has been a sizeable amount of data gathered concerning quantitative trait loci (QTL) governing various domestication traits, especially

focussing on fruit traits, but also the presence/absence of prickles, anthocyanin content and pest resistance (Doganlar et al. 2002; Portis et al. 2014; Salgon et al. 2017). To understand the genes responsible for these economically important phenotypes, the locations of eggplant QTL have been compared to orthologous regions of the tomato genome. In some cases, eggplant QTL overlap with known domestication genes from tomato. For example, fruit weight and shape QTL on eggplant linkage groups (LGs) 2, 3, 7, 8 and 12 (Portis et al. 2014) and anthocyanin QTL on LGs 5 and 10 (Barchi et al. 2012) overlap with cloned genes or candidates for the same traits in tomato.

Sequencing the eggplant genome will improve our understanding of the genetic basis of domestication phenotypes. In addition, several eggplant wild relatives contain potentially adaptive alleles, for example salt tolerance (*S. linnaeanum*; see Daunay et al. 1991) and drought tolerance (*S. incanum*; see Daunay 2008). With a complete published eggplant genome, the time taken to move from phenotypes and QTL, which are already available in some cases, to candidate genes will be considerably shorter. For example, recent efforts to create and phenotype introgression lines (*S. incanum* in the background of *S. melongena*) are underway (Gramazio et al. 2017) and could yield important genes for traits such as fruit size and shape, drought tolerance and pathogen resistance. The parallel domestication of the three eggplant species for similar uses and phenotypes sets the stage for comparative analyses of the domestication process.

As the genomic revolution gains momentum, the pace of research into the genetics and breeding of eggplant and other non-model species will increase with it, bringing a new generation of crops with greater nutrition, yield and resilience.

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Conclusions and Future Directions 13

Mark A. Chapman

Abstract

In this summary, I bring together the work detailed in this volume, highlight the research directions currently being traversed and combine this with examples from other Solanaceous crops to suggest where the future of eggplant research could take us. The sequencing of the eggplant genome, which is likely to be published publicly in the next few months, will set the stage for more detailed, targeted research into all of the topics covered in this volume. It will advance our knowledge of crop domestication in general, but the parallels between eggplant and tomato domestication provide a clear pathway for the investigation of parallel domestication.

13.1 Summary of Current Eggplant Work

As mentioned in earlier chapters, research into eggplant has lagged behind that of other crops in the Solanaceae. This probably stems from the global economic importance of eggplant being a fraction of that of tomato or potato (Chap. 1), and consequently, research priorities have focussed on the latter two species. QTL and linkage maps were being produced for tomato and potato in the 1980s and early 1990s (Bernatzky and Tanksley 1986; Gebhardt et al. 1991; Helentjaris et al. 1986), whereas for eggplant, this was not carried out until the early 2000s (Doganlar et al. 2002a; Nunome et al. 2001; see Chap. 5). A search of Web of Science (http://wok.mimas.ac.uk; accessed August 2018) highlights this disparity, with an order of magnitude greater number of publications using tomato or potato versus eggplant. For example, searching for the keywords "eggplant and (QTL* or linkage map*)" results in 70 publications, whereas the equivalent for tomato and potato return 1771 and 675. Similarly for "eggplant and (candidate gene*)", there are 36 publications, whereas for tomato and potato, there are 969 and 539.

The importance of a genome sequence in stimulating research is highlighted by the number of citations of the tomato genome publication (Sato et al. 2012, >1200 citations) and the potato genome publication (Xu et al. 2011, >700 citations). With the publication of the tomato genome, recent work in tomato has been able to analyse fruit ripening (Zhong et al. 2013), stress tolerance (Bolger et al. 2014) and fruit colour variation (Zhu et al. 2014). All of these are potential traits for crop improvement in eggplant, but the genetic basis in eggplant has yet to be resolved.

The situation, however, is not so bleak when one considers current research activities. At the

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present stage, there is much information from investigations employing a range of approaches, all of which could provide the basis for follow-up genomic investigations and the identification of causal genetic polymorphisms. QTL studies in eggplant have been employed extensively and have identified regions of the genome corresponding to traits of ecological, evolutionary and economic importance. Key traits have been fruit shape and size (Doganlar et al. 2002b; Nunome et al. 2001), fruit colouration (Barchi et al. 2012), pathogen resistance (Salgon et al. 2017) and yield (Portis et al. 2014). Genome-wide association studies (GWAS) have also identified candidate genomic regions for similar traits (e.g. Cericola et al. 2014; Ge et al. 2013; Portis et al. 2015). Utilising high-throughput RNA sequencing (RNA-Seq), candidate genes underlying anthocyanin accumulation (Li et al. 2017, 2018) and disease resistance (Na et al. 2016) have been identified. QTL and GWAS investigations are often a prerequisite for finding causal genes, and a genome sequence expedites gene discovery. In tomato, domestication genes underlying fruit shape and size have been cloned (Chakrabarti et al. 2013; Frary et al. 2000; Xiao et al. 2008), and all relied on initial QTL mapping studies to identify the genomic regions to fine map. This means that once the genome is available, we may see a surge of papers identifying causal genes for a range of phenotypes in eggplant.

