Compendium of Plant Genomes
Series Editor: Chittaranjan Kole

Sudhakar Pandey
Yiqun Weng
Tusar Kanti Behera
Kailiang Bo *Editors*

The Cucumber 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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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 both 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 plants. 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 the 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 the 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, the 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, 8 crop and model plants, 8 model plants, 15 crop progenitors and relatives, and 3 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, particularly Dr. Christina Eckey and Dr. Jutta Lindenborn for the earlier set of volumes and presently Ing. Zuzana Bernhart for all their timely help and support.

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

Foreword

Cucumber (*Cucumis sativus*) is an important vegetable crop of both new and old world. Compared to field crops, the genetics and genomic resources in cucumber are limited. Recent advances in Next-Generation Sequencing (NGS) tools and analytics are providing exciting opportunities to expedite genomics-assisted cucumber breeding. Relatively small genome size, low percentage of repetitive DNA, and short breeding cycle are some of the advantages associated with cucumber. The first draft genome sequence of cucumber was released in the year 2009, and since then, significant progress has been made in our understanding about the cucumber genome.

This book entitled *The Cucumber Genome* presents a comprehensive picture describing botany, genetic resources and diversity, genetics and traditional breeding, tissue culture and genetic transformation, mapping of useful genes/QTLs associated with quality traits, biotic and abiotic stress resistance, and genomics of this important crop.

This book is expected to be useful to the students, teachers, and researchers in the academia and relevant private industries interested in various aspects including genetics, breeding, pathology, entomology, physiology, transgenics, molecular genetics, and genomics of this crop.

I appreciate the efforts and congratulate the authors, Drs. Sudhakar Pandey, Yiqun Weng, Tusar Kanti Behera, and Kailiang Bo, in bringing out this book collating the present status of cucumber breeding and genomics which will hopefully catalyze further research and facilitate promotion and utilization of the crop.

New Delhi, India
December 2020

T. Mohapatra

Preface

Cucumber is most precious vegetable crop grown for its immature fruits worldwide. Among vegetables, after tomato and watermelon, cucumber is cultivated more broadly than any other vegetable and also very popular in kitchen garden and protected condition. Most of the cucumber improvement has been successfully implemented using conventional breeding, which has some of the unavoidable limitations like huge time and resource consumption and therefore, alternative approaches are always sought by researches in order to fasten the crop improvement programs. However, important traits like biotic and abiotic stress required more time for classical breeding necessitates the application of modern biotechnological tools for cucumber improvement. Many new cultivars were developed by using modern breeding practices like marker-assisted selection, which have enhanced the speed and efficiency of breeding programme. Modern biotechnological tools such as transgenic and genome editing has revolutionized the crop improvement programs presently due to its precious target. After release of first cucumber draft genomes, considerable advancement has been made in molecular mapping and cloning of important genes and quantitative trait loci (QTLs), which are helping for marker-assisted selection in cucumber breeding.

The Cucumber Genome book is a part of Compendium of Plant Genomes is a first book on cucumber compiled the comprehensive information on botany, genetics, genome draft, QTL mapping for abiotic stress, disease resistance, quality, plant architecture and fruit traits; classical breeding, transformation, gene editing, genome evaluation and other biotechnological interventions. The students, teachers, scientists, academicians, as well as seed companies and pharmaceutical industries can find cucumber current achievements related to cucumber genome and future direction of its use in advance research at one place.

The editors are grateful to the authors for providing authoritative and updated account in the knowledge of their interest. Our thanks and gratitude goes to all of them and all the co-authors for their useful contributions.

Dr. Sudhakar Pandey, Dr. Yiquen Weng, Dr. T. K. Behera, and Dr. Kailiang Bo express their thanks and high gratitude to Prof. Chittaranjan Kole Series Editor of the “Compendium of Plant Genome” for giving the opportunity to edit this book and for his constant support and guidance right from the inception till publication of this book *The Cucumber Genome*.

We also wish to mention here our thanks and gratitude to the Springer staff, particularly Mr. Prashanth Ravichandran for their timely help and support in achieving this volume.

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Abbreviations

%	Percent
µM	Micrometre
^{0}C	Degree centigrade
6-BA	6-benzylaminopurine
ABA	Abscisic Acid
ACC	1-Aminocyclopropane-1-Carboxylic Acid
ACK	Ancestral Cucurbitaceae Karyotype
ACO	1-Aminocyclopropane-1-Carboxylic Acid Oxidase
ACS	1-Aminocyclopropane-1-Carboxylic Acid Synthase
AFLP	Amplified Fragment Length Polymorphism
ALS	Angular Leaf Spot
AR	Adventitious Root
AR	Anthracnose
ARF	Adventitious Root Formation
ATT	Aspartate Aminotransferase
BC	Backcross
BR	Brassinosteroid
BSA	Bulked Segregant Analysis
Bt	*Bacillus thuringiensis*
CCYV	Cucurbit Chlorotic Yellows Virus
CFMMV	Cucumber Fruit Mottle Mosaic Tobamovirus
CGC	Cucurbit Genetics Cooperative
Chr	Chromosome
cm	Centimeter
cM	Centimorgan
CMV	Cucumber Mosaic Virus
CNV	Copy Number Variant
CRISPR/Cas9	Clustered Regularly Interspaced Short Palindromic Repeats/Crispr-Associated Protein 9
CYSDV	Cucurbit Yellow Stunting Disorder Virus
DM	Downy Mildew
ELISA	Enzyme-Linked Immuno Sorbent Assay
EMS	Ethyl Methane Sulfonate
ERF	Ethylene Response Factor
EST	Expressed Sequence Tag
EST-SSR	Expressed Sequence Tag-Simple Sequence Repeat

ETR	Ethylene Receptor
FAO	Food and Agriculture Organization
FISH	Fluorescence in Situ Hybridization
FW	*Fusarium* Wilt
g	Gram
GA	Gibberellins
GISH	Genomic *in situ* Hybridization
GM	Genetically Modified
GSB	Gummy Stem Blight
GST	Glutathione S-Transferase
GWAS	Genome-Wide Association Study
HPLC	High-Performance Liquid Chromatography
HR	Homologous Recombination
IAA	Indole-3-Acetic Acid
IAN	Indole-3-Acetonitrile
K Cal.	Kilocalorie
KT	Kinetin
LD	Linkage Disequilibrium
LOD	Logarithm of Odds
LRR	Leucine-Rich Repeats
LTG	Low-Temperature Germination
LTRs	Long Terminal Retrotransposons
MALDI – ToF	Matrix-Assisted Laser Desorption Ionization-Time of Flight
MAPK	Mitogen-Activated Protein Kinase
MAS	Marker-Assisted Selection
Mb	Megabases
Mbp	Megabase Pair
MCS	Multiple Cloning Sites
MDH	Malate Dehydrogenase
mg	Milligram
mg/kg	Milligram per kilogram
mg/l	Milligrams per liter
mm	Millimeters
mm^2	Square millimeter
MS	Murashige and Skoog
MYA	Million Years Ago
MYSV	Melon Yellow Spot Virus
NAA	1-Naphthaleneacetic Acid
NBS	Nucleotide-Binding Site
NHEJ	Non-Homologous End Joining
NSG	Next-Generation Sequencing
OD	Optical Density
ORF	Open Reading Frame
PAP	Protein Pokeweed Antiviral
PCD	Programmed Cell Death
PM	Powdery Mildew

PPR	Pentatricopeptide Repeat
PPT	Phosphinothricin
PRSV	Papaya Ringspot Virus
PVE	Phenotypic Variation Explained
QTLs	Quantitative Trait Loci
RAM	Root Apical Meristem
RAPD	Random Amplified Polymorphic DNA
RGA	Resistant Gene Analogues
Ri	Root-inducing
RIL	Recombinant Inbred Lines
RLN	Relative Leaf Numbers
ROS	Reactive Oxygen Species
RWC	Relative Water Content
SAM	S-adenosylmethionine
SAR	Systematic Acquired Resistance
SCAR	Sequence Characterized Amplified Region
SDWw	Shoot Dry Weight
SLAF-seq	Specific Length Amplified Fragment Sequencing
SMRT	Single Molecule, Real-Time
SNP	Single-Nucleotide Polymorphisms
SOD	Superoxide Dismutase
SSAP	Sequence-Specific Amplification Polymorphism
SSR	Simple Sequence Repeat
SU	Survival Rate
TALENs	Transcription Activator Like Effector Nucleases
TF	Transcription Factor
Ti	Tumour
TLS	Target Leaf Spot
TOL	Tolerance Score
ToLCNDV	Tomato Leaf Curl New Delhi Virus
TSP	Tonoplast Sugar Transporters
UDT	Uridine Diphosphate Glucuronosyl Transferase
USDA	United States Department of Agriculture
Vir	Virulence
VLHw	Waterlogged Vine Length
WGD	Whole Genome Duplication
WGS	Whole Genome Sequences
WMV	Watermelon Mosaic Virus
ZFNs	Zinc Finger Nuclease
ZT	Zeatin
ZYMV	Zucchini Yellow Mosaic Virus

Importance, Distribution, Botany and Genetics

1

Sudhakar Pandey and Shubhra Natasha Kujur

Abstract

Cucumber (*Cucumis sativus* L.) is one of the important vegetable crop indigenous to India grown for its immature fruits eaten raw as salad and also can be cooked as vegetable or processed. Demand of fresh market cucumber is very high due to consumption preference as compared to pickling cucumber. Monoecious or gynoecious are dominant sex forms in cucumber, whereas androecious, andromonoecious hermaphroditic and tri-monoecious forms are also found. Five of the cucumber's seven chromosomes arose from fusions of ten ancestral chromosomes after divergence from *Cucumis melo*. The morphological diversity is mainly exhibits in fruit colour, sex type and plant growth habit. Several new genes (73) has been listed in new gene list includes major-effect QTLs bringing total number of genes to 199. Knowing the gene action of qualitative and quantitative traits governed by QTLs and single gene is very important for the improvement of the cucumber. The present article attempts to provide comprehensive information on significance, origin, botany and genetics of cucumber for further research.

1.1 Introduction

Cucumber belongs to family Cucurbitaceae family having 825 species of 118 genera. In total production of vegetable in India, cucurbits contribute about 5.6% and cultivated in 76.6 thousand hectare area and production is about 9516 thousand tons (FAO 2018). Worldwide area under cucumber and Gherkins is 198000 ha with an annual production of 75,219,000 tons (FAO 2018). In India, total cultivated area under cucumber and Gherkins is 31 thousand hectare with an annual production of 195 thousand tons (FAO 2018). Among the cucurbits, cucumber (*Cucumis sativus* L.) is precious vegetable crop grown for its immature fruits. It is commonly grown under open field as well as protected conditions and now available round the year for fresh consumption as well as export. After watermelon, cucumber is cultivated more widely than any other cucurbit crops. Usually, cucumber are eaten raw as salad, but it can also be eaten cooked as vegetable. Pickling cucumber is used as pickled. Cucumber is also beneficial in preventing constipation, jaundice and indigestion. Cucumber is a good source of valuable nutrients, i.e. vitamin C (2 mg/100 g), iron (1.5 mg/100 g) and also contains 0.4% protien and 2.5% carbohydrate.

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1.2 Uses

Cucumber is highly useful in both high and low blood pressure alleviation because of its high content of potassium (50–80 mg/100 g) (Kashif et al. 2008). This fruit is rich in polyphenolics and cucurbitacins, which have multiple medicinal properties such as anti-carcinogenic, antioxidant, anti-elastase, anti-hyaluronidase, anti-inflammatory, diuretic, anti-hyperglycemic, amylolytic, antimicrobial and analgesic effects (Uthapala et al. 2020). Besides, cucumber is extensively used in the beauty products worldwide which include soap, face cream, shampoo, etc. Cucumber seeds are used in a variety of ways for human consumption and also for oil extraction.

1.3 Cultivar Group Based on Market Segment

Based on the market segment, cucumber is classified into two groups, i.e. slicing cucumber (for fresh consumption) and pickling cucumber (for processing purpose). Pickling cucumber is generally smaller in size in relation to length and diameter, has a light green rind colour with prominent tubercles at the immature stage. Slicing cucumber is used mostly for fresh consumption. The fruit colour of processing cucumber is creamy, light green with white tinge and dark green. The consumer preference for colour of fruit varies from region to region. Slicing as well as pickling cucumber has one more segment called parthenocarpy primarily grown under the protected structure. The development of fruit with or without pollination but without fertilization is called parthenocarpy. The parthenocarpy cucumber lines also linked with gynoecious gene, bears only female flowers. Lietzow et al. (2016) detected seven QTLs related to the parthenocarpy fruit set. The parthenocarpic cucumber varieties/hybrids are now grown commercially due to more yield and better quality.

1.4 Origin and Distribution

The cucumber (*Cucumis sativus* L.) has been domesticated from its progenitor *Cucumis sativus* var. *hardwickii* (Qi et al. 2013) about 3,000 years ago in India and spread quickly for cultivation in south and east Himalayas. The primary centre of origin of cucumber is India (Whitaker and Davis 1962; Jeffrey 1980; de Candolle 1886; Robinson and Decker-Walters 1997). China is considered as secondary diversity centre as it spread to China ~2000 years ago and to European countries 700–1500 years ago (Keng 1974; Paris et al. 2012). In the second century, Romans brought cucumber to Greece and Italy. The cucumber introduced to Haiti in 1494 by Spanish, and in Montreal, Canada by Cartier, in Florida, U.S., by Desoto, and in Virginia, U.S., by Amidas and Barlow in 1535, 1539 and 1584, respectively (Whitaker and Davis 1962; Robinson and Decker-Walters 1997). Swiader et al. (1992) confirmed based on record that cucumber cultivation in France and England started in the ninth and fourteenth centuries, respectively.

1.5 Crossability and Domestication

The *Cucumis sativus* has four botanical varieties, which are cross compatible, i.e. *Cucumis sativus* var. *hardwickii* (wild cucumber), *Cucumis sativus* var. *xishuangbannesis* (semi-wild Xishuangbanna cucumber), *Cucumis sativus* var. *sikkimensis* (Sikkim cucumber) and *Cucumis sativus* var. *sativus* (cultivated cucumber). Among these botanical varieties, *C. sativus* var. *hardwickii* possesses a multiple branching and fruiting habit (Horst and Lower 1978) and have considerable variability in *C. sativus* germplasm (Dijkhuizen et al. 1996), thus has better prospect for generating genetic variation in cultivated cucumber (Staub et al. 1992).

The genus *Cucumis* has about 66 species, and only chromosome in cucumber is $2n = 2x = 14$. While its sister species, *C. hystrix*, have

2n = 2x = 24 chromosomes. The wild species *C. hystrix* is found only in the Yunnan Province of Southern China which has distinctive genetic attributes that make its taxonomic determination complex (Chen et al. 1995; 1997a and b). Through dysploid chromosome reduction, cucumber evolved from its extinct 2n = 24 ancestor, in which several chromosome reorganization process includes inversions, fusions and translocations takes place with the exclusion of cucumber chromosome 7, which remained largely uninterrupted during the evolution of *Cucumis* species (Weng 2021). Other distinct species of *Cucumis* is *Cucumis melo* which include annual and perennial species with chromosome number 2*n* = 24 (Kirkbride 1993).

The relationship among the *Cucumis* species has been worked out based on morphology, crossability and protein analysis (Deakin et al. 1971; Staub et al. 1987 and 1992; Perl-Treves and Galun 1985) to know the biosystematics and phylogeny which have been mainly established by nuclear DNA analysis (Jobst et al. 1998; Zhuang et al. 2004). Garcia-Mas et al. (2004) demonstrated the phylogenetic relationships among *Cucumis* species utilizing ribosomal internal transcribed spacer sequences and SSR markers. Earlier report obtained using isozyme and restriction fragment markers (Staub et al. 1992; Perl-Treves and Galun 1985; Jobst et al. 1998) to study the genetic relationships did not agree with the previous findings. It is believed that wild species of *Cucumis* has originated from Africa. On the other hand, Southern Asia was primary sites of domestication for cucumber and germplasm of same origin have been utilized for transfer of several traits in breeding programme (Dane et al. 1980; McCreight et al. 1993; Staub et al. 1999).

1.6 Botany

(1) Habit: It is annual in nature and viny in growth. Plants are climbing herb and can be trail through support.

(2) Root: Plant has strong tap root system penetrate in the soil up to 50–100 cm. Secondary root system is profusely branched having superficial growth in the upper 50 cm of the soil.

(3) Stem: The stem is herbaceous, hairy, angled and stout. Plant has primary as well as secondary branches with simple tendril. The internodal length depends upon the growth habit of the plant. Some plant has small internodal length due to compact growth habit.

(4) Leaf: Leaves are simple with deeply cordate base and acuminate apex. The shape is triangular, ovate has 7–20 cm length. The petiole length is 5–15 cm and unbranched tendril developed at every leaf axil.

(5) Inflorescence: Solitary, female flowers born in leaf axil and male with large peduncle.

(6) Flower: Cucumber was originally monoecious, subsequently gynoecious and andromonoecious cultivar bred. Other sex forms such as androecious, hermaphroditic and tri-monoecious are also reported. In monocious, male and female flowers are born at different nodes on same plant. The number of male flowers are much high as compare to female flowers in monoecious plant. The gynoecious plants beard only pisttilate flowers at every node.

(7) Calyx and Corolla: Sepals are five lobed and five partite petals, yellow, round, 3–4 cm in diameter fused at the base.

(8) Staminate flower: Staminate flowers are predominant (more numerous) in axillary clusters and have three stamens. Anthers of two stamens are bilocular and the third is unilocular. Each stamen has stalk called filaments and it is free to each other, while all stamens are approximately united by their anthers.

(9) Pistillate flower: The arrangement of the pistillate flowers on the plants is generally solitary, axillary and borne on short thick pedicels. Multi-pistillate flowers are also reported in cucumber. The female flowers are epigynous (other floral parts are above the ovary) and hermaphrodite flowers are perigynous (having the stamens and other floral parts at the same level as the carpels). The pistil consists of one to five (but usually

three) carpels which in turn, produce ovaries with a corresponding number of locules. The pistillate flowers contain up to five stigmas. The ovary of pistillate flower has vestiture either white or black on its surface.

(10) Fruit: The fruit shape is generally oblong, cylindrical, oval and elongated and called many seeded pepo. The blossom end fruit shape is acute, obtuse and round, whereas peduncle end fruit shape is generally obtuse. The fruit is many seeded pepo having pale green flesh. The colour of fruit skin at edible stage is varied from yellow, mottled light green to dark green. At immature fruit stage, suture (slightly depressed in relation to the fruit surface) is present on fruit surface. Undulation of the surface of the fruit independently on the position of the carpel is knows as creasing, generally found in Japanese genotypes. Small raised growth on fruit surface is known as wart also present in few genotypes. The colour of fruit skin at ripening (seed harvest) stage is generally brown or yellow.

(11) Seed: The seeds are flat in shape, white in colour, 8–10 cm × 3–5 mm in size and approximately 1000 seeds are counted in 20–23 g. The number of seeds in a single fruit varies from 75 to 250.

1.7 Floral Biology

The floral biology of cucumber crop is very important for the researchers and breeders. Flowering starts after 20–30 days of sowing depending upon the cultivars. The ratio of male and female flower varies from 7:1 to 17:1 in monoecious lines (Ekeke 2018). The anthesis of flower takes place between 5.30 am and 7.00 am, whereas the anthers dehisce from 4.30 am to 5.00 am. The temperature and other environmental conditions influenced the anther dehiscence in cucumber. The optimum temperature for anthers dehiscence is 20.5–21.5 °C. Pollen remains viable up to 2.00 pm after anther dehiscence and become unviable by the evening. Receptivity of stigma starts 12 h before opening of flower and continues to be till 6–7 h after that. Due to short duration stigma receptivity, pollination should be done within two hour after anthesis. Early drying of stigmatic secretion occurs due to increase in temperature. Lack of pollination is the major causes of fruit abortion, deshaped fruit and poor fruit setting particularly in monoecious lines. Most important pollination agent is insects (bees). It is advisable to have at least one beehives per acre for getting better yield and quality fruits. Under protected condition growing of parthenocarpic cultivars of cucumber are suitable.

1.8 Genetic Resources and Variability

Genetic diversity in the accessions of any crop species is the key of improvement programmes. Several thousand accessions of cucumber are being maintained at different location worldwide (Weng and Sun 2011). Assessment of diversity based on morphological traits has limitations, since most of them are influenced by the environmental factors. Therefore, molecular approaches are more important to assess of genetic diversity. The morphological traits which have major diversity are immature fruit skin colour (creamy white, mottled light green, mottled dark uniform green to dark green), fruit ribbing (ribbed and non-ribbed), ovary colour of vestiture (white and black), spine size (large, intermediate, small), fruit wart number (absent, few, many), fruit glossiness (dull, glossy) and fruit strips (absent, present). The information on the extent and makeup of genetic variability of a crop is important for framing the strategies to conserve and utilize of biodiversity.

Reduction in genetic diversity is major bottleneck in the populations of cultivated cucumber. Previous studies suggested that cultivated cucumber has a much narrower genetic base as compared to wild cucumber (Weng et al. 2010; Li et al. 2011; He et al. 2013). Qi et al. (2013) re-sequenced the 115 accessions of cucumber and

reported about 3.6 million nucleotide variants. The targeted 115 cucumber accessions were grouped in 4 clusters, i.e. the Indian, the XIS (Xishuangbanna), the Eurasian and the East Asian group. In compared to the other three groups, Indian group had the significantly higher nucleotide diversity and the large numbers of private variants (Qi et al. 2013). The genetic diversity of Indian collection using EST-SSR was assessed by Pandey et al. (2018). Wang et al. (2018) used SNP to assess the population structure of 1234 cucumber accession comprising India, East Asia and Eurasia collection. The Indian, south Asia and East Asia group had the highest level of diversity, while collection of North America, Turkey Europe, central/west Asia and Africa have less diversity (Wang et al. 2018).

In India, two cucumber lines IC 257296 (IGNR No. 18030) and IC 420405 (IGNR No. 18029) have been identified for bearing two female flower per node with small fruit size and high caroteniod content with orange flesh colour, respectively (Pragya et al. 2019). Several other germplasm either indigenous or exotic contributed to in the improvement of cucumber (Tatlioglu 1993). Indian origin germplasm PI 183056 (large root size), PI 183967 (nematode resistance, sequential fruiting, multiple lateral branching) and PI 197087 (downy mildew and gummy stem blight resistance) used widely for cucumber improvement programme worldwide. Other important introduced lines are PI 200815 (resistant to downy mildew and gummy stem blight), PI 200818 (bacterial wilt), PI 212233 (powdery mildew resistance), PI 220860 (gynoecious), PI 418962, PI 419008, PI 419009 and PI 419135 (multiple disease resistance) used for specific breeding programme (Stoub et al. 2008).

1.9 Genetics and Gene Action

The whole genome of cucumber is first time sequenced (Huang et al. 2009). This study indicated that out of seven chromosomes, five chromosome of cucumber is derived from merging of ten ancestral chromosomes after speciation from *Cucumis melo*. The *sequenced* cucumber genome is useful in the study of traits, i.e. sex expression, biosynthesis of cucurbitacin, disease resistance and 'fresh green' odour. The genomic information may be used for breeding better cultivar, studying the function and development process of the plant vascular system. After release of genome draft of cucumber, significant development has been made in improving genetic resources of cucumber. Several mutants have been reported and many genes or QTLs have been identified, which offers an improved understanding of the inheritance and genetic basis of commercially important traits. The mutant genes are related to cotyledon, hypocotyl, stem, leaf, flower and fruit. Several single-gene mutants affect sex expression in cucumber. Cucumber gene catalog (2017 version) is an updated form of cucumber gene list 2010 (Call and Wehner 2010). In 2017 cucumber gene catalog, major revisions have been made and only cucumber mutations having distinct, noticeable phenotypic variations or of horticultural significance were included. Genes related to isoenzymes were eliminated from the list. QTL with large effects and qualitatively inherited genes for disease resistances and other agriculturally important traits are included (Weng and Wehner 2017). In 2017 version, 73 new genes or major QTLs were included, which brings overall number of genes to 199. For a more comprehensive list of cucumber genes and sources, see the Cucurbit Genetics Cooperative (CGC) 2016–2017 (http://cuke.hort.ncsu.edu/cgc). Newly added gene/ major effect-QTL are briefly described trait wise.

1.9.1 Flower-Related

Many mutants have been isolated and several genes/ QTLs have been identified to understand the inheritance pattern and genetic mechanisms of essential traits in cucumber. Although monoecious or gynoecious are main sex type, but androecious (only staminate flowers), andromonoecious (staminate and perfect flowers), hermaphroditic (perfect flowers) and tri-monoecious

(staminate, perfect and pistillate flowers) sex types also found in cucumber. Due to this great variation of sex types, cucumber serves as a prototype for studies of sex determination. The main mechanism of sex determination is proposed to be controlled by three major genes *F, M* and *A* (Kubicki 1969a and b; Li et al. 2008; Yamasaki et al. 2001; Tan et al. 2015). The degree of female flower expression is governed by *F/f* gene, whereas the gene controlling bisexual flower expression is *M/m.* Dominant *F* allele, suppresses *A/a* gene; however, the recessive *a* allele produces staminate flowers (Kubicki 1969b). Another male flower promoting gene *CsACO2* (*a-1*), encoding ACC oxidase gene is identified by Chen et al. (2016). Subgynoecious (with exclusively female flowers at later stage) is a type of monoecious cucumber. Two subgynoecious gene, i.e. *Mod-F1* and *Mod-F2*, independent of *F* and *M* loci, enhances the intensity of femaleness in cucumber by producing high proportion of female to male flowers were identified by Chen et al. (2011). Time of flowering is an important trait, it plays critical role in the environmental adaptation of most crops during domestication. The Xishuangbanna cucumber (XIS), a semi-wild cucumber is a useful resource of novel genes that could be used in cucumber improvement. Four QTLs, i.e**.** *qFt1.1, qFt5.1, qFt6.1* and *qFt6.2* for flowering time are identified using a Xishuangbanna (XIS) cucumber (Qu et al. 2014; Pan et al. unpublished data; Bo et al. 2015). A major effect QTL, *qEf1.1* for early flowering was tagged by Lu et al. (2014) in a population derived by crossing early flowering genotype, Muromskij and late flowering genotype 9930.

1.9.2 Leaf-Related

Hair-like structure present all over the above ground parts of the plant is called trichome. Plants trichomes may play a significant part to tolerate from biotic and abiotic stresses like high and low temperature, high UV rays and also from insects and herbivorous animals (Wagner 1991). Three new glabrous mutants, i.e. *Glabrous 1* (*gl-1, csgl1*), *Glabrous-2* (*gl-2, csgl-2*) and *Glabrous-3 (gl-3, csgl-3)* are identified by different workers and were added to the new gene list (Weng and Wehner 2017). The three mutants were morphologically different and controlled by dissimilar genetic mechanisms. Cucumber mutant, *csgl1* exhibited trichomes only on hypocotyls and emerging leaves, present on chromosome 3 (Cao et al. 2001; Li et al. 2015b). Mutant *csgl-2* is showing trichomes on the flower sepals, fruits and fruit peduncle, however, leaf, petiole and stem were mostly glabrous, mapped on chromosome 2 (Yang et al. 2011).While *csgl3* was located on chromosome 6 and having trichome-free plant morphology (Cui et al. 2016). *CsGL3* was epistatic to *CsGL1* and encode a class IV and I HDZIP transcription factor, respectively; however, function of *CsGL2* is unknown. Another glabrous mutant identified as trichome-less (*tril*), CGN19839 derived from European greenhouse cucumber, showing completely glabrous phenotype, inherited by single recessive gene (Wang et al. 2016). Most of the cucurbits are having bitter foliage due to the presence of cucurbitacin. Shang et al. (2014) identified a gene for bitter leaf (*Bl*), located in chromosome 5, which regulates biosynthesis of bitterness in leaves of cucumber by triggering transcription of *Bi* that regulates cucurbitacin biosynthesis. Leaf colour mutants are commonly used to understand the chlorophyll biosynthesis pathways and chloroplast development mechanism. A cucumber virescent leaf mutant 9110Gt conferred by the *v-1* gene, was identified and mapped in chromosome 6 showing yellowish green leaves at seedling stage and turned into normal green at later stage of plant growth. *CsaCNGCs* encoding channel protein is identified as putative candidate gene for *v-1* gene (Miao et al. 2011 and 2016). Yellow plant (*yp*) mutant is identified by Abul-Hayja and Williams (1976), mutant plant is having light yellow-green leaves and showing slow growth habit. A similar golden leaf mutant C528, having chlorophyll deficiency is isolated by EMS-induced mutagenesis (Li et al. 2015a; Gao et al. 2016). Photosynthesis efficiency is critically affected by leaf area and it is a major trait affecting crop yield.

A major QTL, *ll2* (*littleleaf-2)* is mapped in wild cucumbers (*Cucumis sativus* var. *hardwickii*) for bearing little leaves, located in chromosome 7 (Shi et al. 2014).

1.9.3 Fruit-Related

Bitterness in cucumber fruit occurs due to cucurbitacin, which is a cucumber beetle attractants and an undesirable property for consumption purpose but it act as protectants against most of the pests. However, cucumber cultivar having low cucurbitacin content in the fruit is preferred by the breeders. Several genes have been identified which control the bitterness/non-bitterness in cucumber, such as recessive gene *bi (bi-1)* producing bitter-free fruit and foliage and dominant gene *Bt (Bt-1)* making highly bitter fruit. Zhang et al. (2013) identified recessive *bi-3* gene and *bi-1* gene is recessive and epistatic to the *bi-3* gene, which produce cucumber plants having non- bitter fruit and foliage. Fruit length is an important agronomic trait in cucumber that affects yield as well as consumer preference. Cucumber exhibits tremendous variation in fruit size, from 5 to 60 cm in length (Yang et al. 2012). Commercially, there are different standard for fruit length of cucumber, for example, pickling cucumbers of the U.S. have short and blocky fruits, while slicing cucumbers have relatively long fruits. Longer cucumber are preferred by Chinese fresh market, while Beit Alpha types popular in the Mediterranean region are thin and shorter ((Robinson and Decker-Walters 1997). To understand the underling genetic mechanism of fruit length, several investigations has been made. Jiang et al. (2015) identified gene *fl-1(fruit length-1*) governing fruit length by using two near-isogenic lines, 408 and 409 having difference in fruit length. Two EMS-induced mutant, *long fruit* (*lfr*) and *short fruit (sfr)* were isolated by Wang et al (2014) from cucumber line "Shannong No. 5". The appearance of fruits of cucumber is one of the essential aspects of cucumber breeding due to the processing purpose and consumer acceptance. Thick tough skin in cucumber is dominant to thin tender skin (Strong, 1931) small spine size (*ss*) is linked with tender fruit (*te*) (Fanourakis and Simon 1987). Fruit ribbing in cucumber is studied by Miao et al. (2011) and they revealed that fruit ribbing is controlled by single, dominant gene *Fr* and four fruit epidermal trait associated genes, *u* (fruit colour), *d* (glossiness), *H* (fruit netting) and *fr* (no ribbing) were found to be strongly linked loci in chromosome 5. Spine density in cucumber fruit is a major quality attribute for marketing. Zhang et al. (2016) isolated recessive mutant, *few spines 1* (*fs1*) from CNS2 cucumber line. Spontaneous mutant line, 06–2 named as microtrichome (*mict*), is derived from the North China inbred line 06–1, is having spineless fruit (Zhao et al., 2015a). The colour of cucumber fruit also largely influences consumers' preference. Colour of cucumber fruit is determined by the content of chlorophylls. Two mutants, light *green fruit* (*lgf, CsYcf54*) and *light green peel (lgp*) for fruit colour in cucumber were isolated by different researchers using EMS-induced mutation from cucumber line 406. Lun et al. (2016) identified a recessively inherited mutant, *CsYcf54* having light green fruits and foliage. Mutation in CsaARC5 gene results into the recessive mutant, *lgp* exhibiting light green exocarp (Zhou et al. 2015b). Orange endocap in ripe fruits of Xishuangbanna (XIS) cucumber is due to the presence of high level of *ß*-carotene, a metabolic precursor of vitamin A, which is recessive to white endocarp (no *ß*-carotene); the *ore* locus governing orange endocarp, encodes β-carotene hydroxylase gene is located on chromosome 3 (Bo et al. 2011). The presence of fragrance is a value added trait in many food crops like rice, soybean and sorghum. A few cucumber cultivars with pandan-like fragrance in their fruits and leaves were found in Thailand. Pramnoi et al. (2013) identified a single recessive gene, *fgr* responsible for pandan-like fragrance in the fruits and leaves of PK2011T202. *fgr* is located on chromosome 1 and encodes betaine aldehyde dehydrogenase 2 (BADH2) (Yundaeng et al. 2015). Cucumber is a highly perishable crop and postharvest losses of crop are of great concern to farmers. Improved shelf life of cucumber helps to reduces postharvest deterioration susceptibility.

Chemically induced mutant identified by Dirks et al. (2013), controlled by single recessive gene (*res*), showing less sensitive ethylene mutant, which maintains fruit firmness consequently resulting in longer shelf life after fruit harvest.

1.9.4 Growth Habit

Breeding cucumber plant with compact (short) structure is an important trait. As the dwarf plant architecture needs less labour intensive cultivation while providing more fruits per plant. Regulatory molecular mechanisms of plant growth and development can be interpreted by dwarf plant mutants. Genes underlying dwarf mutations in a number of plant species have been studied by several researchers. In cucumber, gene catalog 2017, Weng and Wehner added four new mutants having dwarf or compact plant height including *Compact-3 (cp-3)* (Crienen et al. 2009), *Super compact-1 (scp-1)* (Wang et al. 2017), *Super compact-2 (Scp-2)* (Li Zheng unpublished data) and *Short internode (Si)* (Lin et al. 2016). The *cp-3, scp-1* and *scp-2* have extremely reduced plant height. Mutant, *scp-1* and *scp-2* are produce by mutation in genes of brassinosteroid (BR) biosynthesis pathway. Dwarfism in mutant, *si* is associated with truncated F-Box protein, exhibiting short internode (*si*), smaller fruit and more wrinkled leaves. Hypocotyl elongation is affected by environmental conditions in commercial cucumber cultivars. Sometimes high temperature or low light intensity may cause increased hypocotyl length, causing poor quality seedling for transplanting. Short hypocotyl in semi wild Xishuangbanna (XIS) cucumber is controlled by a recessive allele, *sh1*, which is insensitive to UVB-free light and temperature fluctuation. *sh1* encodes a human SMARCA3-like chromatin remodelling factor (Bo et al. 2016). The *tendrilless (ten)* mutation that forms branches instead of tendrils is isolated from a cucumber landrace, CG9192. The affected gene *TEN* encodes a TCP (*TB1*, *CYC*, and *PCF*) transcription factor (Wang et al. 2015). Another mutant, *tendrilless-1* (*td-1*) has been tagged on chromosome 6, exhibits tendril less, dwarf trichome-free plant BM007 (Li YH personal communication). Strong association was observed among branches per plant and yield in cucumber (Carmer and Wehner 2000). However the development of many lateral branches escalates the compactness of the plants, which decreases ventilation and can causes diseases. Non-lateral branch (*nlb*) gene, was mapped on the chromosome 1 of cucumber in F_2 population derived from the crossing of non-lateral branch line 419 and branch line SB-2 (Jiang et al. 2008; Ren et al. 2013). Darker green colour in cucumber is an indicator of extended shelf life. Haaring (2014) invented new cucumber plant having dark green stem (*Gs*) in the seedling stage, which is indicator of a darker green fruits. Dark green stem (*Gs*) is simply inherited trait and dominant or incomplete dominant over regular green stem.

1.9.5 Disease Resistance

Major diseases of cucumber include powdery mildew (PM), downy mildew (DM), target leaf spot (TLS), angular leaf spot (ALS), Fusarium wilt (FOC), anthracnose (AR), scab and various viral diseases like cucumber mosaic virus (CMV), zucchini yellow mosaic virus (ZYMV), papaya ringspot virus (PRSV) and watermelon mosaic virus (WMV). Several investigations have been done for resistances to these economic important diseases of cucumber.

1.9.5.1 Simply Inherited Disease Resistance Genes

Three recessive gene, *cca-1*, *caa-2* and *caa-3* governing resistance to *Corynespora cassiicola* causing target leaf spot (TLS) in cucumber have been tagged on Chromosome 6. A molecular marker, CSFR33 linked with *cca-1* locus has been identified in TLS-resistant cucumber line, Q5 by Wang et al. (2010). Yang et al. (2012) found a single recessive gene *cca-2,* governing TLS resistance in PI 183967. Resistance in D31 cucumber line for TLS is governed by *cca-3* gene, Csa6M375730, a CC-NB-ARC type resistance gene analog is identified as putative

candidate gene for *cca-3* (Wen et al. 2015). Earlier investigations suggested that in cucumber there is strong linkage between resistances genes of three potyviruses including PRSV, WMV and ZYMV, as all these resistance genes are located on chromosome 6. Resistance in Chinese inbred line, '02245' for papaya ring spot virus (PRSV) and watermelon mosaic virus (WMV) are governed by a single recessive gene $prsv^{02245}$ and wmv^{02245}, respectively (Tian et al. 2015, 2016). Zucchini yellow mosaic virus (ZYMV) resistance in cucumber inbred lines 'A192-18' is controlled by a single recessive gene $zym^{A192\text{-}18}$. VPS4-like protein encoding gene is putative candidate for the $zym^{A192\text{-}18}$ (Amano et al. 2013). Resistance to Zucchini yellow fleck virus (ZYFV), was found in inbred lines developed from cucumber genotype, Taichung Mou Gua' (TMG). Resistance to ZYFV was determined to be governed by a single recessive gene, *zyf* (Gilbert-Albertini et al. 1995; Kabelka and Grumet 1997).

1.9.5.2 QTLs Providing Resistance to Following Diseases and Abiotic Stress

Powdery mildew resistance in cucumber line, Jin5-508 is governed by major QTL *Pm1.1,* mapped in chromosome 1 by Xu et al. (2015). *Pm1.1* is dominantly inherited *QTL.* However, Nie et al. (2015) identified a recessively inherited QTL, *pm5.1,* for PM resistance on chromosome 5. de Ruiter et al. (2008) identified resistance in PI 250,147 for cucurbit yellow stunting disorder virus (CYSDV) and mapped three QTL. Major QTL, *qCYSDV5.1* for CYSDV resistance was located in chromosome 5; this QTL is near to the QTL conferring resistance to powdery mildew (de Ruiter et al. 2008). Zhang et al. (2014) identified one major QTL *Foc2.1* governing resistance from fusarium wilt on chromosome 2. Resistance to fusarium stem and root rot was identified in cucumber line URS 189 by de Milliano et al. (2012). Two linked QTLs for FW resistance is mapped on chromosome 6. Weng and Wehner 2017 designated these two QTLs as *qFoc6.1* and *qFoc6.2*. A cross between *C. hystrix* and *C. sativus* produced three gummy stem blight (GSB) resistant introgression lines (ILs), i.e. HH1-8–1-2, HH1-8–5 and HH1-8–1-16. Three QTLs, *qGsb1.1*, *qGsb4.1* and *qGsb6.1* were identified for GBS resistance (Lou et al., 2013). QTLs conferring resistance to MYSV (melon yellow spot virus) were identified in F_2 population developed by crossing 27,028,930 (resistant) and Tokiwa (susceptible) genotypes. Resistant parent 27,028,930 contributes two major QTLs, *qMYSV1.1* (*qSwf1.1*) and *qMYSV3.1* (*qSwf3.1*) and one minor QTL, *qMYSV7.1* (*qSwf7.1*) present on 1, 3 and 7 chromosome, respectively, while susceptible parent Tokiwa also contribute one minor QTL *qMYSV4.1* (*qSwf4.1*) present on chromosome 4 (Sugiyama et al. 2015). One abiotic stress resistance (waterlogging tolerance) QTLs was also included in 2017 gene list (Weng and Wehner 2017). More hypocotyl-derived adventitious roots (AR) were produced by Zaoer-N, a waterlogging tolerant cucumber cultivar under waterlogging condition. QTL analysis revealed that AR number (ARN) is controlled by a major QTL, *ARN6.1* and two minor effect QTLs *ARN3.1* and *ARN 5.1* (Xu et al. 2016).

1.10 Conclusion

The biotic and abiotic factors are the major environmental stresses constantly reducing the crop yield and fruit quality of cucumber. The white fly, aphid, thrips and mites are becoming new challenges for growing of cucumber under protected conditions. Among biotic stress, vector borne viral diseases such as Tomato Leaf Curl New Delhi Virus (ToLCNDV) and Zucchini yellow mosaic virus (ZYMV) are becoming the challenges for the researchers. Other important diseases are gummy stem blight, powdery mildew, downy mildew, wilt and root knot nematode. There is need to develop the cucumber lines for better adoptability, which has capacity to set the fruit under high temperature (heat set) and can tolerate wet condition. There are two major segments in cucumber for consumer point of view. First is for fresh market and second one is for processing. Cucumber is grown under open-

field conditions and under protected conditions (glass house). Partehnocarpy cucumber is only varieties which can be grown under glass house production as it does not requires pollination for fruit setting and have very high yield potential. Development of stable parthenocarpy cucumber variety in different segment (based on skin colour) is very important because liking of colour varies from one region to other region.

Screening of germplasms for various abiotic and biotic stress and using those identified resistance lines in a breeding programme is one of the way to combat the new challenges particularly climate change. For developing multiple disease resistant lines, available molecular markers needs to be validated to check the feasibility. Conventional breeding as well as molecular approaches are used for development of new varieties/lines. CRISPR-Cas9 and gene editing new technology are being used for development of new lines with targeted gene particularly for complex traits.

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Genome Editing and Its Applications for Improvement

2

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Abstract

Worldwide, cucumber fruit is an important part of salads and is consumed for its nutritional qualities. Cucumber breeding programs aim primarily at improving its fruit quality and disease resistance. Traditional breeding has developed several improved varieties and hybrids for various traits. Modern breeding practices like marker assisted breeding have enhanced the speed and efficiency of developing new cultivars. However, it is limited by availability of genetic resources. Recently, modern biotechnological tools such as transgenics and genome editing have revolutionized the crop improvement programs. Clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 system is the latest genome editing tool which has been successfully implemented in various crops including cucumber for improving desired traits. Compared to earlier genome editing tools like ZFNs and TALENs, CRISPR/Cas9 is simple and highly efficient technique and therefore, it is widely applied. Availability of complete genome sequence and genetic transformation methods in cucumber makes the CRISPR/Cas9 highly potential for its improvement. The chapter discusses present status and potential future applications of genome editing in cucumber.

2.1 Introduction

Cucumber (*Cucumis sativus* L.) is an important vegetable salad crop of Cucurbitaceous family, mainly grown for its immature tender fruits. The choice and preference of cucumber fruits vary according to various horticultural types in different countries. The shorter, lighter skinned, "Beit alpha" type fruits are favored in the Middle East and Turkey, whereas in Europe long, dark-green skinned, slicing type fruits are preferred. Based on their use, cucumbers are categorized as fresh market (slicing) and processed (pickling) (Staub and Bacher 1997). Nutritionally, cucumber is low-calorie (113–148 K Cal.) vegetable with high mineral and vitamin content and it is also rich in several easily digestible nutrients. Cucumber also contains "Cucurbitacins" which is primarily responsible for the bitterness found in fruits and roots. All these traits are highly important in genetic improvement of cucumber fruits.

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Cucumber is genetically distinct within the genus as it is not readily cross compatible with any other species due to the difference in number of chromosomes (x = 7) from other Cucumis species (x = 12) (Lower and Edwards 1986). Due to cross-incompatibility, it is difficult to expand the limited genetic base of the cucumber, which is supported by several forms of genetic markers, revealing that the percentage of individuals polymorphism in an adapted germplasm is relatively low (3 to 8 percent on average) compared to other allogamous Cucumis species (10 to 25%) (Knerr et al. 1989; Dijkhuizen et al. 1996; Serquen et al. 1997a; Horejsi and Staub 1999). Cucumber is cross-compatible only with a wild or feral form of the same species botanical variety [*C. sativus* var. *hardwickii* (R.)] in the primary gene pool of *C. sativus*. *C. sativus* var. *hardwickii* has unique features of multiple branching and fruiting habit, diseases and pests resistance which are not present in *C. sativus*, signifying considerable potential for increasing the genetic diversity for improvement of cucumber. Multiple lateral branching, earliness, and gynoecious sexual expression characteristics are some of the important traits that significantly contribute in yield of cucumbers. For sustainable production of high quality fruits, it is necessary to keep plants free from diseases and pests which is again the critical objective of breeding programs.

Several traditional breeding techniques have been successfully implemented in cucumber improvement programs. However, lack of desirable traits like disease resistance, particularly viruses in available gene pool and the huge time required for classical breeding necessitates the application of modern biotechnological tools for cucumber improvement.

2.2 Crop Improvement in the Era of Genome Editing

Crop improvement for various traits such as higher yield, nutrition, processing qualities, and resistance to biotic and abiotic stresses is an important part of almost all the breeding programs. Crop improvement through conventional breeding practices has some of the unavoidable limitations like huge time and resource consumption and therefore, alternative approaches are always sought by researches in order to fasten the crop improvement programs. In conventional breeding, simultaneous improvement of all the desired traits and avoiding undesired traits is a challenging task due to number of factors such as genetic correlations between different characters, population structure, linkage disequilibrium, and linkage drag (Hartl and Clark 1997). More recent and advanced methods of molecular breeding like marker assisted selection, marker assisted backcrossing and genome wide selection have overcome some of the limitations and significantly reduce the required time for genetic improvement of crops (Ribaut et al. 2010). However, traditional breeding and molecular breeding do not allow to exploit the species showing crossing barrier for the betterment of cultivated species. Although it can be overcome to some extent by utilizing embryo rescue technique, it is not always successful and needs skills or expertise as well as significant time. On the other hand, transgenic technology has potentials to overcome the limitation of crossing barrier where a DNA fragment responsible for desired trait from any source can be integrated in plant genome. Development of transgenic crops has been instrumental as they are grown on 189.8 million hectares in 24 countries (ISAAA 2017). However, acceptance of GM crops is restricted to only few countries and commercial cultivation of food crops is hardly approved. Insect resistant Bt Cotton is the only transgenic crop being grown in India and continuous opposition to transgenic crops made it difficult to commercialize the first transgenic food crop Bt brinjal, approved for commercialization by Genetic engineering appraisal committee. Although the Bt brinjal has been commercialized in Bangladesh recently, commercialization of transgenic crops is a difficult task in several countries. This again warrants some alternative strategies for commercialization of transgenic crops or novel research methodologies for crop improvement that can develop

transgene free crops. Recently, genome editing technology has opened a new avenues for a better alternative to overcome issues related to transgenic technology, and it has the potential for crop improvement by developing transgene free crops.

2.2.1 Genome Editing Tools

Genome editing is primarily used for creating mutations in genome as it was developed for; however, more advancement in the field has widened its applications in number of ways. The era of genome editing was started with the development of artificial hybrid zinc finger nuclease (ZFNs) by combining a non-specific DNA cleavage domain of FoK1 endonuclease with DNA binding zinc finger protein which provides specificity function (Carroll 2011). This hybrid nuclease was further modified by replacing zinc finger protein with transcription activator like effector to develop transcription activator like effector nucleases (TALENs) (Mahfouz et al. 2011; Li et al. 2012). Designing of TALENs was comparatively more simple and easy than designing of ZFN. Although ZFN and TALEN were widely used for crop improvement, the most recent Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR associated 9 (CRISPR/Cas9) tool has become a method of choice for genome editing due to its great efficiency, accuracy, and simple way of designing. CRISPR/Cas9 tool consists of a nonspecific Cas9 endonuclease and a guide RNA required for specific cleavage of target region (Jinek et al. 2012). The Cas9 nuclease causes double strand breaks at the target site known as protospacer which is complementary to guide RNA and is characterized by a protospacer adjacent motif NGG at 3' end. The double strand break is subsequently repaired by cellular mechanisms either non-homologous end joining (NHEJ) or homologous recombination (HR) pathway. Development of CRISPR/Cas9 tool for the purpose of genome editing has revolutionized the field of genome editing to a greater extent where it has been utilized not only for creating mutations or gene knock-out but also for various purposes such as gene knock-in, base editing, chromosome engineering, karyotype engineering, chromatin imaging, RNA imaging, protein imaging, RNA editing, gene repression, gene activation, chromatin looping, chromatin remodeling, and the list is increasing every year with demonstrations of new applications (Lau 2018).

2.3 Genome Editing in Cucumber

CRISPR/Cas9 based genome editing has been successfully demonstrated in several crops including cucumber (Karkute et al. 2017; Chandrasekaran et al. 2016). Efficient methods of transformation and regeneration are prerequisite for development of either transgenic crops or transgene free crops by genome editing. This is the limitation of several crops where standard regeneration protocols are not available and thus, genome editing cannot be applied in such crops until transformation and regeneration protocols are developed. An efficient regeneration system in cucumber is most important for transformation. Well established protocol for cucumber regeneration has been reported by several groups and is successfully used to develop transgenic cucumbers (Gal-on et al. 2005; Yin et al. 2005). Another most important requirement for genome editing is availability of sequence information of complete genome or at least the sequence of gene of interest. Fortunately, complete genome of the 'Chinese long' inbred line 9930 of cucumber has been completely sequenced almost a decade ago (Huang et al. 2009). This complete genome sequence information is an invaluable resource for biological research.

2.3.1 Cucumber Transformation

Regeneration of cucumber could be obtained in different ways such as from cotyledon, leaf callus, or from protoplast as well (Yin et al. 2005). A protocol for efficient isolation of cucumber protoplast and transformation for transient expression has been developed by Huang et al.

(2013). The standard protocol included use of cellulase R-10 (1.5%) and macerozyme R-10 (0.4%) enzymes for breakdown and loosening of cell wall. Other components used were 0.4 M mannitol, 20 mM 2-morpholinoethanesulfonic acid, 10 mM $CaCl_2$, and 0.1% bovine serum albumin. The method requires pH 5.8 and 8 h time of enzymolysis. It is reported to yield 6–7 $\times$ 10^6 protoplasts per gram of fresh weight. Nevertheless, direct organogenesis from cotyledon or hypocotyl explants is one of the most efficient methods of regeneration in cucumber which is routinely used (Burza et al. 1995).

Transformation of cucumber has been carried out using *Agrobacterium rhizogenes* initially and then by *Agrobacterium tumefaciens*. Microprojectile bombardment method has also been used for successfully transforming embryogenic callus of cucumber (Chee and Slightom 1992). Among all these methods, *Agrobacterium tumefaciens* mediated transformation is the most commonly used method for cucumber transformation. The standard protocol for *Agrobacterium* mediated transformation of cucumber as described by Galon et al. (2005) is discussed below.

2.3.1.1 Agrobacterium Mediated Transformation of Cucumber

This is one of the most commonly used protocols for transformation of cucumber which includes below mentioned steps. Grow the appropriate strain of *Agrobacterium* overnight at 28 °C in LB medium with suitable antibiotic as selection marker and 100 μM acetosyringone. Subculture it under same conditions for 4 h without using any selective antibiotic.

1 Harvest the bacteria by centrifugation and re-suspend in liquid MS media supplemented with 3% sucrose at a final density of 0.5 OD.
2 Surface sterilize the peeled seeds of cucumber in 70% ethanol for 1 min and then in 2% hypochlorite solution for 2 min.
3 Wash the seeds properly to remove ethanol and hypochlorite solution and incubate the seeds on MS medium with 3% sucrose and 0.8% Oxoid agar. Keep it in dark at 25 °C.
4 Now, dissect the embryos out from the incubated seeds and incubate individual cotyledons for 1–2 days on regeneration medium (MS medium, 3% sucrose, 2 mg/l benzylaminopurine, 1 mg/l abscisic acid, 0.8% Oxoid agar supplemented with 200 μM acetosyringone) at 25 °C in dark.
5 Co-cultivate the cotyledons with *Agrobacterium* by dipping them in *Agrobacterium* suspension for 5 min and drying on filter paper. Co-cultivation is carried out on same plates for two more days in same conditions.
6 Transfer the explants to selection medium and incubate with 16/8-h photoperiod regime until shoots regenerate. The selection medium contains 500 mg/l Cefotaxim and 100 mg/l kanamycin. It is required to subculture it once in a week or two.
7 Transfer the regenerated shoots to elongation medium. The composition of elongation medium is MS, 3% sucrose, 1 mg/l gibberelic acid, 0.1 mg/l BAP, 0.1 mg/l ABA, 0.8% Oxoid agar, 500 mg/l Cefotaxim, and 100 mg/l kanamycin.
8 Transfer it to root induction medium of MS supplemented with 3% sucrose, 0.5 mg/l indole butyric acid, 0.8% Oxoid agar, 500 mg/l Cefotaxim, and 100 mg/l kanamycin.
9 Transplant the rooted plantlets for hardening and then transfer them into green house for further screening or phenotypic analysis.

This protocol of cucumber transformation has been used recently in genome editing of cucumber by Chandrasekaran et al. (2016).

2.3.2 Cucumber Genome Sequence

Cucumber harbors as many as 26,682 genes and function of all or many important genes can be studied by utilizing genome editing as one of the components of different molecular biology tools. Genome sequence information provides us the number of genes involved in different cellular and metabolic pathways which forms the basis

for manipulation of particular pathway for desired output. For example, cucumber genome contains a total of 61 nucleotide-binding sites (NBS)-containing resistance (NBS-R) genes (Huang et al. 2009). These genes can be explored for providing resistance to different pathogens either by mutation or over-expression. Similarly, three *EIF4E* and three *EIF4G* genes have been identified in cucumber that could be responsible for recessive resistance against plant viruses such as zucchini yellow mosaic virus and watermelon mosaic virus. Besides that, cucumber presents a model system to study the formation of tendrils which is considered as a key event in plant evolution. Gibberellin has been reported to play a key role in regulation of tendrils development (Galun, 1959). This in turn provides the opportunity to unravel the genetic pathway of tendril development with the help of genome editing tools.

Cucumber genome provides the molecular information about the genes involved in cucurbitacins biosynthetic pathway a compound that imparts bitter taste to cucumber and is toxic to most organisms. Ethylene is one of the most important hormones in plant and is responsible for stimulating femaleness in cucumber, and therefore, cucumber is a model system for sex determination studies. Complete genome sequencing of cucumber revealed 137 genes that are involved in biosynthesis and signaling pathway of ethylene. Besides this, there is huge information available in cucumber genome and one need to explore it for improvement of quality and yield. This primary sequence information of genes involved in various pathways is sufficient for carrying out genome editing work to improve all these pathways.

2.3.3 Cucumber Improvement Through Genome Editing

Genome editing or genome engineering is currently one of the most widely used methods in the field of crop improvement as well as in functional genomics. Genome editing has been implemented principally in major crops like rice and tomato; however, it has been demonstrated in numerous other crops such as wheat, soybean, maize, cotton, potato, *Brassica napus*, citrus, cucumber, grape, and watermelon (Bao et al. 2019). Application of genome editing is not limited to any single trait and different traits like disease resistance, oil quality, yield, metabolite synthesis, starch quality, shelf life, etc. have been targeted in different crops. Thus, genome editing can have numerous applications in order to improve several traits of cucumber. Although there is only one report of genome editing in cucumber using the latest CRISPR/Cas9 tool by Chandrasekaran et al. (2016), there is huge potential for its use in cucumber improvement programs.

2.3.3.1 Improvement of Disease Resistance

In several crops, the first trait targeted by genome editing is disease resistance. In cucumber, the first report of genome editing demonstrated the development of virus resistance using CRISPR/Cas9 tool. Efficient genome editing of the recessive eIF4E (eukaryotic translation initiation factor 4E) gene was achieved by targeting its N and C termini. The recessive *eIF4E* gene is required for maintaining the life cycle of viruses. Therefore, disruption of its function lead to effective immunity against *Cucumber vein yellowing virus* (Ipomo virus) infection and resistance to the *potyviruses* Zucchini *yellow mosaic virus* and *Papaya ring spot mosaic virus*-W. This report also established the method for producing non-transgenic cucumber plants having resistance against multiple RNA viruses. An alternative approach for developing resistance against DNA viruses using CRISPR/Cas9 has been demonstrated in tomato where tomato plants were engineered to express Cas9 protein and guide RNA targeting the coat protein gene and replicase gene loci of the Tomato yellow leaf curl virus genome (Tashkandi et al. 2018). This approach however results in transgenic plants. Nevertheless, similar strategy could be utilized in cucumber for achieving multiple virus resistance by expressing Cas9 and multiple guide RNAs targeting genome loci of all the potential viruses. This kind of virus

resistance is durable and remains active across multiple generations. The another economically important disease of cucumber posing a major threat for yield and quality is powdery mildew caused by *Podosphaera xanthii* and *Golovinomyces cichoracearum* (Perez-Garcia et al. 2009). Loss of function mutations in *CsMLO1* gene which encodes a cell membrane protein provides durable resistance against powdery mildew in cucumber. *CsMLO1* gene is induced by infection of powdery mildew pathogens at an early stage of host pathogen interaction and thus is considered as a gene responsible for host susceptibility. *CsaMLO8* gene is another susceptibility gene responsible for downy mildew in cucumber and transposon insertion in it causes hypocotyl resistance to powdery mildew (Berg et al. 2015). Schouten et al. (2014) have identified as many as 13 *MLO*-like genes in cucumber that are required for susceptibility to powdery and downy mildew. Site directed mutagenesis of *MLO*-like gene with the help of CRISPR/Cas9 could cause the loss of function mutation to develop transgene free powdery mildew resistant cucumber plants. This strategy has been already utilized in other crops such as wheat, tomato, grapes, for development of powdery mildew resistant plants (Mushtaq et al. 2019). Functional knockdown of susceptibility genes with the help of genome editing technology makes it an effective approach for developing cucumber having resistance against number of diseases where susceptibility genes have been characterized. For example, in case of downy mildew in cucumber, *CsDMR6* (Downy Mildew Resistant) is identified as susceptibility gene and its functional knockdown could modify the plants into downy mildew resistant ones (Van Damme and Van Den Ackerveken 2010). Similarly, another susceptibility gene *STAYGREEN* (*CsSGR*) has been identified in cucumber whose loss of function mutation is responsible for resistance to oomyceteous downy mildew, bacterial angular leaf spot and fungal anthracnose pathogens (Wang et al. 2018). The natural loss of function mutation could be easily replicated in high yielding cucumber cultivars with good quality fruits with the help of new genome editing tools.

2.3.3.2 Improvement of Fruit Quality

Quality of fruits is extremely important trait in cucumber. The quality traits in cucumber primarily includes absence of bitterness, flesh color and firmness, flesh juiciness, fresh smell of fruits, more shelf life, small and soft seeds or seedless fruits, density of spines on fruit and the most important trait is high nutritive value (Gajc-Wolska et al. 2003). High quality cucumber fruits are more remunerative and fetch higher price in the market helping farmers to earn more. Genome editing has the potential to speed up the breeding programs intended to improve cucumber fruit qualities. Bitterness in cucumber is due to the presence of cucurbitacins which are highly oxygenated, tetracyclic triterpenes. Although their presence in cucurbit species is beneficial to plants for repelling the insects due to insecticidal activities (Balkema-Boomstra et al. 2003), their presence in fruits is not desirable. Cucurbitacin C biosynthesis in cucumber involves nine different genes which are regulated by two transcription factors, Bi and Bt (Shang et al. 2014). Fruit specific knockdown of these transcription factors or biosynthetic genes of cucurbitacin using modified CRISPR/Cas9 tools which specifically inhibits the expression of target gene could result in blocking of cucurbitacin synthesis in fruits. Alternatively, transgene free cucumbers can be developed by completely blocking the biosynthesis of cucurbitacins by mutating the biosynthetic genes using CRISPR/Cas9. Cucumber fruits without spines are preferred by consumers in some part of the world like USA and Europe and therefore breeders are aimed at developing cucumber genotypes that do not produce spines on cucumber fruits. A recessive gene, *numerous spines* (*ns*), is responsible for development of large number of spines on fruits (Zhang et al. 2016). In order to develop spineless fruits, *ns* gene is an ideal target for genome editing. Similarly, genes responsible for other non-desirable traits can be knocked out by genome editing for development of better cucumber fruits. Improvement of any fruit quality trait is only limited by characterization of genes that regulates the respective traits. Once such genes are fully characterized, genome editing can be

undertaken for improvement of those traits more quickly and efficiently.

2.3.3.3 Improvement of Yield

Yield is a complex trait and many factors such as genotype, sufficient supply of macro and micro nutrients, and environmental conditions contribute for enhanced yield. Therefore, improvement in crop yield is a difficult task. In cucumber, yield is dependent on number of fruits per plant and size of the fruit. However, there is inverse relation between size of the fruits and number of fruits per plant. Nevertheless, it is prerequisite to identify the genes responsible for yield related traits in order to utilize them in breeding programs. Next generation sequencing technologies and modern computational and genomics tools have increased the use of single nucleotide polymorphism (SNP) based breeding methods in crop improvement. SNPs are presently the most widely used molecular markers for identifying the genes and QTLs. The most ideal SNPs are the gene based SNPs or functional SNPs because transfer of gene based SNPs guarantees the transfer of gene responsible for the particular trait of interest. Thus there are only few SNPs in desirable gene between high yielding and low yielding genotypes. Genome editing can be very effectively used for converting the SNPs in the gene of low yielding genotype into the SNPs of the gene of high yielding genotype. This can be achieved by using recently developed CRISPR/Cas based base editors which are able to create mutations at a single base resolution. Thus, a single nucleotide can be changed to another nucleotide in a highly précised manner. For example, cytosine base editor can carry out C-G to T-A mutation whereas, adenine base editor changes A-T into G-C base pairs (Molla and Yang 2019). In cucumber, a rare SNP have been identified in TCP gene responsible for tendril formation. Plants with this SNP do not develop tendrils. In order to demonstrate base editing in cucumber for converting one SNP into another, the SNP of TCP gene can be targeted. Once successful, the similar strategy can be utilized for other SNPs linked to yield related traits. The only limitation is identification of gene based SNPs responsible for yield related traits. Wei et al. (2014) have identified 9 QTLs and SNPs linked to fruit length and fruit weight which are directly contributing to yield of cucumber. Base editing in genotypes with other desirable traits like high quality of fruits, resistance to diseases, etc. for these SNPs will help in enhancing the cucumber yield of such genotypes.

2.3.4 Genome Editing for Functional Genomics in Cucumber

Since the complete genome sequence of cucumber has been deciphered, functional genomics in cucumber has gained pace to characterize the novel genes. However, over-expression of the gene or site directed mutagenesis of the gene for its characterization takes considerable time. The diversity in CRISPR tools and the speed of exploration in this area for developing novel techniques for versatile applications makes it the most potential technique for functional genomics. Gene hunting or functional validation of genes is much easier with genome editing. Availability of efficient protoplast transformation protocol in cucumber fastens the process of genome editing where multiple genes can be targeted. RNA editing using CRISPR-Cas13 provides the opportunities to knockdown the expression of genes (Cox et al. 2017) which normally could not be knocked out owing to their lethality. The transient expression of CRISPR/cas9 components without involving any tissue culture helps to assess the efficiency of genome editing by gRNAs and allows to validate the function of targeted gene quickly and efficiently (Fister et al. 2018). These techniques could be effectively applied for assigning functions to all the uncharacterized genes of cucumber.

2.4 Conclusion

Genome editing with engineered nucleases is a potential technology with several kinds of applications. CRISPR/Cas9 based genome editing has played a crucial role in crop improvement since its inception. The focused research on

CRISPR system in prokaryotes further lead to identification of novel Cas proteins with different properties that are being used for number of applications. Cas13 can be used to target RNA molecules rather than DNA molecules, and thus it is a potential enzyme to use for RNA editing, RNA knockdown or even to develop transgenic plants resistant to RNA viruses. All these tools can be effectively used for functional characterization of cucumber genes and their utilization in breeding programs. The narrow genetic base of cucumber and lack of availability of sexually crossable species warrants the use of genome editing tools for its improvement. Although there is only one example of genome editing in cucumber so far, availability of whole genome sequence information and standard transformation and regeneration protocol makes it one of the attractive vegetable crops for improvement through genome editing techniques.

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The Cucumber Genome—An Update

3

Yiqun Weng

Abstract

Cucumber, *Cucumis sativus* L. (2n = 2x = 14), is both an economically and biologically important vegetable crop. Cucumber was the first horticultural crop with a publicly released genome. Draft genomes with varying qualities of four cucumber genotypes ('9930', 'Gy14', B10, and PI 183967) were developed using Illumina short-read sequencing or 454 technologies. In recent years, high throughput long-read sequencing and new scaffolding technologies have significantly improved assembly quality of genomes. The low cost of sequencing also allows most crops to afford a draft genome. In cucumber, using single molecule, real-time (SMRT), and Illumina sequencing and 10 × Genomics and Hi-C scaffolding methods, new versions of genome assemblies have been developed for both 9930 (v3.0) and B10 (v3.0). In this chapter, I will summarize major improvements and new insights from these new assemblies. I will also review recent progress in cucumber organelle genomes. I will discuss the cucumber genome in the context of comparative analysis with other cucurbits, cucumber genome evolution, domestication, and population genomics perspectives.

3.1 Introduction

Cucumber, *Cucumis sativus* L. (2n = 2x = 14), was the first among major horticulture crops with a publicly available draft genome. The genotype used for sequencing the genome is a north China fresh market (Chinese Long) type inbred line '9930' (Huang et al. 2009; Li et al. 2011). Subsequently, draft genomes for three more cucumber lines were reported including the North European pickling type variety B10 (v1.0), the US pickling cucumber inbred line Gy14 (v1.0), and the wild cucumber (*C. sativus* var. *hardwickii*) line PI 183967 (Wóycicki et al. 2011; Yang et al. 2012; Qi et al. 2013). These initial assemblies used Illumina short reads or 454 Technologies with total sequences in seven pseudomolecules ranging from 197 and 205 Mb (Table 3.1). The cucumber genome was estimated to be around 367 Mbp in size (Arumuganathan and Earle 1991). This means 55% of the cucumber genome is missing in the early assembles. In addition, mis-orientation and mis-assemblies of scaffolds are common, which is due primarily to the repetitive sequences in the genome that are intractable with the sequencing technologies employed. In recent years, the

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Table 3.1 Key statistics of cucumber draft genome assemblies of four genotypes

Genotypes	9930 V2.0	9930v3.0	PI 183967	Gy14 V1.0	B10v1.0	B10v3.0
Platforms	Sanger + Illumina	SMRT + Illumina	Illumina (62x)	Roche/454	Roche 454	SMRT + Illumina
Scaffolding	de novo + BES	10xGenomics, Hi-C	de novo	de novo	de novo	de novo
N50 contig size (kb)	37.9 kb	8.9 Mbp	118 kb	37.6 kb	27.1 kb	858 kb
Total # Scaffolds	12,845	85	unknown	4,219	4,173	8,035
Scaffold N50	488.2 kb	31.1 Mbp	4.2 Mbp	993 kb	232.40 kb	Not specified
# Scaffolds in Chr1-7	Unknown	89	no data	244	169	119
Sequences in assembly (Mbp)[a]	197.3	211.0	204.8	192.6	206.73	196.6
Repeats in assembly (/%)	24.0	37.7	Unknown	Unknown	Unknown	50.0
BUSCO, complete (%)[b]	No data	1314 (91.3%)	1390 (90.9%)	1286 (89.3%)	No data	1318 (91.7%)
BUSCO, fragmented	No data	38	45	53	No data	27
BUSCO, missing	No data	88	86	101	No data	95
Annotated genes	23,248	24,317	23,836	21,491	26,587	27,271
References	Huang et al. (2009); Li et al. (2011)	Li et al. (2020)	Qi et al. (2013)	Yang et al. (2012)	Woycicki et al. (2011)	Osipowski et al. (2020)

[a] In Chr1-7 excluding Chr0
[b]Based on 1440 BUSCOs

employment of improved Illumina sequencing (McCoy et al. 2014), and long read sequencing with the PacBio single molecule, real-time (SMRT) (Levene et al. 2003), and Oxford nanopore (Jain et al. 2016) sequencing have dramatically improved the read length and throughput. There has been also wide spread use of new scaffolding technologies such as optical mapping (e.g., BioNano), 10X Genomics; Hi-C/Chicago (Lieberman-Aiden et al. 2009; Putnam et al. 2016) which provides long-range contiguity information ranging from ~50 kb to several megabases (reviewed Ghurye and Pop 2019). Combined use of these technologies has significantly improved the assembly quality of plant genomes. In cucumber, using combined SMRT and Illumina sequencing and different scaffolding methods, new version of genome assemblies have been developed for both 9930 (v3.0) (Li et al. 2020), and B10 (B10v3.0) (Osipowski et al. 2020). A new version of Gy14 (Gy14v2.0) was also publicly accessible (https://cucurbitgenomics.org/; Weng et al. manuscript in preparation) but no associated details are available. The cucumber draft genomes for 9930v2.0, Gy14v1.0, and B10v1.0 were reviewed in Weng (2016). Therefore, in this chapter, I will update the newly released genome assemblies for 9930 (v3.0) and B10 (v3.0), summarize major improvements and new insights from these new

assemblies. Since draft genomes of a number of other cucurbit have also been publicly released, I will make discussions in the context of comparative analysis of cucurbit genomes.

3.2 The 9930v3.0 and B10v3.0 Draft Genomes

Li et al. (2020) reported the 9930 v3.0 assembly which was developed with long-read SMRT sequencing facilitated with 10 × Genomics and Hi-C in scaffolding. The SMRT sequencing generated 16.2 gigabases of sequences (46.2 × genome coverage) with average read length of 7,713 bp. De novo assembly of these reads resulted in 232.3 Mbp sequences in 174 contigs with N50 contig length of ~8.9 Mbp. Further scaffolding with 10 × genomics and Hi-C enabled assembly of 89 contigs of 211.0 Mbp into seven pseudomolecules corresponding to seven cucumber chromosomes. Main statistics of available cucumber draft genome assemblies for four genotypes are compared in Tables 3.1 and 3.2.

As compared with v2.0, the 9930v3.0 assembly adds 19.1 Mbp more sequences in the seven pseudomolecules. However, the continuity of the new assembly is much better than the early versions which could be reflected from the N50 contig and scaffold sizes (Table 3.1). In addition, 9930v3.0 also confirmed findings from genetic mapping studies (e.g., Yang et al. 2012) that identified mis-placements of a number of scaffolds in 9930v2.0 including a large chunk of sequences in the upper distal end of Chr5, and two inversions on Chr4 and Chr6. Analysis of the newly added sequences indicated that they have on average ~35.0% a guanine-cytosine (GC) content which is higher than shared sequences between the two assemblies (~32.8%) (Li et al. 2020). This suggests that the long-read SMRT sequencing is able to access those high GC content sequences than the Illumina technology. Meanwhile, there are 15.2 Mbp sequences in 78 super-scaffolds that failed to be anchored on the seven chromosomes suggest that these scaffolds are composed primarily of repetitive sequences.

Annotation of the 9930v3.0 genome assembly identified 82.0 Mb (37.7%) repetitive sequences, which were ~27.6 Mb more than predicted in 9930v2.0 (Huang et al. 2009) (Table 3.2). Among these repetitive sequences, long terminal retrotransposons (LTRs) are the most abundant (12.6%). However, almost half of all repetitive sequences (16.3%) were not classified suggesting the complexity of repetitive sequences in the cucumber genome. Examination of the distribution of scaffolds across each chromosome indicated that most relatively small scaffolds are concentrated in centromeric region of the seven chromosomes.

The 9930v3.0 assembly was annotated using combined methods (ab initio, homology-based, and transcriptome sequencing), and 24,317 protein-coding genes were predicted with on average 112 gene per Mbp genomic DNA sequences (1 gene per 11 kb) (Table 3.3). Although the numbers of genes in the two versions were only 153 in 9330v2.0 and v3.0, 1,078 genes were newly assembled in v3.0, and 2,693 were newly predicted in v3.0 but were not predicted in v2.0 which was probably due to sequencing gaps, errors, or annotation pipeline bias (Li et al. 2020).

In addition to 9930v3.0, Osipowski et al. (2020) reported a new version of draft genome for the B10 cucumber (B10v3.0). De novo assembly of PacBio SMRT sequencing reads resulted in 343.6 Mbp sequences in 8,096 contigs with N50 contig length of 842 kb (Table 3.1). The seven pseudomolecules of B10v3.0 consisted of 196.7 Mbp in 119 contigs with ~50% repetitive DNA sequences, which is much higher than that in 9930v3.0 (Table 3.2). While 27,271 protein-coding genes were annotated in B10v3.0, the details are unknown. No evaluation of the sequencing and assembly quality for B10v3.0 was reported.

The BUSCO (Benchmarking Universal Single-Copy Orthologs) scores are often used for quantitative assessment of genome assembly and annotation completeness (Simao et al. 2015). Based on 1,440 orthologous groups from plant lineages, the 9930v3.0 and B10v3.0 assemblies contained 1314 (91.3%) and 1318 (91.7%) of the

Table 3.2 Percentages of repetitive sequences in two cucumber genome assemblies[a]

Repeat types	9930v3.0	B10v3.0
DNA	3.6	4.0
SINE	0.14	0.0
LINE	2.76	5.0
LTR (Copia + Gypsy)	12.16	21.0
Others LTR	0.31	0.0
Low_comple xity	0.54	
Simple_repeat	1.63	
RC/Helitron	0.13	1.0
Retroposon	0.04	
Satellite	0.01	
RNA	0.09	
Unknown	16.3	19.0
Sum	37.7	50.0

[a] Based on Li et al. (2020) (for 9930v3.0) and Osipowski et al. (2020) (B10v3.0)

plant core genes/orthologues; the rest are either incomplete or missing (Table 3.1). Several other cucurbit genomes have also been sequenced including multiple genotypes of melon (*C. melo*) (Garcia-Mas et al. 2012; Ruggieri et al. 2018; Casacuberta et al. 2020; Yano et al. 2020; Yang et al. 2020), watermelon 97,103 (*Citrullus lanatus*) (Guo et al. 2013, 2019), and bottle gourd (*Lagenaria siceraria*) (Wu et al. 2017), which have approximately 450, 430, and 334 Mbp estimated genome size, respectively. Major statistics of these assemblies are provided in Table 3.4. The melon Harukeiv1.41 draft genome has the highest BUSCO score (95.3%), whereas cucumber 9930v3.0, melon HSv1.0, and watermelon 97103v2.0 have similar values (~91.5%). Cucumber, watermelon, and bottle gourd all have around 22,000 to 24,000 predicted genes and the newest three melon draft genomes have much higher numbers of annotated genes (28,000–33,000 per genome). Cucumber and melon were diverged ~10 million years ago and are highly conserved at genome sequence level. Is it not known if the 24,317 annotated genes in the 9930v3.0 is an under estimate.

A significant difference between cucumbers 9930v3.0 or B10v3.0 with other cucurbit draft genomes listed in Table 3.4 is the coverage of the assembly in proportion to the estimated genome size. Despite similar sequencing and scaffolding technologies used for these assemblies, the 226.2 Mbp sequences placed in seven pseudomolecules of 9930v3.0 is only 61.7% of the estimated 367 Mbp genome size, which is much lower than those in other cucurbit assemblies (Table 3.4). The estimated genome size based on k-mer analysis of Illumina short sequencing reads of B10 was larger than previously estimated 367 Mbp by Arumuganathan and Earle (1991), which was around 414 Mbp (Osipowski et al. 2020). Our k-mer analysis and flow cytometry in the picking cucumber inbred line Gy14 also suggested a genome size around 400 Mbp (unpublished data). If this is true, then nearly 174 Mbp (43.5% of 400 Mbp) genomic DNA sequences are missing from the 9930v3.0 genome. Based on the annotated number of genes, the 9903v3.0 assembly likely contains the majority of coding regions of the cucumber genome. This may suggest that a significant portion of repetitive sequences are not accessible with the PacBio SMRT sequencing technology. It is a challenge to assemble those sequences.

Table 3.3 Physical lengths and annotated genes on seven cucumber chromosomes in different draft genome assemblies

Pseudo Molecules		9930 v2.0*			9930 v3.0*		Gy14v1.0**	9930v1.0**	B10v1.0**
	Physical Length (Mbp)	# Gene models (%)	Mean gene density (# genes/Mbp)	Length (Mbp)	# Gene models (%)	Mean gene density (# genes/Mbp)	Physical Length (Mbp)	Physical Length (Mbp)	Physical Length (Mbp)
Chrl	29.15	3376 (14.5%)	115.8	32.8	3560 (14.6%)	108.5	28.1	26.0	28.6
Chr2	23.17	2850 (12.3%)	123.0	24.81	2848 (11.7%)	114.8	21.3	22.4	25.2
Chr3	35.5	4750 (20.4%)	133.8	40.86	4705 (19.3%)	115.1	39.3	34.1	39.1
Chr4	23.42	2761 (11.9%)	117.9	26.7	2777 (11.4%)	104.0	19.6	22.1	33.2
Chr5	28.01	3053 (13.1%)	109.0	31.9	3240 (13.3%)	101.6	19.5	28.5	29.9
Chr6	29.06	3831 (16.5%)	131.8	31.1	3936 (16.2%)	126.6	28.6	26.8	30.3
Chr7	24.48	2373 (10.2%)	96.9	22.45	2456 (10.1%)	109.4	16.6	17.5	20.4
Chrl-7 sum	192.79	22,994 (98.8%)	119.3	210.62	23,522 (96.7%)	111.7	173	177.4	206.73
Chr0	5.41	270 (1.2%)	49.9	15.2	795 (3.2%)	52.3	30.4	66.1	15.9
Total	198.2	23,264 (l00%)	ll7.4	225.82	24,317 (100%)	107.7	203.4	243.5	225.1

[a] Cauculated based data depostied in the database from http://cucurbitgenomics.org/

[b]From Huang et al. 2009 (9930v1.0), Yang et al. (2012) (Gy14v1.0), and Woycicki et al. (2011). Data for B10v3 were not available.

Table 3.4 Comparison of draft genome assemblies in four cucurbit crops

Crops	Cucumber	Melon	Melon	Melon	Watermelon	Bottle gourd
Genotype/version	9930v3.0	DHL92v4.0	Harukeiv1.41	HSv1.0	97103v2.0	USVL1VR-Ls_v1.0
Chromosome	2n = 2x = 14	2n = 2x = 24	2n = 2x = 24	2n = 2x = 24	2n = 2x = 22	2n = 2x = 22
Estimated genome size (Mbp)	367	454	454	454	430	334
Repeats %	37.7%	45.5%	57.5%	43.0%	55.6%	46.9%
Assembly size (Mbp)	226.2 (61.7%)	359.4 (79.1%)	366.7 (80.8)	359.4 (79.1%)	362.7 (84.3%)	308.1 (92.2%)
Annotated genes	24,317	28,299	33,314	28,898	22,596	22,472
BUSCO score[a]	91.3%	87.3%	95.3%	91.0%	90.1%	85.6%
References	Li et al. (2020)	Ruggieri et al. (2021)	Yano et al (2020)	Yang et al. (2020)	Guo et al. (2019)	Wu et al (2017)

[a]Only complete genes (Yano et al. 2020)

3.3 Cucumber Organelle Genomes

The chloroplast (cp) and mitochondrial (mt) genomes contain genes that play a critical role in eukaryotic cells by ensuring aerobic respiration (mtDNA) and photosynthesis (coDNA), respectively. Cucumber (as well as melon, and *Cucumis hystrix*) has interesting organellar genetics because its three genomes show different modes of transmission: maternal for chloroplast, paternal for mitochondrial, and biparental for nuclear genes which provides a nice system to study the role of intergenomic transfer in the evolution of extremely large mitochondrial genomes (Ward et al. 1981; Havey 1997; Havey et al. 1998; Shen et al. 2013).

A number of cucumber chloroplast genome sequences have been reported (e.g., Kim et al. 2006; Chung et al. 2007; Plader et al. 2007; Lee et al. 2017). The size and structure of the cucumber chloroplast genome are similar to most other angiosperms with a large (LSC) and a small (SSC) single-copy regions separated by two inverted repeats (IRa and IRb) (Palmer 1982; Perl-Treves and Galun 1985; Lim et al. 1990). The sizes of the cucumber chloroplast genomes reported so far are all close to 155 kb, with the SSC, LST, IR being around 18.3, 86,6, and 25.2 kb, respectively. Approximately 130 genes are encoded by cpDNA for chloroplast proteins, tRNAs, and ribosomal RNAs (rRNAs). Variations in cpDNA sequences in different varieties such as SNPs, SSRs, and small indels have been reported (e.g., Chuang et al. 2007; Lee et al. 2016), which have been explored in phylogenetic analysis (e.g., Kocyan et al. 2007; Schaefer et al. 2009).

Unlike the chloroplast genomes in plants which are highly conserved in size and structure, the mitochondrial genome varies significantly in sizes. For example, the estimated sizes of the mitochondrial genomes for the watermelon, zucchini (*Cucurbita pepo*), and cucumber are 379 kb, 983 kb, and 1.8 Mb, respectively; they have $\sim$200 kb of sequences in common, a similar set of genes, and little or no conserved synteny (Alverson et al. 2010). Genes encoded by plant mitochondrial genome consist of rRNAs, tRNAs, ribosome synthesis proteins, and oxidative phosphorylation proteins (Adams et al. 2002; Burger et al. 2003; Sloan et al. 2010). Alverson et al. (2011) reported sequencing of the 1,685-kb mitochondrial genome of cucumber (CV Calypso). Most plant mitochondrial genomes have a single, circular chromosome, but the cucumber mitochondrial genome contains three chromosomes that are 1556, 84, and 45 kb in size, respectively. The three chromosomes are nearly identical in nucleotide composition (GC

content 44.2–44.6%) and replicate independently of one another. The two smaller chromosomes are devoid of known functional genes, and all 62 intact mitochondrial genes are on the main 1556-kb chromosome including 40 protein-coding genes and 21 tRNA genes from mitochondrial (9), chloroplast (13), or unknown origin (1). Alverson et al. (2011) showed that the origins of the large size of the cucumber mitochondrial genome may be due to the proliferation of dispersed repeats, expansions of existing introns, and the acquisition of sequences from diverse sources, including the cucumber nuclear and chloroplast genomes, viruses, and bacteria.

While most plants show maternal transmission of their organelles, the cucumber, *C. hystrix*, and melon are unique in organellar transmission with paternal transmission of the mtDNA, and maternal transmission of cpDNA (Havey 1997; Havey et al. 1998; Shen et al. 2013). However, more recently, Shen et al. (2019) observed evidence for occasional maternal and biparental transmission of mtDNA in cucumber. The transmission of specific regions of the maternal mtDNA could be as high as 17.8%, although the amounts of these maternal regions were often much lower relative to paternally transmitted regions.

3.4 Cucumber Genome Under Evolution and Domestication

Among the 50 plus species in the genus *Cucumis* (Sebastian et al. 2010), cucumber is known to be the only one with $2n = 2x = 14$ chromosomes, and the rest including its sister specie *C. hystrix* have $2n = 24$ chromosomes or its multiples. Analysis of genome sequencing data revealed no recent whole genome duplication (WGD) events in all major cucurbits like cucumber, melon, watermelon, and bottle gourd (Huang et al. 2009; Garcia-Mas et al. 2012; Guo et al. 2013; Wu et al. 2017), and most annotated genomes contain similar number of genes (Table 3.4). Phylogenetic analysis supported the evolution of seven cucumber chromosomes from a $2n = 2x = 12$ ancestor through dysploid reduction (reduction of basal chromosomes but not gene contents). Comparative analysis between cucumber and melon genomes revealed extensive genome sequence homology, and synteny. For example, Huang et al. (2009) speculated that five of the seven cucumber chromosomes may arise from fusions of 10 ancestral chromosomes after divergence from melon. Comparative mapping in cucumber and melon revealed that cucumber Chr7 was syntenic to melon Chr I except for a large inversion; cucumber Chr2 and Chr6 each contained genomic regions that were syntenic with melon chromosomes III + V + XI and III + VIII + XI, respectively. Likewise, cucumber Chromosomes 1, 3, 4, and 5 each was syntenic with two melon chromosomes previously designated as II + XII, IV + VI, VII + VIII, and IX + X, respectively (Li et al. 2011b). Yang et al. (2014) further conducted comparative mapping among cucumber, *C. hystrix*, and melon, and identified 14 inversions and a *C. hystrix* lineage-specific reciprocal inversion between *C. hystrix* and melon. Based on integrated genome sequence, genetic, and cytogenetic analyses, Yang et al. (2014) proposed a model to explain the mechanisms of dysploid chromosome reduction from n = 12 to n = 7 which involved five fusions, four translocations, and 50 inversions.

Cucumber is native to the Southern Asia continent. Four botanical varieties of *C. sativus* have been recognized including cultivated cucumber *C. sativus* L. var. *sativus* (CSS) the wild cucumber *C. sativus* L. var. *hardwickii* (CSH), the semi-wild Xishuangbanna cucumber, *C. sativus* L. var. *xishuangbannanesis* (XIS), and the Sikkim cucumber (*C. sativus* var. *sikkimensis*) (Sikkim). There are significant differences between CSH and CSS in the amount and distribution of heterochromatin, as well as chromosomal rearrangements. Both linkage mapping and cytogenetic genetics revealed six large inversions, five paracentric and one pericentric, on Chr4, Chr5 and Chr7 between the two taxa; and the paracentric inversion on Chr7 occurred during domestication of cucumber (Yang et al.

2012). The work by Yang et al. (2012) also supported the subspecies status of CSH as the progenitor of cultivated cucumber.

The semi-wild XIS cucumber endemic to the tropical southwest China and surrounding regions is characterized by high tolerance to low light, large fruit size and heavy fruit weight, as well as orange flesh color in mature fruits that are very useful for cucumber breeding. Comparative mapping and QTL analysis of these traits revealed that the XIS cucumber shares the major chromosomal rearrangements in chromosomes 4, 5, and 7 between the wild and cultivated cucumbers suggesting the origin of the XIS cucumber through diversification selection after cucumber domestication (Bo et al. 2015). The Sikkim cucumber is featured with some morphological traits like black spine, brown fruit with fine and heavy netting, as well as large hollow in mature fruit. It was established as a botanical variety almost 150 years ago, but little is known about its taxonomic status, genome differentiation, and genetic basis of those characteristic traits. Wang et al. (2021) conducted QTL mapping in populations derived from two Sikkim cucumber accessions, and identified simply inherited genes and quantitative trait loci underlying morphologically characteristic traits of the Sikkim cucumber. No structural changes at the chromosomal level were found between Sikkim and CSS cucumbers. It was suggested that the Sikkim cucumber is an ecotype of cultivated cucumber not worthy of formal taxonomic recognition.

3.5 Population Genomics

India is the center of diversity for cucumber, which has been cultivated there for over 3,000 years (see Weng 2021 for references). From India, cucumber was brought eastward to China at ~2,000 years ago and westward to Europe around 500–1300 CE (Paris et al. 2012). Long-term natural and artificial selections have resulted in many ecotypes, landraces, and market groups with adaptation to local environments, production systems, specific processing requirements, or consumer preferences. Hundreds of cucumber accessions have been collected in various gene banks around the world (Weng and Su 2011). The knowledge of genetic variations in germplasm collections is essential for their conservation and efficient use. The first comprehensive evaluation of worldwide cucumber collection using molecular markers was conducted by Lv et al. (2012) who fingerprinted 3,345 accessions from worldwide cucumber collections with 23 SSR markers. Marker-based clustering analysis classified these accessions into three geographic distinct groups: China/East Asia, Europe/America/Central and West Asia, and India/XIS with the Indian population having the highest genetic diversity. Lv et al. (2012) developed a core cucumber collection consisting of 118 accessions that capture >78% SSR alleles. Qi et al. (2013) further characterized this core collection with re-sequencing data from 115 accessions, which could be divided into four geographic groups: the Indian group, the XIS group, the Eurasian group, and the East Asian group. The Indian group has the highest nucleotide diversity (π) and largest numbers of private alleles. The Estimated π ($\times 10^{-3}$) for the four groups was 4.48, 1.06, 1.85, and 1.03, respectively. The respective linkage disequilibrium (LD) decay (to r^2 of 0.2) was only 3.2 kb in the Indian group, which was 140.5, 55.2, and 56.4 kb for the XIS, Eurasian, and East Asian groups, respectively.

Most recently, based on 23,000 SNPs, Wang et al. (2018) examined the genetic diversity, and population structure of 1,234 cucumber accessions in the United States National Plant Germplasm System collection. While tier 1 grouping of this collection (India, East Asia, and Eurasia) were consistent with early work described above (Lv et al. 2012; Qi et al. 2013), this dataset allowed further subgroupings. For example, the Europe and North America accessions are subpopulations of the Eurasia group, which is reasonable because most North American cucumbers have a strong European heritage. Accessions from Turkey are distributed in two subclades: Central/West Asia group and European group reflecting Turkey as the geographic link between

the two groups during the westward dispersal of cucumber (Paris et al. 2012). In addition, significant number of accessions in the Eurasia groups showed genetic admixture, but most East Asia accessions were more homogeneous in their genetic backgrounds (Wang et al. 2018). The India/South Asia group and the East Asia group had the highest and lowest nucleotide diversity (π), respectively. The pairwise fixation index (F_{ST}) reflecting divergence between two groups was 0.28 between East Asia and India groups, and 0.27 between East Asia and the Western group (North America, Europe, Africa, and Central/West Asia). There was much less divergence among the North America, Europe, Turkey, Africa, and Central/West Asia groups. These observations suggest independent diversifying selection between the East Asian and Eurasia cucumbers after domestication (Wang et al. 2018).

3.6 Perspectives

The availability of genome sequences has revolutionized cucumber research and provided exciting new opportunities to address fundamental issues and advance cucumber breeding with applied genomics tools, which could be evidenced by the exponential growth of publications in cucumber in the last decade. However, it is clear that the quality of the present cucumber draft genomes need to be improved in more complete genome coverage and more accurate annotation. In particular, it is interesting to investigate why so much of the cucumber genome (>40%) is still missing with the state-of-the-art sequencing and scaffolding technologies. On the other hand, a single reference genome sequence is not enough. Pan-genome sequencing of multiple genotypes may reveal a more complete picture on the scope of genetic variation and trait-DAN variant associations in natural cucumber populations. Community standards for gene ontology, gene, and QTL nomenclature are needed (e.g., Wang et al. 2019; Pan et al. 2020). An efficient genetic transformation system is indispensable for functional characterization of genes, which is lacking in cucumber. More mutant libraries will help investigation of gene functions. Development of these tools will allow leveraging the cucumber genomic resources to address important biological processes. There is still a large gap between the quickly accumulating genomic resources and their practical use in cucumber improvement. Limited numbers of genes or QTL have been cloned, and their functions are largely unknown. A public platform to integrate and host the genetic and genomic resources is also critical for efficient use of such information for the community.

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4 Molecular Mapping of QTLs and Genes for Plant Architecture and Fruit Traits in Cucumber

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Abstract

Cucumber (*Cucumis sativus* L.) is a multipurpose vegetable crop cultivated worldwide. China is the largest producer. Cucumber plants vary in plant architecture and fruit traits. Starting from the time of the release of cucumber draft genomes, substantial improvement has been done in molecular mapping and cloning of agriculturally and biologically important genes and quantitative trait loci (QTLs), which may help in cucumber breeding. QTLs for hypocotyl length, plant height, primary branch number, leaf size/shape and fruit-related traits in cucumber are identified. Hence, in this chapter, QTLs and genes identified to date in cucumber are reviewed for each of the architecture- and fruit-related trait of cucumber. From this paper, readers may understand the current knowledge about mutants, QTLs and genes identified so far in plant architecture- and fruit-related traits and could help for farther experimental evidences and as an academic reference material.

4.1 Introduction

Cucumber (*Cucumis sativus* L.) is one of the major cucurbit crops widely cultivated in the world. It is an agriculturally/economically important vegetable possessing vast genetic diversity (Yang et al. 2012; Pan et al. 2017, 2020). Cucumber is also a model crop to learn many important biological systems in plants (Weng and Sun 2012). It is native to Southern Asia and believed to be originated and domesticated in India (Sebastian et al. 2010). China is the secondary center of diversity (Valcárcel et al. 2018). China produced 74.84% of the world's total cucumber in 2018 (http://www.fao.org/faostat/en/#data/QC).

Cucumber varieties are known for their diverse plant architecture- and fruit-related traits. Hypocotyl length, plant height or vine length, branch number, leaf shape, leaf size, fruit shape and fruit size, flesh thickness, fruit neck length and other architectural traits are considered as the main focus of cucumber breeding (Fig. 4.1). These traits are qualitative traits. Cucumber architectural traits directly or indirectly affect

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cucumber fruit yield or quality and influences customers' choice and market value. For instance, the size of fruit neck (length/diameter) trait accounts a major quality concern in fresh cucumber market value as it affects appearance and taste of cucumber fruits.

Some plant architectures may be associated to each other. For example, the hypocotyl length was found to influence plant height of cucumber (Bo et al. 2012). For cucumber, the ideal plant architecture for production should have a strong main stem for nutrient transport, short internode length and few lateral branches to promote high yield, short petiole and compact structure to improve space utilization (Li et al. 2016; Fig. 4.2).

Since one decade ago, the whole genome of cucumber including 9930 (Chinese Long inbred line) (Huang et al. 2009), Gy14 (North American pickling type) (Cavagnaro et al. 2010) and B10 (European landrace) (Wóycicki et al. 2011) are publicly available (http://cucurbitgenomics.org/) which have clear differences in their architecture traits.

At present, the basic genetic information of cucumber has been roughly understood, such as the 367 Mb genome size and thousands of functional genes distributed on seven chromosomes (Xie and Wehner 2001; Ren et al. 2009). Vast sources of genetic diversity of cucumber combined with environmentally influenced genotype effects are accelerating QTLs (quantitative trait loci) study on important cucumber traits including plant architecture. Several main agronomic attributes in plant species are studied and were found as quantitative in nature (Paterson et al. 1988). The detection and characterization of QTLs and genes

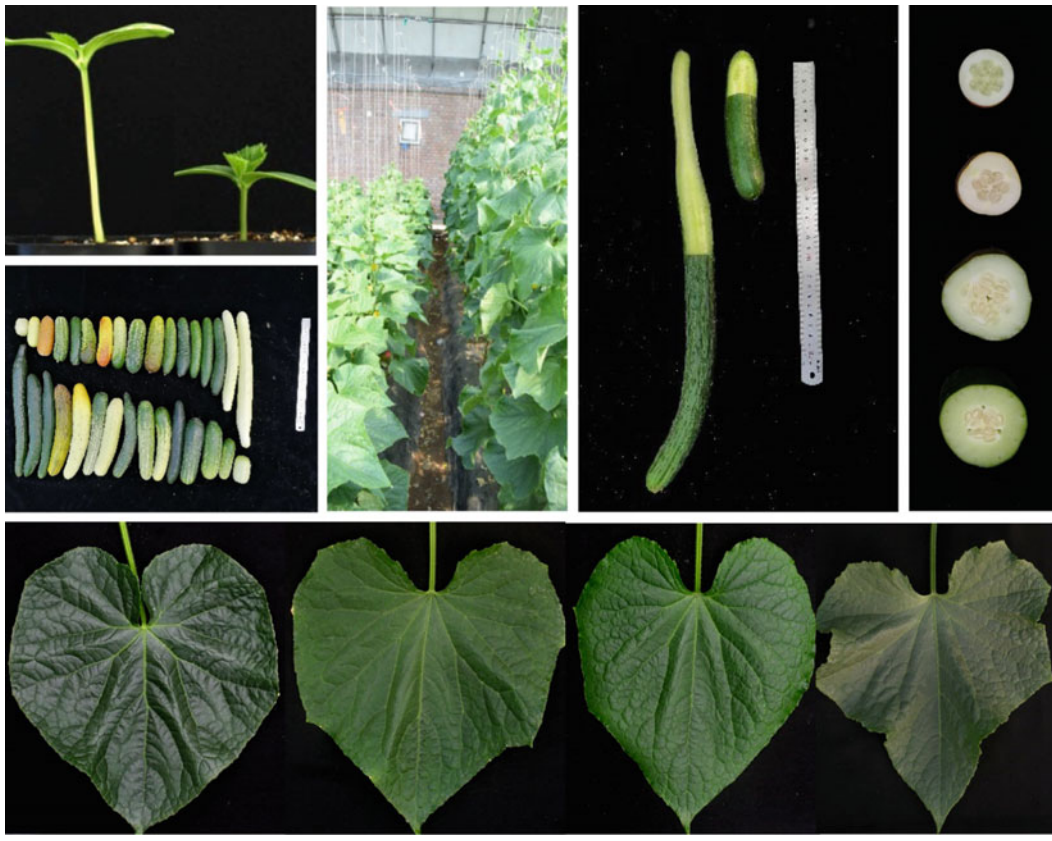
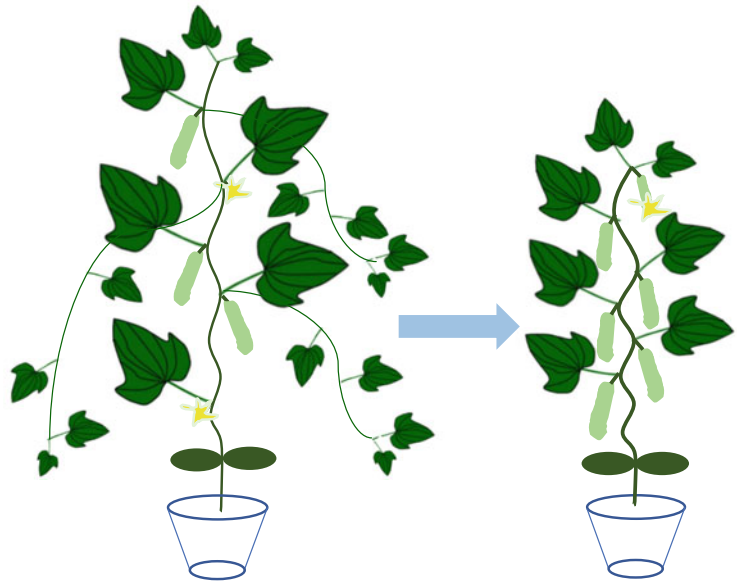


Fig. 4.1 Different traits from cucumber, including hypocotyl length, plant height or vine length, fruit neck length, fruit size and shape, flesh thickness, leaf size and shape (photo by the authors)



(a) Ordinary architecture (b) Compact architecture

Fig. 4.2 **a** Ordinary architecture with gracile stem, long internode length, long petiole length and strong lateral branch and **b** compact architecture with stronger main stem, shorter petiole length, shorter internodes length and fewer lateral branch (drawing by the authors). b is preferable by farmers for manual type of cucumber production

of architecture traits in cucumber are important stages to recognize the associated functional mechanisms and utilize the genetic potential in cucumber crop selective breeding strategies.