13.2 Pathways for Future Research

As alluded to above, we have information on the genetic basis of a range of adaptive traits in eggplant, which could provide the basis for genome-scale investigations and the identification of individual genes responsible for said traits. Even without identifying the specific causal genetic changes, being able to pinpoint small regions of the genome conferring the trait means that molecular breeding approaches, for example, marker-assisted selection (MAS; Collard and Mackill 2008), can be employed to introgress the trait between accessions or between species. In MAS, markers flanking the locus of interest are identified and used to genotype a backcross population such that only the progeny with the required genotypic combinations are retained. To introgress a trait between accessions, the only progeny retained are those containing markers flanking the locus of interest from the donor parent, but few other markers from the donor. This speeds up and reduces the cost of growing and phenotyping large numbers of progeny. The benefits of this type of study are clear as they will aid in crop improvement in eggplant.

Further, if the causative genetic changes can be pinpointed, then it is possible that what is learned in eggplant can be transferred across to other species. Below I highlight two avenues of future research that will be aided by the sequencing of the eggplant genome.

13.2.1 Eggplant Improvement Through Introgression from Wild Species

Using wild relatives as donors of adaptive traits has been successful in many crops (reviewed in Dempewolf et al. 2017) and has been discussed in eggplant (Daunay et al. 1991). However, little research has been carried out until recently on this topic, which is unusual given that one of the first and most well-studied eggplant mapping populations is derived from an interspecific cross (*S. linnaeanum* × *S. melongena*; Doganlar et al. 2002a, 2014). With the availability of a genome sequence, MAS can be used to introgress traits from wild relatives into the eggplant.

A range of wild eggplant species have adaptive traits that may be of use in the crop. In particular, both *S. insanum*, the wild progenitor, and *S. incanum*, a closely related wild taxon (see Chap. 12 and references therein), are known to exhibit greater drought tolerance than *S. melongena* (Daunay 2008; Ranil et al. 2017). With climate change, we are set to see an increasing frequency and magnitude of heatwaves and droughts (Lobell et al. 2008); for crops to survive and continue to thrive, we should be looking to

improve drought tolerance (Deikman et al. 2012). It could be that wild relatives hold adaptive alleles for these traits.

In addition, crop protection from pests and pathogens is another target for crop improvement, and pest and pathogen dynamics may well also be affected by climate change (Bebber et al. 2013). Verticillium wilt is one of the most damaging fungal pests of eggplant, yet several relatives of eggplant show resistance to the causative fungus (Collonnier et al. 2001). Only one of these wild relatives known to show resistance is closely related enough for crosses to be made (*S. linnaeanum*), and this has been suggested as a suitable donor of Verticillium resistance (Liu et al. 2015). This also highlights that an understanding of the genetic relationships between taxa can be important when identifying wild donors because not all wild species might be cross-compatible with the crop.

Another potential target for introgression into eggplant from wild species relates to putative medicinal properties of wild relatives. A range of health-promoting compounds, including phenolics, are present in eggplant and its wild relatives, and eggplants and the wild relatives are often consumed for health benefits (Meyer et al. 2014). In particular, the wild progenitor of eggplant, *S. insanum*, is consumed in South and South East Asia for a range of perceived benefits (Meyer et al. 2014; Ranil et al. 2017), plus it has a greater phenolic content than the domesticated eggplant (Meyer et al. 2015). Other species, for example, *S. incanum*, have phenolics present in the fruit that are absent from domesticated eggplant (Ma et al. 2011).

Recently, extensive work crossing eggplants with wild relatives has been carried out, revealing much about crossability and heterosis (Kaushik et al. 2016; Kouassi et al. 2016) and an introgression line (IL) population using *S. incanum* as the genome donor is well-developed (Gramazio et al. 2017). These resources provide extensive information and germplasm that will be of interest and use for breeding. The ILs developed for tomato in the 1990s (Eshed et al. 1992; Eshed and Zamir 1995) have been used extensively to identify QTL and, coupled with the availability of a genome sequence, the genetic basis of dozens of traits (reviewed in Causse and Grandillo 2016).

It is important to note that some eggplant wild relatives are poorly represented in genebanks; two of the three species named above as being useful for crop improvement through introgression (*S. linnaeanum* and *S. insanum*) were listed as "high-priority species" based on gap analysis and a disparity between natural occurrence and the number of collections available in genebanks (Syfert et al. 2016).