QTL mapping is the main method in quantitative trait genetic analysis, which gives an initial point for mapping, cloning and marker-assisted selection of related genes (Xu et al. 2015). It is an essential method to discover association between genome and phenotype of organisms under various environmental conditions (Soda et al. 2015). Most of the phenotypic differences in quantitative trait loci have been identified due to few loci with great effect and many loci with minor effects (Maki–Tanila and Hill 2014). In QTL mapping, population size of the sample is a major factor since small sample size could fail to detect minor-effects of QTLs (Svishcheva et al. 2012; Belonogova et al. 2013). The remarkable diversity in morphology or architecture in cucumber crop varieties or lines provides tremendous research input potentials to explore and to understand molecular mechanisms driving for the agronomic, fruit yield and quality related parameters of cucumber which can help to facilitate efficient exploitation of important traits in breeding.

Currently, some cucumber plant architecture-related studies on QTLs and genes are identified, cloned or fine mapped (Bo et al. 2016; Pan et al. 2017, 2020; Wang et al. 2017a, 2020). However, there is lack of an organized record of the mutants, cloned genes or QTLs related to cucumber architecture. Moreover, naming used in several research studies is not similar. Therefore, it is important to collect consistent names and records for further investigation and as to be used as references as a teaching material. Hence, the objective of this chapter is to organize these research findings on mutants, QTLs and genes for plant architecture- and fruit-related molecular experiments.

4.2 Plant Architecture-Related Traits

4.2.1 Hypocotyl Length

Hypocotyl length is an important agronomic trait of cucumber. The hypocotyl is the embryonic stem connecting the cotyledons and the radicle in dicotyledonous plants such as in cucumber

seedlings. The hypocotyl is a kind of plastic organ in which many external and internal factors such as hormones, light, gravity and temperature strongly influence its growth and development (Scheres et al. 1994).

The simplicity of hypocotyl morphology and growth behavior are ideal for studying the mechanisms of cell elongation, and mutants of this organ are readily available, and the easy availability of mutants in this organ provides material conditions for further understanding the mechanism controlling cell elongation (Vandenbussche et al. 2005; Boron and Vissenberg 2014; Wang et al. 2017a; Bo et al. 2019).

Miao et al. (2012a) and Wang et al. (2016) performed QTL mapping study for hypocotyl length (*hl*) and identified 11 QTLs on chromosome 1, 2, 3, 5 and 6 of cucumber. However, from these two research results, seven consensus QTLs of cucumber hypocotyls length are inferred (Wang et al. 2020). These QTLs of cucumber hypocotyls length identified so far are: *hl1.1, hl2.1, hl3.1, hl3.2, hl5.1, hl6.1 and hl6.2* (Table 4.1). QTLs of *hl5.1, hl5.2 and hl5.3* were identified by Miao et al. (2012b) with the similar PVE% and position as well as on the same chromosome 5. Hence, scientists have made consensus to be known as *QTL 5.1* (Wang et al. 2020) after reviewing research results published until the end of 2019. Similarly, *hl6.1* and *hl6.2 QTLs* identified by Miao et al. (2012a) were found to have all the same PVE and position as well as detected on the same chromosome 6. However, Wang et al. (2016) identified hl6.1 with different PVE (%) value and position but on the same chromosome 6 (Table 4.1). As a result, the two QTLs identified by (Miao et al. 2012a) have been given consensus QTL name of *hl6.2* (Wang et al. 2020). Therefore, currently seven consensus QTLs for hypocotyl length in cucumber are identified, namely, *hl1.1, hl2.1, hl3.1, hl3.2, hl5.1, hl6.1* and *hl6.2*. Of the seven hypocotyl length QTLs identified in two RIL populations, six have moderate effect (PVE ~ 10%) and one (*hl6.2*) has a large effect (PVE = 22.6%) (Miao et al. 2012a; Wang et al. 2016), but none of them is co-localized with the mutant *short hypocotyl length* (*sh1*).

There is a clear understanding about the hypocotyl length and its association with environmental factors such as the temperature. Environmental conditions play an important role in hypocotyl elongation of modern commercial cucumber (Xu et al. 2015). Too long hypocotyl length will lead to poor transplanting quality of seedlings, and the factors that cause the increase of hypocotyl length include high temperature or low light. Two cucumber varieties, namely, the Xishuangbanna (*C.s.* var. *xishuangbannesis*, XIS) and the wild HARD cucumbers, are known for their amplified *short hypocotyl1* (*sh1*) allele, which gives hypocotyl elongation not sensitive to UVB-free light and temperature changes (Bo et al. 2016) (Fig. 4.3). *Sh1* (*CsSH1*) encodes a human SMARCA3-like chromatin remodeling factor. The *short hypocotyl1* mutation in cucumber may have been taken as an advantage to use for mass seedlings production by farmers in protected environmental condition.

The slow cucumber seedling growth habit is associated with *short hypocotyl* trait which contributes to compact growth habit which is desirable for fast seedling establishment, and easy management practices such as for harvesting and application of pesticides in cucumber production. The *short hypocotyl* characteristics may be the very reason why it was selected against other cucumbers during domestication. An SNP in the *Sh1* locus causes (Low Dosage Ultraviolet B) LDUVB insensitive hypocotyl elongation in the XIS cucumber. However, since LDUVB is always present in nature, it does not mean the *Sh1* allele in the LDUVB insensitive varieties are free from the stress of LDUVB, because LDUVB may also suppress *Sh1* expression (Bo et al. 2016).

4.2.2 Plant Height (Vine Length)

Plant height/vine length is one of the major plant architecture attributes in crop breeding. Short plant height or dwarf growth habit is considered as an essential characteristic in cucumber crop improvement. Mutants and their QTLs have been identified for plant height, which were often

Table 4.1 QTLs for Hypocotyl length (*hl*) and vine length (*vl*) of cucumber

Consensus QTL	QTLs used in Literature	Gy14 V2.0 Location					References
		PVE (%)	Chr	Position Left	Position Right	# Populations or Environments	
hl1.1	*hl1.1*	10.30	1	27,367,230	29,537,810	PI 183,967 × 931 RILs	Wang et al. (2016)
hl2.1	*hl2.1*	11.30	2	10,842,002	16,266,343	PI 183,967 × 931 RILs	Wang et al. (2016)
hl3.1	*hl3.1*	15.10	3	3,068,319	7,680,267	PI 183,967 × 931 RILs	Wang et al. (2016)
hl3.2	*hl3.2*	12.70	3	27,203,767	29,471,880	PI 183,967 × 931 RILs	Wang et al. (2016)
hl5.1	*hl5.1, hl5.2, hl5.3*	13.40	5	24,601,636	26,431,293	9110Gt × 9930 RILs	Miao et al. (2012a)
hl5.1	*hl5.1*	14.80	5	23,964,780	24,325,543	PI 183,967 × 931 RILs	Wang et al. (2016)
hl6.1	*hl6.1*	11.40	6	23,915,023	24,417,940	PI 183,967 × 931 RILs	Wang et al. (2016)
hl6.2	*hl6.1, hl6.2*	22.60	6	27,227,724	31,212,546	9110Gt × 9930 RILs	Miao et al. (2012a)
vl1.1	*ph1.1*	32.10	1	27,367,230	28,141,503	9110Gt × 9930 RILs	Miao et al. (2012b)
vl6.1	*ph6.1*	9.60	6	17,987,073	22,022,192	9110Gt × 9930 RILs	Miao et al. (2012b)

Fig. 4.3 Variation in hypocotyl length, where the CC3 and F_1 seedlings are sensitive to LDUVB but SWCC8 seedlings were not affected by LDUVB application (photo by the authors)

associated with hormones like BR (brassinosteroids). The dwarf mutants associated with BR would not undergo skotomorphogenesis, and these mutants show a decreased hypocotyl length and de-etiolated phenotype characteristics with open cotyledons (Tanabe et al. 2005). To date, reports indicated that only six mutants which have short internode length have been identified in cucumber such as *compact* (*cp*) (Van der linden 2018), *compact-1* (*cp-1*) (Li et al. 2011), *short internode* (*si*) (Lin et al. 2016), *super compact-1* (*scp-1*) (Wang et al. 2017a), *super compact-2* (*scp-2*) (Hou et al. 2017) and *dwarf* (*dw*) (Xu et al. 2018).

The internodes of *scp-1*, *scp-2, dw* and *cp-1* mutants are naturally found as very dwarfed and have little importance in practical application (Wang et al. 2020). The *scp-1* and *scp-2* mutants result from mutations in different genes involved in BR (brassinosteroid) hormone biosynthesis pathway, and the former encodes BR-C6-oxidase (*CsCYP85A*), while the latter encodes steroid 5-

alpha-reductase (*CSDET2*) (Wang et al. 2017b; Hou et al. 2017).

The *scp-1* is extremely dwarfed as a result of practically no internode elongation. Wang et al. (2017a) found *scp-1* could be a BR-deficient mutant, because exogenous application of brassinolide (BL) can make the mutant undergo skotomorphogenesis in dark growing condition. Furthermore, Wang et al. (2017a) identified the Cytochrome P450 gene, *CsCYP85A1,* might be a possible candidate gene for *scp-1* mutation in cucumber. *SI* mutants have short internode (about 50% of WT) and small fruit, which encodes part of the VIER F-BOX PROTEIN member of the F-Box protein (*CsVFB1*) (Lin et al. 2016). Two QTLs, namely, *ph1.1 and ph6.1* were identified by Miao et al. (2012b) for Vine length (plant height). The two QTLs have been given consensus QTLs names of *vl1.1* and *vl6.1,* respectively, to have standard nomenclature of QTLs in cucumber vine (Wang et al. 2020).

A relationship between vine length (plant height) and hypocotyl length may exist. Vine length (vl) is influenced by hypocotyl length (hl) in cucumber. To determine the linkage connecting the vl and hl traits, QTL mapping was carried out using vine length (at 5-true leaf stage) and hypocotyl length in the 124-RIL, the result indicated that the pleiotropic effect of *sh1* allele showed to slow down seedling growth on vine length (Bo et al. 2012). Hence, seedlings with shorter hypocotyl length may lead to shorter vine length.

4.2.3 Lateral or Primary Branch Numbers

The number of lateral or primary branches per cucumber plant is an important agronomic trait affecting the productivity and quality of fruits. Lateral branches of plant can directly influence the size and density of the canopy and consequently may affect disease incidence, yield and quality of fruits in cucumber. For instance, plants with more open canopies, compact growth habit or reduced branch architecture can decrease disease occurrence. Gevens et al. (2006) observed that lower disease occurrences was recorded from cucumber line with compact growth habit and provided reduced young fruit off to the ground. The reduced disease incidence was not due to natural resistance, but significantly due to the architectural traits which permit less contact of fruit with the soil which are practical for *Phytophthora capsici* management of pickling cucumber (Ando and Grumet 2006). Therefore, knowledge of the molecular mechanism of lateral branch number is important for cucumber breeding improvement.

Considerable variations exist among plants in measurements of the lateral branch numbers (LBN). QTL mapping for this trait was conducted using primarily a RIL population from the cross of S94 × S06 (Qi et al. 2013), in which six QTLs were identified including two major-effects QTLs (*lbn1.2* and *lbn6.2*). The LBN consensuses QTLs in cucumber identified so far are *lbn1.1*, *lbn1.2, lbn3.1*, *lbn6.1*, *lbn6.2* and *lbn7.1* (Table 4.2).

The *littleleaf* (*ll*) mutant H19 has several lateral branches, possibly due to the pleiotropic effect at the *ll* locus (Yang et al. 2018). The two QTLs of lateral branch number (*lbn1.2* and *lbn6.2*) of cucumber are not located near to the *ll* locus which shows multiple mechanisms may regulate LBN in cucumber.

4.2.4 Leaf Size/Shape

The leaf size trait plays a crucial role affecting plant architecture, photosynthesis ability, yield and fruit quality (Tsukaya 2006; Feng et al. 2020). There are ten leaf mutants that have been identified in cucumber, including *yellow mutant* (*yp*), *virescent-yellow leaf* (*vyl*), *virescent leaf* (*v-1*), *variegated leaf* (*vl*), *paternal sorting of mitochondria* (*psm*), *curly-leaf* (*cul*), *round leaf* (*rl*), *little leaf* (*ll*) (Weng et al. 2010), *glabrous3* (*gl3*) and *small and cordate leaf* (*scl-1*).

The chlorophyll deficient *yellow mutant* (*yp*) C528 is identified to have a golden leaf color trait. The possible gene for this mutation was reported as *CsChlI*, and the gene was identified

Table 4.2 QTLs for lateral branch number and their chromosomal locations in cucumber

Consensus QTL	QTL name in Literature	Gy14 V2.0 Location				# Populations
		PVE (%)	Chr	Position—Left	Position—Right	
lbn1.1	*lbn1.1, flbn1.1*	27.60	1	3,435,063	8,393,188	S94 × S06 RILs
lbn1.2	*11 qtl*	6.60	1	28,341,342	31,105,895	S94 × S06 RILs
lbn3.1	*lbal5.1*	3.50	3	4,419,164	5,339,800	S94 × S06 RILs
lbn6.1	*4 qtl*	8.40	6	10,767,736	19,391,504	S94 × S06 RILs
lbn6.2	*15 qtl*	32.30	6	22,994,885	27,270,372	S94 × S06 RILs
lbn7.1	*lbal7.1, lbtl7.1*	3.80	7	3,274,145	11,236,180	S94 × S06 RILs

Source Jiang et al. (2008); Wang et al. (2020)

to encode the CHLI subunit of cucumber Mg-chelatase (Gao et al. 2017). *CsVYL* encodes a DnaJ-like zinc finger protein; mutation in this gene results in reduced pigment content and delays chloroplast development (Song et al. 2018). The *virescent leaf* (*v-1*) mutant has light yellow cotyledons; the first 2 to 3 true leaves are also light yellow in color but turn later to green color when fully grown. The color is also temperature dependent. The *v-1* locus was putatively shown to encode cyclic nucleotide-gated ion channel (CsCNGC) proteins (Miao et al. 2016).

The *Csvl* mutant (*vl*) shows green-yellow-white phenotypes throughout the growth cycle with defective chloroplasts in mesophyll cells. The *Csa6G405290* (*Cscs*) is the putative gene for the *Csvl*, which encodes chorismate synthase (Cao et al. 2018). *Pentatricopeptide repeat (PPR) 336* was proposed to be the candidate for *psm*, which influences the predominant mitochondria transmitted to offspring (Del Valle–Echevarria et al. 2016). The leaf edges of *cul-1* and *cul-2* rolled upward to form a shallow cup-shaped form, both of which are due to allelic mutations in the HD-Zip III type transcription factor gene, *CsPHB*. The *TEN* genes encoding the TCP transcription factor results in tendril-free mutations. In Chr6, another *tendrilless1* (*td-1*) mutation is mapped on the 190 kb region (Rong et al. 2019).

Three round leaf mutants, *rl-1*, *rl-2* and *rl* have been mapped, which are all due to mutations in the *PINOID (CsPID)* gene encoding for the auxin polarity transporter PIN (pin-formed) (Song et al. 2019; Zhang et al. 2016). The round *leaf mutant* is regulated by a single recessive gene (*rl*) and the candidate gene is identified as *Csa1M537400* encoding PINOID kinase protein which is involved in auxin transport (Song et al. 2019). Hence, auxin signaling pathways may have role in regulating the molecular mechanism of cucumber leaf shape.

The *little leaf* (*ll*) gene that controls leaf size was identified on chromosome 6 between SSR02355 and SSR03940 markers (Weng et al. 2010). The *ll* locus was mapped in 57 cM proximal to *de* (Fazio 2003). Zhu et al. (2016) identified 11 QTLs for *determinate (de)* and *little leaf (ll)* traits. Using mapping of 145 RILs of two markers, SSR21758 and UW083795 were found closest to *LL locus*, at 1.0 and 0.6 cM, respectively. Using these two markers, 29 recombinants were identified from F_2 individuals obtained from H19 and G421 parents (Feng et al. 2020). According to these studies, marker-assisted molecular tools as breeding strategy can be effective for *ll* genes-related variation in cucumber.

The glabrous (*gl3*) mutant of leaf is controlled by a single recessive gene and *Csa6M514870* is identified as the candidate gene of *gl3* mutant (Cui et al. (2016). Gao et al. (2017) isolated the *scl-1* gene which regulates leaf size and shape of cucumber using BSA-seq and the production of *scl-1* mutant was caused by single nucleotide polymorphisms, which may involve in a putative nucleoside bisphosphate phosphatase.

Although limited works have been done, leaf-related molecular-based research results may

contribute great impact in modern molecular breeding to improve the yield and quality traits of cucumber.

4.3 Fruit Quality-Related Traits

4.3.1 Fruit Length/Diameter

There is remarkable diversity of fruit length and fruit diameter architectural traits among cucumber varieties. Fruit size is usually measured by Fruit Length (FL) and Fruit Diameter (FD) ratio, which is a quantitative trait in nature (Weng et al. 2015). FL and FD are usually used to describe fruit elongation and radial growth (Pan et al. 2020). The fruit shape and size traits are highly influenced by the FL/FD ratio.

Wild cucumber (*C. s.* var. *hardwickii*) typically produces small and round-shaped fruits having 3–5 cm in diameter and weighing 25–35 g per fruit, whereas cultivated cucumber varieties weigh up to 5 kg having different fruit diameter and length. Fruit length and fruit diameter affects consumers' choice and market demand. Consumers in North China prefer fresh cucumber with 25–30 cm fruit length and 2.5–3.1 cm of diameter, considered as premium grade in the market (Zhou et al. 2005). In Canada for the Dutch type of greenhouse cucumber, fruit should have more than 28 cm of fruit length and more than 4.1 cm of diameter to be considered as first grade cucumber fruit (Canadian Food Inspection Agency Cucumber Standards, http://www.inspection.gc.ca/). The pickling cucumbers are ideally preferred to have FL/FD ratios of approximately 3.0 (Wenzel et al. 1995).

Fruit length generally has relatively high narrow-sense heritability (Strefeler and Wehner 1986). The unique qualitative variations in cucumber fruit length and diameter have attracted breeders to investigate the fundamental genetic base and molecular mechanisms of breeding. The fruit size and shape of nine typical cucumber inbred lines were studied, then fruit length trait has strong positive correlation to the cell number in the longitudinal section, but negatively correlated results were observed between fruit length and fruit cell size (Liu et al. 2020).

The first QTL mapping study on fruit quality traits, such as fruit diameter, fruit length, peel color, fruit length/diameter ratio, seed cavity diameter and seed cavity/fruit diameter ratio in cucumber, was conducted by Wenzel et al. (1995). One complicating factor of QTL records is the inconsistent names QTL used in various studies (Pan et al. 2020; Wang et al. 2020).

Yuan et al. (2008) identified 5, 6 and 15 QTL for FW (*fruit weight*), FSI (*fruit size index*) and FL/FD ratio, respectively. Recently, Pan et al. (2020) reported three QTLs: *CsFW3.1*, *CsFS1.2* and *CsFSI5.2* for FW, FS (*fruit size*) and FSI, respectively. Twelve QTLs (*ldr1.1, ldr1.2*, *ldr1.3*, *ldr4.1, ldr4.2*, *ldr4.3,* ldr4.4*, ldr6.1, ldr6.2, ldr6.3, ldr6.4* and *ldr7.1)* were also detected for FL/FD ratio. Most of these QTLs were found to have LOD scores of $\geq$ 3.5. Five QTLs, namely, *ldr1.1*, *ldr1.3*, *ldr6.1*, *ldr6.3* and *ldr7.1*, indicated consistent in response to magnitude and effect of test environments (Fazio 2003). The QTLs of *ldr1.2* and *ldr1.3* were associated with determinate *(de)* and *little leaf (ll)* habit.

Zhu et al. (2016) identified five QTLs for fruit length (*fl3.1*, *fl3.2*, *fl4.1*, *fl6.1* and *fl7.1)* and six QTLs (*fd1.1*, *fd3.1*, *fd5.1*, *fd5.2*, *fd6.1* and *fd7.1*) for fruit diameter. Moreover, Weng et al. (2015) found three QTLs associated with fruit length located on chromosomes 3, 4 and 6; and three QTLs related to fruit diameter which were located on chromosomes 2, 5 and 6, whereas Zhou et al. (2015) found two QTLs for fruit length which were mapped on chromosomes 5 and 7.

The immature and mature fruits diameter variation in cucumber may be independently regulated. Recently, one QTL (*fd3.2*) for *immature fruit diameter* and four QTLs (*mfd1.1, mfd1.2, mfd3.1* and *mfd3.2*) for *mature fruit diameter* were identified in cucumber (*Cucumis sativus* var. *sikkimensis*) (Wang et al. 2021). QTLs of *cFL4.1* for immature fruit length and *cFD6.1* for immature fruit diameter are detected on chromosomes 4 and 6, respectively, while the QTLs for matured fruit length and fruit diameter are identified as *FL2.2* and *FD3.1,* respectively

(Pan et al. 2020). This indicates that immature and matured fruits have different QTLs for the same trait. Four QTLs were detected for *immature fruit length*: *fl1.1*, *fl2.1*, *fl3.1* and *fl5.1*, and four QTLs for *mature fruit length*: *mfl1.1*, *mfl2.1*, *mfl3.1* and *mfl5.1*, where both *mfl3.1* and *mfl5.1* had large effects on fruit elongation (PVE > 15%), while the other two had relatively smaller effects (Wang et al. 2021). This research provided evidence that the *immature fruit length* and *mature fruit length* on each chromosome are co-localized telling the same set of QTL controls fruit elongation throughout the developmental stages of fruits in cucumber.

The *fruitful1* (*CsFUL1*) gene regulates fruit elongation in "9930" (Chinese Long) cucumber line (Zhao et al. 2019). Pan et al (2020) recently studied QTLs related to fruit size (FS), fruit shape index (FSI) and fruit weight (FW) and revealed wide-ranging conservation of fruit shape/size genes homologs in *Cucurbitaceae*, in which fruit shape/size candidate genes *CsTRM5* and *CsSUN25-26-27a* alignment were amplified in cucumber. Wang et al. (2021) identified 15 QTLs for fruit size, variation in *Cucumis sativus* var. *sikkimensis*. Pan et al. (2017) found eight FS/FSI QTLs, among which *FS5.2* showed the major effect to cause round fruit shape trait in cucumber.

Two *short fruit* mutants, *short fruit-1* (*sf-1*) and *short fruit-2* (*sf-2*), are known so far (Xin et al. 2019; Wang et al. 2017b), where the later encodes a cucurbit specific RING-type E3 ligase, which enhance self-ubiquitination and degradation, and increases expression of *ACS2* which regulates fruit shape elongation. The fruit elongation may result due to this expression where an alellic mutation may occur in the *m* locus (*m-1*) on the andromonoecious plants that frequently set round fruits (Tan et al. 2015).

The change of cucumber fruit size was also affected by the number of fruit carpel (CN) (Tan et al. 2015). CN difference (3 vs 5) is regulated by the *Cn* gene which encodes the CLAVTATA3 protein (Li et al. 2016).

Cucumber fruit traits are not always round or oval, but also may be oblong, ellipsoid, long or very long (Bisht et al. 2004; Yang et al. 2012; Pan et al. 2020; Fig. 4.4).

Fig. 4.4 FNL variation in cucumber natural population (photo by the authors)

Mango-shaped fruit (*mango fruit, mf*) traits emerge due to an SNP in the *WUSCHEL-related homeobox1 (CSWOX1)* gene (Niu et al. 2018).

The genetic diversity in fruit length and diameter which influences the fruit size/shape trait in cucumber may provide opportunity to explore the underlying genetic basis and molecular mechanisms driving fruit development, which will help efficient exploitation of fruit size and shape for cucumber yield and quality improvements through molecular breeding.

4.3.2 Fruit Neck Length (FNL)

FNL is an important fruit quality trait of cucumber that may directly impact market acceptance and price. Variation of FNL in commercial harvesting stage among cucumber varieties may vary from 1–12 cm accounting up to 35% from the total length of the fruit (Zhao et al. 2019) (Fig. 4.4). Cucumber fruits with long fruit necks are not preferred by consumers due to the fact that it affects fruit appearance and has unwanted bitter taste because of the absence of fleshy tissue in the neck part (Fanourakis and Tzifaki 1992; Zhou et al. 2005; Che and Zhang 2019). Moreover, the

neck is also undesirable during harvesting and postharvest handling. It can easily break during harvesting and postharvest handling including packing and transporting (Fanourakis and Tzifaki 1992). In north China, cucumber fruit neck length with more than 14.3% or 1/7 ratio from the total length of the fruit is not preferred in fresh market cucumbers (Zhou et al. 2005).

FNL is a quantitative trait, and only limited work has been done on its genetic basis. Using an F_2 population by crossing YN (short-necked) and Jin5-508 (long-necked), Xu et al. (2020) identified a candidate gene for the major-effect FNL QTL *Fnl7.1* on chromosome 7, which encodes a late embryogenesis abundant protein. Xu et al. (2020) further showed that variation in the promoter of this candidate gene between YN and Jin5-508 can lead to *CsFnl7.*1 expression increase in long neck Jin5-508. The function of *CsFnl7.*1 was confirmed by overexpression of the gene in transgenic cucumber, and it was found that *CsFnl7.*1 regulated fruit neck growth by regulating cell expansion rather than changing cell number.

4.3.3 Flesh Thickness

Fruit flesh thickness (fft*)* is the main fruit trait which directly determines the yield and quality of cucumber fruits. Thicker fruit flesh has greater edible portion. The fruit flesh thickness growth starts to fill and is escorted by expanding the vacuolization between 4 and 12 days after anthesis (Ando et al. 2012).

For cucumber fruit thickness, the molecularly tagged gene and QTL are identified using SSR marker-based QTL mapping. Xu et al. (2015) identified *fft2.1* on chromosome 2 which controls fruit flesh thickness. Higher expression level of *Csa2M058670.1* (SET domain protein–lysine methyltransferase) was found in thicker fruit parent, D8, compared with thin fruit flesh parent, XUE1, during fruit flesh development. Hence, *Csa2M058670.1* gene is considered as the putative candidate gene that controls cucumber fruit flesh thickness (Xu et al. 2015). Wang et al. (2021) also reported four QTLs for fruit flesh thickness variation in two populations obtained from Sikkim-type inbred lines (WI7120 and WI7088D). QTLs: *fth3.1* and *fth5.1* for *immature fruit thickness* and *mfth3.1* and *mfth5.1* for *mature fruit thickness* were identified (Wang et al. 2021), in which larger-effect QTLs, *fth5.1* and *mfth5.1*, were indicated in both immature and mature fruit stages (Table 4.3). Thicker flesh (mesocarp) was found in WI7088D and *fth3.1/mfth3.1* and *mfth5.1/fth5.1* alleles of WI7088D were identified to cause opposite effects that increase or decrease flesh thickness, respectively. Little works have been done on

Table 4.3 Some QTLs of cucumber fruit traits

Traits	QTL	Reference
Fruit size	*CsFSI5.2*	Pan et al. (2020)
Fruit length-diameter ratio	*ldr1.1*, *ldr1.3*, *ldr6.1*, *ldr6.3* & *ldr7.1*	Fazio (2003)
Immature fruit length	*cFL4.1*	Pan et al. (2020)
Mature fruit length	*FL2.2*	Pan et al. (2020)
Immature fruit diameter	*cFD6.1*	Pan et al. (2020)
Immature fruit diameter	*fd3.2*	Wang et al. (2021)
Mature fruit diameter	*mfd1.1, mfd1.2, mfd3.1* & *mfd3.2*	Wang et al. (2021)
Mature fruit diameter	*FD3.1*	Pan et al. (2020)
Fruit shape index	*CsFS1.2*	Pan et al. (2020)
Fruit weight	*CsFW3.1*	Pan et al. (2020)
Fruit flesh thickness	*fft2.1*	Xu et al. (2015)
Immature fruit thickness	*fth3.1* & *fth5.1*	Wang et al. (2021)
Mature fruit thickness	*mfth3.1* & *mfth5*	Wang et al. (2021)

flesh thickness genetic and molecular mechanism in cucumber and research in this need to be fostered.

4.3.4 Fruit Spine Density

The cucumber fruit spine density has a vital influence on the marketable values, and spine density of different ecotypes of cucumber was different (Fig. 4.5). Fruits with few or smooth spines are preferred in the US and European market but in China, the north China-type cucumber with dense fruit spines is popular (Zhang et al. 2016b). Most customers prefer cucumber fruits with no or few spines, this may be due to the advantages of these fruits suitable to pack, store, transport and clean (Zhang et al. 2010; Li et al. 2015; Chen et al. 2016).

The effect of cucumber breeding for decades in different regions of the world has resulted in four main types of spines, namely: smooth (no) spines, few spines, dense spines and numerous spines. Furthermore, the number of spines on the lower, middle and upper surface of a cucumber fruit can be differently scattered (Bo et al. 2019a). Even for these cucumber fruits with dense spines, the fruit spine number may vary from variety to variety (Bo et al. 2019a).

The few-spine trait is dominant over the numerous-spine trait. The numerous-spine trait is regulated by a single gene, *ns* (*numerous spines*) (Zhang et al. 2016b), in which the preliminary genetic mapping of the *ns* gene was detected on chromosome 2 of cucumber. The genetic distance between the two closest flanking markers of *ns* gene, SSR22338 and SSR11596, was 10.2 cM and 1.7 cM, respectively (Zhang et al. 2016b). In combination with fine mapping, genetic diversity and transcriptome analysis, Xie et al. (2018) found that auxin transporter gene *ns* plays an important role in the development of cucumber fruit spine.

Zhang et al. (2016a) reported that the *fs1* gene regulates the few spines trait in cucumber fruits. Bo et al. (2019a) conducted QTL mapping on spine density, and identified three QTLs: *fsd4.1, fsd6.1* and *fsd6.2* (Fig. 4.6). *Csgl3* that regulates high density of fruit spine is predicted at the *fsd6.2* locus in natural cucumber plants. Moreover, a novel locus *fsd6.1*, which controls ultra-high density of fruit spines together with *Csgl3*,

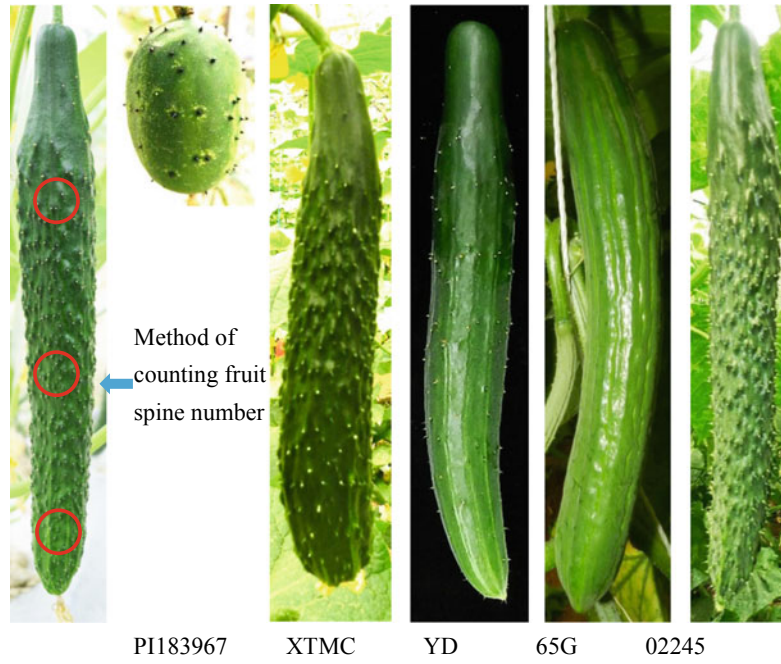


Fig. 4.5 Fruit spine density counting method and the phenotype variation of spines in five cucumber varieties (photo by the authors)

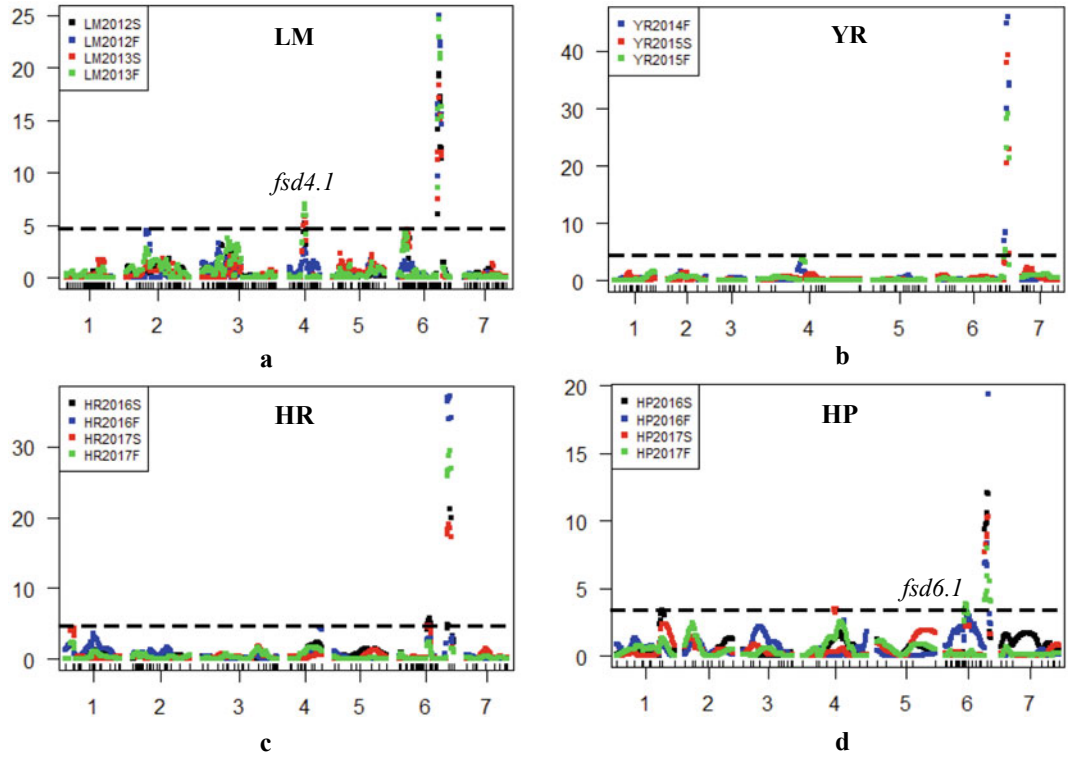


Fig. 4.6 Whole-genome examination of QTL mapping of fruit spine number in LM (**a**), YR (**b**), HR (**c**) and HP (**D**) cucumber populations. Fifteen trials were conducted according to CIM model in R/qtl. The *X*-axis indicates the linkage map of all the seven chromosomes, and the *Y*-axis represents the LOD score; the horizontal dotted line shows the LOD threshold achieved with 1000 permutation tests ($P = 0.05$) (drawing by the authors)

was identified by GWAS, and this locus evolved with cucumber domestication (Bo et al. 2019a). According to this evidence, *Csa6G421750* is the possible gene that controls ultra-high density of fruit spine in natural cucumber population.

4.3.5 Flesh Color and Peel Color

Both fruit flesh color and peel color are vital cucumber fruit quality traits which influence consumers' choice. The cucumber fruit flesh color can be Orange (O), Light Orange (LO), Yellow (Y), Yellow Green (YG), Light Yellow (LY), Green Yellow (GY), Light Green (LG), Green (G) or White (W) (Chart 1966; Cuevas et al. 2010). There are obvious differences among immature and mature fruit colors (Fig. 4.7a, b) in cucumber on its external (peel) fruit which is also known as exocarp and internal (flesh) fruit color which includes the mesocarp and endocarp part of the fruit.

Fruit flesh color is a main feature of cucumber breeding (Adami et al. 2013; Bo et al. 2019b). Most cucumber fruits have white flesh color. Some such as the semi-wild XIS cucumber fruits have *orange flesh* (Qi et al. 2013) due to accumulation of high-level β-carotene at mature fruit stage (Bo et al. 2012). The *ore* (*orange endocarp flesh*) gene that regulates the β-carotene biosynthesis in XIS cucumber fruits has been cloned and the *ore* gene is located on chromosome 3. The candidate gene is identified as *CsBCH1* (Qi et al. 2013). The *yellow flesh* (*yf*) locus was also fine mapped (Lu et al. 2015). Carotenoid and chlorophyll are the major pigments in flesh color formation (Li and Yuan 2013).

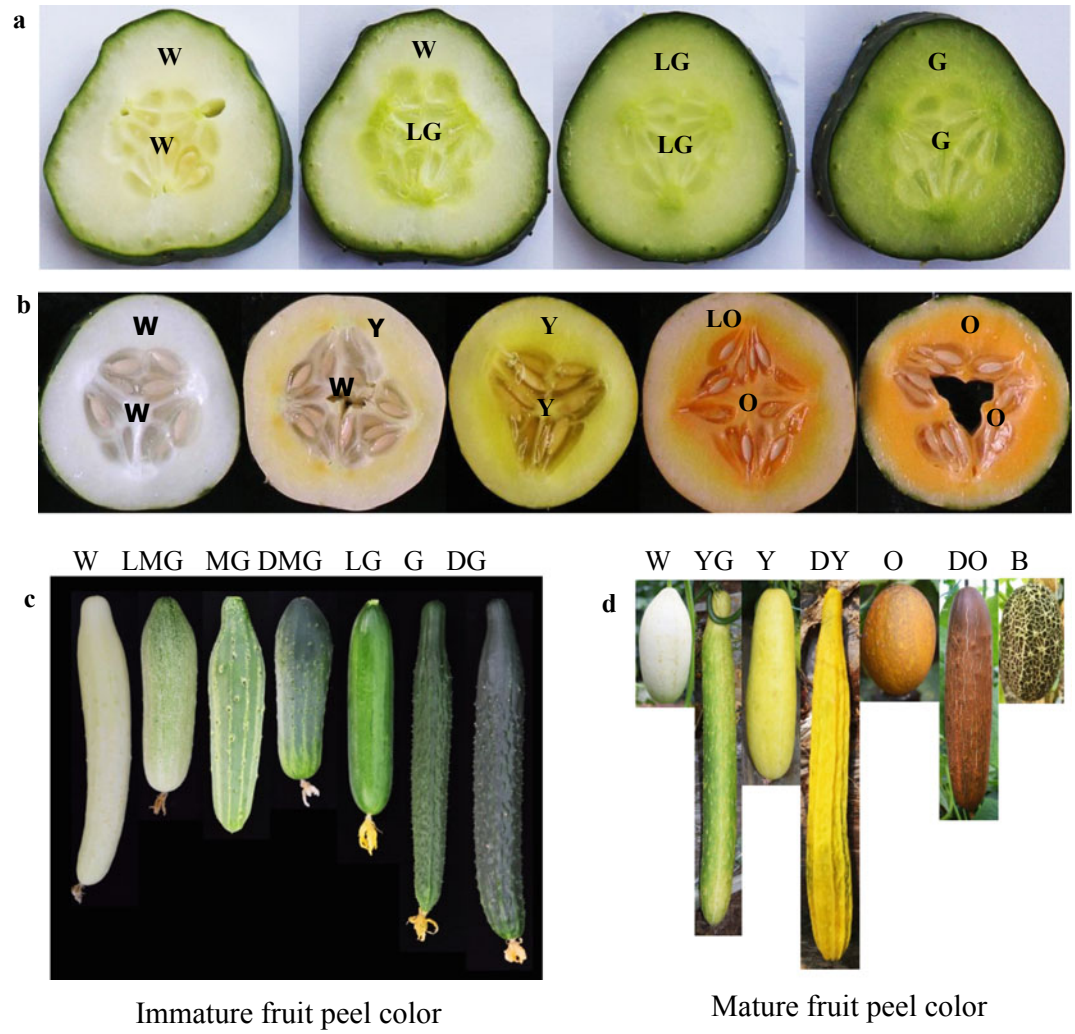


Fig. 4.7 Immature flesh (**a**), mature flesh (**b**), immature peel (**c**) and mature peel (**d**) fruit color in cucumber labeled as white (W), light green (LG), green (G); yellow (Y), light orange (LO), orange (O), light mosaic green (LMG), mosaic green (MG), dark mosaic green (DMG), dark green (DG), yellow green (YG), dark yellow (DY), dark orange (DO) and brown (B) color (photo by the author)

Yellow and orange flesh colors appear on matured fruits of some cultivars. A single recessive gene controls orange endocarp, whereas two recessive genes regulate orange mesocarp (Cuevas et al. 2010). Kooistra (1971) reported the yellow flesh color in cucumber and suggested that flesh color including yellow, orange, white, dingy and intense white may be regulated by two genes. The *yellow flesh* trait of cucumber is regulated by a recessive gene (*yf*) mapped on chromosome 7 between yfSSR108 and yfindel29 with physical distance of 149.0 kb containing 21 candidate genes (Lu et al. 2015).

Song et al. (2010) identified QTLs related to orange flesh color where two genetic linkage maps were constructed using seven markers. According to this report, the QTLs detected for orange mesocarp (*mc*)/endocarp (*ec*) were *mc6.1*/*ec6.1* and *mc3.1*/*ec3.1*. A locus for yellow skin in watermelon (*Citrullus lanatus* L.) was also identified by Dou et al. (2018) using BSA-seq and GWAS in F_2 and BC_1 populations.

Table 4.4 QTLs and genes of flesh fruit color and peel color in cucumber

Trait	QTL/mutant	Gene	Reference
Orange flesh	*Ore*	*CsBCH1*	Bo et al. (2012), Qi et al. (2013)
Green flesh color	*qgf5.1 & qgf3.1*	*Csa5G021320*	Bo et al. (2019b)
Yellow flesh	*yf*	*N/A*	Lu et al. (2015)
Orange mesocarp flesh	*Mc3.1, mc6.1*	*N/A*	Song et al. (2010) Yuan et al. (2015)
Orange endocarp flesh	*ec3.1, ec6.1*	*N/A*	Yuan et al. (2015)
White peel	*w*	*CsAPRR2*	Liu et al. (2016)
Light green peel	*lgp*	*CsYcf54, CsARC5*	Lun et al. (2016), Zhou et al. (2015)

QTL mapping experiment revealed that two novel loci: *qgf5.1* and *qgf3.1* were associated with green fruit flesh color development in cucumber (Table 4.4). The green flesh color is controlled by a major-effect *qgf5.1* (Bo et al. 2019b, Table 4.4). According to this experimental evidence, *Csa5G021320* is the putative gene of *qgf5.1* controlling green flesh color in cucumber in natural population which is found more in higher latitude region of the world.

According to Hao et al. (2018), the yellow green peel (*ygp*) is due to mutation in the *Csa2G352940* gene encoding *MYB36* transcription factor. While, the white immature fruit peel color is caused by *Csa3G904140* gene in cucumber plants (Tang et al. 2018). However, there is lack of evidence whether the peel color genes/QTLs also regulate the flesh colors.

There was a wide spectrum of peel colors in cucumber fruit, and immature cucumbers have a very different peel color from ripe cucumbers (Fig. 4.7C, D). The *white* peel color (*w*) is caused by mutation in the *CsAPRR2* gene, which influences the fruit pigment accumulation (Liu et al. 2016). The mutations of *lgp* (*light green peel*, *CsARC5*), *lgf* (*light green fruit*, *CsYcf54*) genes and *w* (*white*, *CsAPRR2*) cause change of dark green fruit color into light green or loss of green (Zhou et al. 2015; Liu et al. 2016; Lun et al. 2016). In some cucumber varieties, the mesocarp and endocarp flesh colors are independently regulated, displaying different colors while some other varieties have similar colors. Currently, cloned genes and mapped QTL such as *CsBCH1, yf and qgf5.1* indicated to control both mesocarp and endocarp colors (Qi et al.2013; Lu et al. 2015; Bo et al. 2019a). However, little is known about the relationship and biological mechanisms of fruit flesh color (mesocarp vs endocarp) and fruit peel color (epicarp) variation.

Cucumber crops have diverse genetic resource distributed worldwide, having great variation in architectural and fruit-related traits. These variations may provide opportunities to improve fruit yield and quality using modern breeding strategies. Recent molecular mapping studies revealed new clue to improve cucumber architectural and fruit-related traits. Significant progress has been made in molecular mapping and cloning of economically important genes and QTLs, which may help for cloning mediated and marker-assisted breeding strategies in cucumber improvements in the era of climate change to cope up with food security and high fruit quality demand.

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5 *Agrobacterium Tumefaciens*-Mediated Genetic Transformation in Cucumber

Hanqiang Liu and Yiqun Weng

Abstract

Genetic transformation is a versatile platform that is playing increasingly important roles in functional genomics studies and crop improvement. Cucumber (*Cucumis sativus* L.) is an economically important vegetable crop, and a model of choice to understand several biological processes. Although many genetic transformation studies have been carried out in the last four decades, the transformation efficiency and reproducibility of cucumber transformation remain low limiting its widespread and routine use. In this chapter, we reviewed the work in cucumber on *Agrobacterium tumefaciens*-mediated genetic transformation, and discussed factors affecting its efficiency in each step including genotypes, explant types, the time, temperature and bacterial concentration for co-cultivation, as well as growth regulators and selective agents for transgenic plant regeneration. We emphasized the importance to develop an efficient, reliable and reproducible cucumber genetic transformation system through collaborative and community efforts.

5.1 Introduction

Genetic transformation is a process by which a cell takes up naked DNA from the surrounding medium and incorporates it to acquire an altered genotype that is heritable (Smith et al. 1981). The transgenic plants are expected to show the target phenotype after expressing the alien gene in plant cells. This approach can be used to introduce interested genes into specific crop varieties for crop improvement, as well as verifying the function of a cloned gene.

Trulson et al. (1986) were the first to report genetic transformation in cucumber. Since then, significant efforts have been made to improve genetic transformation efficiency in this crop. Plant regeneration and the integration of alien genes into the recipient plant genome are two main steps in gene transfer. Plant regeneration can be achieved from somatic embryogenesis (Chee 1990a) or direct organogenesis (Ganapathi and Perl-Treves 2000). Cucumber cotyledonary disks or root sections have been used for embryo induction and plantlet generation (Akasaka-Kennedy et al. 2004; Chee 1990b; Hu et al. 2017; Kim et al. 1988; Lou and Kako 1994; Schulze et al. 1995; Trulson and Shahin 1986). More recently, cotyledons and hypocotyls were used as explants for direct plantlet induction (Xu

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et al. 2018; Zhao et al. 2019). Among several methods to deliver target genes into recipient genomes, *Agrobacterium tumefaciens*-mediated transformation has been shown to be the most effective in both monocot and dicot plants (Xu et al. 2018; Yang et al. 2014). In cucumber, many factors have been examined that affect the transformation efficiency, which may include genotypes, explant types, *A. tumefaciens* concentration, inoculation time, temperature and light conditions, selective antibiotics, and selection pressure (e.g., Gal-On et al. 2005; Sun et al. 2017; Zhang et al. 2017). A few 'optimized' cucumber transformation protocols have been reported (e.g., Miao et al. 2009; Sun et al. 2017; Vasudevan et al. 2002; Zhang et al. 2017). However, the transgenic efficiency from these studies varied significantly, and a reliable, and reproducible protocol achieving acceptable transformation efficiency is still lacking (Wang et al. 2015a). In many cases, those protocols lack necessary experimental details for others to reproduce. Some protocols use specific cucumber genotypes or plasmid vectors but the seeds of those lines or vectors are not publicly accessible, which constitute some challenges to their widespread adaption by the cucumber research community.

With the release of the whole genome sequences of cucumber (Huang et al. 2009; Yang et al. 2012; Li et al. 2019; Osipowski et al. 2020) (also see Chap. 3), many candidate genes for horticulturally important traits have been cloned (Wang et al. 2019; Pan et al. 2020; also see Chapter y of this book). Functions for most of those genes have not been characterized due in part to the lack of an efficient genetic transformation system in cucumber. The objective of this chapter is to review the current status of cucumber genetic transformation, and discuss key factors affecting transformation efficiency.

5.2 Overview of Cucumber Genetic Transformation

The methods to deliver the alien genes to plant genome could be broadly classified into two categories: non-biological (including chemical and physical methods) and biological (Chee and Slightom 1992; Rui et al. 2015; Keshavareddy et al. 2018). The *Agrobacterium*-mediated gene transfer is the most widely used and most efficient technique for cucumber genetic transformation (Xu et al. 2018). The genus *Agrobacterium* has five species based on symptoms and plant host range (Gelvin 2003), two of which, *A. tumefaciens* harboring the Ti plasmid and *A. rhizogenes* harboring the Ri plasmid have been reported to mediate transformation in cucumber (Bevan and Chilton 1982; He et al. 2008). Of the two, *A. rhizogenes*-mediated transformation generates transgenic plants from hairy roots, which is technically laborious, used in cucumber nowadays (e.g., van der Mark et al. 1989; Trulson et al. 1986). The *A. tumefaciens* bacterium has the natural ability to transfer the tumor-inducing plasmid DNA (T-DNA) into plant cells through the assistance of its *virulence* (*Vir*) genes during infection (Simpson et al. 1986). The infection machinery facilities the passage of T-DNA through the barriers of plant cell wall, cell membrane and entrance into the nucleus, and final integration into the plant genome (Demirer and Landry 2017). Thus an alien gene could be put into an engineered T-DNA which is used as a vehicle to deliver the target gene into the recipient plant genome (Bevan 1984; Lee and Gelvin 2008). This system makes it possible to transfer and express the alien transgenes in different plant species, even animals (Bulgakov et al. 2006).

In general, a typical *Agrobacterium*-mediated transformation protocol includes the following five main steps. 1) Culture of seeds in Murashige & Skoog basal medium (MS) (1–2 days). 2) Dissect explants (cotyledons) and infect with *Agrobacterium* solution (1–2 min). 3) Co-cultivation of explants with *Agrobacterium* (several days). 4) Shoot regeneration in selective MS media. 5) Rooting of shoots, generation of plantlets, and validation of transgenic plants. In the following sections, we will summarize results from cucumber genetic transformation studies and discuss factors affecting transformation efficiency in each step (also see summary in Table 5.1).

Table 5.1 Summary of main factors influencing transformation efficiency in cucumber

Genotypes	Explant type	Agrobacterium strains	ODsoo or cocn	Growth media	Inoculation time (min)	Cocultivation			AS	Selection agent		Transgene constructs	TransformationEfficiency (%)	References
						Tm(C)	Period	Light	(uM)	Antibiotic	Conc			
Straight 8	Hypocotyl	A. rhizogenes	–	MS	–	27	–	Light	–	Km	25	pnos::nptII	–	Trulson et al. (1986)
Gy3	Hypocotyl	A. rhizogenes	–	MS	–	–	–	–	–	–	–	–	–	van der Mark et al. (1989)
Poinsett 76	Cotyledon	C58z707	$2 *10^8$	–	–	26	4	Dark	100	Km	–	pnos::nptII	–	Chee (1990a)
Endeavor	Petiole	EHA105	10^8	MS	5	27	2–4	Dark	100	Km	50	pnose::ptII	–	Raharjo et al. (1996)
Endeavor	Petiole	MOG101	10^8	MS	5	27	2–4	Dark	100	Km	50	p35S::CH5B	–	Raharjo et al. (1996)
Poinsett 76	Cotyledon	EHA105	0.8–1.0	MS	10–15	25 ± 2	2	–	50	Km	100	ptII, GUS, Bar	–	Ganapathi and Perl-Treves (2000)
Greenlong	Cotyledon	EHA105	–	MS	10	27	2	Dark	100	Km	25	p35S:: nptII + GUS,	12	Vasudevan et al. (2002)
Cengel Koy	Cotyledon	EHA101	0.8–1.0	MS	15–20	25 ± 1	2	Dark	100	Km	50	p35S:: nptII + GUS	16	Kose and Koc (2003)
Cengel Koy	Hypocotyl	EHA101	0.8–1.0	MS	15–20	25 ± 1	2	Dark	100	Km	50	p35S:: nptII + GUS	0.5	Kose and Koc (2003)
Poinsett 76	Cotyledon	EHA105	1	AB	10	–	2	–	200	Km	100–200	p35S::nptII,	1.6	Rajagopalan and Perl-Treves (2005)
Poinsett 76	Cotyledon	EHA105	1	MS	0	25 ± 2	2	Dark	20	Km	150	p35S:: nptII + GUS	14	Sureshkumar et al. (2005)
Poinsett 76	Cotyledon	EHA104	1	MS	10	25 ± 2	2	Dark	20	Km	150	p35S::bar	7.4	Sureshkumar et al. (2005)
Greenlong	Cotyledon	LBA4404	1	AB	10	25 ± 2	3	Dark	50	PPT	2–6	nptII, p35S:: bar,	1.1	Vengadesan et al. (2005)
Jinyan no.7	Cotyledon	EHA105	–	MS	25	–	3	Dark	100	–	–	p35S::PMI	23	He et al. (2006)
Poinsett 76	Cotyledon	EHA105	1	MS	10	25 ± 2	2	16 h/8 h	0	PPT	2	p35S::GUS,	–	Vasudevan et al. (2007)
Poinsett 76	Cotyledon	EHA105	1	AB	10	25	2–5	–	0	PPT	1–4	p35S:: i > gfp-byg	21	Selvaraj et al. (2010)
Poinsett 76	Cotyledon	LBA4404	–	–	–	–	–	–	–	Km	50–150	p35S::bar, nptII	8.5	Selvaraj et al. (2010)

(continued)

Table 5.1 (continued)

Genotypes	Explant type	Agrobacterium strains	ODsoo or cocn	Growth media	Inoculation time (min)	Cocultivation			AS	Selection agent		Transgene constructs	TransformationEfficiency (%)	References
						Tm(C)	Period	Light	(uM)	Antibiotic	Conc			
S516	Cotyledon	EHA3101	–	MS	15	25	3	Dark	–	Km	100	nptII	8 transformants	Liu et al. (2010)
Guonong No.25	Cotyledon	LBA4404	–	MS	12	28	2	Dark	–	Hyg	10	p35S:: antisense	20 transformants	Sui et al. (2012)
Xintaimici	Cotyledon	LBA4404	0.2–0.3	MS	20	25	2–3	Dark	–	Km	150	nptII, p35S: CsNMAPK	4.8	Wang et al. (2013)
Shinhokusei 1	Cotyledon	EHA105	0.5	MS	10	25	3	Dark	200	Mer	10	nptII, p35S:: GFP	11.9 ± 3.5	Nanasato et al. (2013)
Shinhokusei 1	Cotyledon	EHA105	0.5	MS	10	5	3	Dark	200	Km	50	p35S::HPF	–	Nanasato et al. (2013)
Guonong No.25	Cotyledon	LBA4404	–	MS	12	28	2	Dark	–	Km	100	p35S:sense:: antisens	17 transformants	Wang et al. (2014)
S06	Cotyledon	GV3101	–	MS	15	25	3	Dark	–	Km	100	p35S::Tu	4 transformants	Yang et al. (2014)
Xintaimici	Cotyledon	LBA4404	0.3–0.5	1/2MS	15	28	2	Dark	–	Km	50	p35S:sense:: antisens	2 transformants	Cheng et al. (2015)
Shital	leaf, nodal, internodal callus	LBA4404	0.6	LB	5	25 ± 2	2	16 h/8 h	–	Km	0	p35S::CIPK	–	Faisal et al. (2015)
Cs0601	Cotyledon	EHA3101	–	–	15	–	2	Dark	100	Km	100	p35S:: CsEXP1, GUS,	–	Sun et al. (2017)
Xintaimici	Cotyledon	EHA105	–	–	15	28	2	Dark	–	Km	50	p35S:: CsARN6.1	2 transformants	Xu et al. (2018)
Poinsett 76	Cotyledon	AGL1	0.7	1/2MS	12	23	3	Dark	–	Km	100	p35S::LL P35s::npt II,	21 transformants	Yang et al. (2018)
CU2	Cotyledon	EHA105	–	MS	–	–	3	–	–	–	–	CRISPR-CAS9	0.1	Hu et al. (2017)

5.3 Key Factors for *Agrobacterium*-Mediated Transformation in Cucumber

5.3.1 Vectors

The T-DNA of Ti plasmid vector is defined by left border (LB) and right border (RB), and the T-DNA is inserted into plant cells from right border to left border (Wang et al. 1984). Any sequences between the two borders are able to be integrated into plant nuclear DNA (Bevan 1984). There are three critical elements in a T-DNA construct: multiple cloning sites (MCS) used to park the target gene, a selectable marker gene (*NPT II*, *Hpt II*, *Bar*., etc.), and a report gene (*GUS*, *GFP,* etc.) (Fig. 5.1a). It is preferable to select a vector that can insert the target gene between the right border and selectable marker gene such that the target gene can be transferred ahead of selectable marker gene (Fig. 5.1b, c) (Barrell and Conner 2006).

The widely used plasmid vector, pBI121 has MCS located to the left of the selectable marker gene (*NPT II*), which may potentially result in the insertion of *NPT II* but not the target gene if the delivery process is disrupted (Fig. 5.1d). For example, Yang (2014) developed a construct of pBI121-CaMV35S:*Tu* aiming to overexpress *Tu* in cucumber. Among four tentative transgenic plants obtained, plant #2 had a single copy insertion but showed no expression of *Tu,* which was probably due to the interruption of the delivery of T-DNA to the plant cell (Yang et al. 2014). On the other hand, most pCAMBIA vectors have the MCS between RB and selectable markers (Fig. 5.1) (Miao et al. 2009; Yang et al. 2018).

5.3.2 Factors Affecting Co-Cultivation Efficiency

During co-cultivation of Agrobacterium and explants, the salt strength in the MS media seems to have an important effect on the T-DNA delivery efficiency. In canola and wheat, it was found that reduced salt strength in the inoculation and co-cultivation media promoted T-DNA delivery (Cheng et al. 1997; Opabode 2006). When one-tenth strength of MS media was used in both inoculation and co-cultivation media,

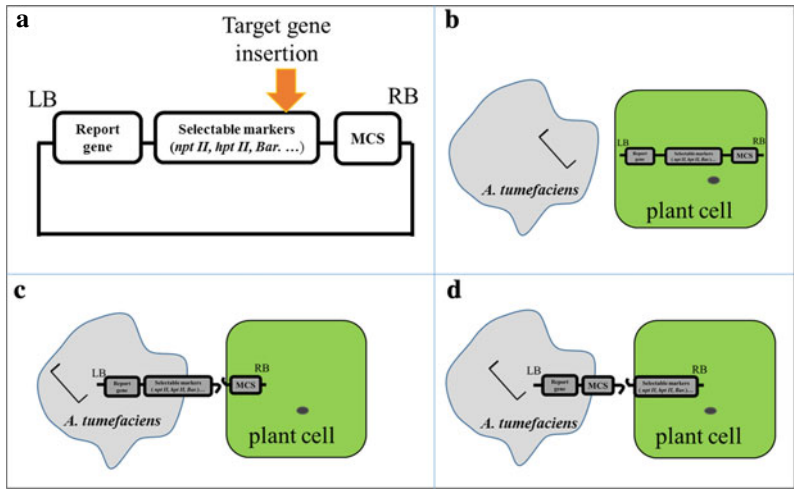

Fig. 5.1 The structure of an optimum expression vector and the delivery of T-DNA from *A. tumefaciens* to host plant cell. A typical construct contains the MSC between the right border (RB) and selectable marker (**a**). Ideally, the whole T-DNA can be delivered from *A. tumefaciens* into a plant cell (**b**), but only part of the T-DNA enters a plant cell if this process is interrupted (**c**, **d**). Abbreviations: *npt II*, *neomycin phosphotransferase II*; *hpt II*, *hygromycin phosphotransferase II*; *bar*, *bialaphos resistance gene*; MSC, multiple cloning sites; LB, left border

transient *GUS* expression on the freshly isolated immature embryos was significantly higher than that in the full-strength MS media in wheat (Cheng et al. 1997). The effect of different MS medium strengths on the transformation efficiency in cucumber has not been systematically evaluated. Most reports on cucumber transformation used full strength MS media as the buffer to dilute the *Agrobacterium* culture (Table 5.1) with reported success of transgenic plant generation (Fan et al. 2019; Lü et al. 2017; Li et al. 2018; Liu et al. 2019, 2018; Ma et al. 2019; Shen et al. 2019; Sui et al. 2018, 2017; Sun et al. 2019; Wen et al. 2019; Zhao et al. 2019, 2018). The half-strength MS was also successfully used to produce transgenic cucumber lines (Cheng et al. 2015; Yang et al. 2018). In a few cases, other types of growth media, such as autoinducer bioassay (AB) minimal medium, were reported for successful cucumber transformation (Chilton et al. 1974; Prem Anand and Rafael 2005; Vengadesan et al. 2005).

It is commonly believed that higher *A. tumefuciens* cell density and longer inoculation/ co-cultivation time yield more efficient T-DNA delivery on various tissues or cells. However, higher bacterial cell density may kill the explants directly. Low bacterial density will result in low success in T-DNA delivery to explants (Wang et al. 2015a). An 'optimal density' of *A. tumefaciens* has been reported to range from OD_{600} of 0.2 (Cheng et al. 2015; Wang et al. 2013) to 1.0 (Selvaraj et al. 2010; Sureshkumar et al. 2005; Vasudevan et al. 2007) in cucumber transformation. Of the 20 studies listed in Table 5.1, six used OD_{600} of 1.0. It was reported that re-suspension of *A. tumefaciens* inoculum by gently shaking at 28 °C for about 2 h before inoculation may facilitate efficient induction of *vir* genes (Nanasato et al. 2013). However, longer infection time could lead to necrotic explants and failure of transformation. The infection time from different studies varied from 5 to 25 min in cucumber, and the optimal time was suggested to be 10–15 min (Zhang et al. 2017).

Acetosyringone (AS) is the main compound in wounded plant cells to induce the expression of *Vir* genes in *A. tumefaciens* (Charles et al. 1992). The presence of AS during infection and co-cultivation was reported to enhance the infection frequency (Table 5.1). Vasudevan et al. (2007) suggested that the optimal concentration of AS is 20 μM for the Poinsett 76. Two other significant measures to increase transformation efficiency are the use of a surfactant in the inoculation medium and vacuum infiltration. Surfactants such as Silwet 77, Pluronic acid F68, and Tween 20 may enhance T-DNA delivery by increasing attachment of *A. tumefaciens* to the explants (Opabode 2006). Vacuum infiltration may serve a similar function as the surfactants to promote delivery of *A. tumefaciens* cells to closed wound of explants. It was found that GFP signals were stronger in the deeper cell layers of explants applied with vacuum infiltration than the control in cucumber transformation (Hu et al. 2017).

5.3.3 Cucumber Genotypes and Explants

Plant regeneration and transformation frequencies vary widely among genotypes and explants (Table 5.1). Galperin et al. (2003) compared regeneration abilities of 30 melon genotypes, and found 24 had no detectable normal shoots and 5 exhibited low efficiency of regeneration. Most North China type cucumbers (Chinese Long), such as 'Guonong 25' (Sui et al. 2012; Wang et al. 2014), 'Xintaimici' (Cheng et al. 2015; Xu et al. 2018) seem to have a high regeneration rate (80–97%) (Zhang et al. 2017). Poinsett76, an American slicing cucumber, also has a high transformed efficiency (Table 5.1). Different genotypes respond very differently to phytohormone concentrations, bacterial density and inoculation period, co-cultivation conditions and antibiotic selection pressure. Wang et al. (2018) conducted QTL mapping to understand the genetic basis of variation in regeneration ability in cucumber, and identified a candidate gene *Csa1G642540* which may play a role in regulating regeneration ability in cucumber. However, additional work is needed to validate this. In addition, the *heme oxygenase* (*CsHO1)* was

reported to be involved in adventitious rooting in cucumber (Li et al. 2011).

The source of explants is another key factor affecting transformation efficiency. Transgenic plants can be obtained from shoot organogenesis directly (Vasudevan et al. 2007), or by inducing somatic embryogenic calli which then are differentiated into plantlets (Raharjo et al. 1996). Regeneration has also been reported from cotyledons, hypocotyls, leaves, petioles, protoplasts (Colijn-Hooymans et al. 1988), roots (Trulson and Shahin 1986), stem (Maciejewska-Potapczykowa et al. 1972), and suspension cultures (Schulze et al. 1995) in cucumber, but successful *Argrobacterium*-mediated genetic transformation has not been reported from protoplasts, root or suspension cultures. So far, the cotyledon nodes are the most widely used explants in cucumber transformation (Wang et al. 2015a).

The age of explants seems to be the most determining factor for regeneration and gene-delivery competency. Regeneration efficiency is highly correlated with developmental stages of the seedlings. However, it is difficult to ascertain the exact age of the seedling best suited to excise the explants, which is also genotype-dependent. One-day-old (Vasudevan et al. 2007) to 5-day-old (Selvaraj et al. 2007) cucumber seedlings have been used as resources for explants. In a regeneration ability assay with 5 developmental stages in cucumber, the seedlings at a stage with the similar length between cotyledon and hypocotyl produced more buds and shoots (Colijn-Hooymans et al. 1994). Seed coat is another key factor that influences the explant age. The seed coat delays germination and results in uneven germination. In the cucumber varieties Straight 8 and Sumter, when seed coat is present, it appears that 4 to 6 days is the optimum germination period for shoot regeneration from cotyledon tissue (Cade et al. 1990). Mechanical wounding on explants facilitates bacterial infection and was found to enhance transformation frequency (Vengadesan et al. 2005).

5.3.4 Temperature for Co-Cultivation

Early studies showed that development of tumors on infected plants by *A. tumefaciens* was optimal at ~22 °C which did not occur at >29 °C (Fullner and Nester 1996). When T-DNA integrates into the host genome is unknown (Mysore et al. 2000), which may occur during co-cultivation (Sonti et al. 1995). T-DNA is integrated via homologous recombination (Bundock et al. 1995), but the integration sites in the genome do not share extensive homology (Gallego et al. 2003). No plant genes involving T-DNA insertion have been identified (Mysore et al. 2000). Processing and transfer of the T-DNA depend upon the expression of *vir* genes. Although *vir* gene induction is maximal at 25–27 °C (Jin et al. 1993; Turk et al. 1991), it may be beneficial to co-cultivate explants with *A. tumefaciens* at lower temperatures during the initial few days (Gelvin 2003), which seems to enhance the *A. tumefaciens*-mediated transformation efficiency in *Phaseolus acutifolius* and *Nicotiana tabacum* (Sunilkumar and Rathore 2001). Co-cultivation at 21 °C resulted in higher transformation frequencies in cotton than at 25 °C (Sunilkumar and Rathore 2001). A temperature of 22 °C was found to be optimal for T-DNA delivery in tobacco. Co-cultivation of tomato with *A. tumefaciens* suspension at 24 °C can obtain 76% of kanamycin-resistant shoots, which was 66%, and 52% at 28 °C and 20 °C, respectively. In cucumber, there are no reports on evaluation of the effects of temperature on transformation efficiency during co-cultivation, or shoot induction. An optimal temperature for co-cultivation is of great importance for successful gene delivery in cucumber. In most studies, 25 °C was used for co-cultivation (Liu et al. 2010; Nanasato et al. 2013; Selvaraj et al. 2010; Wang et al. 2013). On the other hand, there are also reports to develop transgenic cucumber lines with co-cultivation at 28 °C (Cheng et al. 2015; Wang et al. 2014; Xu et al. 2018). In our work on genetic transformation in

Poinsett 76, we obtained satisfactory results using 23 °C for co-cultivation, which was better than that under 28 °C (Yang et al. 2018).

5.3.5 Growth Regulators

The concentrations and combinations of phytohormones in initiation and shoot induction media affect shoot regeneration frequency of cucumber. The commonly used phytohormones include auxins such as 2,4-D, 1-naphthaleneacetic acid (NAA), and indole-3-acetic acid (IAA), cytokinins such as 6-benzylaminopurine (6-BA), kinetin (KT) zeatin (ZT), etc. Somatic embryos have been reported to form on 2,4-D/ BA (Rajasekaran et al. 1983; Ziv and Gadasi 1986), 2,4-D/ kinetin (Chee 1990a), NAA/ kinetin (Lou and Kako 1994) or 2,4-D/ BAP (Schulze et al. 1995) from various cucumber explants. Regeneration through organogenesis was previously noted on media containing 2,4-D/ BAP and NAA/ BAP (Nishibayashi et al. 1996), and leaf explants (Seo et al. 2000), respectively. In cucumber, the combined use of 6-BA and ABA is popular for shoot induction (Liu et al. 2019; Sun et al. 2014; Wang et al. 2014). There is an increasing trend in using ABA in plant regeneration in vitro (Torrizo and Zapata 1986). ABA was able to stimulate adventitious bud formation in begonia leaves, or shoot bud induction (Heide 1968; Singh et al. 2014). In cucumber, the addition of ABA into shoot induction medium increased the efficiency of shoot organogenesis and induced multiple shoots (Tabei et al. 1998). Other reports showed that ABA plays an important role during embryogenesis and germination in diverse plant species (Prewein et al. 2004; Singh et al. 2014). However, the molecular mechanism of ABA-stimulated shoot induction in cucumber remains unknown (Nanasato et al. 2013). It was postulated that ABA may stop the vitrification of explants by regulating water content and/ or activating stress-tolerance genes, resulting in improved regeneration efficiency (Nanasato et al. 2013).

Silver ion (Ag^+) is capable of inhibiting or suppressing the growth of *A. tumefaciens* cells (Charles and Jyoti 2001; Opabode 2006; Zhao et al. 2002). Ethylene (C_2H_4) is produced during in vitro plant tissue culture (Finkelstein et al. 1988). Poor regeneration response may be associated with ethylene produced during tissue culture (Chi and Pua 1989; Chi et al. 1991). Silver nitrate, a silver-containing compound, is known to be an inhibitor of ethylene (Mohiuddin et al. 1997), and significantly suppresses the *A. tumefaciens* growth during co-culture without compromising T-DNA delivery and subsequent T-DNA integration. This inhibitor of *A. tumefaciens* growth and ethylene production can facilitate plant cell recovery and result in increased efficiency of transformation (Cheng et al. 2003). The increased efficiency of transformation by adding silver nitrate was also found in wheat and maize (Opabode 2006; Zhang et al. 2003). This beneficial effect of silver nitrate is concentration-dependent, which is 20–50 μM in corn during co-cultivation (Charles and Jyoti 2001). Addition of $AgNO_3$ to the regeneration media could significantly enhance the frequencies of shoot regeneration and the number of shoots per explant in cucumber (Mohiuddin et al. 2005). The regeneration responses in relation to the optimum concentration of $AgNO_3$ are explant and genotype-dependent. Mohiuddin et al. (1997) found that addition of $AgNO_3$ at concentrations of 10, 30 and 50 μM to the shoot regeneration media increased number of shoots per explant in both proximal and distal cotyledon as well as proximal hypocotyl of 'Spring Swallow' and 'Tasty Green' cucumber lines; however, shoots failed to be induced in distal cotyledon of 'Suyo Long' upon the same treatment of $AgNO_3$ (Mohiuddin et al. 1997).

5.3.6 Pre-Selective Agents

A. tumefaciens-mediated transformation usually only results in gene delivery into one, or only a few cells in the targeted tissue. Typically, a selective agent is applied post-transformation to kill un-transformed cells in the target tissue. An optimal selection pressure is important for the transformation of a cucumber variety to reduce false positives. Genes frequently used to select

transformed cucumber tissues include *npt II*, *hpt II* and *bar* (Vengadesan et al. 200; 5Sui et al. 2012) that confer resistance to kanamycin, hygromycin and phosphinothricin (PPT), respectively. Typically, transformed cells in these systems are able to survive with appropriate concentration of selection agent, while non-transformed cells will be killed (negative selection) (He et al. 2006).

Kanamycin is the most often used antibiotic in cucumber transformation studies (Table 5.1). Kanamycin allows plants to be much more vigorous when transferred into the soil, whereas hygromycin disturbs plant development considerably. The concentration of the selection agents always influences the selective efficiency. Low concentration may cause non-transgenic escape shoots (Sarmento et al. 1992; Yin et al. 2005a; Yin et al. 2005b). A large number of escapes were encountered when using kanamycin as the selection agent (Schulze et al. 1995; Yang et al. 2014). High concentration may kill the true transgenic cells during differentiation. Gradually increasing concentration of hygromycin for the cotyledon and cotyledon node explants at different developmental stage could boost transformation rate. From the literature, 25–200 mg/L of kanamycin (Prem Anand and Rafael 2005), 7–25 mg/L of hygromycin, and 2–6 mg/L of PPT (Vengadesan et al. 2005) have been reported in various cucumber transformation studies. It is important to note that fresh antibiotics should always be used; the antibiotics, alone or in combination, do not absolutely prevent the occurrence of false positive shoots (Estopà et al. 2001).

5.4 Verification and Validation of Transgenic Plants

A successful transgenic assay includes the integration of the alien gene into plant genome, the expression of the alien gene and the appearance of the target phenotype. Many approaches can be performed to verify at each step.

5.4.1 The Integration of the Alien Gene

The integration of the alien gene can be verified by 3 steps. 1) Applying an optimal concentration of selection pressure at the differentiation period. 2) After moving the plantlets to soil, PCR verification with DNA extracted from newly merged true leaves as the template. 3) Southern hybridization with genome DNA.

High selection pressure (antibiotics, PPT, etc.) significantly decreases putative transgenic lines. The effective concentration of kanamycin for selecting transformants varies in different genotypes. From a screening with 100 mg/L of kanamycin in Poinsett 76 cucumber, 55.8% and 59.0% shoots were regenerated as escapes and 33.8% and 37.0% of shoots as chimeras for EHA105 and LBA4404 strains, respectively. However, no escapes were found when 2 mg/L of PPT was used (Selvaraj et al. 2010).

PCR is particularly useful as a preliminary screening tool to identify positive transformants from a large number of putative transgenic lines. Gene-specific primers, such as antibiotic (e.g., *NPTII, Hpt II, Bar*), *Vir* and reporter genes (*GUS, GFP*, etc.) can be used to evaluate the transgenic plants (Liu et al. 1995; Miao et al. 2009; Selvaraj et al. 2010). Alternatively, in assays of overexpressing an alien gene with coding region only (not including intro region), a bigger and a smaller amplicons can be amplified from the genome and inserted T-DNA, respectively, when primers specifically anchored at different exons spanning at last one intron.

Thermal asymmetric interlaced PCR (Tail-PCR) (Liu et al. 1995; Liu and Whittier 1995), is an alternative approach for the initial screening of transgenic events. By characterizing the genomic DNA regions flanking the T-DNA insertion sites, the sequencing result of Tail-PCR is a direct evidence to show successful integration of the target gene in the recipient genome. Tail-PCR has been used in Arabidopsis (Wu et al. 2015), and *Fusarium oxysporum*

(Mullins et al. 2001) to verify the transgenic plants. No such work has been done in cucumber though.

Southern hybridization analysis is usually carried out following the PCR to verify positive transformants, which is time-consuming though. Xu et al. (2018) reported three putative transgenic T0 cucumber plants from independent transformation events; two were verified by PCR. The T3 progenies were further verified by Southern hybridization using the digoxigenin-labeled *NPT II* probe to determine the number of fragments. In another study, Miao et al. (2009) examined 12 putative transgenic cucumber plants; a single band was observed in six plants, while 2 and 3 bands were observed from 4 and 2 plants, respectively. The further GUS staining assay showed 11 of 12 GUS positive plants (Miao et al. 2009).

5.4.2 Expression of Target Alien Gene

The CaMV35S is generally considered to be a strong constitutive promoter and it drives high levels of RNA production in transgenic plants. In transgenic cucumbers carrying the $CsFUL1^{A}$ transgene, significant increase and decrease of expression levels of the target gene were observed in the overexpressed and RNAi transgenic plants, which was consistent with its role as a negative regulator of cucumber fruit elongation (Zhao et al. 2019). Northern hybridization was carried out in some experiments to evaluate the expression of a target gene in transgenic cucumber. For example, the four true transgenic plants for the cucumber gene *phytoene synthase-2a carotene desaturase* (*PAC*), all four transgenic plants constitutively accumulated *PAC* mRNA which was not detected in non-transformed control plants (Jang et al. 2016).

Many engineered plasmid vectors contain *β-glucuronidase* (*GUS*) or *green fluorescent protein* (*GFP*) as reporter genes to identify successful transformation. Young leaves from transgenic lines showed a dark blue coloration, indicating *GUS* gene expression, whereas the leaves from non-transgenic lines did not show any signals (Sun et al. 2017). Expression of the *GFP* gene from the jellyfish does not need any exogenous substrate, which can be monitored in vivo without an additional cofactor (Wang et al. 2015a). GFP fluorescence can be observed at a very early stage of transformation and help identify escapes and chimeric shoots to increase the growth of transformed shoots on cotyledon explants. Fluorescent shoots can be visually tracked in vivo for GFP expression with a hand-held UV lamp throughout the tissue culture process (Selvaraj et al. 2010). Occasionally, the GFP fluorescence was difficult to detect in fully expanded mature leaves due probably to the accumulation of chlorophylls (Nanasato et al. 2013).

5.4.3 Phenotypic Characterization

In functional characterization of target genes, some phenotypes coded by the transgenes are expected in true transgenic plants. For example, in cucumber, the *CsCER1* (*Cucumber ECERIFERUM1*) gene encodes an enzyme in alkane biosynthesis. In transgenic RNAi plants, the expected glossy fruit phenotype could be observed; in transgenic plants overexpressing this gene produce waxy fruit, which are both inheritable (Bourdenx et al. 2011; Wang et al. 2015b). When the *little leaf* (*ll*) gene was overexpressed in the wildtype (large leaf) Poinsett76 cucumber, bigger leaves at most nodes were observed (Yang et al. 2018). However, since the locations of T-DNA integration into the recipient genome are largely random (Sonti et al. 1995), genetic transformation may often produce unexpected phenotypes due to T-DNA insertion mutagenesis (Bundock et al. 2002; Koncz et al. 1992; Yang et al. 2018), which may lead to discovery of novel genes or gene functions.

5.5 Perspectives

Although recent biotechnological advances are providing more options for alien gene transfer, *A. tumefaciens*-mediated genetic transformation is

still the dominant approach to produce genetically modified plants (Wang et al. 2015a). At present, the transformation efficiency in cucumber is far from satisfactory preventing its widespread and routine use (Hu et al. 2017). With the advent of the post-genomic era, more and more genes or QTLs are expected to be cloned in cucumber and their functions need to be characterized. The emerging CRISPR-Cas9-based gene-editing technology has the potential to revolutionize plant biology and crop improvement (Doudna and Charpentier 2014; Jaganathan et al. 2018). Till date, only limited reports claimed the editing of genes in cucumber with the CRISPR/Cas9 system (Chandrasekaran et al. 2016; Hu et al. 2017). Success of all these exciting developments in cucumber relies on an efficient and reliable genetic transformation system. Work so far suggests that the transformation and regeneration efficiencies vary significantly among genotypes. It may be beneficial to focus representative genotypes in each market class (for example, the US slicing cucumber Poinsett 76) to optimize the conditions and standardize the parameters in each step. Reproducibility could be tested across multiple labs. It is particularly important that the protocols, vectors/constructs and seeds of the genotypes are publicly accessible. From the experiences in field crops, we can optimistically expect that a concerted community effort will overcome the difficulties in the low cucumber transformation efficiency. In addition to *A. tumefaciens*-mediated transformation, other methods that are more genotype-independent have been widely used in field crops, which should also be explored in cucumber.

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QTL Mapping for Abiotic Stress

6

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Abstract

Abiotic stress is one of the severe stresses of the environment that threats to the growth and production of any plant worldwide. During the past decade, several studies have been published on molecular mapping of abiotic stress resistance in cucumber. Here, we summarize the relevant quantitative trait loci (QTL) linked to temperature stress, waterlogging stress, drought stress and salinity. These data provided valuable information for future genetic studies and marker-assisted selection and gene cloning for abiotic stress tolerance of cucumber.

6.1 Introduction

Plants are largely dependent on certain environmental and climatic conditions known as abiotic stresses. Vegetables growth and productivity are generally affected by genetic (internal) factors and external factors (such as abiotic stress). Abiotic stress occurs when plants face any variation from their optimum growing environment which alters their normal metabolic homeostasis (de Oliveira et al. 2013).

Abiotic stresses are major limiting factors in crop production, play an indispensable role in plant growth and development, provide the extent to which crop plants attain their potential values and it also determines the scientific principles on which production technology need to be based. Abiotic stresses cause significant amount of yield loss in agricultural crops including cucumber. Abiotic stresses cause molecular, and biochemical and physiological changes in plants as a result generate morphological modifications (Sahoo et al. 2014) which farther affect crops production.

In the era of climate change, the world food security is threatened by the fast increase in human population density (Lesk et al. 2016). To overcome the food security challenges, having abiotic stress resistant crops varieties is crucial. The utilization of the genetic differences within the existing varieties for stress tolerance can be done using natural selection under stressful environment or by QTLs mapping followed by marker-assisted selection approach (Ashraf et al. 2008). Usually, crops response to stresses differs depending on crop species, age of the plant, timing of stress application, stress intensity and genotypes (Gall et al. 2015). Inheritance of abiotic stress resistance is complex, the most universal approach to identify genes or QTLs

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associated with stress tolerance is based on linkage or genetic mapping (Fleury and Langridge 2014).

To cope with abiotic stresses, genetic maps based on molecular markers and identified QTLs have been used for developmental and breeding programs for crops (Shivhare and Lata 2017; Jiang et al. 2017; Hu et al. 2016) to enhance its productivity and quality.

QTL detection is the observation of a significant relationship or genetic linkage between the alleles at a specific locus or genomic region and the variation in a quantitative trait (Fleury & Langridge, 2014). It determines the heritable variations in phenotypes (Collins et al. 2008). QTL is also important to understand the genetic architecture of crops including cucumber to breed desirable traits (Bo et al. 2014). This approach is also used to dissect the genetic factors underlying source-sink communication under abiotic stress (Welcker et al. 2007).

QTL mapping is a useful biological procedure to locate regions/genes and its functional genomics. It is an important method to discover the correlation between genome and phenotype of an organism subjected to diverse stresses (Soda et al. 2015). Most of the phenotypic differences in QTLs are caused by few loci with major effects and many loci with smaller effects (Maki-Tanila and Hill 2014). In qualitative trait locus mapping, the size of the population is a limiting factor since low sample size could fail to calculate the accurate effects of QTLs (Svishcheva et al. 2012; Belonogova et al. 2013).

To reveal the abiotic stresses resistant QTLs, a lot of works was done by plant breeders in field crops such as Rice, Wheat, Maize, etc. (Farooq et al. 2004; Hu et al. 2016; Jiang et al. 2017). However, in cucumber, most QTL mapping studies have focused on traits for disease resistance and fruit development (Miao et al. 2011; Wang et al. 2016). There is lack of studies about QTL mapping related to resistance of cucumber to abiotic stresses such as high temperature, salinity, drought, soil toxicity and humidity. Recently some QTL studies were conducted for waterlogging and low temperature in cucumber (Martin et al. 2018; Xu et al. 2017; Song et al. 2018; Yagcioglu et al. 2019). In this chapter, we will discuss about the QTLs studies in plants focusing in Cucumber for different abiotic stresses that is important to know the desirable QTLs associated with the abiotic stress tolerance and may help for future cloning abiotic stress tolerance genes in cucumber.

6.2 QTLs for Temperature Stress

Cucumber (*Cucumis sativus* L.) is a thermophilic crop that is originated in the tropics, in the Himalayas. Hence, this vegetable species is sensitive to low temperatures (Cabrera et al. 1992).

High temperature causes to inhibit growth, discolor leaves and fruits as well as burning of wigs and leaves with clear visual of sunburn symptoms (Vollenweider and Gunthardt- Goerg, 2005). Exposure of plants to high temperature typically results in reduce the biosynthesis of chlorophyll (Dutta et al. 2009). Chlorophyll biosynthesis was decreased by about 60% in cucumber grown at 42 °C primarily because the 5-aminolevulinate synthesis under high-temperature regimes was inhibited (Tewari and Tripathy 1998). From seed germination to fruit harvesting stages, temperature fluctuation largely affects cucumber.

The optimal temperature for Cucumber seed germination is from 24 to 28 °C (Staub and Wehner 1996), thus, extended low temperature may significantly affect stand count, delay growth and affect overall performance of Cucumber crops (Wahid et al. 2007).

The ability of Cucumber to resist low-temperature germination (LTG) and high temperature is a desirable trait for cucumber breeding (Gu et al. 2002; Wahid et al. 2007; Song et al. 2018; Yagcioglu et al. 2019). Hence, recently LTG have been interest of researchers including QTL studies (Gu et al. 2002; Song et al. 2018; Yagcioglu et al. 2019). Tolerance to low temperature is a complex quantitative trait in cucumber and a QTL mapping approach would be an effective means to detect genes controlling traits concerned in seed germination in low-

temperature condition (Song et al. 2018). Gu et al. (2002) indicated low-temperature germination traits which includes relative germination energy, germination index and radicle length fitted additive-dominant model, and the relative germination rate under low temperature was controlled by two major dominant genes.

A number of studies have been conducted on *Arabidopsis* and cereal crops revealing a large number of genes or QTLs that function in response to low temperature. At least 30 LTG QTLs were detected that are distributed in all of the 10 chromosomes in rice (Fujino et al. 2008; Dixit et al. 2012; Li et al. 2013; Jiang et al. 2017). Six QTLs which control low-temperature germination rate were detected in maize, (Hu et al. 2016). Fujino et al. (2008) identified and cloned a major QTL firstly, *qLTG3-1*, controlling low-temperature germination inability in rice, which was strongly expressed in the embryo during seed germination. Wang et al. (2018) performed genome-wide association analysis on LTG capacity among 187 accessions, and detected 53 QTLs underlying LTG. Among them, the *stress associated protein 16* (*OsSAP16*) was found to be the candidate gene in rice, which encodes a zinc finger domain-containing protein.

Three QTLs, namely *qLTG1.1*, *qLTG2.1* and *qLTG4.1*, were identified linked to low temperature during seed germination in cucumber using low-temperature-sensitive and low-temperature-tolerant recombinant inbred lines (Song et al. 2018). According to this experiment, *qLTG1.1* was linked as the major one for germination rate, germination energy and germination index, explained more than 50% of the observed variability of phenotype (Song et al. 2018).

Yagcioglu et al. (2019) identified also identified three QTLs, *qLTG1.2*, *qLTG2.1* and *qLTG4.1*, which were detected in multiple experiments and explained 27.3–52.5% observed the phenotypic variation.. Pan et al. (2017) identified three flowering time QTLs, *FT1.1*, *FT5.1* and *FT6.2* from which *FT6.2* contributed less photoperiod sensitive and early flowering. These photoperiod-related QTLs may have linkage with temperature tolerance as duration of sunlight correlates with temperature intensity. No QTL mapping studies have been carried out on resistance by cucumber to high temperature, thus, no genes or QTLs have been identified yet for high-temperature tolerance, and the mechanism for tolerance is still not well understood (Song et al. 2018).

6.3 Waterlogging

Waterlogging is one of the major abiotic stresses that severely restrict crop growth and productivity. Cucumber is susceptible to waterlogging because of its shallow roots and strict oxygen requirements (Qi et al. 2012; Xu et al. 2014, 2016). Cucumber roots can easily deteriorate if there is a lack of oxygen, which consequently leads to an energy shortage (Xu et al. 2014). Factors such as torrential rainfall, irrigation, floods, lack of sufficient drainage application and poor management of drainage systems causes waterlogging (Valipour 2014). Waterlogging has been the main challenge in some parts of the world, like in South China, South East Asia, some parts of USA and Australia (Setter and Waters 2003).

Waterlogging accounts about 16% of the productive areas of the world annually and causes yield reductions of 15 to 80% (Shabala 2011). About 24.3 million hectares of lowland areas are vulnerable to waterlogging which accounts for about 20% of cultivated land in China (FAO 2004). As a result, waterlogging is a major problem of cucumber production in China particularly in the Yangtze River basin which has been frequently affected by flooding during periods of high rainfall (Jiang et al. 2000).

To reduce the damage of waterlogging problem in cucumber production, introducing tolerance cultivars is economically feasible. But breeding for the waterlogging stress tolerance is not an easy task as it is a complex quantitative trait and the measurement of the stress tolerance can be simply affected through environmental conditions (Broughton et al. 2015; Xu et al. 2017). The identification of waterlogging tolerance-related QTLs in cucumber can help to

solve environmental stresses. The QTL studies in cucumber on waterlogging inheritances are limited, compared with the significant number of studies conducted on field crops.

The cucumber germplasm shows a significant variation in waterlogging tolerance. For example, a cucumber lines known as Zaoer-N and PW0832 are comparatively more tolerance to waterlogging whereas line Pepino and PW0801 are low waterlogging stress-tolerant. The waterlogging tolerance performance in some cucumber accessions is due to their adventitious root (AR) number formation from hypocotyls, comparatively more shoot dry weight and waterlogged vine length under waterlogging conditions compared with the waterlogging susceptible lines (Yeboah et al. 2007; Xu et al. 2017). An experiment confirmed that Zaoer-N seedlings produced a number of adventitious roots on the hypocotyls to acclimatize to waterlogging stress, while in Pepino seedlings, a waterlogging stress-sensitive line, almost no ARs were generated (Xu et al. 2017). To restore the damaged original root system during flooded conditions, a key response in cucumber is the emergence of adventurous roots (Qi et al.2011; Xu et al. 2018).

Few qualitative trait locus-related studies were studied in Cucumber for waterlogging stress (Martin et al. 2008; Bo et al. 2014, Xu et al. 2016; Xu et al. 2017). Through QTL analysis using a F_2 population derived from a cross between Zaoer-N and Pepino, a major effect QTL, *ARN6.1*, was identified in cucumber which can explain about 17.6 to 24.0% of the phenotypic variance of AR numbers(Xu et al. 2017).

Martin et al. (2008) identified 14 QTLs in cucumber which were associated with four waterlogging tolerance traits: tolerance score (TOL), adventitious root formation (ARF), Shoot dry weight (SDWw) and waterlogged vine length (VLHw). Three QTLs namely tol1_1, tol4_1 and tol5_1 were associated with TOL of which QTL tol4_1 was specific to TOL and not associated with other traits while, the other two QTLs tol1-1 and tol5-1 accounted for 17.0 and 10.4% of the total phenotypic variation (Martin et al. 2008). Moreover, According to this result, a waterlogging tolerance (ARF) was found to have two QTLs namely arf2_1 and arf5_1. From these QTLs arf5.1 was specific to ARF but arf2_1 was also mapped to shoot dry weight trait of waterlogging QTL in linkage group two. Moreover, five QTLs namely vlh_w1_1, vlh_w 2_1, vlh_w 4_1 and vlh_w 5_1 were found to be associated with VLHw while four QTLs sdw_w1-1, sdw_w 2–1, sdw_w 4_1 and sdw_w 5–1 were found to be associated with SDWw (Martin et al. 2008). The success of waterlogged stress tolerance in cucumber plants depends on the genetic difference available in a population as well as values of heritabilities. Yeboah et al. 2007 projected that the narrow-sense heritability for adventitious root formation and waterlogging tolerance score were reasonably high.

The QTL for the waterlogged traits accounted for 7.9–33.2% of the phenotypic variations (Martin et al. 2008). The detection of QTLs through molecular marker-assisted technique could help to provide important information for future genetic studies including necessary for future cloning of waterlogging stress-tolerant genes of cucumber. Recently Xu et al. (2018) identified a candidate gene for *ARN6.1* that was responsible for waterlogging tolerance due to increased AR formation in the cucumber line Zaoer-N. Moreover, the major effect QTL *ARN6.1* was predicted to encode an AAA ATPase domain that contains protein. All of these waterlogging stress tolerance studies are conducted at seedling stages. Hence, further studies beyond seedling stages could be the most suitable approach related to performance of cucumber plants for their productivity and quality of products to confirm their tolerance under field as well as greenhouse conditions need to be investigated.

6.4 Drought Stress

Drought stress has been indicated as one of the most detrimental environmental stress affecting agriculture worldwide and responsible for the greatest loss of yield of crops (Hu and Xiong, 2014). Drought stress can influence plants at any

growth stage and can affect the productivity and quality of produce depending on the degree, duration and intensity of the stress. Lack of sufficient water in the soil has several negative consequences including decrease of relative water content as well as water potential of plants. Drought stress causes osmotic stress and limits the uptake nutrient from the soil then results in leaf growth inhibition, reduce photosynthesis activity stomata closure and oxidative stress.

The occurrence and severity of drought stress is expected to augment as a result of environmental changes (Tester and Langridge 2010; Shabala 2013). The decrease in precipitation and change in rainfall patterns are causing the frequent onset of droughts around the earth (Lobell et al. 2011; Fahad et al. 2017). Thus identifying QTL and genes from drought-tolerant cultivars will be crucial breeding in crops including cucumber. Drought tolerance in plants is a complex trait being controlled by a combination of minor genes and QTLs (Mohammadi et al. 2005). Many morphological and physiological phenotypes were applied as indicators (Fan et al. 2015). Among which relative water content (RWC), leaf wilting and proline contents are among the most frequently used indicators for drought tolerance (Richards et al. 2002; Condon et al. 2002; Teulat et al. 2003). Wang et al. (2015) found the cucumber *CsCER1* in transgenic plants had strong effects on cuticle permeability and drought resistance. But till now, the QTLs for cucumber related to drought resistance is not yet identified.

The main problem associated with the selection of a proper QTL for drought tolerance is a high degree of interaction between environment and QTL (Tuberosa and Salvi 2006). Therefore, once a drought tolerance QTL is identified, isogenization is mandatory for its proper characterization (Salvi and Tuberosa 2005). Mapping of QTL for traits related to drought tolerance has been done in a number of crop species (Dixit et al. 2012; Farooq et al. 2014; Lata et al. 2015; Fahad et al. 2017).

Inadequate population size used in detection of QTL might lead to underestimation of QTL number and overestimation of QTL effects. The number of identified QTL can be increased with increased population size but most of the increased QTL are with small effects (Vales et al. 2005). With the development of molecular markers and linkage maps, marker-assisted selection technology has led to find desirable traits linked to drought tolerance in field crops. However, the accuracy in QTL analysis is problematic as significant genetic and environment interactionand use of wrong mapping populations(Ashraf et al. 2008). Very few QTLs have been successfully applied in commercially breeding programs due to the relatively lower heritability (Fan et al. 2015).

There is scarcity of QTL studies for drought stress tolerances in cucumber. In cucumber, most QTL mapping studies have focused on traits for disease resistance and fruit development (Zhang et al. 2013; Tian et al.2015; Wang et al. 2016; Wang et al. 2016; Weng et al. 2015;). No QTL mapping study related to the resistance of cucumber to high temperature have been reported thus, no genes or QTLs have been identified yet for high-temperature tolerance, and the mechanism for tolerance is still not well understood (Song et al. 2018) (Table 6.1).

6.5 Salinity

Due to irrigation application, salinity is becoming serious issue. It was estimated that about 1.5 million hectares of arable land is affected by salinity annually (Foley et al. 2005). Like drought the occurrence and severity of salinity stresses to crops is expected to augment due to the global environmental changes, negatively influencing food supply (Tester and Langridge 2010; Shabala2013).

Salt stress tolerance in plants is a developmentally regulated observable fact and the stress tolerance at one growth stage may not associate with that of other stages (Kumar et al. 2015), which has brought difficulties for the genetic research of salt tolerance. Phenotypes used as selection criteria for salinity tolerance include seed germination, relative water content, chlorophyll content, wet and dry weights, shoot

Table 6.1 Abiotic stress-related stresses tolerance QTLs identified in cucumber

	QTL	Genome size	Abiotic stress-related to	Reference
1	ARN6.1	550.8 cM	Waterlogging tolerance	Xu et al. (2017)
2	*qLTG1.1* and *qLTG4.1*		Low-temperature tolerance	Song et al. (2018)
3	*FT6.2*		to confer less photoperiod sensitive for early flowering during domestication of cucumber	Pan et al. (2017)
4	tol4_1 & arf5.1	992.2 cM	Waterlogging	Martin et al. (2008)
Genes				
5	5787 genes were differentially expressed	880 cM	Identification of waterlogging stress-inducible genes	Qi et al. (2012)

sodium content and survival rate (Chen et al. 2005; Shavrukov et al. 2010; Fan et al. 2015).

Plant abiotic stress tolerance is conferred by many interrelated mechanisms. The cell's ability to maintain membrane potential is considered to be the most crucial trait, because of a positive relationship between the ability of plants to maintain highly negative membrane potential and its tolerance to salinity stress (Gill et al. 2017). When the plants are subjected to salt treatment for a long time, the growth of the plant is slow, the membrane permeability is increased and the photosynthesis is blocked (Wang et al. 2006).

Salt stress-related QTLs were identified in plants such as Sunflower, wheat, rice and *Brassica species* (Lexer et al. 2003; Lindsay et al. 2004; Ahmadi and Fotokian 2011; Su et al. 2013; Lang et al. 2017). However, the QTLs studies for traits related to salinity stress tolerances are not well understood in cucumber. The current evidence on cucumber salinity stress tolerance was evaluated by visual scoring (TOL), relative leaf numbers (RLN14t) and survival rate (SU). The TOL, SU and RLN14 were higher in the tolerant parent than sensitive one (Kere et al. 2017).

Bo et al. (2014) concluded that molecular mapping can reveal structural rearrangements and QTLs underlying traits with local adaptation in semi-wild Xishuangbanna cucumber where he identified 13 QTLs for domestication and diversifying selection-related traits.

Salt stress tolerance in cucumber is controlled by both environmental as well as genetic factors.