Finally, it would not be prudent to ignore recent advances in genome editing techniques, as an alternative to traditional transgenic modification (Feng et al. 2013). CRISPR genome editing allows targeted gene modification without inserting transgenes into the genome; any inserted material is essentially degraded. In eggplant, a search of Web of Science (http://wok.mimas.ac.uk; accessed August 2018; keywords "eggplant and CRISPR"), however, reveals no published work using CRISPR in our focal species. The equivalent search for tomato and potato, however, reveals 80 and 35 hits, respectively (acknowledging that some of these are not direct reports, only reviews). In tomato, genome editing has been used to modify fungal tolerance (Nekrasov et al. 2017) and fruit ripening (Ito et al. 2015), traits which would be of interest to investigate and potentially manipulate in eggplant. Without the genome sequence, however, it would be almost impossible to design the required guide RNAs for CRISPR whilst reducing the off-target effects which need to be eliminated as far as possible.

13.2.2 Ecological and Evolutionary Research

The majority of this volume considers the agronomic attributes of eggplant, and the ways in which a genome sequence can aid in understanding these economic aspects of eggplant biology. However, eggplant and its wild relatives

have been shown to possess other attributes making this group interesting for the study of general ecological and evolutionary phenomena.

Firstly, the contribution of phenotypic plasticity to evolution is a contentious topic, and some of the first work to document the role and consequences of plasticity in plants was carried out in (distant) wild relatives of eggplant (Diggle 1994; Miller and Diggle 2003). More recently, QTL analyses carried out across multiple locations have revealed that a number of traits are plastic and dependent on the environment. For example, some yield-related QTLs identified in a cross between two eggplant cultivars were present at two sites, but others only at one site (Portis et al. 2014), and an analysis of the same population for fruit phenolics revealed a similar story (Toppino et al. 2016). Further, an analysis of eggplant and other crops revealed "bimodality" in the plasticity of agronomic traits, with some traits plastic and others fixed (Fisher et al. 2017).

Another major question in evolutionary research is the role of parallel and convergent evolution. Under this scenario, a trait evolves more than once; in some instances, this could be due to similar changes in the same genes, whereas in others it could result from completely different genetic mutations (Elmer and Meyer 2011). In grass crops, independent mutations in the same gene have given rise to the non-shattering phenotype in sorghum, rice and maize (Lin et al. 2012), backing up work based on co-location of QTLs for equivalent traits across these crops (Paterson et al. 1995). In eggplant, 40% of the QTL involved in fruit size, shape and colour overlap with those found in at least one other Solanaceous crop (tomato, potato and/or pepper (Doganlar et al. 2002b), suggesting that to some degree parallel genetic changes could underlie these domestication phenotypes. Preliminary work has shown some potential overlaps between eggplant QTL and tomato genes involved in analogous traits (e.g. fruit colour and yield; Barchi et al. 2012; Portis et al. 2014), but the absence of an available eggplant genome has hampered further validation of these candidate genes.

Finally, the evolution of feral weeds from domesticated species has both evolutionary and economic consequences (Gressel 2005). Weeds can arise from crops through the breakdown of domestication traits (which would otherwise be selected against in the wild) or through the introgression of wild and weedy traits from local wild species. Early taxonomic work in eggplant identified differences between wild and weedy eggplants and described them as two taxa (*S. melongena* groups E and F; Lester and Hasan 1991); however, more recent work has suggested these to be the same taxon (Knapp et al. 2013; Meyer et al. 2012). Our unpublished work using genotyping-by-sequencing (see Chap. 12) suggests an intermediate story whereby some of the "group E" individuals are genetically similar to the "group F" eggplants; however, others designated group E appear to be phylogenetically nested within the domesticated eggplants with evidence of admixture. These latter samples exhibit wild-like traits (smaller fruit, spreading habit and often spines on the leaves), but their genetic ancestry indicates they are derived from domesticated eggplant. Thus, in eggplant, feral weeds have evolved from the crop, representing an exciting natural scenario to investigate how weeds evolve. Their admixed ancestry suggests gene flow with true wild eggplant, but whether this is neutral or adaptive (i.e. whether the introgressed loci control wild-like traits) has yet to be examined. It is noteworthy that feral chickens on the Hawaiian island of Kauai appear to have evolved from domesticated ones through admixture with wild red junglefowl; however, the genomic regions controlling feralisation and domestication are largely non-overlapping (Johnsson et al. 2016). Gene flow between wild and crop eggplants in India has been identified recently (Davidar et al. 2015), further suggesting that ongoing gene flow may be affecting both wild and crop eggplant.

13.3 Conclusions

The absence of a genome sequence can hamper the fine-scale analysis and identification of causative genes underlying adaptive traits, yet in eggplant much research has been completed so far without a genome sequence. We, as eggplant researchers, are at the stage where the availability of a reference genome is just around the corner and the information we have gathered so far positions us to very soon be carrying out genome-scale investigations and identifying individual genes which confer traits of interest. Research so far, without a reference genome, demonstrates that eggplant is not only worth studying as a crop, and therefore, because of only its economic value, but it is also an excellent model to study ecological and evolutionary processes.

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