Low heritability for salt stress tolerance in cucumber shows that hybrid breeding for salt stress tolerance development in cucumber may not be sufficient. Salinity reduces germination, seedling growth, biomass production and yield of cucumber (Tiwari et al. 2010). Though, there is genetic differences in salt stress tolerance of cucumber cultivars, improvement of salinity stress-tolerant cucumber is not easy due to narrow genetic base and lack of reliable morphological markers (Tuberosa and Salvi 2004; Ashraf et al. 2008). Recently, Kere et al. (2017) identified three microsatellite markers with significant relationship with specific quantitative traits in cucumber. These markers could be used in marker-assisted selection for salinity tolerance improvement in the cucumber breeding program.

Salt stress tolerance is quantitatively inherited trait controlled using several genetic loci. Consequently, the technique used to find new alleles and genes for salt tolerance to a specific stress situation should be well designed for the species and target trait in well-defined environments. In this respect, cucumber salinity tolerance may be significantly improved through marker-assisted selection where the underlying QTLs are stacked

into one reliable genotype. Kere et al. (2017) understood that phenotypic markers alone do not fully explain the underlying genetic factors affecting salinity tolerance in cucumber. Whereas single marker analyses showed a significant association between the traits and corresponding loci, the multiple interval mapping detected only one QTL for TOL with relatively low LOD (2.5). A major QTL, qSPAD5 was detected in Brassica where a salt stress-tolerant-related gene *Bra003640* was primary identified as the candidate gene (Lang et al.2017). In Arabidopsis, some QTLs and salt stress tolerance-related genes were identified, like *RAS1*, a gene responsible to encode a plant-specific protein which involves in plant salt stress tolerance during germination as well as seedling stages (Ren et al. 2010).

Four traits of salt tolerance in cucumber; visual scoring (TOL), the survival rate (SU), relative leaf numbers (RLN14) and green leaf (% GL) were evaluated. SSR20710 located on chromosome 3 was found to be associated with four traits after six markers (SSR20710, SSR13312, SSR1667, SSR23627, SSR13021 and SSR 00,398) were subjected to simple regression analysis (Kere et al. 2017). SSR20710 contributed 16.5, 7.1, 5.6 and 7.8 of variations observed in SU, TOL, RLN14 and %GL, respectively. While, marker SSR13312was strongly associated with RLN14 with a 25% contribution to the phenotypic variation observed. SSR 16,667 accounted for 58.7% of the TOL but was not related to the remaining phenotypes.

This study reveals that TOL and RLN14 are controlled by two loci on chromosome 3.

This study provided valuable information for future genetic studies of salinity tolerance in cucumber. The recent QTL study may provide important information for future genetic studies of salinity stress tolerance in cucumber and marker-assisted selection and gene cloning for salinity stress tolerance improvement in cucumber.

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QTL Mapping for Disease Resistance in Cucumber

7

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Abstract

Cucumber (*Cucumis sativus* L.) is an important vegetable crop cultivated world widely. Many pathogens can infect cucumber to cause great losses of fruit yield and quality, including bacteria, fungi and viruses. Because of the complex system of plant, pathogen and environment, the disease resistance is complicated and involved many genes, researched commonly as quantitative trait. In cucumber, recent studies focus on mapping QTLs (quantitative trait locus) of disease resistance. We try to make a brief summary of QTL mapping for disease resistance in cucumber, excluding those disease resistances as quality traits.

7.1 Viral Diseases

Many plant viruses can infect cucumber to damage leaves and fruits, make serious loss in yield and quality, which including *Cucumber mosaic virus* (CMV), *Melon yellow spot virus* (MYSV), *Papaya ringspot virus* (PRSV-W), Watermelon mosaic virus (WMV), *Zucchini yellow mosaic virus* (ZYMV), *Cucurbit yellow stunting disorder virus* (CYSDV), *Cucurbit chlorotic yellows virus* (CCYV).

According to the reports, several viral disease resistances were studied as quality traits controlled by one or two resistant genes. The recessive resistant gene of ZYMV, *zym*, from the cucumber inbred lines TMG-1, Dina-1 and A192-18 (Kabelka et al. 1997; Amano et al. 2013), was mapped on the chromosome (Chr) 6 of cucumber, which candidate gene was *Csa6G152960* encoding vacuolar protein sorting-associated protein 4-like (Amano et al. 2013). The recessive resistant gene of WMV, wmv^{02245} from cucumber inbred line '02245' was located between markers SSRWMV60-23 and CAPS-W1 on the Chr6 (Tian et al. 2016). The PRSV resistant gene, recessive $prsv^{02245}$ from '02245' was mapped into the interval of SSR markers SSR11-177 and SSR11-1 on the Chr6 of cucumber (Tian et al. 2015).

7.1.1 *Cucumber Mosaic Virus* (CMV)

CMV is a member of the genus *Cucumovirus* of the family *Bromoviridae*, which is an important plant virus with wide range of hosts. In cucumber, CMV infection causes leaves and fruits mosaic and distortion, stunting, which seriously threatens cucumber production (Kooistra 1969; Huang 2007; Wang et al. 2010).

The studies for CMV-resistant genetics of cucumber are inconsistent because of the

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complex resistant mechanism, and different materials. Based on the reports, the CMV resistance was regulated by a single dominant or recessive gene, or three recessive genes (Kooistra 1969; Munshi et al. 2008).

With resistant line F-3 and susceptible line HZL04-1, a recessive CMV-resistant gene was identified, which was linked to the AFLP marker E22/M88-204 bp (8.57 cM) (Huang et al. 2007). Using the same materials, 17 QTLs of CMV resistance were mapped on Chr3, Chr4, Chr5 and Chr6 of cucumber, among of which the primary locus, *cmv17*, explained 67.3% of the phenotypic variance (Wang et al. 2010).

With RILs derived from the cross of cucumber resistant line '02245' and susceptible line '65G', a QTL of CMV resistance, *cmv6.1*, was detected on Chr6 between SSR9-56 and SSR11-177, explained 31.7% and 28.2% of the phenotypic variations (2016 and 2017, respectively), which locus was mapped in an interval of 1,624.0 kb with 9 annotated genes, and *Csa6M133680* was considered as candidate gene (Shi et al. 2018).

7.1.2 *Cucurbit Yellow Stunting Disorder Virus* (CYSDV)

Whitefly-transmitted CYSDV belongs to the *Crinivirus* of *Closteroviridae* family, can infect and produce yellowing symptoms, interveinal chlorotic leaves and stunting in cucumber (Aguilar et al. 2006; Eid et al. 2006; Ruiz et al. 2006). It threatens cucumber production reducing yield of 30%–50% (Eid et al. 2006). From 300 cucumber accessions, only A1 and A2 were resistant to CYSDV (Aguilar et al. 2006). In another study of 124 cucumber accessions, none was immune and 7 accessions had part resistance to the virus (Eid et al. 2006). It indicates that CYSDV-resistant cultivar breeding is important for cucumber production.

With 391 individuals of F_2 population from 'PI 250,147' (CYSDV-resistant) and a breeding line (CYSDV-susceptible) crossing, three QTLs were detected, a major QTL (*QTL1*) flanking by amplified fragment length polymorphism (AFLP) markers on linkage group 4 (LG4), showed 42% phenotypic variation, which resistance of CYSDV was recessive (de Ruiter et al. 2008).

7.1.3 *Melon Yellow Spot Virus* (MYSV)

MYSV is a member of *Tospovirus*, transmitted by thrips, infects cucurbit plants causing leaves chlorotic, mosaic and yellowing, fruit mottling to reduce fruit yield and quality (Kato et al. 2000; Sugiyama et al.2009; Sugiyama et al. 2015).

There were two isolates of MYSV in Japan, MYSV-S and MYSV-FuCu05P (Sugiyama et al. 2009). MYSV-S can infect cucumber, melon, watermelon, squash and bottle gourd; the infection of MYSV-FuCu05P is restricted on cucumber (Kato et al. 1999; Sugiyama et al. 2015).

Cucumber accession '27028930' from Thailand is resistant to MYSV-S and MYSV-FuCu05P, which is useful for MYSV-resistant breeding (Sugiyama et al. 2009). Using sevaral F_2 populations derived from crosses between '27028930' and MYSV-susceptible parents 'Tokiwa', 'High Green 21' and 'Kyuri Chukanbohon Nou 4', four QTLs of MYSV-FuCu05P-1 resistance were detected, 2 major QTLs, *Swf-1* on Chr1 and *Swf-2* on Chr3; 2 minor QTLs, *Swf-3* on Chr4 and *Swf-4* on Chr7, which explained phenotypic variances of 20.1%, 22.1%, 13.8% and 9.4%, respectively (Sugiyama et al. 2015). The MYSV-FuCu05P-1 resistance was incomplete-dominant, which *Swf-1*, *Swf-2 and Swf-4* were contributed from 27,028,930, *Swf-3* from 'Tokiwa'; and the resistance of MYSV-S was performed by a single dominant gene, *Sws* on Chr3 linked to SSR marker SSR12383 (Sugiyama et al. 2015).

7.2 Bacterial Disease

7.2.1 Angular Leaf Spot

Angular leaf spot (ALS) is one of the important bacterial diseases in cucumber because of widespread distribution and often occurrence (Carsner 1918). The first report describing ALS resistance

of cucumber was published by Carsner (1918). The worldwide disease caused severe economic losses (Carsner 1918; Dessert et al. 1982). The bacterium *Pseudomonas syringae* pv. *lachrymans* is the generally causal agent of ALS, which mainly affects leaves, petioles, tendrils and fruits of cucumber. On leaves, the lesions appear light-green and water-soaked, which later expands into a polygonal shape (with or without a chlorotic halo). On fruits, water-soaked lesions often caused fruit to turn misshapen (Dessert et al. 1982).

The disease caused cucumber leaves presenting necrotic lesions with or without chlorotic halo. Dessert et al. (1982) considered that the non-halo lesion phenotype was resistant reaction to ALS. With the F_1, F_2, backcross and F_3 populations of resistant lines MSU9402 and Gyl4, and susceptible materials SMR18 and National Pickling, the result of genetic analysis showed that the non-halo lesion type of resistance was regulated by a single recessive gene, *psl* (*pl*) (Dessert et al. 1982). Above halo or non-halo lesion types on the infected leaves, Olczak-Woltman et al. (2009) analyzed the inheritance of resistance to ALS adding another parameter: the number and size of necrotic and chlorotic lesions. The symptom severity of ALS was related with multiple genes, which resistance heritability was measured as 53% in the F_2 generation (Olczak-Woltman et al. 2009).

Using a recombinant inbred line (RIL) population of Gy14 (containing resistance gene *psl*) and susceptible line B10, two near QTLs of resistance to ALS (*psl5.1* and *psl5.2*) were detected in Gy14, beside the *psl* on Chr5; the *psl5.1* (LOD = 23.1/26.3, two experiments) and *psl* locus were located in the same interval of two markers, IS_16325300 and UW085415, which explained 25.6%/27.6% of phenotypic variations (S omnicka et al. 2018). The R^2 of *psl5.2* was 14.4/10.7 with LOD of 9.6/7.3 (two experiments), flanking markers 16,327,616 and IS_16326693 (S omnicka et al. 2018).

Wang et al. (2019) also mapped the QTL of *psl* on Chr5 and identified its candidate gene *STAYGREEN* (*CsSGR*) for ALS resistance in Gy14, WI2757, H19, G421, Gy8 and 2A, which gene performs broad-spectrum resistances to downy mildew and anthracnose. Two other QTLs were detected, *psl1.1* located between markers UW084541 and SSR05817 on Chr1, $R^2 = 15.37$; *psl3.1* located the interval of SSR15043 and SSR00311 on Chr3, $R^2 = 7.60$ (Wang et al. 2019).

Zhang et al. (2019) detected a single recessive locus *psl-1* that was responsible for conferring resistance to ALS in *Cucumis hystrix* introgression line IL52 (immune to ALS), and located in the 3,118,855–3,951,558 bp interval on Chr1 of cucumber.

7.3 Fungal Diseases

7.3.1 Gummy Stem Blight

Gummy stem blight (GSB) is a destructive disease of cucumber that involves three species of fungi, *Stagonosporopsis cucurbitacearum* (syn. *Didymella bryoniae*), *S. citrulli*, and *S. caricae*, which makes symptoms of gummy stem, blight leaves and black rot fruits (Stewart et al. 2015). It affects root, stems and leaves of cucumber, often causing losses in production of 15 to 30%, and above 80% in severe infections (Zhang et al. 2017).

With 5 crosses of 4 GSB-resistant lines and 2 susceptible lines to detect GSB-resistant genes, no major effective gene was found, and at least 5 effective factors were estimated in the cross of 'Slice' x 'Wis. SMR 18' (Amand et al. 2001).

Using 160 RILs derived from the cross of GSB-resistant line PI183967 and GSB-susceptible line 931, six QTLs of GSB resistance in seedling stage were identified, *gsb3.1*, *gsb3.2*, *gsb3.3*, *gsb4.1*, *gsb5.1*, and *gsb6.1*, which phenotypic variations (R^2) were 6.0%, 7.4%, 5.7%, 6.4%, 15.1% (17.9% and 8.1% at 3 seasons), 6.5%, respectively (Liu et al. 2019). With the same RILs, 5 QTLs of resistant to GSB in stem were detected, *gsb-s1.1*, *gsb-s2.1*, *gsb-s6.1*, *gsb-s6.2* and *gsb-s6.3*, R^2 of 8.7%, 6.7%, 10.3%, 22.7% and 8.7%, respectively (Zhang et al. 2017). Among these QTLs from the same RILs, all were located at different intervals. It indicates that the GBS-resistance genes are complicated.

With *Cucumis sativus-hystrix* introgression lines and 3 mapping populations, 2 QTLs of GBS-resistance were detected on Chromosome 4 and 6, which fragments should be from *Cucumis hystrix* (Lou et al. 2013).

7.4 Fusarium Wilt

Fusarium wilt (FW) is a serious fungal disease in cucumber, which pathogen is *Fusarium oxysporum* f. sp. *cucumerinum* Owen (FOC), the soil-borne, infecting from roots and injuring vascular bundle to make whole-plant wilt (Armsrong et al. 1978). The pathogen can survive in soil for years, difficult to deal with. So, for cucumber production, using FW-resistant cultivars is necessary to control this disease in FW prevalent areas. To date, at least 4 races of FOC are isolated in cucumber, race 1 to race 4 from America, Israel, Japan and China, respectively (Armsrong et al. 1987; Weng et al. 1989; Netzer et al. 1977).

The FW resistance of cucumber was considered as a trait controlled by a single gene: *Foc*, a dominant gene from inbred line 'WIS-248' (Netzer et al. 1977); or *Fcu-1/Foc* from cv. 'SMR-18', dominant resistant to FOC race 1, 2 and 3 (Vakalounakis 1995; Vakalounakis 1996; Vakalounakis et al. 2018); or *Foc-4* dominant resistant to FOC race 4 from 'WIS2757' (Mao et al. 2008; Zhou et al. 2015).

In some reports, FW resistance was studied as a quantitative trait in cucumber (Pierce et al. 1990; Zhang et al.2014; Dong et al.2019). With RILs of the cross of '9110Gt' (FW resistant line) and '9930' (FW susceptible line), a major QTL of FW resistance, *Foc2.1*, was detected on chromosome 2 (Chr2) of cucumber, which locus was located between two SSR markers, SSR03084 and SSR17631, with phenotypic variances of 64.2%, 32.2% and 38.8% for 3 years testing, respectively (Zhang et al. 2014). In the study of Chen's group, the *fw2.1*, with F_2 population of the cross between 'Superina' (FW susceptible line) and 'Rijiecheng' (FW resistant line), a major QTL was mapped to a 0.6 Mb interval on Chr2 (from 1,248,093 to 1,817,308 bp), which location is different from the *Foc2.1* of SSR03084-SSR17631 (Dong et al. 2019). According to the mapping results, *Foc2.1*, *Foc-4* and *fw2.1* were detected at different intervals of Chr2. We need to isolate them for FW resistant breeding, and find they are the same or not.

7.5 Powdery Mildew

In cucumber, powdery mildew (PM) is one of the most popular diseases, infecting leaves and stems, causing serious yield reduction and economic loss. The disease can be caused by three different fungal species of the family Erysiphaceae: *Podosphaera xanthii* (synonym *P. fusca,* formerly *Sphaerotheca fuliginea*), *Golovinomyces cichoracearum* (formerly *Erysiphe cichoracearum*) and *Leveillula taurica* (Braun, 1995), which are obligate biotrophic ectoparasites, and make the same symptoms but can be discriminated easily with light microscopy (Braun et al. 2002). Of the three pathogens, *P. xanthii* is the most common and widespread, which causes a great problem for cucumber production, especially in greenhouse (PÉREZ-GARCÍA et al. 2009).

The environmental factors, temperature, relative humidity and light, can directly influence PM development in cucumber (Lamsa et al. 2011). Under proper environmental conditions (temperature 75～85 °F, relative humidity 80～95%, no rainfall), PM pathogens can infect cucumber and proliferate very quickly, making the disease develop and prevail fast in field and greenhouse (Askary et al. 1997). The symptom of PM is easy to recognize with white, powdery spots of spores and hyphae on leaves, petioles, stems and fruits, which seriously affects photosynthesis, respiration and vitality of cucumber plant (de Ruiter et al. 2008).

In cucurbit production, fungicide application is common to control PM effectively, which would make the pathogen produce fungicide-resistant strains, and bring chemical pollution (McGrath 1991; PÉREZ–GARCÍA et al. 2009). Therefore, high-level PM-resistant cucumber germplasms and cultivars are useful for breeding

and production with environment-friendly and efficient disease management. There are many cucumber materials that show resistance or susceptible to PM. For example, cucumber lines PI250147 (de Ruiter et al. 2008), WI2757 (He et al. 2013; Mao et al. 2005), PI197088, PI200815, PI200818, 'Natsufushinari', 'Asomidori' (Morishita et al. 2003), Jin5-508 (Xu et al. 2016), S06 (Liu et al. 2008), S1003 (Nie et al. 2015b) are high resistant to PM. Cucumber inbred lines 'Santou' (Sakata et al. 2006), D8 (Xu et al. 2016), True Lemon (He et al. 2013), S94 (Liu et al. 2008), S1001 and S05 (Nie et al. 2015a) are high susceptible to PM.

Because PM occurs commonly in cucumber production everywhere, there are many studies on the genetics and QTL mapping of PM resistance in cucumber (Fig. 7.1). Due to different PM pathogens, cucumber materials, environments and resistance evaluations, the inheritance of cucumber PM resistance is complicated, as a quantitative trait controlled by genes. Mao et al. (2005) considered that PM resistance was controlled by 3 genes in cucumber, one major recessive gene, one dominant gene and another recessive gene. Munger et al. (1979) found a dominant gene for PM resistance in 'Spartan salad' and 'PI197088', which parents were crossed with 'Marketmore' to generate F_1 of intermediate PM resistance. However, Morishita et al. (2003) reported that the temperature-independent PM resistance in PI197088-5 (a derivative line of PI197088) was governed by 2 genes, a recessive and an incompletely dominant gene. With RILs population of 'Santou' (PM-susceptible line) and PI197088-1 (PM-resistant line) crossing, the PM resistance showed as a quantitative trait governed by temperature-dependent (26 and 20°C) QTLs, which were mapped on Chr1, 5, 6 and 7 of cucumber (Sakata et al. 2006). However, Wang et al. (2018) identified 4 QTLs of PM resistance from PI197088 on Chr1, 2, 5 and 6 of cucumber by using 148 RILs. Liu et al. (2008) detected 4 QTLs (*pm1.1, pm2.1, pm4.1* and *pm6.1*) for PM resistance by using the $F_{6:7}$ populations derived from PM-susceptible line S94 and resistant line S06. With PI250147, de Ruiter et al. (2008) identified two major-effect QTLs for PM resistance and the two major-effect QTLs conferred leaf resistance and hypocotyl resistance, respectively. Zhang et al. (2011) detected 3 QTLs on Chr5 and one on Chr6 by using the $F_{2:3}$ family lines derived from the cross of K8 (PM-resistant line) × K18 (PM-susceptible line). Using the 132 $F_{2:3}$ families derived from WI2757 (PM-resistant line) and 'True Lemon' (PM-susceptible line), He et al. (2013) performed 3-year QTL mapping study and identified 6 QTLs, *pm1.1*, *pm1.2*, *pm3.1*, *pm4.1*, *pm5.1* and *pm5.2*, for PM resistance in cucumber, of which the major QTLs, *pm1.1* and *pm1.2,* conferred leaf resistance of PM and the minor QTLs, *pm3.1* and *pm4.1* contributed to disease susceptibility. Using 111 RILs derived from the cross between 'CS-PMR1' and 'Santou', Fukino et al. (2013) identified 9 QTLs for PM resistance, which were distributed on 6 cucumber chromosomes, 7 QTLs contributed by 'CS-PMR1' and 2 QTLs contributed by 'Santou'. Using an F_2 population developed from the cross between S1003 (PM-resistant line) and S1001 (PM-susceptible line), the major-effect QTL, *pm5.1*, detected by de Ruiter et al. (2008) and He et al. (2013) was also detected by Nie et al. (2015a), which was on the long arm of Chr5. Xu X et al. (2016) developed a single-segment substitution line, SSSL0.7, and confirmed a dominantly inherited major-effect QTL conferring PM resistance on Chr1, *Pm1.1,* with a segregating population of 3600 F_2 plants derived from the SSSL0.7 (PM-resistant line) × D8 (PM-susceptible line). Zhang et al. (2018) developed a *C. hystrix* introgression line of cucumber, IL52, which possessed PM resistance. Using 155 RILs and 193 F_2 individuals derived from the cross between 'Changchunmici' (PM-susceptible line) and IL52, one QTL for PM resistance was detected in Chr5 and a single recessive gene *pm* was mapped to an approximately 468 kb region (Zhang et al., 2018). Recently, with F_2 population from two Korean cucumber inbred lines crossing, PM-R (resistant) and PM-S (susceptible), 2 QTLs (*pm5.2* and *pm6.1*) for PM resistance were detected on Chr5 (16.35–24.99 Mb) and Chr6 (11.01–12.42 Mb), of 30% and 11% R^2, respectively (Zhang et al. 2020).

In recent years, due to next-generation sequencing technologies and genomic sequence

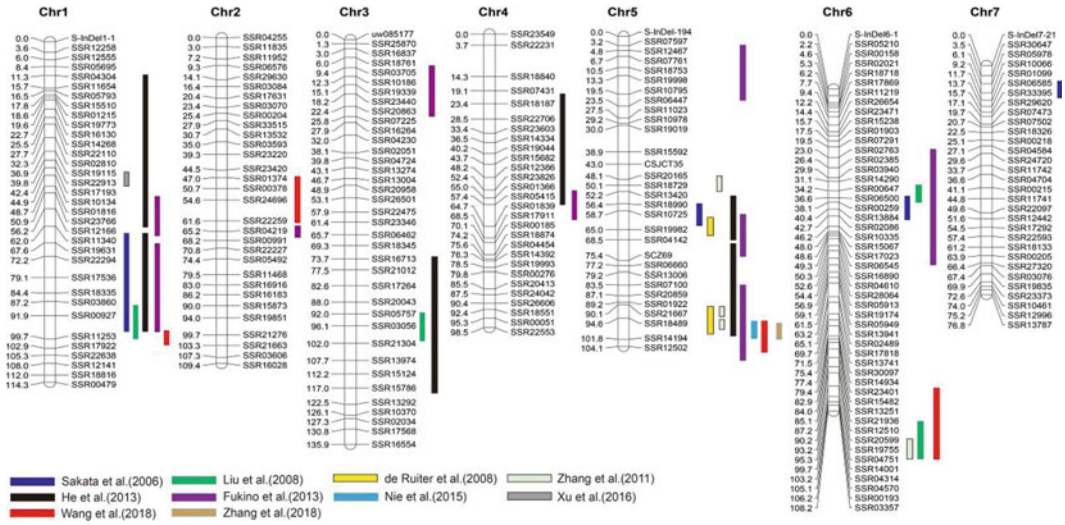

Fig. 7.1 Locations of QTLs for powdery mildew resistance in cucumber (2006–2018)

of cucumber, several PM resistance genes of cucumber have been cloned. The first PM resistance gene of cucumber was isolated and reported by Nie et al. (2015a, b). Based on marker-assisted selection (MAS), the BC_3F_1 and BC_2F_2 populations segregating only at *pm5.1* region were developed and the PM resistance gene, *Csmlo1,* was isolated by map-based cloning, which confers PM resistance due to the loss-of-function of *CsMLO1*, verified by the transgenic complementation of *Arabidopsis* mutant and tomato mutant (Berg et al. 2015; Nie et al. 2015b). *CsMLOs* were considered as susceptible genes for PM or downy mildew in cucumber (Schouten et al. 2014). With super-BSA (bulked segregant analysis) and SLAF-seq (specific length amplified fragment sequencing), 2 main intervals for PM resistance were found on Chr1 and Chr6 of cucumber, 5 candidate genes, *Csa1M568500.1*, *Csa1M568560.1*, *Csa1M569110.1*, *Csa6M406530.1* and *Csa6M407080.1* (Zhang et al. 2015). Through map-based cloning, two concatenated genes, *Csa1M064780* and *Csa1M064790* encoding the same function of a cysteine-rich receptor-like protein kinase, were cloned by using 3600 F_2 plants developed from the cross between SSSL0.7 and D8 (Xu et al. 2016). Based on the genomic resequencing of PM-susceptible cucumber line D8 and its chromosome segment substitution line, SSL508-28 (PM-resistant line), *Csa2M435460.1* and *Csa5M579560.1* were considered as candidates of PM resistance genes (Xu Q et al., 2016). In 2018, other 3 candidate genes for PM resistance gene in cucumber were isolated by fine mapping, *Csa5M622800.1* (encoding a UDP-glycosyltransferase family protein), *Csa5M622830.1* (encoding a GATA transcriptional factor) and *Csa5M623490.1* (encoding a serine carboxypeptidase-like protein) (Zhang et al. 2018). Through QTL-seq and mapping, *CsGy5G015660* was isolated as PM-resistant candidate gene, which encodes a putative leucine-rich repeat receptor-like serine/threonine-protein kinase and has rapid and specific expression after pathogen inoculation in PM-resistant line (Zhang et al. 2020).

7.6 Downy Mildew

Cucurbit downy mildew (DM) is a major foliar disease of cucurbit plants, which pathogen is an obligate oomycete, *Pseudoperonospora cubensis* (Berkeley and Curtis) Rostovzev. It is a worldwide and serious disease in field and greenhouse, threatening for the productions of cucumber, melon, watermelon, squash and pumpkin with up to 95% yield losses (Lebeda 1991; Savory et al. 2011; Kozik et al. 2013). When relative humidity is high, near to 100%, DM pathogen would

attack cucumber plants suddenly, and causes yellow lesions on leaves, which are angular due to leaf veins, and developing to brown and dry, dark 'down' (mildew masses) should be observed on the backside of infected leaf. The ideal temperature for sporulation and subsequent infection is 15°C, but a range between 5 and 30° C will suffice (Call et al. 2012b). The pathogen can be spread by wind, rain splash or physical transfer on equipment within a field. The infection rates are associated with higher relative humidity, leaf wetness and also temperature (Cohen 1981). Under ideal conditions, high humidity and temperature, the sporangia produced by pathogen were directly germinate or produce zoospores in the presence of free water on the surface of leaves. Zoospore becomes encysted upon sensing the location of stomata and forms a germ tube, which could enter the host plant through the stomata (Lebeda et al. 2011). Hypha grow intercellularly and form haustoria to adsorb nutrients from host cells. On the underside of leaves, the pathogen produces fuzzy, gray to black sporangia, which can be dispersed by wind (Fan et al. 2019). DM infection first appears as small, slightly chlorotic to bright yellow areas on the upper surface of the leaves. Initially, lesions may be in round shape. As these lesions expand, they may remain chlorotic or, depending on environmental conditions, became necrotic and brown with angular shape (Call et al. 2012a; Lindenthal et al. 2005). The pathogen overwinters through active mycelia in the hosts infected, and thick-walled oospores in plant debris and soil (Bains et al. 1976; Runge et al. 2009).

Because of oomycete pathogen broad variability, there were many pathotypes of *P. cubensis* among isolates from North American, Asia and Europe, which had different infection in species of *Cucurbita*, *Citrullus* and *Cucumis*, had close relationship from Europe and North American except Asian pathotypes (Thomas et al. 1987; Cohen et al. 2003; Lebeda et al. 2001; Shetty et al. 2002). It causes DM resistance of cucurbit very complex, involving different genes.

According to disease resistance evaluation, some cucumber lines or cultivars are resistant to DM, such as PI197088 (India), PI330628 (Pakistan) and PI605996 (VandenLangenberg et al. 2016; Wang et al. 2016; Wang et al. 2018), 129 (Ding et al. 2007), S94 (Bai et al. 2008), PI197085 (Szczechura et al. 2015), TH118FLM (Win et al. 2017) and Ames2354 (Kozik et al. 2013), which resistances were *P. cubensis* isolate dependent, PI197088 and PI330628 showing highest resistant to many isolates (Chen et al. 2020).

The inheritance of DM resistance in cucumber has been studied by many research groups. Some studies reported that resistance to DM in cucumber was a quantitative trait, controlled by QTL. About the DM-resistant gene(s) in cucumber, there were 1 or 2 major genes and one or several minor genes (Jenkins 1946; Zhang et al. 2007; Kozik et al. 2013), or several genes (Bai et al. 2008). Some studies reported that DM resistance in cucumber was controlled by a single gene (Ding et al. 2007; Fanourakis et al. 1987). In the work of Cohen's group, the DM-susceptible line SMR-18 was crossed with PI197088 or PI330628 to produce F_1, which F_1 plants exhibited partial resistance, F_2 populations showed the normal distribution of quantitative trait with different segregation ratios of resistant, moderately resistant and susceptible, depending on the pathogen isolate inoculated (Chen et al. 2020). Different results for the inheritance of DM resistance in cucumber were likely due to 4 factors: different pathogen isolates, different environments of growth, different evaluation of DM resistance, different populations from DM-resistant and susceptible lines.

In recent years, several studies have reported QTL mapping for DM resistance in cucumber (Fig. 7.2). Using a RILs population derived from the cross between S94 (DM- resistant line) and S06 (DM-susceptible line), three putative QTLs controlling DM were mapped on linkage group 1 and 6 of cucumber (Bai et al. 2008). With the F_2 population developed from Lucinde (DM-susceptible line) and PI197088, three QTLs of DM resistance were identified on Chr2, Chr4 and

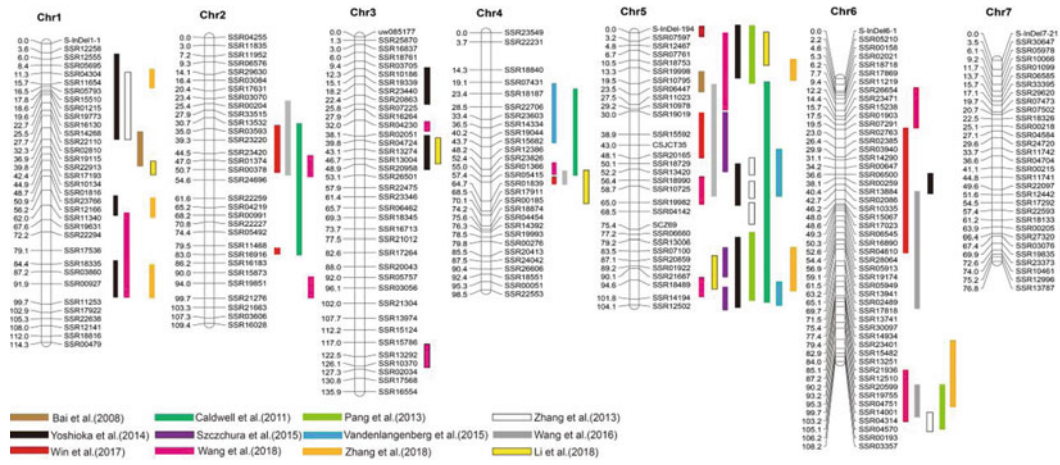

Fig. 7.2 Locations of QTLs for downy mildew resistance in cucumber (2008–2018)

Chr5 (Caldwell et al. 2015). Using an F_2 population developed from the crossing between DM-susceptible cucumber line 'Changchunmici' and introgression line IL52 (DM-resistant, from *C. hystrix*), 3 QTLs were detected on Chr5 and Chr6, *DM_5.1*, *DM_5.2* and Necr_6.1, explaining 17.9%, 14.2% and 13.9% of phenotypic variation, respectively (Pang et al. 2013). With cucumber line K8, Zhang et al. (2013) detected 5 QTLs (*dm1.1*, *dm5.1, dm5.2*, *dm5.3* and *dm6.1*) contributed DM resistance on Chr1, Chr5 and Chr6 of cucumber. Using the RILs from the crossing between Japanese cucumber cultivar 'Santou' (DM-susceptible) and line CS-PMR1 (DM-resistant, derived from PI197088), Yoshioka et al. (2014) identified 10 QTLs of DM resistance (*dm1.1*, *dm1.2*, *dm1.3*, *dm3.1*, *dm3.2*, *dm5.1*, *dm5.2*, *dm5.3*, *dm6.1* and *dm7.2*) on chromosome 1, 3, 5, 6 and 7 of cucumber, the major-effect QTL was located on Chr5.

With the F_2 population derived from the cross between DM-resistant line PI197085 and susceptible line PI175695, Szczechura et al. (2015) detected 3 QTLs of DM resistance, *DM1*, *DM2*, *DM3*, which were mapped on Chr5 of cucumber, explaining 11.67%, 12.75% and 13.21% of phenotypic variation, respectively. With a 157 RILs population of PI197088 and 'Coolgreen' (DM-susceptible), Vandenlangenberg (2015) identified 3 QTLs for DM resistance, explaining 52.29% of total variation, which were closely linked to marker SSR15321 (Chr5), SSR18489 (Chr5) and SSR17911 (Chr4), respectively.

Using 243 $F_{2:3}$ families developed from the cross between 9930 (DM-susceptible) and WI7120 (PI330628) for 4 field experiments at 3 locations, total 5 QTLs for DM resistance were detected, *dm2.1*, *dm4.1*, *dm5.1*, *dm6.1* and *dm6.2-Chl*, which explained 2.5–7.3%, 20.3–40.6%, 8.5–20.8%, 3.1–3.7% and 2.9–4.5%, respectively (Wang et al. 2016). Using a F_2 population derived from the cross between TH118FLM (DM-resistant line) and WMEJ (DM-susceptible line), Win et al. (2017) conducted next-generation sequencing (NGS)-assisted BSA for DM-resistant QTL mapping, and detected total 5 QTLs, *dm2.2*, *dm4.1*, *dm5.1*, *dm5.2* and *dm6.1*, among which *dm2.2* and *dm5.1* explained 10.8–24.0% and 14.0–27.2% of phenotypic variation, respectively.

With 183 $F_{2:3}$ families developed from the cross between 'Changchuanmici' (DM-susceptible) and PI197088 for QTL mapping in 7 independent trials, five QTLs for DM resistance were identified on Chr1, 3, 4 and 5 of cucumber, which explained 10.62% (*dm1.1*), 7.00% (*dm3.1*), 27.00% (*dm4.1*), 7.60% (*dm5.1*) and 12.46% (*dm5.2*) of R^2, respectively (Li et al. 2018). Using a RILs population derived from a cross between 'Coolgreen' (DM-susceptible line) and PI197088, Wang et al. (2018) detected 11 QTLs for DM resistance on chromosomes of

cucumber except Chr7, among which the major-effect QTLs were *dm5.1*, *dm5.2* and *dm5.3* (19.68%, 27.76% and 30.97% of R^2, respectively), three QTLs (*dm2.1*, *dm5.2* and *dm6.1*) were co-localized with three PM resistance QTLs (*pm2.1*, *pm5.1* and *pm6.1*), respectively.

Based on the development of next-generation sequencing and BSA-seq, several DM resistance candidate genes had been reported in cucumber. Through linkage analysis and BSA-seq with RILs and F_2 populations from the cross between IL52 and 'Changchunmici', 6 QTLs for DM partial resistance were detected, *dm1.1* (R^2 = 6.35%), *dm1.2* (R^2 = 3.85–4.20%), *dm1.3* (R^2 = 4.91%), *dm5.1* (R^2 = 5.72–32.89%), *dm5.2* (R^2 = 20.60–31.25%) and *dm6.1* (R^2 = 3.69%), among which *dm5.2* co-localized with the PM resistance QTL *pm,* was the major-effect QTL, *Csa5M622830.1* considered as a candidate gene for *dm5.2*/*pm* (Zhang et al. 2018). Based on fine mapping, Wang et al. (2019) cloned a DM resistance gene, *CsSGR* (*STAY-GREEN*), conferring triple-disease resistances to DM, ALS and fungal anthracnose (AR).

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Mapping for Quality Traits

8

Han Miao and Yue Peng

Abstract

The genome of cucumber was released in 2009. The cucumber genome has been useful for the isolation and characterization of important genes. In the last few years, significant progress has been made in quality traits molecular mapping and gene cloning in cucumber. This chapter summarizes the quality trait genes including fruit traits (fruit epidermal features, fruit flavor, trichomes, fresh color, carpel number, etc.), leaf-relate traits (leaf color and shape), stem-relate traits, sex expression, and disease resistance, which have been molecularly mapped or cloned in cucumber.

8.1 Introduction

Cucumber (*Cucumis sativus* L, $2n = 2x = 14$) is one of the most important cultivated cucurbits with a global production of 75.2 million tonnes in 2018 (www.fao.org/ faostat/en/). Cultivated cucumber exhibits large variation in traits such as fruit epidermal features (ridges, warty, colors, speckling, spines, fruit size, flesh bitterness, and flesh cilor), growth habit (vine length, hypocotyl, and branching), leaf (size, shape, and colors), sex expression, and so on.

Improving horticultural quality in regionally adapted cultivated cucumber and its wild relatives is challenging due to complex genetic control of traits affecting morphology, development, and yield. Mapping horticultural quality traits to genomic sites is an important step in the improvement work mentioned above. An in-depth study of the internal mechanisms of horticultural quality will be helpful in the selection of improved, resilient, and regionally adapted C. *sativus* germplasm using multi-trait markers.

Up to now, more than 150 single gene traits have been reported in cucumber (Weng and Wehner 2017). In the last few years, significant progress has been made in quality traits molecular mapping and gene cloning in cucumber. This chapter summarizes the quality traits genes that have been molecularly mapped or cloned in cucumber.

8.2 Mapping for Fruit Traits

8.2.1 Fruit Skin Color

Cucumber fruit exhibits a wide spectrum of skin colors that can vary from light green, yellow green, green, dark green, to creamy, white, yellow, brown, orange, or red, and so on (Wang et al. 2020).

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The skin color is a crucial external trait to judge whether the cucumber is mature or not. And the white skin is shared by limited numbers of commercial cultivars. Genetic analysis showed that the white fruit skin trait in cucumber was controlled by a single recessive nuclear gene *w*. The *w* gene was mapped to a linkage group with 14 SSR markers, corresponding to chromosome 3 of cucumber (Dong et al. 2012). Wang et al. (2013) studied the genetics of the white immature fruit color trait in cucumber and identified the molecular marker SSR15312, which was located on Chr.3. The white immature fruit color gene *w* was mapped to a 33.0-kb region. The *CsAPRR2* gene encoding peroxidase was found in this region, and it plays an important role in fruit pigment accumulation (Liu et al. 2016). Tang et al. (2018) found other white immature fruit skin color gene *Csa3G904140*.

The peel color of fruit is one of the important commercial traits in cucumber. Zhou et al. (2015) Genetic analysis showed the light green peel (*lgp*) is conferred by a single recessive gene, identified the candidate gene Csa7G051430 encoding ACCUMULATION AND REPLICATION OF CHLOROPLASTS 5 (ARC5) that is the causative mutation for the light green peel. A recessive mutant showing light green fruits and leaves was identified in an M2 population derived from ethylmethane sulfonate mutagenized elite cucumber line406. MutMap identified the candidate gene Csa6G133820 encoding Ycf54-like protein (*CsYcf54*) that is the causative mutation for the light green fruit *lgf* (Lun et al. 2016).

Ncg127 (red ripe fruit) and 9930 (yellow ripe fruit) were used as parents for genetic analysis and gene mapping of cucumber red ripe fruit. The results showed that the red mature fruit trait in cucumber was controlled by a single dominant nuclear gene *R*. The *R* gene was mapped to a linkage group corresponding to chromosome 4. The flanking markers UW019319 and UW019203 were linked to the *R* gene with genetic distances of 0.8 and 0.7 cM, respectively (Liu et al. 2014).

Whether the fruit color of immature cucumber is uniform or not is also a very important external quality trait that affects the market value of cucumber. Genetic analysis of different populations revealed that one single recessive gene, *u* (uniform immature fruit color), determines the uniform immature fruit color trait in cucumber. The *u* locus is located on chromosome 5 (Miao et al. 2011). The *u* gene was finely located between markers SSR10 and SSR27 with an interval of 313.2 Kb. The genetic distances were 0.8 and 0.5 cM, respectively (Yang et al. 2014a).

8.2.2 Fruit Epidermal Features

Consumers attach great importance to the appearance of the fruit when choosing agricultural products in the market. Several simply inherited genes determine fruit epidermal features, some of which are tightly linked on Chr5 including warty fruit (*Tu/tu*), glossy fruit skin (*G/g*, *D/d*), Ribbed fruit (*Fr/fr*), Heavy netting (*H/h*) and palisade epidermis (*Pe/pe*), and black spine color (*B/b*).

Warty fruit is one of the highly valuable fruit epidermal features related to the market values of cucumber. Genetic analysis has shown that the warty fruit trait of cucumber was determined by a single dominant gene, *Tu* (Tuberculate fruit) (Zhang et al. 2010b). The tuberculate fruit gene *Tu* has been identified by cloning. And it encodes a transcription factor (TF) with a single *C2H2* zinc finger domain. The *Tu* was expressed specifically in fruit spine cells during the development of fruit tubercules, but it could not be expressed in glabrous and tubercule-free mutant containing *Tu* (Yang et al. 2014b).

Glossy fruit skin is closely related to the market value of cucumber, which is a highly valuable appearance quality trait. Inbred line1101 (glossy fruit skin) and inbred line1116 (dull fruit skin), 9930 (dull fruit skin), 1107 (dull fruit skin) were used as experiment materials for genetic analysis and gene mapping. Genetic analysis showed that the glossy fruit skin trait in cucumber was controlled by a single dominant nuclear gene, *G* (glossy fruit skin). *G* gene was mapped to a linkage group with 30 SSR markers, corresponding to chromosome 5 of cucumber

(Dong et al. 2013). The dull/glossy fruit skin is also an important external quality trait that affects the sales of cucumber. Genetic analysis showed that the dull fruit skin trait of cucumber was determined by one single dominant gene, *D* (dull fruit skin).

The *D/d* gene was fine-mapped between markers SCZ69 and SSR16203 on chromosome 5, and the possible candidate gene *D* was identified as *Csa016880* or *Csa016887* (Yang et al. 2014c).

Ribbing fruits are very common in Northern China ecotype cucumber lines such as 9930. Miao et al. (2011) first reported that ribbing fruit was controlled by a single dominant gene. They propose this gene be designated as *Fr* (fruit ribbing). The *fr* (no fruit ribbing) and *H* (heavy fruit netting) were clustered in a 2.4 cM region in Chromosome 5, flanked with microsatellite markers SSR15818 and SSR06003.

Heavy netting of mature fruit is one of the important criteria to measure the appearance quality of cucumber. Genetic analysis showed that the heavy netting of mature fruit trait was controlled by a single dominant nuclear gene (*H*). The *H* locus is located on chromosome 5 (Miao et al. 2011). *Csa5G591790* was speculated as a possible candidate gene of heavy netting trait (Wang et al. 2014).

Firmness of skin is a feature of the palisade epidermis in these types compared to flat epidermis in fresh cucumbers. It is an important trait to measure the quality of pickling cucumber.

The palisade epidermis (*Pe/pe*) trait is controlled by a single gene, which is dominant over flat epidermis. It was mapped to cucumber chromosome 5 between SSR14611 and Indelpe12. The physical distance between the two markers is 227.5 kb (Zhang et al. 2017).

The fruit spine color is an important trait for cucumber fruit quality. Genetic studies indicated that black or brown spines are dominant to white and controlled by a single gene *B* (Li et al. 2013; Strong 1931; Tkachenko 1980; Walters et al. 2001) confirmed that a single, dominant gene B controlled both black spine color and orange mature fruit color. The *B* locus was mapped in the distal region of the short arm of cucumber chromosome 4. R2R3-MYB gene may be a candidate gene predicted by the *B* locus conditioning black spine and orange mature fruit colors of cultivated cucumber.

8.2.3 Fruit Flavor

The presence of cucurbitacin causes bitterness in fruits and leaves of cucumber. The cucurbitacins are tetrocylic terpenes that are present widely in cucurbits. Several genes have been described that control the bitterness trait, with *Bi* making fruit and foliage bitter free (Chi et al. 2007; Gu et al. 2005; Zhang et al. 2013), *Bt* making the fruit highly bitter (Zhang et al. 2011), and *Bl* making the foliage bitter (Shang et al. 2014).

Genetic analysis showed that one single dominant gene, *Bt* (fruit bitter), determines the fruit bitterness trait in cucumber. The *Bt* gene was mapped to the short arm of cucumber chromosome 5 within the region of 3.3 cM (Zhang et al. 2011). The *Bi* gene was mapped to the chromosome 6 within the region of 3.9 cM (Li et al. 2010). The *Csa5G156220* was named *Bl* (bitter leaf), which is used to synthesize bitter organisms in cucumber leaves.

Zhang et al. (2013) cloned *Bi* gene promoter from North China cucumber 9930, and this gene was an important candidate regulator that could control the biosynthesis of bitter material in cucumber. *Bi* encodes a cucurbitadienol synthase that catalyzes the cyclization of 2,3-oxidosqualene into the tetracyclic, which is the first committed step in the cucurbitacin biosynthesis.

Both *Bl* and *Bt* encode two basic helix-loop-helix (bHLH) transcription factors that express specifically in leaves and fruits, respectively. *Bl* binds to the E-box elements of *Bi* promoter and regulates cucurbitacin biosynthesis by activating transcription of *Bi* in cucumber leaves; fruit bitterness requires both *Bi* and the dominant *Bt* (Bitter fruit) gene. *Bt* has similar biochemical function as *Bl* but regulates cucurbitacin biosynthesis in cucumber fruits.

The leaves or fruits of cucumber usually have no fragrance, but there are some varieties from

Thailand, whose leaves or fruits can emit the pandan-like fragrance. Because their fruits and leaves have pandan (Pandanus amaryllifolius L.)-like fragrance (Pramnoi et al. 2013), which is the same as those possessed by fragrant rice, soybean and sorghum. Genetic analysis showed that the fragrance in fruits and leaves is controlled by a single recessive gene, proposed as *fgr* with no xenia effect for the fragrance (Pramnoi et al. 2013). The *fgr* gene was mapped to the chromosome 1, in this region have candidate gene *CsBADH*. The *CsBADH* encodes the betaine aldehyde dehydrogenase, which is responsible for fragrance in cucumber PK2011T202.

8.2.4 Cucumber Trichomes

There are trichomes (spines) on the surface of cucumber, which directly affect the appearance and quality of cucumber products. Trichosome is a hair-like structure on the epidermis. It can resist biotic and abiotic stresses. Six simply inherited genes determine cucumber trichomes features have been reported, including *csgl1*(*glabrous-1*) or 'micro-trichome' (*mict*), *csgl2*, *csgl3* (*tril*), *gl4, ns,* and *ss*.

The *'glabrous-1'* (*csgl1*) or *'micro-trichome'* (*mict*) mutant shows no observable trichomes on all aerial organs except the hypocotyl. *CsGL1* encodes a Class I HD-ZIP transcription factor (Li et al. 2015). The identification of *CsGL1* reveals a novel function for the homeodomain-leucine zipper I gene.

The fruit, sepal, petiole, and flower pedicel of the csgl2 mutant are covered with fine hairs, but there are no hairs on the stem, petiole, and leaf surface. Genetic analysis has shown that the glabrous trait (*gl-2*) of cucumber was determined by a single recessive gene. The *gl-2* gene was mapped to chromosome 2 of cucumber, and two flanking markers closely linked to gl-2 gene were SSR10522 and SSR132751 (Yang et al. 2011).

Tril and *CsGL3* were found to be the same gene *Csa6M514870*, in three different labs (Cui et al. 2016; Li et al. 2015; Pan et al. 2015; Wang et al. 2016; Zhao et al. 2015). The *csgl3* (or *tril*) mutant exhibits a completely glabrous phenotype on all aerial organs, completely glabrous which encodes a class IV HD-ZIP transcription factor. The glabrous phenotype in *csgl3* is due to different SNPs or retrotransposon insertion in the coding region.

The *gl4* mutant has glabrous fruit skin but reduced size and number of trichomes on the stem and leaves; *CsGL4* encodes a C-type lectin receptor-like tyrosine protein kinase (Mengnan et al. 2015).

Guo et al. (2018) elaborated cucumber mutant NC073, possessing tender and soft spines on fruits. The mutant trait was controlled by a single recessive nuclear gene named as tender spines (*ts*). It was located 109.7 kb physical distance on chromosome1. The most likely candidate gene predicted of TS is *Csa1G056960*. The function of this gene is to encode a C-type lectin receptor-like tyrosine-protein kinase during cucumber fruit formation.

The number of spines in cucumber is also a quality trait that consumers pay more attention to in recent years. Cucumber inbred lines NCG-122 with numerous spines. Genetic analysis showed that the numerous spines trait of NCG-122 was a quality trait controlled by a single recessive nuclear gene (*ns*). The few spines trait was dominant over the numerous spines trait. The *ns* encoding an auxin transporter-like protein 3 (CsLAX3) (Xie et al. 2018; Zhang et al. 2016).

Fruit spine size is one of the most important factors affecting the economic benefit of cucumber fruit. Genetic analysis showed that the fruit spine size trait of cucumber was controlled by a single dominant gene. The fruit spine size *SS/ss* locus was located in the region of SE1 and SSR43. The physical distance is about 189 Kb between *Csa5G576680* and *Csa5G577370* (Zhang et al. 2018).

8.2.5 Fresh Color

Cucumber inbred line PI200815 (yellow flesh) and inbred line 931 (white flesh) were used as parents to construct genetic analysis population of cucumber flesh color. The results showed that the yellow fruit flesh trait of PI200815 was controlled by a single recessive gene *yf*. The *yf*

gene has been mapped to chromosome 7. The closest flanking markers linked to *yf* were yfSSR108 and yfIndel29 with genetic distances of 0.6 and 0.3 cM, respectively (Lu et al. 2015).

The semi-wild XIS cucumber has *orange flesh (or)* and accumulates high-level β-carotene during maturing. Segregation analysis revealed that endocarp Q beta C of greenhouse-grown fruit was controlled by a single recessive gene. In addition, marker analysis indicated seven SSR markers on linkage group 3 were linked to the gene controlling Q beta C (Bo et al. 2012).

8.2.6 Carpel Number

Carpel number variation in cucumber was controlled by a simply inherited gene *Cn* with CN = 3 being incompletely dominant to CN = 5. The *CsCLV3* is the candidate gene of the *Cn* locus, which was located in melon chromosome XII (from 14,029,752 to 14,031,746). The physical locations of *CsCLV3* and *CmCLV3* were consistent with the known syntenic relationships between chromosomes of cucumber and melon (Li et al. 2016).

8.3 Mapping for Leaf-Relate Traits

8.3.1 Leaf Color Mutant

The five leaf color mutants have been mapped, including *yp* (yellow plant), *v-1* (virescent leaf-1), *vl* (variegated leaf), *vyl* (virescent yellow leaf), and *Psm* (Paternal sorting of mitochondria).

The cucumber chlorophyll-deficient mutation (yellow plant, *yp*) plant shows golden yellow color throughout the whole development stage; the *Yp* gene (CsCHLI) encodes the Mg chelatase I subunit, which is a rate-limiting enzyme in the chlorophyll biosynthesis pathway (Gao et al. 2016).

8.3.1.1 Virescent Leaf-1 (*v-1*)

Virescent leaf-1 (*v-1*) has light yellow cotyledons; the first 2–3 true leaves are also light yellow but turn to green when fully expanded. The *v-1* was located in the 50.4 Kb genomic DNA region of chromosome 6 by genetic mapping. Many evidences have proved that *CsaCNGCs* is the only candidate gene of *v-1*. The function of *CsaCNGCs* is to encode cyclic nucleotide gated channel protein (Miao et al. 2016).

8.3.1.2 Virescent Leaf (*vl*)

The *Csvl* mutant showed green-yellow-white phenotypes throughout the growth cycle. There are defective chloroplasts in *Csvl* mutant's mesophyll cells. The Csa6G405290 (*Cscs*) was the candidate gene for the *Csvl* leaf mutant in cucumber, which encoded chorismate synthase. The *Cscs* may interacted with some genes that encode zinc finger protein, heat shock protein to control variegated phenotypes in cucumber's leaf (Cao et al. 2018).

8.3.1.3 Virescent Yellow Leaf (*vyl*)

The *CsVYL* encodes a DnaJ-like zinc finger protein, a recessive gene, which resulted in reduced pigment content and delayed chloroplast development in virescent yellow-leaf (*vyl*) mutant. Confirmed by gene expression analysis, there are differences between wild type and mutant plants' transcription level of Csa4G637110 (Song et al. 2018).

8.3.2 The Mosaic Mutants (Psm, Paternal Sorting of Mitochondria)

Pentatricopeptide repeat (PPR) 336 was a nuclear locus, considered to be the candidate gene for Paternal Sorting of Mitochondria (*Psm*), which affects the predominant mitochondria transmitted to offspring. *Psm* was mapped to a 170 kb region on chromosome 3 of cucumber. There will be almost always fatal, if the *Psm* allele combine with mitochondria from MSC16 (Del Valle-Echevarria et al. 2016).

8.3.3 Leaf Shape Mutant

The leaves of wildtype cucumber are flat, with seven lobes, teeth, or smooth edges. There are

three leaf mutants that have been mapped, *rl-1, rl-2,* and *rl*. This is due to mutations in the allele of the PINOID (*CsPID*) gene that codes for the auxin polarity transporter PIN (pin-formed). The *rl-1* candidate gene is *CsPID*. The reason why round leaf phenotype occurs is the *CsPID*'s second exon occurred a nonsynonymous SNP. CsPID is associated with the formation of cucumber leaf shapes, with female sterile flowers and round leaves occurred in the cucumber round leaf (*rl*) mutant, which encodes PINOID (PID) protein kinases. (Song et al. 2019; Zhang et al. 2016).

The leaf edges of *cul-1* and *cul-2* rolled upward to form a shallow cup-shaped shape. All mutations are due to allele mutations in a class of homologous domain—leucine zipper (hd-zip III) transcription factors in the *CsPHB* gene. The *TEN* genes that encoding the TCP transcription factor results in tendril-free mutations. In Chr6, another tendrilless1 (*td-1*) mutation is mapped to a 190 KB region. (Rong et al. 2019).

The tiny leaf (*ll*) mutant was discovered 40 years ago and has a leaf area a quarter the size of the average American pickle. The WD40 repeat domain protein is the product of *ll* coding. QTL analysis showed that the main effect QTL had co-localization on multiple lateral branche fruit size, weight, and seed weight, while the *ll* locus indicated the pleurism of *ll* gene mutation. LL gene plays a significant role in cucumber's lateral branch development and the control of organ size (Yang et al. 2018).

8.4 Mapping for Stem-Relate Traits

8.4.1 Vine

Plant architecture, especially plant height or vine length, is an important trait in cucumber breeding. At present, six mutants with reduced internode length or compact growth habit have been reported including compact (*cp*), compact-1 (*cp-1*), short internode (*si*), super compact-1(*scp-1*), super compact-2 (*scp-2*), and dwarf (*dw*). Both *scp-1* and *scp-2* are due to mutations brassinosteroid (BR) biosynthesis pathway genes encoding the BR-C6-oxidase (*CsCYP85A*) and steroid 5-alpha-reductase (*CsDET2*), respectively. The *si* mutant exhibits short internode (50% of WT) and small fruit, which encodes a member of the VIER F-BOX PROTEIN subfamily of the F-Box protein (*CsVFB1*).

8.4.1.1 Carpel Number (*Cp*)

The *cp* locus was mapped to a 220 kb region by using 1269 F2 strains for fine genetic mapping. This regional gene contains homologous genes of cytokinin oxidase (*CKX*) gene and may be densification's a potential candidate gene. According to the parents' homologs alignment of the *CKX* gene, a 3-bp deletion was revealed in the sequence of exon (Li et al. 2011).

8.4.1.2 Super Compact-1 (*Scp-1*)

The super-compact (*SCP*) mutant, C257, is dwarfed by almost no internode elongation. The SCP's candidate gene is a recessive gene, scp-1, which encodes the BR biosynthesis pathway's BR-C6-oxidase. An SNP of scp-1's exon leads to an *SCP* phenotype. One of three *CsCYP85A* gene copies in cucumber genome seemed active was *scp-1/CsCYP85A1* gene (Wang et al. 2017).

8.4.1.3 Super Compact-2 (*Scp-2*)

The dwarf mutant not only is spontaneous, but the leaves is wrinkle and dark green. Because of steroidal 5-reductase mutation in *CsDET2*, Super compact-2 (*SCP-2*) exhibits a typical BR biosynthesis defect phenotype. *Scp-2* differs from the two mutants previously reported: super compact (*scp-1*) and compact (*cp*). In *CsDET2* gene, the single base insertion and two single nucleotide polymorphisms resulted in the conserved amino being missense mutations, which lead to the truncated protein lacked the conserved catalytic domain and cannot predict the steroid 5 reductase protein (Hou et al. 2017).

8.4.1.4 Dwarfism (*si*)

The mutational locus of *si* dwarf phenotype was identified as SNP4G21398058, located in the gene *Csa4G641640* of cucumber. The production of *Csa4G641640* is an F-box protein, including 14 hypothetic Leucine-rich repeats

(LRR) and an F-box motif. Premature termination results in the shortening of the f-box protein and the deficiency of four LRRs. *Csa4P641640* was named *CsaVFB1*), and the *CsaVFB1* was expressed in various organs of plants (Lin et al. 2016).

8.4.1.5 Dwarf (*dw*)

The mutant *Csdw* was induced by the presence of ethyl methyl-sulfonate in cucumbers, due to the division of cell in the main stem was reduced, which showed a dwarfism phenotype and shortened internode length. Csa3G872760 (CsCLAVATA1), which encodes clavata1 receptor kinase, is a recessive gene and a candidate gene for dwarf mutation in cucumber (Xu et al. 2018).

8.4.2 Tendril

The gene *TEN* plays an important role in cucumber tendril development. Transcriptome Sequencing showed that only *Csa5G644520* in the telomere region of chromosome 5 was specifically expressed in tendril tissue. There is a rare SNP in Csa5G644520, located in the nucleotide of chromosome 5 (Wang et al. 2015).

In mutant B007, the recessive gene *td-1* controlled the tendril-less phenotype, and the td-1 locus was mapped to 190 kb region in chromosome 6 of cucumber. A histone acetyltransferase, *CsGCN5* (*Cucumis sativus* GENERAL CONTROL NONDEREPRESSIBLE 5), was regarded as the most possible candidate gene of *td-1*. The mutation of the first exon in CsGCN5 changed an amino-acid and developed the tendril-less phenotype (Chen et al. 2017).

8.4.3 Hypocotyl

The short hypocotyl phenotype was controlled by a recessive allele, *sh1*, in XIS cucumber. The cucumber which carrying the dominant allele *Sh1* are sensitive to LDUVB, but the carrying homozygous sh1 allele are not. The Sh1 allele originated in wild cucumbers and was screened during domestication to adapt to the local environment. Cultivated cucumber mainly carries the allele thesh1allele, which is almost fixed in semi-wild Xishuangbanna's cucumber. By regulating the UVR8 signaling pathway, *sh1* regulates the LDUVB-dependent hypocotyl elongation in cucumber (Bo et al. 2016).

8.5 Mapping for Sex Expression and Disease

8.5.1 Sex Expression

Genomic genetic studies showed that the three genes responsible for gender separation and expression in cucumber lines were *M/ m*, *A/a,* and *F/f*. *F* gene and *M* gene promote the appearance of female flowers and hermaphrodite flowers respectively, and the two recessive genes f and a combine to promote the appearance of male flowers. Various environmental factors and plant hormones also affect the gender expression of cucumber lines. As a candidate gene for *F/f* site, *CsACS1G* encodes a key enzyme in ethylene synthesis pathway (Li et al. 2009).

In cucurbit cucumbers and melons, female flower development is controlled by ACS11, an enzyme encoded by the androecy gene that limits ethylene biosynthesis. ACS11 is expressed in phloem cells and is associated with flowers that are about to develop into female flowers. If ACS11 is not expressed, male flowers appear. In monoecious species, the gene CmWIP1 promoted the development of male flowers, while CmACS11 achieved the coexistence of female and male flowers by inhibiting CmWIP1 (Boualem et al. 2015).

The *CsACO2* gene in cucumber is mutated into *ACO*, which destroys the enzyme activity of *CsACO2* and reduces the ethylene produced by the plant's shoot tips. Therefore, the cucumber produces only androgens and only male flowers. The expression of *CsACO2* in the pericarp overlaps with that of *CsACS11* at the critical stage of female flower sex identification, providing enough ethylene to allow the normal expression of *CsACS2*. *CTACO3*, a homologous gene of *CsACO2*, has a similar expression pattern

in the central lobe region, indicating the *CsACO2/CmAC03*'s conserved function (Chen et al. 2016).

8.5.2 Disease

8.5.2.1 Scab (*Ccu*)

In cucumber, *Ccu* gene was mapped to linkage group 2 of chromosome 2, with a genetic distance of 1.6 cM and 0.7 cM, respectively, from the flanking markers SSR17631 and SSR03084. The accuracy rate was 98.3% by verifying the two flank marks (Zhang et al. 2010a).

The *Ccu* gene was mapped to the 180 kb region. Through detailed annotation of this region, it was found that this region was a cluster composed of six drug-resistant gene analogues (RGAs), belonging to nucleoside binding site (NBS) type R gene. The four RGAs are located in the areas delineated by the two flanking markers Indel01 and Indel02, which are considered possible candidates for *Ccu* (Kang et al. 2011).

8.5.2.2 *Papaya Ring Spot Virus* (*prsv*)

A simple recessive inherited gene, *PRSV* (02245), makes cucumbers resistant to papaya ring spot virus (*PRSV*). The resistance gene, *PRSV* (02245), is mapped to SSR markers on chromosome 6, SSR11-177 and SSR111. The physical distance between ssr11-177 and SSR111 is 600 kb. The accuracy rate of *PRSV* resistance in cucumber lines was more than 80% with SSR11177 (Tian et al. 2015).

8.5.2.3 *Watermelon Mosaic Virus* (*wmv*)

A recessive gene, *WMV* (02245), makes cucumber line '02245' resistant to Watermelon Mosaic Virus. The recessive single gene is mapped to two molecular markers SSRwmv60-23 and caps-w1 on chromosome 6, which are 0.34 cM and 1.19 cM away from *WMV* (02,245), respectively. In the region between the two markers, there were a total of 21 candidate genes in 134.7 kb. After mark-assisted verification, the accuracy rate of SSRWMV6023 was 94.0% (Tian et al. 2016).

8.5.2.4 *Zucchini Yellow Mosaic Virus* (*ZYMV*)

A recessive gene zyma192-18 (02245) makes cucumbers '02245' resistant to Zucchini Yellow Mosaic Virus. The recessive single gene was located on two molecular markers on chromosome 6, 0.9 cM and 1.3 cM from *ZYMV* (02245). By comparing the sequence of candidate gene coding region, it was found that the sequence of encoding vacuolar protein sorting-associated protein 4-like (*vps4-like*) genes were different in the parental lines, so the candidate gene of zyma192-18 was the gene encoding vps4-like protein (Amano et al. 2013).

There is a protein 4-like (*vps4-like*) in cucumber cell line a192-18, and the gene encoding this protein is used as the candidate gene for ZYMV resistance. In the *vps4-like* gene's coding regions, those haplotypes such as a192-18, dina-1, and tmg-1 encode the same protein sequence, indicating that the genetic origin of ZYMV resistance is consistent in different germplasm (Ramírez-Madera and Havey 2017).

8.6 Summary

New plant genomic technologies and cucumber's resources have allowed for a surge in research leading to QTL mapping and identification of candidate genes associated with a wide array of phenotypic traits. In this work, we recorded simple genetic genes or QTLs associated with quality traits, including fruit, leaf, plant stem, sex expression, and disease resistance in cucumbers, which provided chromosome locations, allelic variants and associated polymorphisms, predicted functions where appropriate.

It will be helpful to clarify the relationship between the genes of different quality traits by sorting out the locus that controlling some quality trait in cucumber. Knowledge of the locations of some quality trait in cucumber will allow the design and implementation of more efficient selection to develop high yield, high resistance, and marketable cucumber genotypes.

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Genome Evaluation of Cucumber in Relation to Cucurbit Family

9

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Abstract

Cucumber is the only crop in Cucurbitaceae family with 2n = 2x = 14 chromosomes, while the majority of other crops in this family have chromosome number of n = 10 ~ 12. The cucumber genome size is estimated to be around 367 Mb, which is relatively smaller than melon and watermelon genomes. Comparative genetics and genomics revealed that the cucumber genome has a high level of synteny among chromosomes and sequence identity when compared to melon and *Cucumis hystrix,* both of which are members of genus *Cucumis*. With the rapid development and reduction in cost of sequencing technologies, the genomes of other Cucurbitaceae family members such as melon, watermelon, pumpkin/squash, wax gourd, bottle gourd, bitter gourd, and sponge gourd, have been sequenced in the recent years. The gene number in cucumber is comparable to that of melon, watermelon, and bottle gourd, which is significantly less than that of the other three cucurbit species, according to gene annotation for these genomes. Furthermore, the availability of these genome sequences has enabled extensive phylogenic and synteny comparison of cucurbit species using comparative genomics, which is critical for understanding the genome structure and evolution of various species with different chromosome number.

9.1 Introduction

Members of the *Cucurbitaceae* family, which includes 118 genera and 825 species, are frequently prostrate or climb via tendrils (Jeffrey 1980). An angular stem (typically 5-angled) is distinguished anatomically by bi-collateral vascular bundles that are often arranged in two concentric rings. The leaves are palmately veined, alternate, exstipulate, simple or occasionally palmately compound, and lobed (Bates et al. 1990; Whitaker et al. 1976). The vast majority of members of this family are herbaceous annual vines, with only a few perennial shrubs having perennial tubers or roots. Cucumber (*Cucumis sativus*), melon (*Cucumis melo*), wax gourd (*Benincasa hispida*), watermelon (*Citrullus lanatus*), pumpkin or squash (*Cucurbita pepo*, *Cucurbita moschata*, and *Cucurbita agyrosperma*), bitter gourd (*Momordica charantia*), and sponge gourd (*Luffa cylindrica*) are among the important members

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consumed as vegetables and salads. Some members of this family contribute to human well-being in a variety of ways. For example, the fruit of monk fruit (*Siraitia grosvenorii*), the whole plants of *Gynostemma pentaphyllum* and *Actinostemma tenerum* are usually used in traditional remedies, while *Ecballium elaterium* and *Lagenaria siceraria* which have attractive fruits, are used as popular ornamentals.

The species in Cucurbitaceae family have different chromosome number, but most of them have a similar chromosome number of around n = 12, which may represent the ancestral karyotype of the cucurbit species. Different species of the same genus may have different chromosome numbers. Cucumber (*C. sativus*) is the only species in the genus cucumber with 2n = 14 chromosomes, is one of the top ten vegetables in the world, and is now widely grown in Asia, Europe, and North America. Melon, which has 2n = 24 chromosomes, is an important fruit crop in the *Cucumis* genus (Garcia-Mas et al. 2012). Melon is one of the most diverse species, whereas cucumber has a narrow genetic base. The higher diversity in melon can be seen in the morphological variations of fruit shape, size, color, texture, taste, and composition (Mliki et al. 2001; Lopez-Sese et al. 2003; Staub et al. 2004; Escribano et al. 2012). Watermelon (*Citrullus lanatus*) with chromosome number of 2n = 22 for diploid species is another important fruit crop in Cucurbitaceae family, belonging to the genus *Citrullus*. The *Citrullus* genus contains four diploid species: *C. lanatus* (Thunb.) Mat-sum. & Nakai, *C. colocynthis* (L.) Schrad, *C. ecirrhosus* Cogn., and *C. rehmii* De Winter (Jarret et al. 1997; Levi et al. 2001). Among these four species, *C. lanatus* includes the cultivated watermelon (*C. lanatus* var. *lanatus*), which grows well in West Africa and is widely grown across the world. Genus *Cucurbita* ($2n = 2x = 40$) includes a number of important species, most notably *C. moschata*, *C. maxima*, and *C. pepo*, which are cultivated all over the globe as pumpkins and squash, primarily for their delicious fruits (Gong et al. 2013). Bitter gourd and Sponge gourd are also two important Cucurbitaceae family vegetable crops that are widely cultivated in tropical and subtropical regions of the world. Bitter gourd (*Momordica charantia*, 2n = 22), also known as bitter melon, is an African native that has been domesticated in Asia over a long period of time (Schaefer and Renner 2010). *Luffa cylindrica* (L.) Roem., also known as sponge gourd or luffa, is a diploid herbaceous plant with 26 chromosomes (2n = 26) and one of the nine genus Luffa. It is widely grown in China, Malaysia, Thailand, India, and Africa (Wu et al. 2014).

Because of the rapid advancement of sequencing technology and the reduction in sequencing cost, whole-genome sequences (WGS) are becoming more widely available. Almost all of the major Cucurbitaceous crops' draft genome sequences have been completed using next-generation sequencing (NGS) technology, and draft sequences are now available in the public domain. The Chinese fresh market type cucumber 9930 was the first vegetable genome to be sequenced using a combination of traditional Sanger sequencing (sequencing by synthesis) and NGS technologies (Huang et al. 2009), with a final draft genome sequence of 243.5 Mb assembled and 26,682 predicted genes. A large number of transposable elements have been predicted in the cucumber genome, and 54.4 Mb repeat sequence have been identified in 9930 draft genome version 1.0, accounting for ~24% of the genome (Huang et al. 2009). The cucumber genome of 9930 was improved further using 10X Genomics and high-throughput chromosome conformation capture (Hi-C) data, resulting in a final assembly of about ~211.0 Mb for version 3.0 (Li et al. 2019). In genome v3.0, the genome annotation revealed some novel repetitive sequences, and 82.0 Mb of repetitive sequences were identified, representing 36.43% of the genome (Li et al. 2019). This is ~27.6 Mb more than the predicted in previous version. Long terminal retrotransposons (LTRs) are still the most abundant of these repetitive sequences, and their sizes have been markedly increased in v3.0. The majority of these full-length LTRs (FL-LTRs) occurred recently in cucumber. Additionally, the draft genomes of inbred lines Gy14, a North American pickling

cucumber and B10, a European pickling type cucumber, were also sequenced and assembled (Woycicki et al. 2011; Yang et al. 2012). The Gy14 genome was sequenced using Roche/454 technology, and 244 scaffolds were anchored on a high-resolution genetic map containing 735 SSR markers. The integration of genetic and physical mapping resulted in a 193 Mb chromosome-level draft genome assembly. The complete genome sequence information of these three cucumber genotypes prompted a comparative genomics study of cucumber to other species of Cucurbitaceae family.

Except for cucumber, the genomes of other Cucurbitaceae species have been sequenced in recent years, including melon (Garcia-Mas et al. 2012), watermelon (Guo et al. 2013), pumpkin/squash (Montero-Pau et al. 2018; Sun et al. 2017), wax gourd (Xie et al. 2019), bottle gourd (Wu et al. 2017; Xu et al. 2014), bitter gourd (Urasaki et al. 2017; Cui et al. 2020), and sponge gourd (Zhang et al. 2020). Because these genome sequences are now available, comprehensive phylogenic and synteny comparisons of cucurbit species are now possible. The genome sequences and genetic maps are powerful tools for understanding the genome organization and evolution of distinct cucurbit species with varying chromosome number, as described in the following sections.

9.2 Comparative Genetics and Genomics in *Cucumis* Genus

9.2.1 Syntenic Relationships Between Cucumber and Melon

There are 52 species in the *Cucumis* genus, with Cucumber (*C. sativus* L., 2n = 2x = 14) and melon (*C. melo* L., 2n = 2x = 24) being the only two important crops (Schaefer 2007; Sebastian et al. 2010). Both species originated in Asia and differentiated from a common ancestor approximately 10 million years ago (Sebastian et al. 2010). *C. sativus* is the only Cucumis species having 2n = 14 chromosomes, while the rest have 2n = 24 chromosomes (Kirkbride 1993).

Despite their distinct evolutionary connection and sexual incompatibility, the genomic sequences of melon and cucumber are remarkably conserved. The melon genome is approximately 425 Mb in size, while the cucumber genome is approximately 367 Mb in size, with a similar number of protein-coding genes in both species (Li et al. 2011b; Garcia-Mas et al. 2012), and the size difference is thought to be primarily due to expansion of intergenic regions and proliferation of transposable elements in the melon genome (Garcia-Mas et al. 2012; Yang et al. 2014). Furthermore, cucumber and melon molecular markers have a considerably better cross-species transferability than other cucurbit species (Neuhausen 1992; Katzir et al. 1996; Danin-Poleg et al. 2000; Park et al. 2004; Gonzalez et al. 2010). Zhu et al. (2016a) developed 28,570 SSR markers from the draft genome of melon DHL92 and tested their cross-species transferability in cucumber and watermelon. 14.01 percent (4002) of Gy14 cucumber draft assembly contained one amplicon, and these cross-species SSR markers spanned 191.53 Mb, accounting for 99.44 percent of cucumber Gy14 assembly. Only 1,085 melon SSR markers have one amplicon in the watermelon draft genome assembly (Zhu et al. 2016a). The number of cross-species SSR markers in cucumber, melon, and watermelon, is consistent with the evolutionary distances between the three species.

The origin of the seven cucumber chromosomes has long been a mystery, and many studies have been focused on it by looking into the syntenic relationship between the cucumber seven chromosomes and the melon twelve chromosomes. Huang et al. (2009) examined the syntenic relationship between cucumber and melon by aligning 348 melon markers on cucumber chromosomes, and discovered that cucumber chromosome 7 corresponded to melon chromosome 1. They proposed that after divergence from melon, the fusion of ten ancestral chromosomes resulted in modern-day five chromosomes in cucumber. A combined melon

genetic map derived from two melon mapping populations with 199 SSR markers taken from the cucumber genome was constructed. Syntenic relationship between melon and cucumber chromosomes was predicted based on correlations between markers on the consensus melon map and cucumber draft genome scaffolds (Li et al. 2011a). It was discovered that cucumber chromosome 7 is syntenic with melon chromosome I. Cucumber chromosomes 2 and 6 are syntenic to melon chromosomes III + V + XI and III + VIII + XI, respectively. Cucumber chromosomes 1, 3, 4, and 5 were syntenic with genomic portions of two melon chromosomes, respectively, II + XII, IV + VI, VII + VIII, and IX + X. However, the marker ordering in many syntenic blocks on these consensus linkage maps was not co-linear, implying that cucumber has undergone more complex structural changes during evolution than simple chromosomal fusion events.

Using a double-haploid line DHL92, Garcia-Mas et al. (2012) sequenced the melon genome and assembled a draft genome of 375 Mb, representing 83.3 percent of the expected melon genome. They also used comparative genomics to align cucumber and melon genomes, and synteny analysis revealed an ancestral fusion of five melon chromosomal pairs in cucumber, as well as numerous inter- and intra-chromosome rearrangements. The high level of synteny at greater resolution revealed several previously unseen shorter areas of chromosomal rearrangements. Melon LG I correspond to cucumber chromosome 7, but with several inversions and a larger overall chromosomal size (35.8 vs. 19.2 Mb). Cucumber chromosome 3 was formed after the fusion of melon LG IV and LG VI, albeit with numerous rearrangements and a decrease in overall size (39.7 Mb vs. 30.4 and 29.8 Mb). Melon LG IV and cucumber chromosome 3 distal 8.5 and 5 Mb are highly collinear, but with a gradual increase in size in melon toward the heterochromatic portion, corresponding to a higher density of transposable elements and a lower density of gene fraction in melon. Due to the lack of orientation of some small scaffolds in both species, the total number of small inversions cannot be easily determined. The whole-genome comparison revealed more complex chromosomal rearrangements between cucumber and melon.

The cucumber/melon synteny was refined further through large-scale comparative fluorescence in situ hybridization (FISH) mapping using 128 fosmid clones comparative genomics, between cucumber and melon. The DHL92 melon genome was divided into 91 syntenic blocks, each of which was larger than 500 kb and shared sequence similarities with the Gy14 cucumber draft genome. 35 of the 91 syntenic blocks were anchored by two or more fosmid loci, providing support to their orientation. At least one inversion between cucumber and melon was discovered in 45 syntenic blocks. Synteny was discovered between cucumber chromosome C2 and melon chromosome V (Li et al. 2011a; Garcia-Mas et al. 2012). Two additional syntenic blocks of melon chromosome V were discovered in cucumber chromosomes C4 and C6, which were verified using pachytene FISH. They specially focused on cucumber chromosome C7, which has a one-to-one whole chromosomal synteny with melon CI. They used comparative FISH mapping to validate this conserved synteny in cultivated cucumber (*C. sativus var. sativus*), wild cucumber (*C. sativus var. hardwickii*, the progenitor species of cultivated cucumber; Yang et al. 2012), *C. hystrix*, melon, and watermelon (Fig. 9.1). With the exception of probe 72.0, which found no signal in melon, all 14 probes detected single hybridization signals in these species. A *C. sativus* specific paracentric inversion covering six fosmid loci (71.1–71.6) was reported in the C7 (Yang et al. 2014). One or two inversions distinguished the short arms of wild cucumber, *C. hystrix* and *C. melo* (defined by fosmid loci 71.1–71.8). The six fosmid loci (71.9–72.4) on the long arm showed complete collinearity across the *Cucumis* species, indicating that this area is highly conserved in *Cucumis*. In the Gy14 cucumber (Yang et al. 2014) and DHL92 melon (Garcia-Mas et al. 2012) draft genomes, the region was around 8.0 and 13.0 Mb, respectively. They also investigated at the extent of conservation of this region in other distant cucurbit species by retrieving sequences

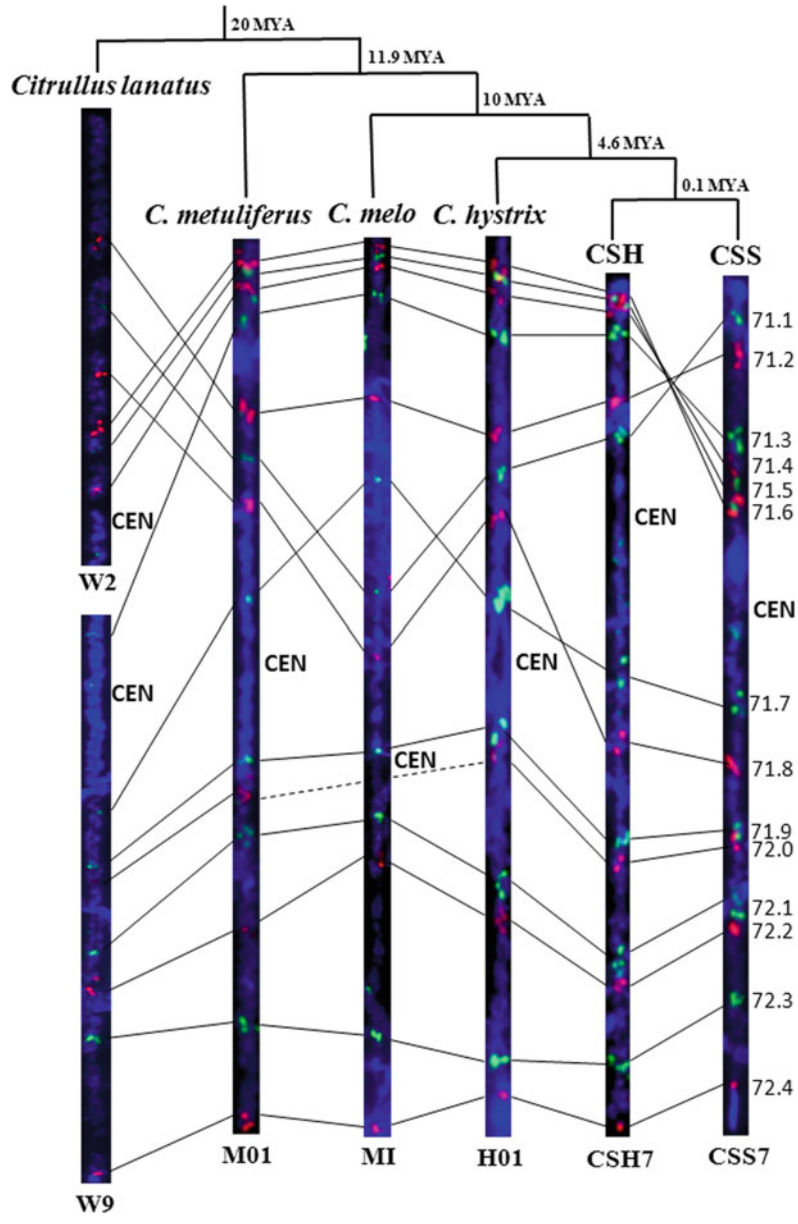

Fig. 9.1 Synteny of cucumber chromosome 7 (C7) with other cucurbit species

in the syntenic block of this region from the watermelon draft genome (Guo et al. 2013). By comparing annotated genes, DNA transposons, and retrotransposons, they discovered that 70% of genes in this area were conserved across the three species, and that the number of transposons in melon had increased 3.6 and 2.5 times when compared to cucumber and watermelon, respectively. Melon gene density was lower than that of cucumber and watermelon, which is consistent with its large size. In contrast, the distribution of mobile elements, was not consistent across the region. The proximal centromeric region of melon expanded significantly, with a greater frequency of mobile elements. The distribution of genes and transposons or retrotransposons in watermelon was fairly consistent, but the highly conserved regions in both melon and cucumber

were gene-dense, with the region corresponding to the expansion in melon also being transposon-dense. Cucumber chromosome C7 has remained mostly intact throughout Cucumis evolution. C7 centromere positions, melon chromosome I centromere positions, and *C. hystrix* H01 centromere positions all appear to be consistent with a centromere repositioning event.

9.2.2 Syntenic Relationships Between Cucumber, *C. Hystrix*, and Melon

According to phylogenetic analysis, *C. hystrix* is the sister species of *C. sativus* (Ghebretinsae et al. 2007; Renner et al. 2007; Sebastian et al. 2010). *C. hystrix* (2n = 2x = 24) is the closest of the 24 Cucumis species to *C. sativus* (2n = 2x = 14), from which it diverged around 4.6 million years ago (Sebastian et al. 2010). Embryo rescue has produced inter-specific hybrids between *C. hystrix* and *C. sativus* (Chen et al. 1997). *C. hystrix* shares a distribution that overlaps with that of wild cucumber (*C. sativus var. hardwickii*) in Myanmar (Burma), North and West Thailand, and Southwest China (Sebastian et al. 2010). *C. sativus* and *C. hystrix* descended from the same progenitor, according to these findings. *C. hystrix* is important in understanding the process of chromosomal reduction in Cucumis because it is the sister species to *C. sativus*. Prior to *C. hystrix* genome sequencing, only limited genetic and genomic data was available for these comparisons. Yang et al. (2014) sequenced two *C. hystrix* accessions, WI7001 and WI7002 using both Roche/454 GS FLX and Illumina-HiSeq 2000 technologies. WI7001 and WI7002 high-quality reads produced by the Roche/454 were 651.8 and 418.0 Mb, respectively. From approximately 252 million raw Illumina-HiSeq 2000 sequences, 5218 Mb WI7001 and 4432 Mb WI7002 sequences were retrieved. A draft genome assembly of 209 Mb sequences for *C. hystrix* including 11,649 scaffolds was generated using a hybrid of Illumina contig sequences and Roche/454 paired-end reads. They generated 128,257 and 117,711 SSR markers from the WI7001 and WI7002 genome assemblies, respectively.

A *C. hystrix* genetic map was constructed using 215 *C. hystrix*, 151 cucumber, and 50 melon SSR markers, with 416 markers covering 1001.5 cM map length, which was comparable to two previous melon genetic maps (Diaz et al. 2011; Li et al. 2011a), implying nearly complete coverage of the *C. hystrix* genome. The 416 SSR markers were divided into 12 linkage groups based on their synteny with the corresponding melon chromosomes, H01–H12 (I to XII). The *C. hystrix* was aligned to 261 of the 416 markers, covering about 294 Mb (93%) of the DHL92 melon genome. The 261 shared markers were used to infer the syntenic relationship between *C. hystrix* and melon. Because 10 of the 12 C. hystrix chromosomes were highly syntenic with melon, thus retaining the ancestral condition. Interestingly, the 53.1–97.5 cM block on C. hystrix chromosome H02 was syntenic to melon chromosome II, whereas the 0–42.8 cM block was about 7.2 Mb syntenic to the distal end of melon chromosome VIII. Seven of eight SSR markers in H08 were shared with chromosome VIII, while one was on the syntenic region of melon chromosome II. Pachytene FISH-based which provided evidence of reciprocal translocation between H02 and H08 confirmed these findings. They used 128 fosmid clones encompassing all chromosomes for large-scale comparative pachytene FISH mapping between *C. hystrix* and melon, and found at least 14 inversions between the two genomes. All fosmid probes examined in chromosomes H09 and H10 were completely collinear with those tested in melon chromosomes IX and X, respectively, indicating that the two chromosomes were highly conserved during evolution. The cucumber genome was found to be aligned to 348 (84%) of the 416 markers in the *C. hystrix* genetic map, covering 95 percent (181/191 Mb) of the Gy14 draft genome assembly. The syntenic relationships between *C. hystrix* and *C. sativus* are based on the chromosome synteny inferred from this alignment and the physical locations of these markers in the Gy14 genome may be expressed

as follows: H01 = C7, H02 = C1 + C6, H03 = C2 + C6, H04 = C3, H05 = C2 + C4 + C6, H06 = C3, H07 = C4, H08 = C1 + C4 + C6, H09 = C5, H10 = C5, H11 = C2 + C6, and H12 = C1. To better understand the syntenic relationships between the two species, the 12 *C. hystrix* chromosomes were divided into 53 syntenic blocks. Each block was defined as an area on the *C. hystrix* genetic map anchored by at least one common marker or fosmid position and matching a continuous length of DNA sequences in the Gy14 genome. The order of shared markers on the *C. hystrix* genetic map and in the Gy14 assembly was used to establish the orientation of each syntenic block in reference to the Gy14 genome assembly, which was then confirmed using pachytene FISH. Twenty of the 53 syntenic blocks were collinear, and 25 included inversions between cucumber and *C. hystrix*. The orientation of eight blocks is unclear. In terms of synteny with *C. hystrix*, the seven cucumber chromosomes were precisely mapped and can be deduced to the following: C1 = H02/H08 + H12, C2 = H03 + H05 + H11, C3 = H04 + H06, C4 = H7 + H8 + H05, C5 = H09 + H10, C6 = H03 + H11 + H08/H02 + H05, and C7 = H01 (Table 9.1). Cucumber C7 has complete synteny with H01, but C1, C3, and C5 appear to be the consequence of the fusion of two *C. hystrix* chromosomes, and C2, C4, and C6 include syntenic blocks from more than two *C. hystrix* chromosomes. The arrangement of *C. hystrix* syntenic blocks in all cucumber chromosomes except the C7 is definitely more evolutionarily complex than a simple *C. hystrix* chromosomal fusion.

Pachytene FISH analysis was performed with 14 fosmid probes of cucumber chromosome 7 in other four *Cucumis* species (CSS, *C. sativus* var. *sativus*; CSH, the wild cucumber *C. sativus* var. *hardwickii*; *C. melo* MI; *C. hystirx* H01 and *C. metuliferus* M01) and watermelon (chromosome W2 and W9). CEN indicates the putative centromere location.

9.2.3 Syntenic Relationships Between Cucumber and Watermelon

Watermelon (2n = 22) is a popular horticultural crop and one of the most popular fresh fruits consumed globally. It belongs to the *Citrullus* genus, which includes four diploid species (Levi et al. 2001). *Citrullus lanatus* includes the

Table.9.1 The syntenic relationship in *Cucumis* genus revealed by different studies

Cucumber (Chromosome)	1	2	3	4	5	6	7
C. hystrix (Yang et al. 2014)	H02, H08, H12	H03, H05, H11	H04, H06	H05, H07, H08	H09, H10	H02, H03, H05, H08, H11	H07
Melon (Huang et al. 2009)	II, XII	III, V	IV, VI	VII	IX, X	III, VIII, XI	I
Melon (Li et al. 2011)	II, XII	II, V, XI	IV, VI	VII, VIII	IX, X	III, VIII, XI	I
Melon (Garcia-Mas et al. 2012)	II, XII	III, V, XI, (I, X)	IV, VI, (V)	VII, VIII	IX, X	III, VIII, XI, (V, XII)	I
Melon (Yang et al. 2014)	II, XII	III, V, XI	IV, VI	V, VII, VIII	IX, X	III, V, VIII, XI, (XII)	I

cultivated watermelon (*C. lanatus* var. *lanatus*), which thrives in West Africa and has been widely cultivated worldwide (also known as 'egusi' melon), the preserving melon (*C. lanatus* var. *citroides*), which is grown in Southern Africa (also known as 'tsamma' melon) (Chomicki and Renner 2015; Paris 2015), and *C. colocynthi* ('bitter apple') is a perennial species grown in sandy areas throughout northern Africa, south-western Asia, and the Mediterranean (Levi et al. 2001). Genome size of watermelon is relatively small and is around 425 Mb. The genomes of elite Chinese watermelon line 97103 (Guo et al. 2013) and the American heirloom watermelon cultivar Charleston Gray have both been sequenced and made available in the cucurbit genomics database (www.icugi.org). The 97103 draft genome sequence was 353.5 Mb in size, and represented 83.2 percent of the projected watermelon genome. These watermelon genomic resources have aided to fundamental research in areas like as molecular marker development, genetic map construction, gene/QTL mapping, and comparative genomics (Ren et al. 2012; Lambel et al. 2014; Reddy et al. 2015). Cucumber and watermelon draft genome assemblies have now been made public, and the availability of a large number of molecular markers has enabled researchers to clearly establish syntenic relationships between the two species. Huang et al. (2009) established the syntenic relationship between cucumber and watermelon by aligning 136 watermelon marker sequences mapped from watermelon linkage groups in 9930 cucumber draft genome. Because the watermelon genetic map they used contained only 18 linkage groups, the syntenic relationship between two species was not reflected at the chromosomal level. Guo et al. (2013) used comparative mapping to examine chromosome-to-chromosome associations within the Cucurbitaceae family and found complex syntenic patterns depicted as mosaic chromosome-to-chromosome orthologous relationships among watermelon, cucumber, and melon.

The syntenic relationship and chromosomal rearrangements between cucumber and watermelon, on the other hand, are still fragmented and incomplete. Zhu et al. (2016b) generated 32,869 SSR markers and found 832 highly conserved cross-species SSR markers between watermelon genome and cucumber Gy14 pseudo-chromosomes using the published 353.5 Mb genomic sequences of East Asia watermelon cultivar 97103. Using these 832 cross-species SSR markers, they deduced the syntenic relationships between watermelon and cucumber chromosomes. Watermelon and cucumber major syntenic chromosomes exhibited complex patterns for different chromosomes. The simplest syntenic pattern with cucumber was seen on watermelon chromosomes W3 and W10, which were predominantly syntenic to two cucumber chromosomes. W3 contained 55 SSR markers in common with cucumber, 52 of which were found on C1, and the remaining three were found on C5. Watermelon chromosomes W2 and W11, which were both syntenic to five cucumber chromosomes, showed the most complex patterns. Watermelon chromosome W9 was syntenic with four cucumber chromosomes, while the remaining six watermelon chromosomes were syntenic with three cucumber chromosomes apiece.

The 11 watermelon chromosomes were further divided into syntenic blocks based on cross-species SSR markers that shared continuous physical locations on both genomes. At least three SSR markers were present in each block. The researchers divided the 11 chromosomes into 84 syntenic blocks, 44 of which were collinear, and the remaining 40 blocks showed inversions between the watermelon and cucumber genomes. There were 5–11 distinct syntenic blocks on each watermelon chromosome. WCB73 (W10) was the longest block on cucumber chromosome C3, measuring 7.70 Mb. Watermelon syntenic block WCB6 contained the most shared SSR markers that were collinear between cucumber C6 and watermelon W1. Using FISH, Zhu et al. (2016b) further confirmed the syntenic relationships between cucumber chromosome C7 and watermelon chromosome W2 and W9. To validate the synteny in watermelon, 14 cucumber fosmid probes situated on cucumber C7 were chosen. In

watermelon, all 14 fosmid probes identified a single hybridization signal, despite being located on two distinct chromosomes, W2 and W9. On watermelon chromosome W2, five probes (71.1–71.2, 71.4–71.6) from the short arm and one fosmid (71.8) from the long arm of cucumber C7 were discovered, while the remaining eight probes (71.1–71.2, 71.4–71.6) were discovered on chromosome W9 (Fig. 9.1). Two inversions were discovered between watermelon W2 and cucumber C7 that were consistent with blocks WCB8 and WCB9, indicating that the results of in silico comparative mapping were in accordance. When compared between the top of W9 and the end of cucumber C7 in watermelon, the large inversion block WCB62 stretched approximately 8 Mb, as validated by FISH mapping using probes 71.9–72.4. In comparison to cucumber, six probes on watermelon W2 were collinear with melon chr I, which was divided into two genomic regions in accordance with blocks WMB8 and WMB9. The remaining fosmid probes detected three inversions between watermelon W9 and melon chr I, which were associated with the blocks WMB56, WMB 58, and WMB60. The findings revealed by comparative mapping utilizing cross-species SSR markers were fully consistent with the results of FISH mapping.

9.2.4 Syntenic Relationships Between Cucumber and Other Cucurbit Crops

9.2.4.1 Cucumber and Wax Gourd

Wax gourd (2n = 24) is a tropical gourd that is commonly cultivated in India, Japan, China, and other tropical places. It is native to the Indo-China region (Robinson et al. 1997). Wax gourd is the sole member of the genus *Benincasa*, which belong to the tribe Benincaseae, which also includes cucumber, melon, watermelon, and bottle gourd. Xie et al. (2019) sequenced a wax gourd inbred line B227 using Illumina and single-molecule real-time (SMRT) sequencing technology, yielding a 913 Mb draft genome assembly with a scaffold N50 of 3.4 Mb. A total of 27,467 protein-coding genes have been predicted in this genome. Despite the fact that the number of protein-coding genes in the wax gourd genome is equivalent to those in cucumber, melon, and watermelon genomes, the wax gourd draft genome is at least double bigger than the other three species of the Benincaseae tribe. They also discovered that the larger size of wax gourd genome is not due to a specific whole-genome duplication (WGD) event, but rather to a significant accumulation of transposable elements in its genome following its separation from other species. The wax gourd genome is predicted to have a high percentage of repetitive sequences (75.5%) (689.5 Mb). Wax gourd has considerably longer DNA transposons and long-terminal repeat (LTR) retrotransposons, including Copia, Gypsy, and other elements, than cucumber, melon, and watermelon. Copia elements in wax gourd, for example, are 20 times longer than those in cucumber and nine times longer than those in melon and watermelon. Furthermore, a lot of species-specific LTRs have been discovered, particularly in the genome of wax gourd, which is one of the reasons for its large genome. A maximum-likelihood phylogenetic tree was built using 463 single-copy conserved gene families, and it revealed that wax gourd diverged about 18.1 million years ago (MYA) with a sister clade to Cucumis species, and the two clades diverged about 18.1 MYA, which is consistent with previous research (Schaefer et al. 2009). According to the distribution of synonymous substitution levels (Ks) of syntenic orthologous and paralogous genes, the wax gourd genome was inferred to have the slowest evolutionary rate among the cucurbit species and the most preserved ancestral karyotype (Xie et al. 2019).

9.2.4.2 Cucumber and *Cucurbita* Species

Pumpkin and squash belong to *Cucurbita* genus (2n = 2 × = 40), which includes at least five domesticated and over ten wild species (Ferriol and Pico 2008). Most *Cucurbita* species are wild resources, but three domesticated species are widely cultivated in the world including

Cucurbita maxima, *Cucurbita moschata,* and *Cucurbita pepo* (Loy 2004). The earliest archeological evidence indicate that *Cucurbita* species were domesticated around 8,000–10,000 years ago (Nee 1990; Smith 1997). The *Cucurbita* genus, native to the America and southern Mexico, was first introduced into Europe and then underwent a great diversification in Asia, which later became the secondary center of domestication (Nee 1990; Ferriol and Picó 2008; Kates et al. 2017). Cucurbita species have a wider range of fruit form, size, and color than other Cucurbitaceae species due to their long history of cultivation and domestication (Savage et al. 2015).

Cucurbita species have relatively small genome sizes and a larger number of chromosomes compared to other Cucurbitaceae crops (Arumuganathan and Earle 1991). The assembled genome sizes are 271.4 Mb for *C. maxima*, 269.9 Mb for *C. moschata*, and 263 Mb for *C. pepo*, respectively (Sun et al. 2017; Montero-Pau et al. 2018). The repeat sequences accounted for more than 40% of both *C. maxima* and *C. moschata* genome assemblies (Sun et al. 2017), and a similar result was found in *Cucurbita pepo* genome, which consisted of 93 Mb repetitive elements, representing 37.8% of the assembly (Montero-Pau et al. 2018). The majority of LTRs in three Cucurbita genomes are copia- and gypsy-type LTRs. Cucumber (Huang et al. 2009), melon (Garcia-Mas et al. 2012), and watermelon (Huang et al. 2012) have all revealed similar trends in their genomes (Guo et al. 2013). Though the genomes of *Cucurbita* species are relatively smaller, the predicted protein-coding genes in *C. maxima* 32,076, *C. moschata* 32,205, and *C. pepo* 34,240 genomes were significantly higher than those in cucumber (23,248; Li et al., 2011b), melon (27,427; Garcia-Mas et al. 2012), and watermelon (23,440; Guo et al. 2013). In addition, Sun et al. (2017) also discovered 2414 and 2336 transcription factors in *C. maxima* and *C. moschata*, respectively, which is nearly double the number found in other sequenced cucurbit genomes. These findings indicated that the genomes of these cucurbit species underwent a WGD event that was not seen in other sequenced cucurbits such as cucumber, melon, watermelon, and bitter gourd.

9.2.4.3 Cucumber with Bottle Gourd

Bottle gourd (*Lagenaria siceraria* (Molina) Standl.) (2n = 22) belongs to the genus *Lagenaria* in the Cucurbitaceae family, which is an edible, medical, container and grafting stock plant cultivated all over the tropics. The African *L. siceraria* ssp. *Siceraria* and the Asian *L. siceraria* ssp. *Asiatica* (Schlumbaum and Vandorpe 2012) are two subspecies that are thought to have originated in Sub-Saharan Africa (Decker-Walters et al. 2004). Bottle gourd is extensively cultivated throughout the globe, notably in East Asian nations, and is one of the oldest crops cultivated by humans (Kistler et al. 2014).

Bottle gourd has 22 chromosomes, with its estimated genome size of about ∼334 Mb (Achigan-Dako et al. 2008). Wu et al. (2017) reported the de novo assembly of the USVL1VR-Ls bottle gourd inbred line, resulting in a total genome size of 313.4 Mb (∼93.8 percent of the predicted genome size). Based on the integration of two genetic maps, 308.1 Mb (98.3%) of the assembled scaffolds were anchored to the 11 linkage group (Xu et al. 2014; Wu et al. 2017). It has 22,472 protein-coding genes predicted (Wu et al. 2017), which is equivalent to cucumber (23,248) and watermelon (23,440) gene numbers (Li et al. 2011b; Guo et al. 2013). Its genome has 46.9% repeat elements and 39.8% of these are LTRs, with copia-type (23.2%) and gypsy-type (13.4%) LTRs being the most common. Similar findings have been found in cucumber, melon, and watermelon.

Several researchers have explored at the bottle gourd's synteny with other cucurbit species. Using 2098 high-quality SNPs with a total length of 1361 cM, Xu et al. (2014) created a high-density genetic map encompassing 11 linkage groups, probably corresponding to the 11 chromosomes of the haploid bottle gourd genome. They used a low-depth genome assembly of scaffolds and a genetic map, with 922 scaffolds

anchored, to detect syntenic areas among related cucumber, melon, and watermelon species. Each bottle gourd LG (Ls-LG) matched with one to three chromosomes of cucumber and melon, and up to four chromosomes of watermelon, as evident from macro-collinearity between bottle gourd and each of the three genomes. Because LsLG8 and LsLG10 are co-linear to just one or two chromosomes of the other genomes, they appear to have suffered the fewest chromosomal break/fusion events.

Even though watermelon is phylogenetically closest to bottle gourd and shares the same haploid chromosome number of 11, their syntenic relationship does not appear to be straightforward. No one-to-one chromosomal correspondence is present between the two genomes and both intra- and inter-chromosomal rearrangements have been observed. Based on the Ks distribution of orthologous genes between species, it suggested that bottle gourd diverged from watermelon around 10.4–14.6 million years ago, from *Cucumis* 17.3–24.3 million years ago, and from bitter gourd 29.2–41.0 million years ago (Wu et al. 2017). They also hypothesized that the ancestral Cucurbitaceae karyotype (ACK) has 12 protochromosomes based on a comparison of the genomes of bottle gourd, watermelon, melon, cucumber, and squash. They also looked at the synteny and paralogy between ACK and the cucurbit species, using the 12 hypothesized protochromosomes as the Cucurbitaceae origin, which showed that melon has preserved its ancestral genome structure, unlike the other species that had shuffling events. ACK gives rise to 11 bottle gourd chromosomes, derived from 19 chromosomal fissions, and 20 chromosomal fusions. The modern cucumber genome (seven chromosomes) was formed by 6 chromosomal fissions and 11 fusions, whereas the watermelon genome (eleven chromosomes) was formed by 27 fissions and 28 fusions. Finally, to attain its current structure of 20 chromosomes, the squash genome underwent chromosomal rearrangements and a specific whole-genome duplication.

9.2.4.4 Cucumber with Bitter Gourd

Bitter gourd (*Momordica charantia*, 2n= 22) is a dicot vine species belonging to the family Cucurbitaceae, and it is characterized by its warty-skinned fruit widely cultivated in tropical and subtropical regions of the world. Bitter gourd is native to Africa, but it was domesticated in Asia over a long period of time, according to recorded Sanskrit documents from the Indo-Aryan culture (2000–200 BC) (Deena et al. 1999; Schaefer and Renner 2010). Urasaki et al. (2017) sequenced a draft genome of the bitter gourd line OHB3-1, resulting in a scaffold-level genome assembly of 285.5 Mb, accounting for 84 percent of the bitter gourd projected genome size (339 Mb) (Urasaki et al. 2015). The OHB3-1 draft genome assembly predicted 45,859 protein-coding genes, which is much higher than the other sequenced Cucurbitaceae genomes. Cui et al. (2020) sequenced the whole genomes of *M. charantia* lines Dali-11 and TR, resulting in *de novo* scaffold assemblies of 293.6 and 296.3 Mb for Dali-11 and TR, respectively. Both of these lines had a genome size of around 300 Mb, which was less than OHB-1 genome (339Mb). They also reported that transposable elements (TEs) made up 41.5 percent (121.8 Mb) and 39.9 percent (118.2 Mb) of the Dali-11 and TR assemblies, respectively. Of these, 31.8 % and 33.1 % were long-terminal repeat (LTR) retrotransposons (Cui et al. 2020). The Dali-11 and TR genomes contain 26,427 and 28,827 protein-coding genes, respectively. The number of genes predicted in both bitter gourd genomes was similar to cucumber, melon, and watermelon genomes, but significantly lower than the OHB3-1 genome (Urasaki et al. 2017).

Synteny mapping of the OHB3-1 scaffolds against pseudomolecule sequences of cucumber, melon, and watermelon was used to compare the bitter gourd genome to that of other Cucurbitaceae crops. Bitter gourd was found to be closely related to watermelon rather than to Cucumis species (Urasaki et al. 2017). The result is also supported by synteny mapping and phylogenetic analysis of internal transcribed spacer sections of nuclear

ribosomal RNA genes or sequences of chloroplast genes (Jobst et al. 1998; Schaefer et al. 2009), proving that the bitter gourd is more closely related to watermelon than cucumber or melon (Jobst et al. 1998; Schaefer et al. 2009).

Cui et al. (2020) did a genome comparison between bitter gourd and other Cucurbitaceae family species. Cucumber, melon, watermelon, bitter gourd, zucchini, pumpkin, and bottle gourd all have mapped to 2248 single-copy orthologous genes. According to phylogeny and molecular clock analyses based on the 2248 shared single-copy genes, *M. charantia* diverged from the distantly related genus *Cucurbita* around 36.5 million years ago, indicating that it is an older species than other cucurbit crops. There has been no recent whole-genome duplication (WGD) in the *M. charantia* genome, as there has been in cucumber, melon, and watermelon. They also identified 992, 807, and 922 large syntenic blocks, and these syntenic regions contained 14,938, 14,567, and 14,804 genes in *C. lanatus*, *C. melo*, and *C. sativus*, respectively.

9.3 Conclusions

Cucurbit species have extremely diverse genomes in terms of genomic size and chromosomal number. Cucumber (2n = 14) has the smallest genome size and the least chromosomes in the Cucurbitaceae family. Despite these considerable variations, comparative mapping revealed that the linear order of genetic markers and genes in distinct cucurbit genomes is well maintained. The advantages of such conservation will be useful in a variety of ways, such as promoting cucumber as a model genome for cucurbit species, implementing cross-species transferable markers in other cucurbit genomes, and developing better positional cloning techniques.

In the last decade, the development of new sequencing technologies has allowed whole-genome sequencing and de novo assembly of many species, and the draft genome assembly of almost all of the important Cucurbitaceae crops is now available, which has greatly prompted comparative studies in this family. To give information on the underlying genome evolution, comparative genomic analysis examines the similarities and unique differences, gene number differences, and repetitive elements between related species.

Cucurbit species have undergone many events of genome expansion and chromosomal rearrangements, such as chromosome fusion and fission, but have retained remarkable overall conservation between the genomes. The structural changes like as insertions, deletions, duplications, inversions, and translocations have been linked to stress tolerance, resistance, increased yields, adaptation, and speciation, and they are a significant driver of genetic and phenotypic diversity.

Though the macro-collinearity is commonly identified among cucurbit species, there are still lots of large and small chromosomal structural variations occurring even in mostly closely related species such as cucumber and melon, which may be the main reasons for the big change and difference of genetic diversity between two species. Furthermore, the sequence comparisons and gene annotation at syntenic blocks or genomic level has revealed that the size difference between different cucurbit species is primarily due to the expansion of repetitive DNA, the majority of which corresponds to retrotransposons. These comparative genomic findings have potential to greatly improve our understanding of molecular function and evolutionary processes in cucurbit species.

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Cyto-Molecular Genetics of the Interspecific Hybridization in Cucumber

10

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Abstract

Cucumber, *Cucumis sativus* L. (CC, 2n = 2*x* = 14), is a valuable vegetable crop widely cultivated and consumed around the world. However, due to the narrow genetic base and lack of resistant genes, cucumber breeding has been hindered, especially in resistance breeding. Cultivated cucumber suffered from a range of devastating diseases, like downy mildew, powdery mildew, root-knot nematode, etc. Therefore, transferring specific traits from the wild relatives through interspecific hybridization has been highlighted for its importance by the breeders for a long time. Among more than 50 wild relatives, *C. hystrix* (HH, 2n = 2*x* = 24) is the only wild *Cucumis* species grouped into the same subgenus together with *C. sativus*, while all others are classified into *Melo* subgenus. Also, bearing multiple disease-resistant characteristics, *C. hystrix* is the only known wild species cross-compatible with *C. sativus* in this genus. The one and only successful synthetic allotetraploid *C.* ×*hytivus* J. F. Chen & J. H. Kirkbr (HHCC, 2n = 4*x* = 38) was obtained via an interspecific hybridization between *C. hystrix* and *C. sativus*. It has been reported that both genetic and epigenetic reprogramming in this *C.* ×*hytivus*, which might be the reason for the novel phenotypic variation, such as delay maturation. Hybridization and allopolyploidization frequently bring a 'genomic shock' that causes rapid genetic and epigenetic changes, due to the merger of two or more divergent genomes, which leads to many problems, like the meiosis abnormality, extensive abnormal chromosome pairing, imbalanced chromosome segregation, and karyotype variations. Still, according to the clear genetic background and small genome size with whole-genome released recently, the *Cucumis* allotetraploid could serve as an excellent system for studying immediate consequences following allopolyploidization. Cyto-molecular genetics and genomic information of this hybrid and its allotetraploid could provide a novel insight into the establishment of allopolyploids with different chromosome bases, as well as provide effective ways to create new species and materials, which can be employed for cucumber and melon improvement.

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10.1 Introduction

Cucumber, *Cucumis sativus* L. (CC, 2n = 2*x* = 14), has been served as one of the model systems for studying sex determination and vascular biology, whose available draft genome was first released publicly among major horticulture crops (Huang et al. 2009). Also, cucumber, one of the valuable vegetable crops, is widely cultivated and consumed around the world (Pitrat et al. 1999; Wang et al. 2020). However, the narrow genetic base hampered cucumber breeding, and lack of resistant genes, whose nucleotide-binding site (NBS)-containing resistant genes are less than one-third of that in *Arabidopsis*, cucumber suffered from a range of devastating fungal, bacterial, viral, and insect diseases, like downy mildew (caused by *Pseudoperonospora cubensis*), powdery mildew (caused by *Podosphaera xanthii*), root-knot nematode (caused by *Meloidogyne* spp.), etc. (Chen and Lewis 2000; Pląder et al. 2007; Huang et al. 2009; Zhang et al. 2018; Cheng et al. 2019). Wild species captured more attention for they possess abundant potential useful genes, which are urgently needed in cultivated cucumber. However, bearing multiple disease-resistant characteristics, *C. hystrix* is the only wild species cross-compatible with *C. sativus* (Chen et al. 2002; Sebastian et al. 2010). A synthetic allotetraploid *C.* ×*hytivus* Chen and Kirkbr. (HHCC, 2*n* = 4*x* = 38) was obtained from the interspecific hybridization between *C. hystrix* and *C. sativus* (Chen and Kirkbride 2000). In this newly synthetic allotetraploid, the merge of two divergent genomes brings a 'genomic shock' that causes rapid genetic and epigenetic changes, leading to many problems in chromosome perturbations given rise to pairing errors at meiosis (Wang et al. 2017b; Zhao et al. 2019; Yu et al. 2021). Yet, the clear genetic background and small genome size make the *Cucumis* allotetraploid serving as an excellent system for studying immediate consequences following allopolyploidization. This chapter provides here a review about the cyto-molecular genetics works on the interspecific hybridization between *C. sativus* and *C. hystrix*, especially on chromosome-doubled progeny, the novel synthetic allotetraploid *C.* ×*hytivus*. Cytogenetic studies, along with the whole genome sequencing, help us to better understand the genetic changes triggered by hybridization and allopolyploidization. It is also stated that with this interspecific hybridization, great potential will be offered elite genes into commercial cultivars for resistance and be provided novel insights into plant polyploid genome evolution.

10.2 A Newly Allotetraploid *Cucumis* Species *Cucumis* ×*hytivus* Synthesized

10.2.1 The First Repeatable Interspecific F1 Hybrid

A common obstacle to utilizing germplasm of wild species for crop improvement was sterility in F1 hybrids. In many cases, this sterility was associated with meiotic abnormalities and was a big problem that followed hybridization and hindered utilization.

By transferring specific traits from the wild relatives, interspecific hybridization is believed to improve crops resistance (Bowley and Taylor 1987). The importance of wild *Cucumis* species has long been recognized as valuable sources of resistance to multiple diseases such as powdery mildew, downy mildew, Fusarium wilt, and root-knot nematode (Kirkbride 1993; Nugent and Dukes 1997; Lou et al. 2013a; Pang et al. 2013; Zhang et al. 2018; Cheng et al. 2019). Intraspecific genetic variation of *Cucumis* is relatively limited, making creation of novel germplasm through interspecific hybridization more and more critical and meaningful.

Successful interspecific hybridization between *C. hystrix* and *C. sativus* was carried out successfully (Chen and Staub 1997). It was the first reproducible cross between a cultivated *Cucumis* species and a wild relative, which represented a breakthrough in interspecific hybridization in

Cucumis. What's even more amazing is that the success of this cross was conducted using the parental species with different chromosome bases. Achieved by embryo rescue following pollination of *C. sativus* by *C. hystrix* (Fig. 10.1), the original F1 hybrid (2n = 19) inherited 7 chromosomes from *C. sativus* and 12 from *C. hystrix* and was both male- and female sterile. To restore fertility, reciprocal crosses were made, and the chromosome numbers of the progeny were successfully doubled (Chen et al. 1998). Finally, a novel allotetraploid *Cucumis* species, named as *Cucumis* ×*hytivus* Chen and Kirkbr. (HHCC, 2n = 4*x* = 38), was proposed in 2000 followed by chromosome doubling of the F1 hybrid (Chen and Kirkbride 2000) (Fig. 10.2).

However, self-pollination and backcrossing of the F1 hybrid plants to either parent indicated that the original hybrid was both male- and female sterile, probably due to meiotic abnormalities caused by lack of homology and the odd chromosome number 2n = 19 (including 7 from cucumber and 12 from *C. hystrix*) (Fig. 10.1). The chromosome number in the hybrid was doubled to restore the fertility. Pollen grains were released from the synthetic amphidiploid and fruits set with viable seeds on the fertility-restored plants (Fig. 10.2).

10.2.2 The Taxonomy Challenged

The classification of *Cucumis* has undergone several renovations. The first taxonomy of *Cucumis* was promoted by Linnaeus in 1753 (Ghebretinsae et al. 2007), in which there are seven cultivated or economically useful species in the genus *Cucumis*. A number of taxonomic placements of *Cucumis* were published since the work of Linnaeus (Pangalo 1950; Jeffrey 1962, 1967, 1980, 1990; Kirkbride 1993; Schaefer 2007; Garcia-Mas et al. 2004) in which Kirkbride proposed the most comprehensive version of *Cucumis* taxonomy (1993). From his investigations, the genus *Cucumis* was divided into two subgenera with different geographical origins and basic chromosome numbers. Subgenus *Melo* (30 spp., n = 12) was originated in Africa and was partitioned into two sections (*Melo* and *Aculeatosi*), whereas subg. *Cucumis* (two spp., n = 7) was originated in Asia. A detailed taxonomic depiction of the genus *Cucumis* elaborated by Kirkbride (1993) is given in Table 10.1. However, the rediscovery of a wild *Cucumis* species *C. hystrix* of Asian origin, possessing 24 chromosomes, broke this taxonomic placement. The interspecific hybridization was successfully conducted between *C. hystrix* (2n = 24) and cultivated cucumber (Fig. 10.3).

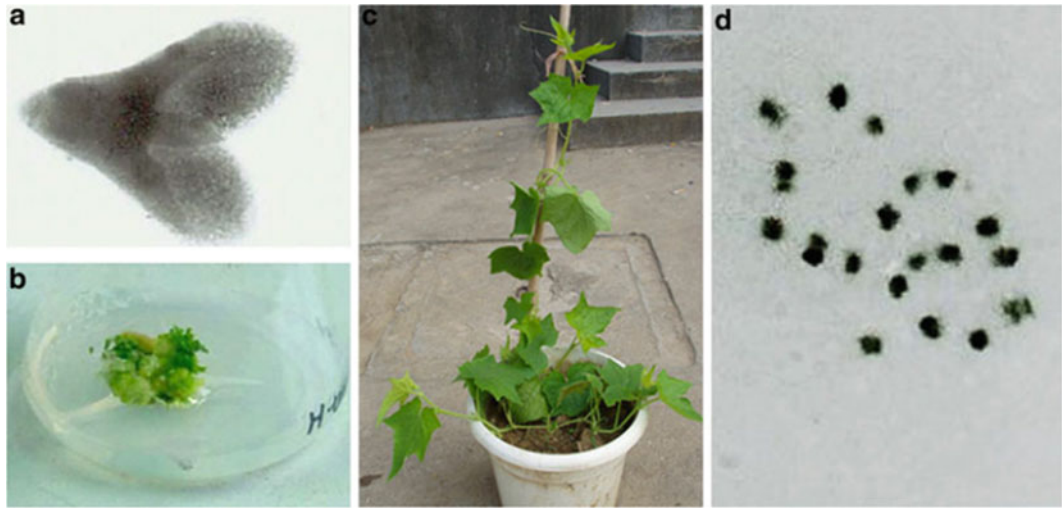

Fig. 10.1 Production and chromosome counting of interspecific hybrids F1 between *C. hystrix* and *C. sativus*. **a** Embryo of hybrid. **b** Regeneration from the young embryo. **c** Acclimatized plants. **d** Metaphase chromosomes of interspecific hybrid F1 (2n = 19)

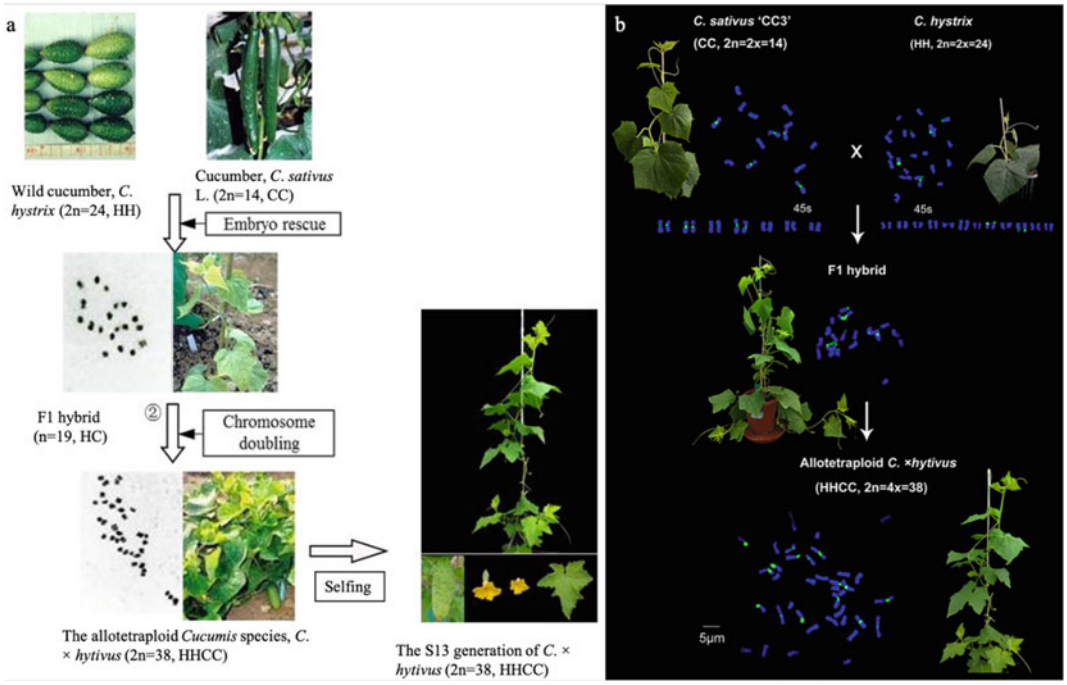

Fig. 10.2 The synthesis of the *Cucumis* allotetraploid *C.* ×*hytivus*. The allotetraploid *Cucumis* ×*hytivus* (HHCC, 2n = 4*x* = 38) was synthesized through interspecific hybridization between cucumber (*Cucumis sativus* L. vs. 'Beijingjietou', CC, 2n = 2*x* = 14) and its wild relatives *C. hystrix* (HH, 2n = 2*x* = 24) followed by chromosome doubling. **a** shows the key biotechnical and breeding methods in the process to synthesize the allotetraploid *C.* ×*hytivus*. (Wang et al. 2017b) **b** shows mitotic metaphase chromosome patterns with 45S rDNA signals were shown for each species. (Zhao et al. 2019)

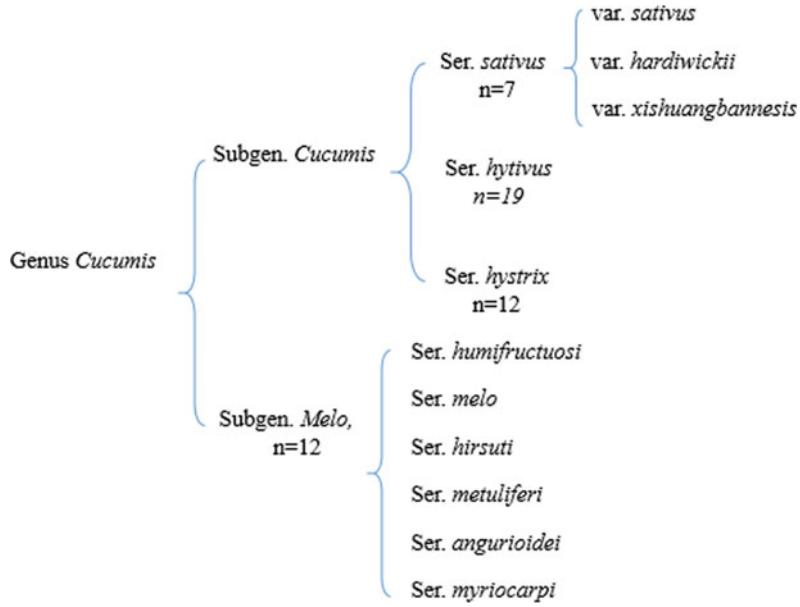

Fig. 10.3 The *Cucumis* systematic system including the synthetic allotetraploid *C.* ×*hytivus* (Kirkbride 1993; Zhuang et al. 2008)

Table 10.1 Taxonomy of the genus Cucumis (Kirkbride 1993)

Cucumis spp.	Chromosome number (*n*)
Subgenus *Melo*	
Section *Aculeatosi*	
Serie *Myriocarpus*	
C. myriocarpus	
subsp. *myriocarpus*	12
subsp. *leptodermis*	12
C. africanus	12
C. quintanilhae	–
C. heptadactylus	24
C. calahariensis	–
Serie *Angurioidei*	
C. anguria	
var. *anguria*	12
var. *longaculeatus*	12
C. sacleuxii	12
C. carolinus	–
C. dipsaceus	12
C. prophetarum	
subsp. *prophetarum*	12
subsp. *dipsaceus*	12
C. pubituberculatus	–
C. zeyheri	12(24)
C. prolatior	–
C. insignis	12
C. globosus	12
C. thulinianus	–
C. ficifolius	12(24)
C. aculeatus	24
C. pustulatus	12, 48, 72
C. meeusei	24
C. jeffreyanus	–
C. hastatus	–
C. rigidus	–
C. baladensis	–
Serie *Metuliferi*	
C. metuliferus	12
C. rostratus	–
Section *Melo*	
Serie *Hirsuti*	
C. hirsutus	12

(continued)

Table 10.1 (continued)

Cucumis spp.	Chromosome number (*n*)
Serie *Humifructosi*	
C. humifructosi	12
Serie *Melo*	
C. melo	
subsp. *melo*	12
subsp. *agrestis*	12
C. sagittatus	12
Subgenus *Cucumis*	
C. sativus	7
C. hystrix	12

(*C. sativus*, 2n = 14) (Chen and Staub 1997). A new species *C.* ×*hytivus* Chen and Kirkbride was proposed in 2000 followed by chromosome doubling of the F1 hybrid (Chen and Kirkbride 2000). Furthermore, the relationship among cultivated cucumber (*C. sativus* L.), a cucumber variety *C. sativus* var *hardwickii* (Royle) Alef. *C. hystrix*, C. ×*hytivus*, *C. melo*, and *C. metuliferus* was investigated by using random amplified polymorphic DNA (RAPD) and simple sequence repeat (SSR) markers. Combining the *Cucumis* taxonomy proposed by Kirkbride, the systematic placement of *C.* ×*hytivus,* a novel species in *Cucumis* were obviously present (Zhuang et al. 2008; Yu et al. 2021).

There are two different hypotheses put forward to explain the relationship between the two basic chromosome numbers (n = 7 and n = 12) in *Cucumis*. One is a 'fragment' hypothesis suggesting that the basic number n = 12 arose from n = 7 progenitor by fragmentation of particular chromosomes followed by *de novo* regeneration of centromeres (Kozhukhow 1930; Whitaker 1933; Bhaduri and Bose 1947; Ayyangar 1967). The other is the fusion hypothesis, saying that the basic number n = 7 might have derived from n = 12 possibly by unequal translocation or fusion of non-homologous chromosomes (Trivedi and Roy 1970), which was verified to become the mainstream. The results, gain through genetic distance estimation by using SSR and RAPD markers, lend support to the fusion hypothesis (Zhuang et al. 2008). Han et al. (2009) proposed the involvement of centromere repositioning in the evolution of cultivated cucumber chromosome C7 based on cucumber/melon comparative fluorescence in situ hybridization (FISH) mapping data. The results of molecular phylogenetic studies suggest that n = 12 is ancestral in the genus *Cucumis* (Ghebretinsae et al. 2007; Renner et al. 2007; Sebastian et al. 2010). Yang et al. (2014) investigated chromosome synteny among cucumber, *C. hystrix* and melon using integrated and complementary approaches, and revealed the mechanisms of dysploid chromosome reduction that led from an n = 12 ancestor to the n = 7 karyotype of cucumber (Yang et al. 2014).

10.3 Phenotype of the New Synthesized Species *C.* ×*hytivus*

10.3.1 Morphology

To investigate the difference in morphology, fertility, and DNA, a set of reciprocal crosses with different chromosome levels made between *C. sativus* and *C. hystrix*. Hybrid plants (2n = 19; 12 from *C. hystrix* and 7 from cucumber) were sterile but morphologically uniform. The multiple-branching habit, densely brown hairs (on corolla and pistil), orange-yellow corolla, and

Fig. 10.4 Morphological comparison between the interspecific hybrid and synthetic allotetraploid *C.* ×*hytivus* **a** and **b**. The interspecific hybrid F1 diploid, sterile, hybrid plant from embryo rescue (left) and its chromosome-doubled tetraploid, fertile plant (right). **c** Female flowers of F1 hybrid (left) and allotetraploid (right). **d** Fruit of F1 hybrid (left) and allotetraploid (right). **e** Seeds harvested from the allotetraploid and its diploid progenitors (Chen et al. 1998)

ovate fruit of F1 hybrid plants were similar to that of the *C. hystrix* parent, and the appearance of the first pistillate flower was more similar to that of *C. sativus* parent (Fig. 10.4). The diameter of stem, the length of petiole, the shape and size of leaves were intermediated when compared with parents. The branching number and the appearance of first female flower showed paternal transmission while the length of internode of main stem showed maternal transmission in all hybrids.

To restore fertility, chromosome doubling of the F1 hybrid plants was carried out (Chen and Staub 1997). Sixty-two chromosome-doubled plants were obtained. The chromosome-doubled F1 plants were morphologically distinct from the parents and other progeny in traits such as a curve on leaf margins and shorter and stronger internodes. The fruits at two ploidy levels differ in morphology (Fig. 10.4). While diploid fruit (2n = 19, seedless) was longer and spindle-like in shape, the tetraploid fruit was shorter and column shaped. After fertility selection, two primary allotetraploids produced fertile flowers and set fruit with viable seeds (Chen et al. 1998). The restoration of fertility in the chromosome-doubled F1 hybrid marks the creation of a new combination of genomes and a new synthetic species that did not exist previously.

10.3.2 Abiotic Stress and Biotic Stress Resistance

Several photosynthetic characters of the hybrid species *C.* ×*hytivus* under weak light conditions were studied (Qian et al. 2002). The light compensation point of allotetraploid was 11.25 μE m^{-2} s^{-1}. After treatment with low-intensity light for 2 weeks, the leaf contents of chlorophyll a and b increased, while the value of chlorophyll a/b decreased, indicating that the allotetraploid has good tolerance to low irradiance. Zhuang et al. (2002) investigated the responses of seedlings of the new species *C.* × *hytivus* and its progenies from backcross with cucumber to chilling injury. The abnormal metabolism was observed in *C.* ×*hytivus* as it was subjected to low

temperature treatment; however, the progenies from backcrossing the new species to cucumber showed high tolerance to chilling injury.

Resistance to downy mildew tests was conducted in *C. hystrix* with disease index 5.3, indicating that it is highly resistant to downy mildew. This resistance was partially transmitted to the *C.* ×*hytivus*, and the progenies from backcross. Compared with the susceptible cucumber cultivar 'Jinlu', all the materials derived from this interspecific hybridization possess at least moderate resistance.

The three groups (*C. hystrix*, *C. sativus*, and reciprocal interspecific hybrids) were evaluated for their responses to inoculation of *M. incognita*. *C. hystrix* had a high level of resistance to *M. incognita* with mean gall index of 1.8 (Fig. 10.5), while cultivated cucumbers (*C. sativus*), which was confirmed as highly susceptible, got a mean gall index of 4.8–5.0. The interspecific F1 hybrid was identified with a mean gall index 3.4, placing in the middle between the two parents (Ye 2011). What's more, the transmission of the resistance was observed in the progeny of *C.* ×*hytivus* (S14, HHCC), compared to that of *C. hystrix* (HH), the resistance to RKN (*Meloidogyne* spp.) of chromosome-double F1 was intermediate, higher than that of *C. sativus* (CC) (Yu et al. 2021).

10.4 Cytological Evidence for 'Genomic Shock'

10.4.1 Chromosome Configuration in Early Progenies

The cytological evidence was found for genomic exchange and rearrangement in early stage of the amphidiploid species *C.* ×*hytivus* (S2). Such as '8' and 'cross' configurations at prophase, chain and ring multivalents at metaphase I, as well as various abnormal chromosome structure including chromosome bridges, lagging, genomic separation, micronuclei etc., probably brought results in unequivalent distribution of genetic germplasm (Fig. 10.6) (Guo and Chen 2005). Such an extensive genomic exchange and rearrangement, leading to abnormal microspores in polyads and microspores with unbalance germplasm, could not develop into normal male gametophytes. This was the main reason for the low fertility of male gametophyte in *C.* ×*hytivus*.

Chen et al. carried out the studies on the cytogenetic characteristics and pollen fertility of the F1 hybrid and selfed progenies (S1–S4) of amphidiploid from *C. hystrix* ×*C. sativus* The results showed that at metaphase I (MI), the chromosome configuration of F1 hybrid was 16.75 I + 0.5 II + 0.25 III + 0.13 IV. Most chromosomes in the F1 investigated were univalent, while in the amphidiploid, they were bivalent. At metaphase II (MII), the F1 could not produce normal tetrads but most polyads, while the amphidiploid mainly produced tetrads. Moreover, the frequency of bivalents at MI, tetrads at anphase II (AII), and fertility of pollen increased during the selfing course that indicated the improved cytogenetic stability of the amphidiploid. It was also found that at anaphase I (AI) and AII, the pollen mother cells (PMC) contained lagging chromosomes, bridge fragment, unequal disjunctions, and nonsynchronized disjunctions in amphidiploid, which might cause the low pollen fertility (Fig. 10.7) (Chen et al. 2006b).

The exchanges and reconstitutions between the two genomes in the allotetraploid *C.* ×*hytivus* were investigated by cytological means. Among the 108 PMC observed, 50 PMC (about 46.3%) had multivalents, indicating the wide genomic exchange and reconstitution. The average chromosome configuration was 0.56 I + 17.36 II + 0.35 III + 0.26 IV + 0.046 V + 0.056VI (Table 10.2) (Zhuang et al. 2005).

Cytological studies were carried out on microsporogenesis and male gametophyte development of asynthetic amphidiploid species, *C.* ×*hytivus*, using chromosome preparation. During microsporogenesis, the result indicated that about 31% of PMCs had chromosome configuration with 19 I, and about 69% of PMCs had complex chromosome configurations (average configuration 0.41 I + 14.69 II + 0.06 III + 0.93 IV + 0.62 VI + 0.07 VIII) Table 10.3). About

Fig. 10.5 *C. hystrix* showing resistance to root-knot nematode *M. incognita* (right), susceptible control 'Beijingjietou' (left), and resistant control *C. metuliferus* (middle) (Ye 2011)

8.78% of tetrads were observed, while the rest were polyads at the tetrad stage. During male gametophyte development, about 10% microspores developed into normal two-celled pollens with three apertures, and 90% were aborted. Additionally, a special phenomenon was also observed such as genome separation at anaphase II and pollen aberration in morphology (Guo et al. 2005).

10.4.2 Meiotic Chromosome Behavior and Pollen Viability

To associate mitotic stability with meiotic behavior, chromosome pairing at different stages of meiosis I in 60 PMCs from allotetraploid *C.* ×*hytivus* was examined (Wang et al. 2017). Genomic DNA from cucumber and *C. hystrix* was labeled with either digoxin-dUTP (red) or biotin-dUTP (green) to distinguish and record the presence and relationship of the parental chromosome sets in genomic in situ hybridization (GISH) experiments. The results showed that chromosome pairing was mostly presented as bivalents (II), with the mean chromosome configurations for *C.* ×*hytivus* as 2.7 I + 11.21 II + 0.97 III + 2.12 IV. Notably, chromosome pairing did not strictly occur among homologous chromosomes; intergenomic bivalents and multivalents were frequently observed (Fig. 10.8a,b). As shown in Fig. 10.8b, two types of intergenomic trivalents were presented: cu/cu/hy and hy/hy/cu (indicated with white arrows). In

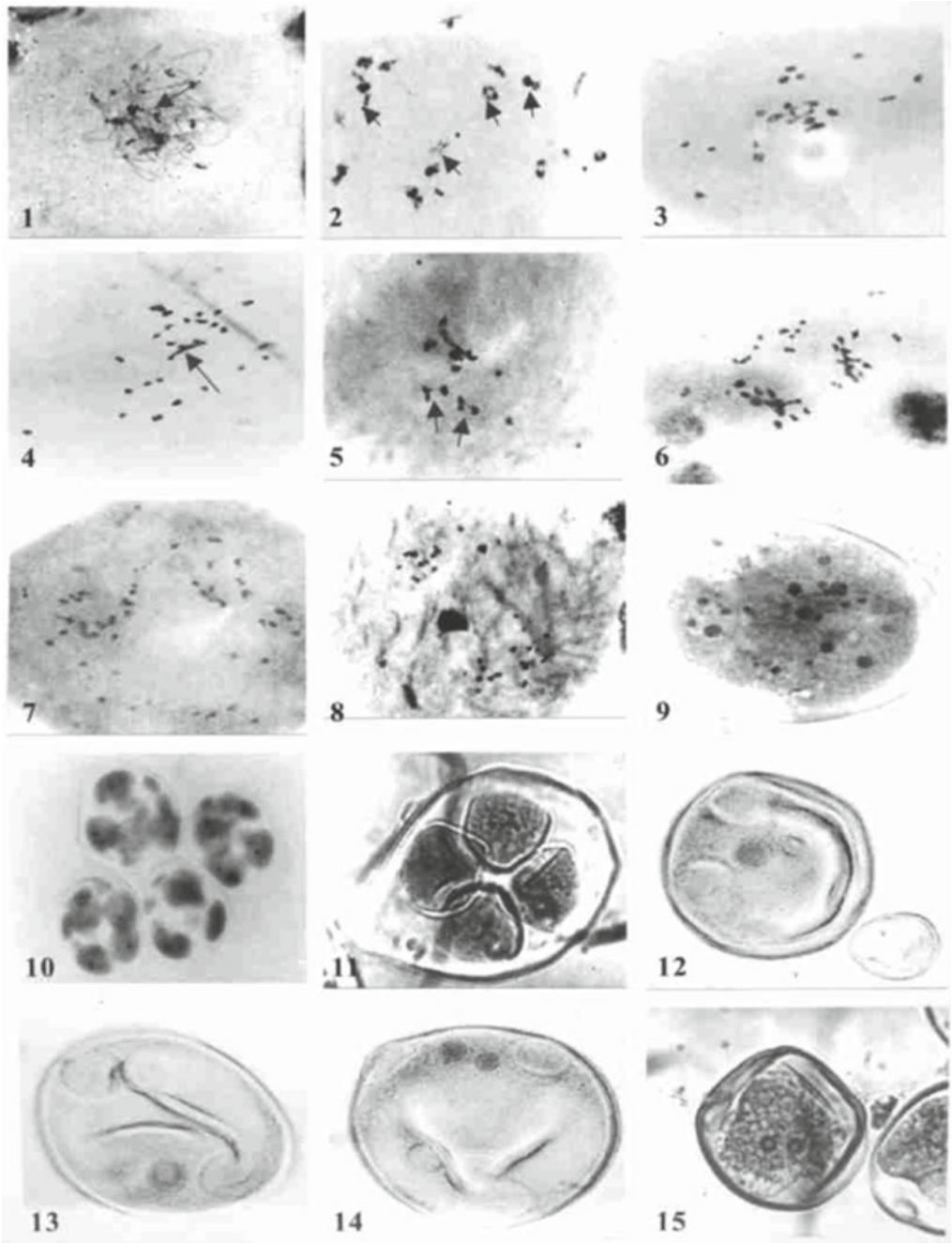

Fig. 10.6 Chromosome abnormalities during meiosis and male gametophyte in *C.* ×*hytivus* (Guo and Chen 2005)

addition, chromosome lagging and conglutination were also observed in some cells (Fig. 10.8c,d). Meiosis pachytene chromosomes exhibited unpaired chromosome strings that might be structural heterozygotes caused by homoeologous recombination (Fig. 10.8e,f), which were not detected in mitotic metaphase chromosomes using fosmid-FISH.

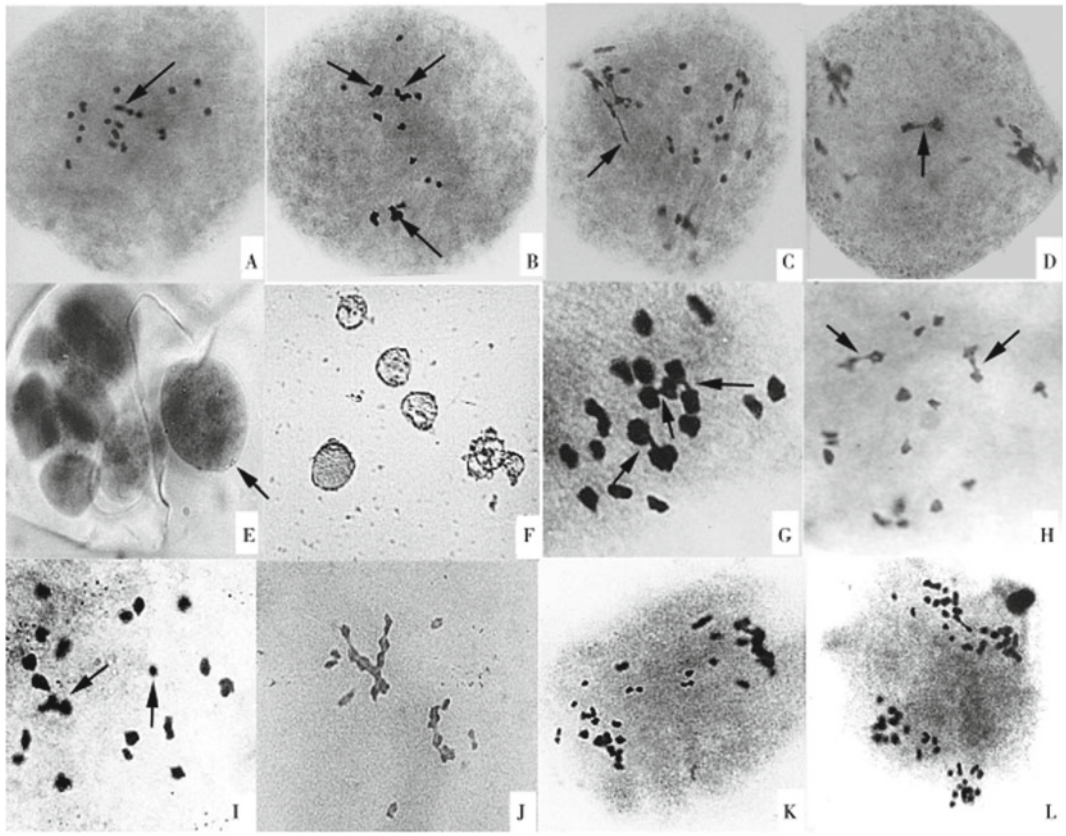

Fig. 10.7 Observation of meiotic behavior in F1 hybrid and S1 to S4 generations of amphidiploid (Chen et al. 2006b)

Furthermore, the latest researches were reported that there are differential subgenome biases in interspecific F1 hybrids and *C.* ×*hytivus* (the 4th and 14th inbred family, S4 and S14, respectively) through oligo-painting and GISH (Zhao et al. 2019; Yu et al. 2021). Both in F1 hybrids and allotetraploids, the individual chromosome has biases for homoeologous pairing and univalent H-subgenome exhibits easier autosyndetic pairings and higher univalent and chromosome lagging frequencies than C-subgenome. Meiotic stability has been increased from S4 generation to S14 generation, including synchronous meiosis behavior, reduced incidents of univalent and chromosome lagging (Fig. 10.9). Above all are the vital evidence for the genomic basis that revealed for the phenomenon of 'diploidization', observed in this *Cucumis* interspecific allotetraploid.

Wang et al. also calculated pollen viability with the mature pollen stained using 1% acetocarmine (Wang et al. 2017a). The round pollen grains that stained were regarded as viable, and the non-stained and lightly stained pollen grains with irregular sizes were considered aborted. The stainability of *C.* ×*hytivus* was 41.84%. This relatively low pollen viability reflected the impact of meiosis irregularities. However, whether those chromosomally viable gametes could produce progeny needs further analysis (Wang et al. 2017a, b).

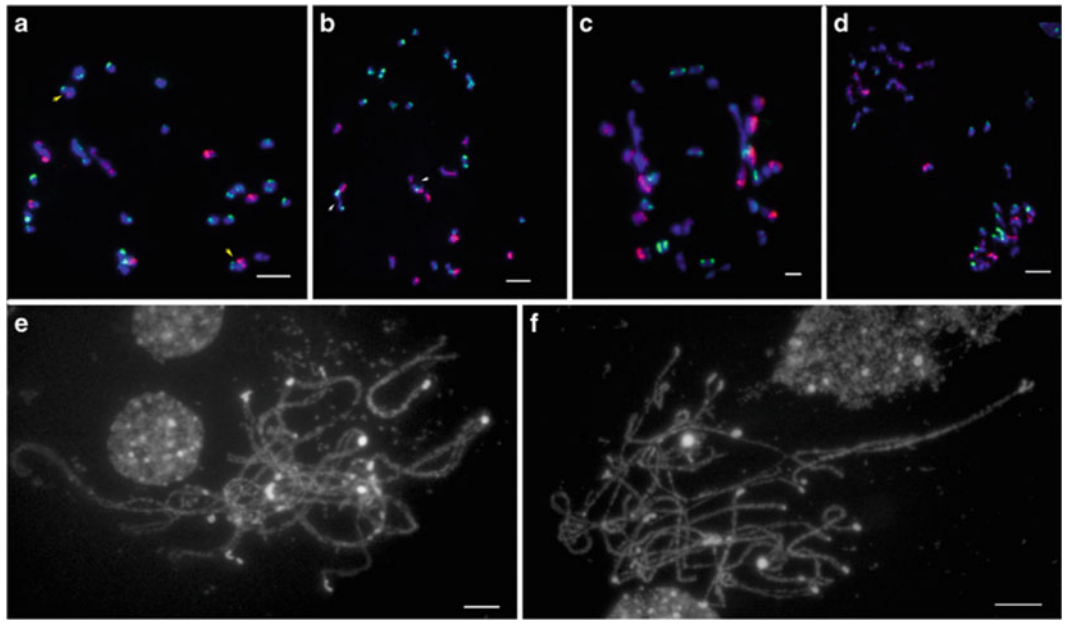

Fig. 10.8 GISH signals of cu-gDNA (red) and hy-gDNA (green) on meiosis I chromosome spreads of the allotetraploid *C.* ×*hytivus*. (Wang et al. 2017a)

Table 10.2 Chromosome configurations of pollen mother cell of cucumber and allotetraploid at meiotic metaphase I (Zhuang et al. 2005)

			Chromosome configurations					
Materials	Number of cells	Cells with multivalent	I	II	III	IV	V	VI
C. sativus	100	0	0	700	0	0	0	0
			0	7	0	0	0	0
Allotetraploid	108	50 (46.3%)	61	1875	38	28	5	6
			0.56	17.36	0.35	0.26	0.046	0.056

Table 10.3 Chromosome configurations at MI of PMCs in the amphidiploid (Guo et al. 2005)

	Chromosome configuration (average value)					
Numbers of cells examined	I	II	III	IV	VI	VIII
95	0.41	14.69	0.06	0.93	0.62	0.07

10.4.3 *Chromosomal Distribution of LTR Retrotransposons in C.* ×*hytivus*

The results of FISH showed that the two types of LTR retrotransposons were distributed throughout all the chromosomes of *C.* ×*hytivus*, with clusters on the terminal regions (Fig. 10.10). Most chromosomes showed clusters only on one terminal region, however, some had clusters on both terminal regions (indicated by arrows Fig. 10.10). Furthermore, among the chromosomes with two clusters, most of them exhibited two intensive signals on both terminal regions,

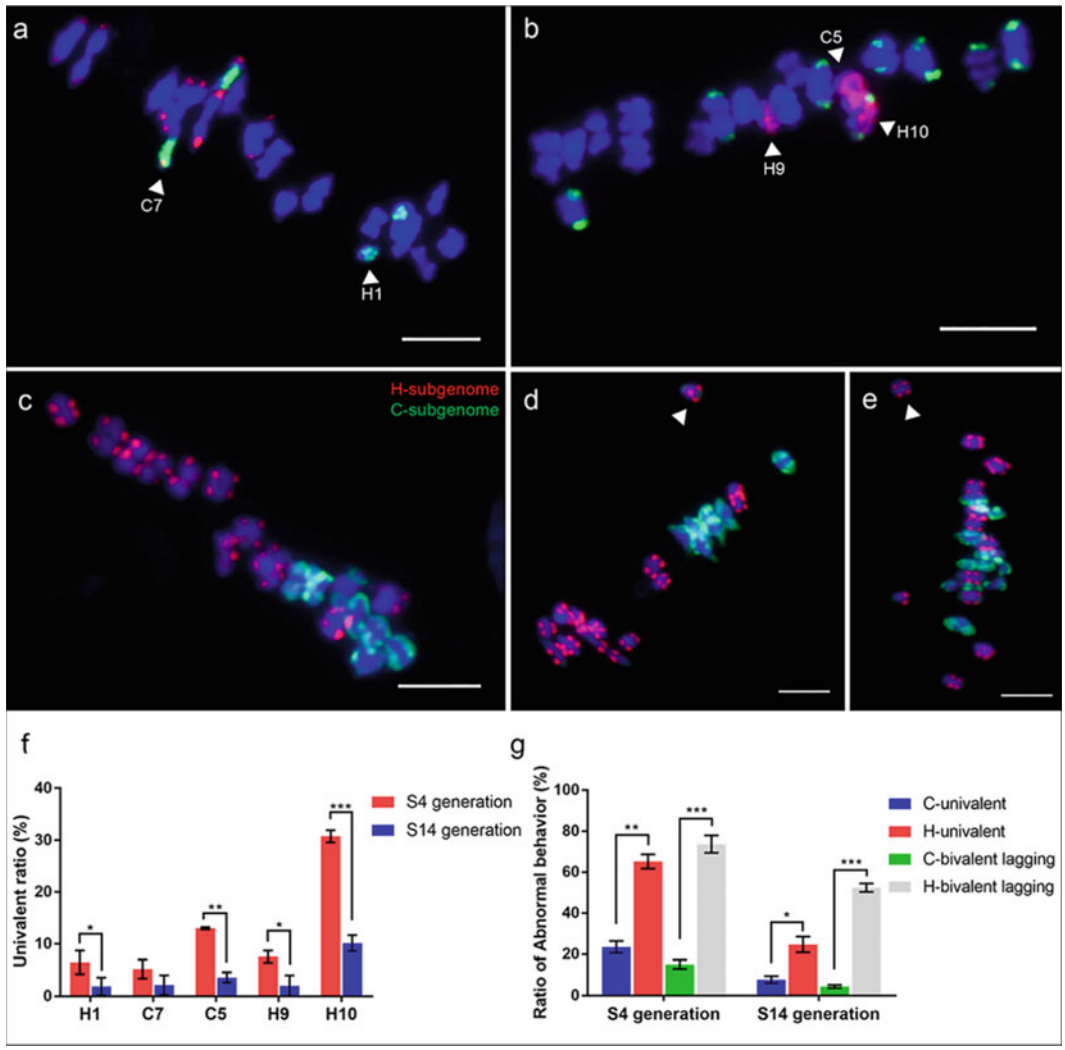

Fig. 10.9 Meiotic behavior at metaphase I in S14 and the biases of chromosomes/subgenomes for univalents and lagged bivalents at metaphase I (Zhao et al. 2019)

and the rest of chromosomes showed two clusters with one intensive signal on one side and one weak signal on the other side (Jia et al. 2014).

10.5 Karyotype of the Allotetraploid *C.* ×*hytivus*

Cyto-molecular genetic researches on synthetic and naturally formed allopolyploids have reported various types of chromosomal variations, including intergenomic translocations, aneuploidy, ribosomal DNA changes, and the loss of repetitive sequences (Pontes et al. 2004; Skalická et al. 2005; Mestiri et al. 2010; Xiong and Pires 2011; Xiong et al. 2011; Chester et al. 2012). Cytogenetics on cucumber has been widely studied using FISH with kinds of probes, including tandem DNA repeats (Type I/II, Type III, and Type IV), ribosomal DNA (45S and 5S), fosmid clones, and single copy genes (Ren et al. 2009; Zhao et al. 2011; Han et al. 2011, 2015; Lou et al. 2013b, 2014; Sun et al.

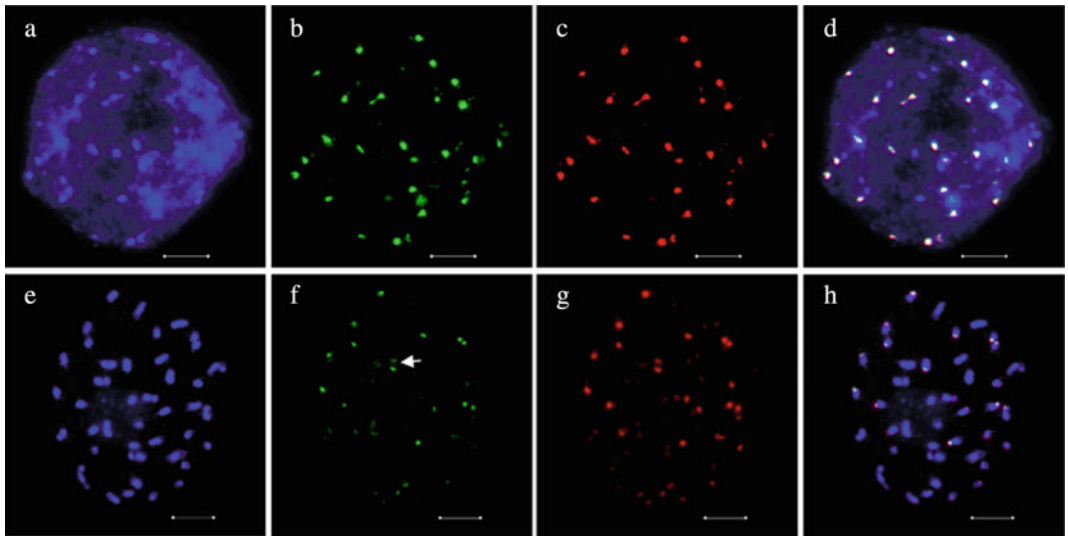

Fig. 10.10 Distribution of two types of LTR retrotransposons on the somatic mitotic chromosome of *C.* ×*hytivus* (Wang et al. 2017a)

2013). Moreover, comparative mapping studies have reported sequence conservation and synteny between *C. hystrix* and cucumber; 53 *C. hystrix* syntenic blocks were orientated on seven cucumber chromosomes (Yang et al. 2014). These achievements provide an opportunity for karyotyping in *C. hystrix* by cross-species fosmid-FISH and further promote our understanding of genetic structure and chromosomal stability of *C.* ×*hytivus*. All homoeologous chromosomes of allopolyploid *C.* ×*hytivus* were identified through GISH and FISH using repetitive sequences. Distribution patterns of cucumber major repeats, 45S and 5S ribosomal DNA (rDNA), and a set of 29 cucumber fosmid clones were investigated to characterize the chromosomal structure of *C.* ×*hytivus* (Fig. 10.11; Table 10.4). No aneuploids were identified in any *C.* ×*hytivus* individuals that were characterized, and no large-scale chromosomal rearrangements were identified, indicating a relatively stable chromosomal structure. Finally, we observed meiotic chromosome behavior, which plays a key role in the genetic stability of allopolyploids. Meiotic irregularities, such as homoeologous pairing, univalents, and intergenomic multivalents, were frequently observed. Analysis of chromosomal stability of *Cucumis* allotetraploid species could provide novel insights into the establishment of allopolyploids derived from a distant chromosome base.

10.6 Molecular Analysis in Early Genomic Changes

The relationship was studied by RAPD markers between cultivated cucumber and its wild varieties, wild *Cucumis* species (*C. hystrix* Chakr.), interspecific hybrids and the BC1S2, and melon cultivars (Zhuang & Chen, 2003). To detected polymorphism, 31 arbitrary primers were screened among the total 23 cultigens tested. Of the 375 sites produced, 90.0% were polymorphic. Each primer amplified an average of 6.3 fragments. The results from UPGMA cluster analysis suggested that 23 cultigens could be classified into four groups, they are: cultivated cucumber and its wild variety, *C. hystrix*, *C.* ×*hytivus*, and melons (Fig. 10.12; Table 10.5).

Chen et al. carried out AFLP analysis in amplification of the DNAs from different generations of the synthetic allotetriploid (*Cucumis* ×*hytivus*,

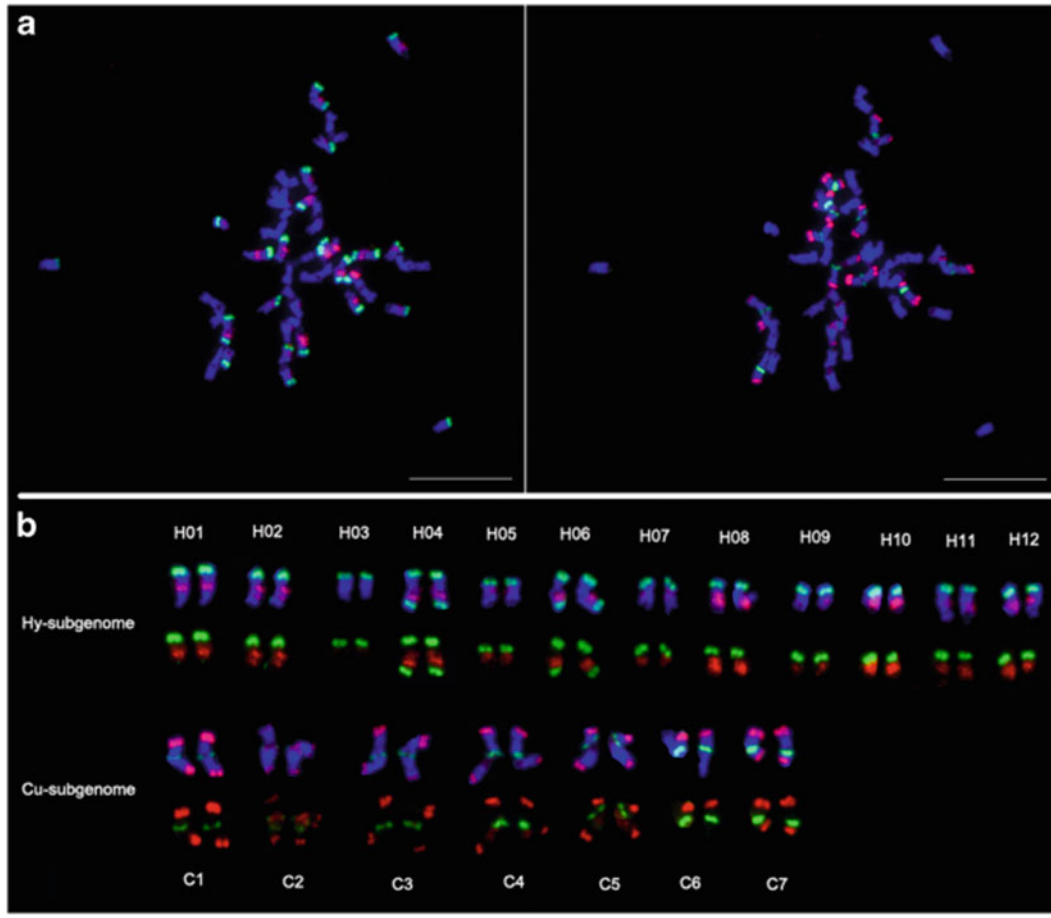

Fig. 10.11 Somatic chromosome karyotype of the allotetraploid *C.* ×*hytivus* using the FISH mixture: cu-gDNA (red), Type III (green), BAC clone 457-F11 (red), and hy-gDNA (green) that could distinguish 19 homoeologous chromosomes. (Wang et al. 2017a)

Table 10.4 Information of the 29 selected cucumber fosmid clones; yellow boxes were the fosmids used in karyotype construction of *C. hystrix*

	Code	Marker	Position (cM)	Genome position (bp)	Fosmid clone	
H01	chr7-78	SSR01898	50.9	15641671- 15673977	gcfbd0_0343_F07.ab1	Short arm
	chr7-80	–	–	–	gcfbe0_0497A02	Long arm
H02	chr1-20	SSR14445	73.029	21848915- 21879630	gcfba0_0007_C04.ab1	Long arm
	chr1-70	SSR23757	4.9	1966203- 2006434	gcfbd0_0464_C11.ab1	Short arm
	chr6-35	–	–	–	gcfbe0_0180_C09.ab1	—
	chr6-60	SSR02906	84.5	24183018- 24219723	rgcfbe0_0257_E12.ab1	Long arm
H03	chr2-7	SSR23732	61.5	16344196- 16377413	rgcfbd0_0252_E04.ab1	Long arm
	chr2-23	SSR20045	74	17928507- 17965119	gcfbd0_1142_B06.ab1	Long arm
	chr2-69	SSR30665	94.3	21968920- 22001624	gcfbd0_0304_A09.ab1	Short arm
	chr6-45	SSR11219	6.2	3346943- 3384961	gcfbe0_0187_G09.ab1	Short arm

(continued)

Table 10.4 (continued)

	Code	Marker	Position (cM)	Genome position (bp)	Fosmid clone	
H04	chr3-10	SSR21454	92.4	32147183- 32188786	gcfbe0_0104_H02.ab1	Long arm
	chr3-43	–	–	–	gcfbe0_0072_E12.ab1	Long arm
	chr3-44	SSR23517	105.1	38050645- 38088342	gcfbe0_0001_D11.ab1	Short arm
H05	chr2-41	SSR11952	5.9	1374855- 1409777	gcfbd0_1078_H03.ab1	Long arm
	chr2-54	SSR00184	0	142789- 184478	gcfbe0_0022_B06.ab1	Long arm
H06	chr3-56	SSR22514	31	10059164- 10101377	gcfbe0_0207_F03.ab1	Long arm
	chr3-72	CSWGATT01B	49.4	16484183- 16510660	gcfbd0_0554_G09.ab1	Long arm
	chr3-74	SSR03049	0	1121698- 1155015	rgcfbe0_0472_B12.ab1	–
H07	chr4-18	–	–	10327544- 10359112	gcfbd0_0108G02	–
	chr4-37	–	–	–	gcfbd0_0802_E07.ab1	Long arm
	chr4-55	–	–	19347769- 19383016	gcfbe0_0098_F07.ab1	Long arm
	chr4-95	–	–	351- 43490	gcfbe0_0315F11	Short arm
H08	chr4-58	SSR23826	7.5	11480614- 11510697	gcfbe0_0243_E12.ab1	Short arm
H09	chr5-40	–	–	3813355- 3851250	gcfbd0_0986F03	Short arm
H10	chr5-36	SSR17975	39	24499019- 24533838	gcfba0_0066_B05.ab1	Long arm
	chr5-52	–	–	11041510- 11076720	gcfbe0_0314B09	Long arm
H11	chr5-59	SSR21918	26.5	22654705- 22698074	rgcfbe0_0257_D06.ab1	short arm
	chr6-57	SSR01903	10.8	5816959–5853441	gcfbe0_0459_C08.ab1	Long arm
H12	chr1-30	SSR12070	33.502	7698959- 7732033	gcfbe0_0544_G05.ab1	Short arm

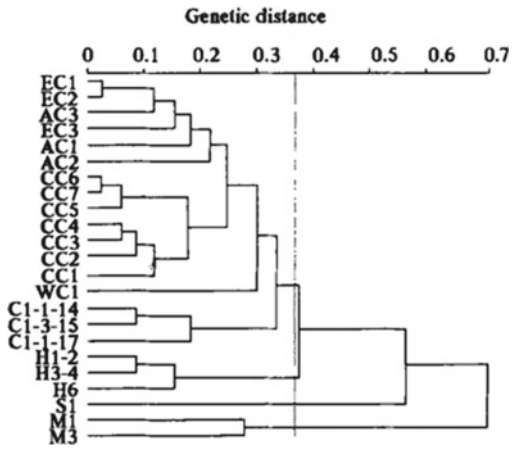

Fig. 10.12 The cluster dendrogram of RAPD in 23 *Cucumis* species. For assigned code, see Table 10.4. (Zhuang & Chen, 2003)

Chen and Kirkbride, 2n = 38), the interspecific F1, and its diploid parents (Chen et al. 2005). The results indicated that extensive genomic changes occurred in the synthetic allotetraploid, and the variation rate was as high as 22.2%. The genomic changes started in the F1 and the highest variation rate observed in *C.* ×*hytivus* S2 generation. Most genomic changes involved the loss of parental fragments, formation of novel fragments, with some fragments disappearing in one generation and reappearing in others (Fig. 10.13). In addition, it was observed that some parent-specific bands were inherited preferentially from the male parent.

Besides, Chen et al. carried out an amplified fragment length polymorphism (AFLP) analysis by using 13 pairs of *Eco*RI-NN/*Mse*I- NNN selective primers in amplification of the DNAs

Table 10.5 *Cucumis* species and varieties in the study

Assigned code	Name	Type	Source	Chromosome number
AC1	A309	美国盐渍型 American pickling	1	2n=2x=14
AC2	GY14	美国雌性系 American gynoecious	1	2n=2x=14
AC3	PI22289	美国盐渍型 American pickling	1	2n=2x=14
CC1	二早子 Erzhaozi	华南型 Southern China	2	2n=2x=14
CC2	白丝条 Baisitiao	华南型 Southern China	2	2n=2x=14
CC3	北京截头 Beijing jietou	华北型 Northern China	2	2n=2x=14
CC4	津研 4 号 Jinyan No. 4	华北型 Northern China	2	2n=2x=14
CC5	西双版纳黄瓜 1 var. *xishuangbannesis* sample 1	西南型 Southwestern China	2	2n=2x=14
CC6	西双版纳黄瓜 2 var. *xishuangbannesis* sample 2	西南型 Southwestern China	2	2n=2x=14
CC7	西双版纳黄瓜 3 var. *xishuangbannesis* sample 3	西南型 Southwestern China	2	2n=2x=14
EC1	戴多星 Deltastar	欧洲温室型 European greenhouse	3	2n=2x=14
EC2	荷蒙 Harmonie	欧洲盐渍型 European pickling	3	2n=2x=14
EC3	康德 Gondesa	欧洲温室型 European greenhouse	3	2n=2x=14
WC1	var. *hardwichii*	野生黄瓜变种 Wild cucumber variety	1	2n=2x=14
C1-1-14	回交自交株系 1 *C.*× *hytivus*× *C. sativus* line 1	遗传改良型 Genetically-improved cucumber	4	2n=2x=14
C1-3-15	回交自交株系 2 *C.*× *hytivus*× *C. sativus* line 2	遗传改良型 Genetically-improved cucumber	4	2n=2x=14
C1-1-17	回交自交株系 3 *C.*× *hytivus*× *C. sativus* line 3	遗传改良型 Genetically-improved cucumber	4	2n=2x=14
H1-2	种间杂交种株系 1 *C.*× *hytivus* line 1	种间杂交种 Interspecific hybrid	4	2n=4x=38
H3-4	种间杂交种株系 2 *C.*× *hytivus* line 2	种间杂交种 Interspecific hybrid	4	2n=4x=38
H6	种间杂交种株系 3 *C.*× *hytivus* line 3	种间杂交种 Interspecific hybrid	4	2n=4x=38
S1	野生种酸黄瓜 *C. hystrix*	近缘野生种 Wild species	4	2n=2x=24
M1	灯瓜 Denggua	甜瓜变种 Melo variety	4	2n=2x=24
M3	黄河蜜 Huanghe mi	栽培甜瓜 Cultivated melon	2	2n=2x=24

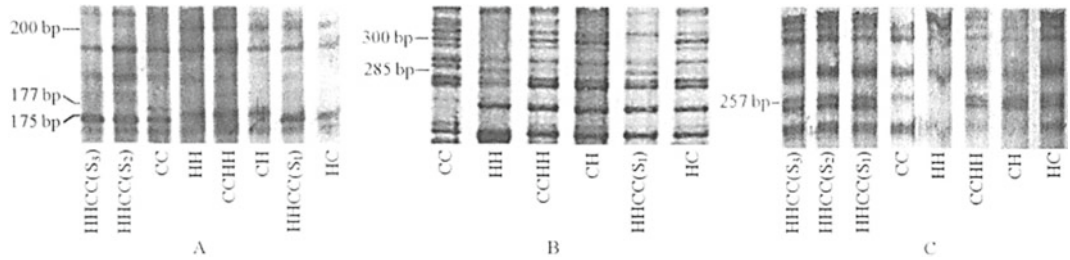

Fig. 10.13 Examples of early genomic changes in alloploypoid of *Cucumis* (Chen et al. 2005)

from reciprocal F1 hybrids of *C. hystrix* and *C. sativus*, the synthetic allotetraploids and the diploid parents. The phenomenon that sequence elimination in reciprocal allopolyploids often appears variable in different species during diploidization was discovered, suggesting that the nuclear–cytoplasmic interactions play different roles in different polyploid plants. This result indicated that extensive DNA sequence elimination was induced by the genomic merge in the allopolyploids. The frequency of elimination of some parental sequences was affected by cytoplasmic factors, the differences from reciprocal crosses were not statistically significant, and the time of elimination (both started in the F1) and the type of elimination were also the same, suggesting that the nuclear–cytoplasmic interactions might not be the main factor causing sequence elimination (Table 10.6). In addition, the direction of sequence elimination was not affected by the reciprocal crosses, and the elimination was more common from the *C. sativus*, which has fewer chromosomes (Chen et al. 2006a).

Luo et al. performed the electrophoresis of aspartate aminotransferase (AAT), malate

Table 10.6 Sequence elimination in allopolyploid and the progenies*

Code	Total Number of bands	Number of genomic-specific bands	Band loss of *C. hystrix*		Band loss of *C. sativus*	
			Band number	Ratio (%)	Band number	Ratio (%)
HH	760	107	–	–	–	–
CC	773	120	–	–	–	–
HC	786	–	44bAB	5.6	82aA	10.4
HHCC	795	–	40bAB	5.2	84aA	10.6
CH	800	–	27bB	3.3	74aA	9.3
CCHH	799	–	29bB	3.6	74aA	9.3

*Using Duncan analysis, with the capital and lower case letters showing $P < 0.01$ and $P < 0.05$, respectively.

dehydrogenase (MDH), and esterase (EST) to characterize and compare the reciprocal interspecific hybrid plants between the wild relative *C. hystrix* (2n = 24, HH) and cultivated cucumber (*C. sativus* cv. 'Changchunmici', 2n = 14, CC), and their parents. The results indicate that the zymograms in the reciprocal F1 were typically expressed by complementary bands from both parents. In addition, four heterodimeric bands (Aat-1-94, Aat-2-104, Mdh-3-102 and Est-5-102) were observed (Fig. 10.14). All the three isozymes investigated could identify the interspecific hybrid plants. The results also reveal the differences of the reciprocal F1s at the number and intensity of the bands in the zymograms of AAT and MDH, which further confirmed the reciprocal differences in the interspecific hybridization between *C. hystrix* and *C. sativus*.

Zhuang et al. conducted the exchanges and reconstitutions between the two genomes in the allotetraploid *Cucumis hytivus* Chen and Kirkbride (2n = 4x = 38) investigation by molecular means (Zhuang et al. 2005). Among 446 arbitrary primers, only 5 primers could produce six specific bands of *C.* ×*hytivus*. Three of them were selected and converted into sequence

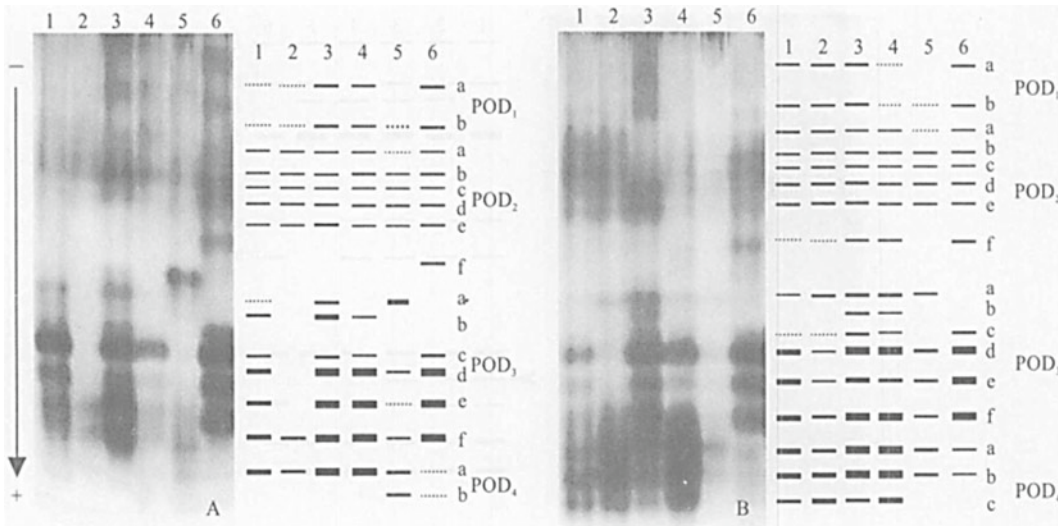

Fig. 10.14 The zymograms and schematic charts of peroxidase (POD) isozymes in the young leaves (A) and flower buds (B) (Luo et al. 2006). 1. Allotriploid in *Cucumis*; 2. Allotetraploid *C.* ×*hytivus*; 3. 'Changchun mici' ×*C. hystrix*; 4. *C. hystrix* ×'Changchun mici'; 5. *C. hystrix*; 6. *C. sativus* cv. 'Changchun mici'

Table 10.7 The primer sequences of SCAR markers converted from RAPDs (Zhuang et al. 2005)

SCAR marker	Sense primer 5′ to 3′	Antisense primer 5′ to 3′
SAP-03/700	GTAAGGCGCATGCAGGTATAG	AATGAAATATGCGTGAGAAAGAAT
SAP-07/640	CCGCTAAAGTGGGAAACAAA	CACCCGCTAGAGGTTCAAGAT
SAP-20/700	CCCGGATACAAAAATATGGTG	CGGATACACGAAGAACACCAT

characterized amplified region (SCAR) markers (Table 10.7). The SCAR markers were used in amplification with 13 *Cucumis* genotypes, and the results showed that only SAP-03/700 marker was specifically amplified (Fig. 10.15). Compared the sequence of three different bands amplified by SAP-03/700 primer pair in *C.* ×*hytivus*, *C. sativus* var. *sativus* and *C. sativus* var. *hardwickii*, the sequences at two ends were similar, while the middle parts were different. In addition, about 200 bp of SAP-03/700 was homologous with cucumber mitochorndrial genome, but the directions were contrary (Fig. 10.16).

Zhuang et al. (2006) investigated the phylogenetic relationships in *Cucumis* species using RAPD. The focus of this research was mainly on the analysis of genetic relationship among *C. hystrix*, *C. sativus* and *C. melon* and the new synthetic species *C.* ×*hytivus*. Based on the results, a modified taxonomic system was proposed that *C. hystrix* should remain in subgen. *Cucumis,* although it had a chromosome number different from that of *C. sativus*. With the

Fig. 10.15 Alignment of the sequences of SAP-03/700 amplified from *C.* ×*hytivus*, *C. sativus*, and *C. hardwickii* (Zhuang et al. 2005)

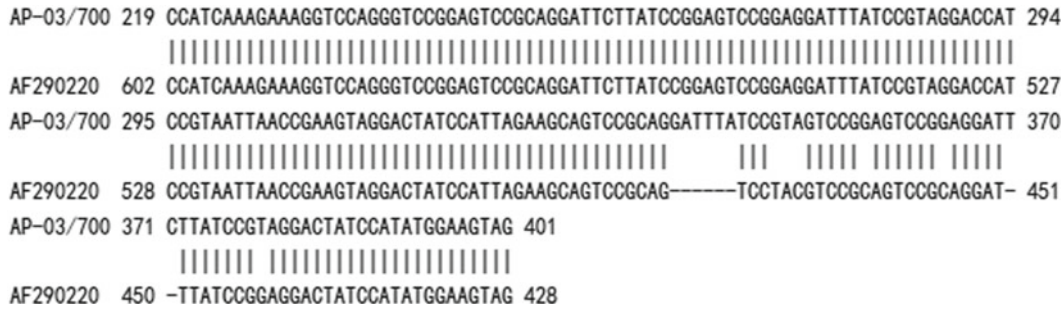

Fig. 10.16 Homologous comparison between SAP-03/700 sequence and cucumber mitochrodial genome sequence (Zhuang et al. 2005)

interspecific hybrids *C.* ×*hytivus* as the third species, subgen. *Cucumis* was, thus, made up by three species. Although the basic chromosome number and geographic location theorized were challenged by the proposed system, the use of it will likely assist in the exploitation of the wild *Cucumis* species in Asia.

Jia et al. employed cDNA-SSAP technique to detect the changes of gene expression, which is induced by the insertion of retrotransposon in the first four generations of the synthetic allotetraploid *C.* ×*hytivus*, S1-S4, compared with its two diploid parents *C. hystrix* (HH) and *C. sativus* (CC) (Table 10.8; Fig. 10.17). It was

Table 10.8 Pre-amplification and selective amplification primer sequences used in the study

Type	Primer/adaptor name	Sequence
SSAP adaptors	*EcoR* I-adaptor 1	5′-CTCGTAGACTGCGTACC-3′
	EcoR I-adaptor 2	3′-CATCTGACGCATGGTTAA-5′
	Mse I-adaptor 1	5′-GACGATGAGTCCTGA-3′
	Mse I-adaptor 2	3′-TACTC-AGGACTCAT-5′
Primers of pre-amplification	E00	5′-GACTGCGTACCAATTC-3′
	M00	5′-GATGAGTCCTGAGTAA-3′
Primers for SSAP	L1	5′-GTTTACGTCGTCATTCGACCAC-3′
	L2	5′-AATCAAGTTTATATTTCAACAC-3′
	L3	5′-ACAATTCTCAATTATACTCCAG-3′
	L4	5′-ATTCTTCTATCCTTGCCCGTGG-3’
	MCAA	5′-GATGAGTCCTGAGTAACAA-3′
	MCAC	5′-GATGAGTCCTGAGTAACAC-3′
	MCAG	5′-GATGAGTCCTGAGTAACAG-3′
	MCAT	5′-GATGAGTCCTGAGTAACAT-3′
	MCTA	5′-GATGAGTCCTGAGTAACTA-3′
	MCTC	5′-GATGAGTCCTGAGTAACTC-3′
	MCTG	5′-GATGAGTCCTGAGTAACTG-3′
	MCTT	5′-GATGAGTCCTGAGTAACTT-3′
	MCGA	5′-GATGAGTCCTGAGTAACGA-3′
	MCGC	5′-GATGAGTCCTGAGTAACGC-3′
	MCGG	5′-GATGAGTCCTGAGTAACGG-3′
	MCGT	5′-GATGAGTCCTGAGTAACGT-3′

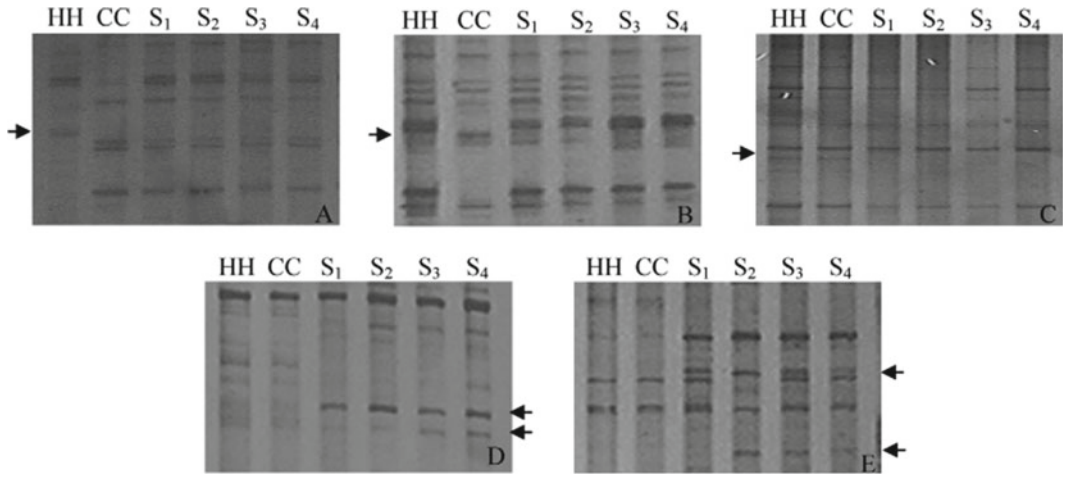

Fig. 10.17 cDNA-SSAP patterns detected in the two diploid parents, *C. hystrix* (HH) and *C. sativus* (CC), and the first four generations of the synthetic allotetraploid *C.* ×*hytivus* (S1-S4) (Jia et al. 2014)

defined that as gene expression alteration only when a new transcript appeared in the allotetraploid that is absent in the diploid parents, or conversely, when a specific transcript present in one of the parents or both disappeared in the allotetraploid (Jia et al. 2014).

10.7 Recommendations for Future Actions and Future Prospects

Wild species, an important reservoir of useful genes, offer great potential to incorporate genes into commercial cultivars for resistance to major diseases, insects, and tolerance to various abiotic stresses. Many of the useful alien genes differ with those in the cultivated species and play a critical role in broadening the sources of resistance/tolerance to various stresses. In genus *Cucumis*, wild relatives are diverse with useful sources of characters desired to improve cultivated *Cucumis* species. However, despite the importance of wild species of *Cucumis*, very little information is available on the studies of wild species. Some wild relatives, such as *C. metuliferus* E. Meyer ex Naudin (nematode resistance) and *C. figarei Naudin* (virus resistance), have long been attractive to scientists. Yet, the limits to utilization of wild relatives depend on the breeders' ability to produce interspecific hybrids, but hybrids may be sterile. Therefore, we hope that, in future, more attention should be paid to research on the wild species, meanwhile; the great effort should be made on producing fertile interspecific hybrids through biotechnological or other approaches.

Furthermore, the utilization of wild relatives is limited due to the cross-incompatiability problems, although many wild species are resistant to pests and diseases or adapted to adverse environments. It is with great difficulty to the use of exotic germplasm for the development of lines and populations with unique traits. The application of genetic markers to germplasm management or for marker-assisted selection has not been clearly defined in *Cucumis*. However, the successful development of the synthetic allotetraploid species *C.* ×*hytivus* Chen and Kirkbride via chromosome doubling of an interspecific hybrid between cucumber and *C. hystrix* pushed forward the progress of transferring desirable genes from the wild species *C. hystrix*. Future works may be emphasized on fine-mapping and the cytomolecular verification of the disease-resistant genes introgressed from *C. hystrix*, like downy mildew and root-knot nematode resistance genes, will be of great value for the improvement of cucumber and melon.

Interspecific hybridization, with the merge of two or more divergent genomes, is believed to cause 'genomic shock', which will bring the subgenome dominance, not only in epigenetic regulation, gene expression, and homoeologous exchanges but also in the meiotic chromosome behavior of the subgenomes. In *Cucumis*, both genetic and epigenetic reprogramming were detected in the synthetic allopolyploid *C.* ×*hytivus*, which obtained via interspecific hybridization. Also, previous studies have shown that there are the meiotic behavior biases between two subgenomes in allotetraploid *C.* ×*hytivus*, including asynchronous meiosis, univalent formation, and lagging chromosomes. The rapid advancement in the cyto-molecular genetics and the application of genome sequencing has now opened a new era of technologies in crop breeding study. Thus, in future, efforts may also be kept on the integrated and comprehensive research on mechanisms for the distinct processes that occur during the phenomenon of 'diploidization' in this interspecific hybridization.

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Cucumber Sex Determination: Aspects of Gene Interactions

11

Zheng Li, Huanhuan Niu, and Yalu Guo

Abstract

Cucumber (*Cucumis sativus*) has been used as a study model of sex determination for decades. In terms of yield and quality, sex determination is a hot topic, and related genes in *Cucumis*, including cucumber and melon, are conservative and highly homologous. In field experiments, according to the distribution of female, male or bisexual flowers of an individual plant, six sex types (monoecy, gynoecy, subgynoecy, androecy, andromonoecy, and hermaphrodite) could be classified. Genetic analysis has identified three major loci, *F*, *M*, and *A* that are associated with sex determination. So far, five genes controlling sex determination have been cloned, and their regulation modes and combinations can explain the observed cucumber sex types. Apart from genetic mechanisms, phytohormone ethylene is also directly involved in sex determination. Recently, two ethylene signaling molecules have been discovered, which were used to study the interaction among the sex-controlling genes. To date, our integrated knowledge from physiological, genetic, and molecular biological researches supports that ethylene is the core regulator in sex determination. Ethylene not only acts as biochemical product of the sex-controlling genes but also involves in the interactions among them. This chapter summarizes the recent progress of sex determination mechanism in cucumber and gives our prospectives for future research in this field.

11.1 Introduction

Flower development is the basis for plant fruit and seed production. In angiosperms, about 90% of species have perfect (or bisexual) flowers with independent male reproductive organs (stamens) and female reproductive organs (carpels), simultaneously. Compared with bisexual flowers, the differential development of carpels and stamens or selective arrest in certain plant species produces unisexual flowers, which leads to the diversity of flower sex types (Tanurdzic and Banks 2004). The different combinations and distributions of the three kinds of flowers lead to the production of hermaphroditic, monoecious, and dioecious plants, thus forming the diversity of plant sex types. These adjustments in flower development are defined as sex differentiation or sex determination. The sex determination of plants has been extensively studied in recent years (Lai et al. 2018a; Pannell 2017; Pawełkowicz et al. 2019b; Tanurdzic and Banks 2004).

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Cucumber (*Cucumis sativus*) has abundant flower and plant sex types, and its yield and quality are regulated by sex determination. According to the distribution or proportion of the three kinds of flowers produced in the plant (Fig. 11.1A), there are six classic phenotypes in normal cucumber, including: monoecy, gynoecy, subgynoecy, androecy, andromonoecy, and hermaphrodite (Fig. 11.1B). The most common type is monoecy, which only grows unisexual flowers. However, the distribution of male and female flowers is variable in different varieties. Generally, male flowers appear in the early or lower nodes in a monoecious plant, female and male flowers appear in a mixed way in the middle nodes, and female flowers dominate the higher nodes. Subgynoecious plants usually produce few male flowers in the lower nodes and female flowers in the higher nodes. The most obvious difference of subgynoecious line from a typical monoecious plant is a lack of the mixed phase comprising of female and male flowers (Galun 1961; Kubicki 1969a, b, d). Only female or male flowers can be found in gynoecious or androecious plants, respectively. Male and bisexual flowers appear in an andromonoecious plant, which can be regarded as bisexual flowers replacing female flowers found in a monoecious line. In addition, a hermaphroditic plant bears only bisexual flowers. In most cucumber varieties studied, female and bisexual flowers are exclusive in the same plant. However, Kubicki (1969c) found in some reported mutant varieties, female, bisexual, and/or male flowers can be produced in a same plant, and the sex type of the plant is named trimonoecy (or gynomonoecy).

In the early stage of cucumber flower development, all buds are morphologically hermaphroditic, which contain staminate and pistillate primordia simultaneously. Selective arrest in either the staminate or pistillate primordia leads to female or male flowers, respectively. If the arrest does not occur, the bisexual flower is formed (Atsmon and Galun 1962). In 2004, Bai et al. used a detailed section assay to divide cucumber flower development into 12 stages (Bai et al. 2004). Flower meristem begins at Stage 1. From Stage 2 to Stage 5, the primordia of sepal, petal, staminate, and pistillate (carpel) start in sequence. Selective arrest occurs after Stage 5. In the male flower bud, stamens differentiate into anthers and filaments at Stage 6, anthers expand at Stage 7, locules differentiate at Stage 8, microsporocytes differentiate at Stage 9, meiosis begins at Stage 10, mononuclear pollens appear in Stage 11, and finally form mature pollens in Stage 12. At the same time, the carpel primordium in the male flower morphologically slightly enlarges from Stage 6 to Stage 12. On the contrary, at Stage 6 of a female flower bud, the carpel primordia begin to elongate; at Stage 7, the stigma elongates and the ovary begins to differentiate; at Stage 8, the ovule and integument primordium begin to differentiate, and macrosporocytes begin at Stage 9, meiosis begins at Stage 10, embryo sacs are formed at Stage 11, and finally at Stage 12, the relevant auxiliary tissues mature. The stamen primordia in the female flower only can differentiate into anthers and filaments structure at Stage 6, but they are smaller than the stamen primordia in the normal male flower. Thereafter, starting from Stage 7, arrest of stamen development is observed by its limited volume growth. In the bisexual flower, stamen primordia and pistil primordium have the same normal morphological differentiation as in the male and female bud, respectively (Fig. 11.2).

In terms of morphology, the sex differentiation after flower meristem differentiation can be classified into two types: (1) pistillate primordium initiation and (2) staminate primordia can continue to grow after pistillate initiation. The former involves the induction of pistillate primordium, and the determination of unisexual or bisexual flowers is dependent on the latter case. In the past two decades, some gene loci related to sex determination (or sex type) have been discovered and studied. Here, we discussed the latest findings in cucumber, starting with genes that control the specific processes.

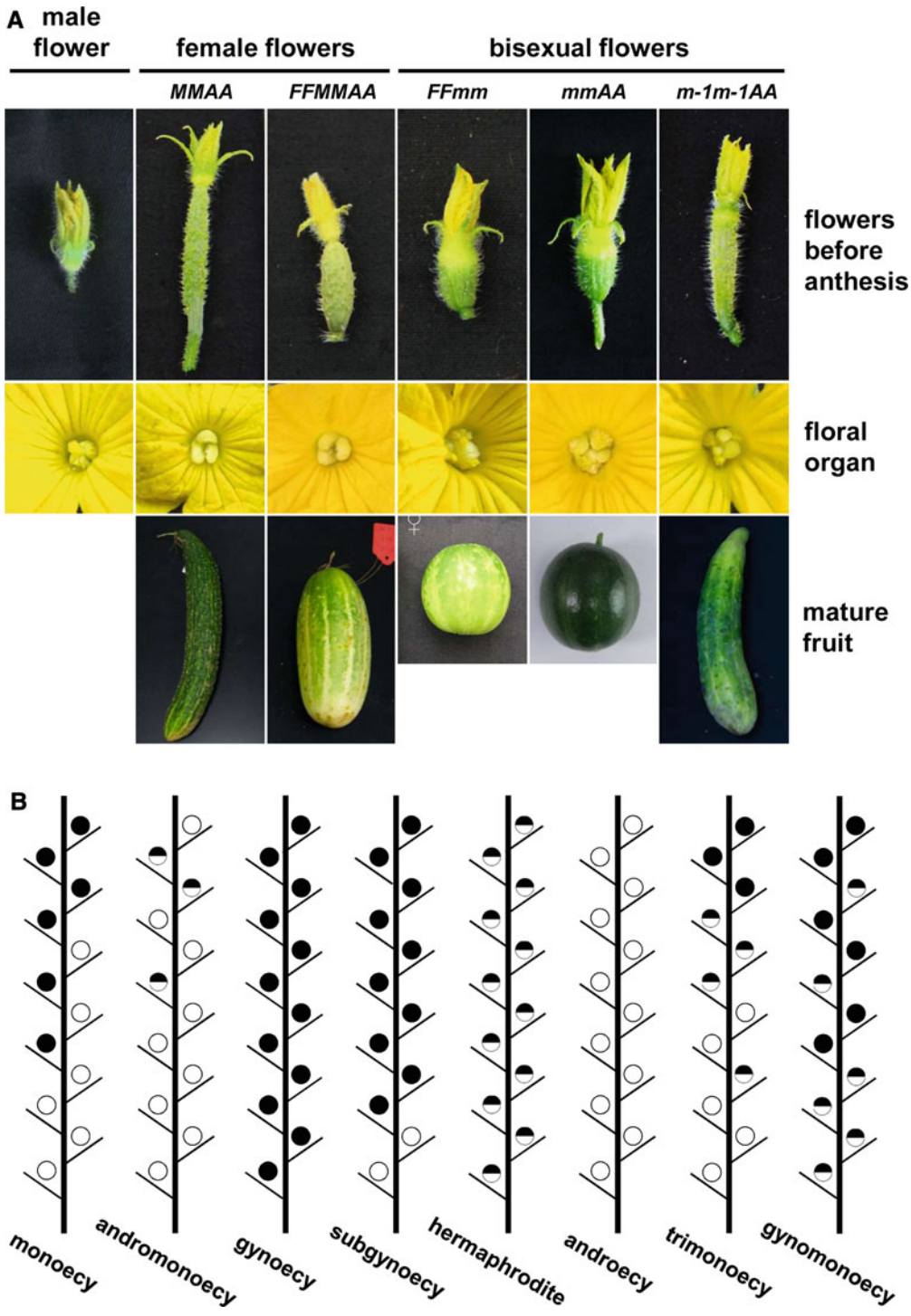

Fig. 11.1 Sex expression in cucumber (main vine). **A** Flower and fruit morphology in cucumber lines. **B** Schematic diagram of sex type in cucumber. Hollow, solid, and mix circles show male, female and hermaphroditic flowers, respectively. This figure is cited from Li et al. (2019), and has been authorized

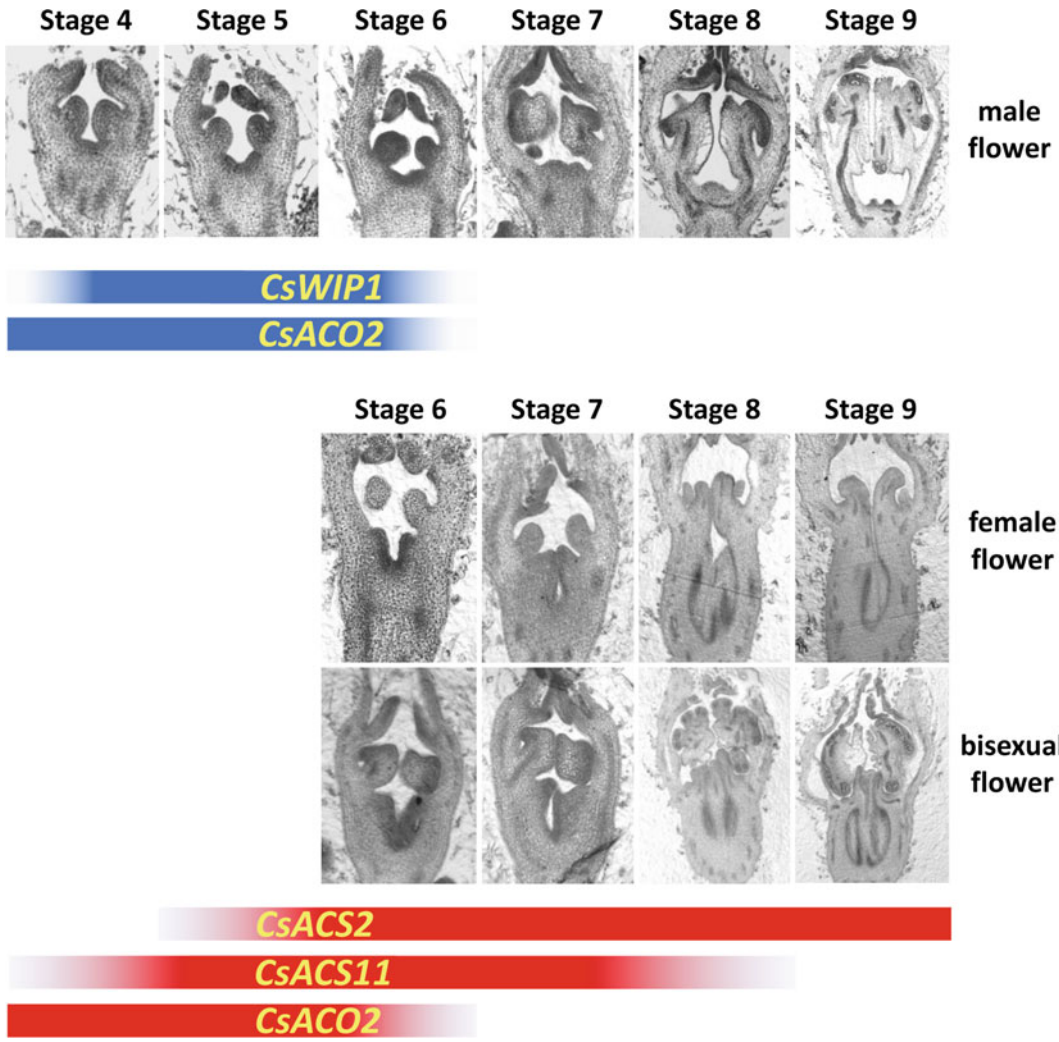

Fig. 11.2 Developmental stages of cucumber flowers, and the detailed expression timing of sex-controlling genes. Before Stage 5, the developmental process of female flowers, male flowers and bisexual flowers are visually similar, and the details list in the text. The mRNA accumulation patterns of sex-controlling genes are showed in blue (in male flower) and red (in female flower) bands, respectively. The graduated darker color shows the mRNA content. This figure is cited from Li et al. (2019), and has been authorized

11.2 Genes Initiating the Pistil Development

In cucumber, gynoecy is an important breeding trait. Immature cucumber fruits are usually harvested a week or 10 days after flowering. Such short growth period means that more female flowers will bring higher potential yield. In cucumber, there are two reported gynoecy-controlling loci, conferring dominant and recessive gynoecy, respectively. Tkachenko discovered the first gynoecious plant from a Korean variety (Kubicki 1969a). Because no male flowers arising on the plant, which prevents self-pollination, homozygous gynoecious line was non-hereditary before 1960s, when gibberellin (GA) was first used to induce male flowers in cucumber (Peterson and Andher 1960). In 1961, Shifriss discovered a gene (named *Acr*) that can

cause female flowers to grow in the lower nodes (Shifriss 1961). Later, Galun (1961) and Kubicki (1969a) named the loci as st^F and Acr^F, respectively. In 1976, Robinson et al. used *F* to define this dominant gynoecy-controlling gene (Robinson et al. 1976). In 1997, Trebitsh et al. found in the gynoecious cucumber plant, *F* gene was associated with a second copy of *CsACS1*, which encodes a 1-aminocyclopropane-1-carboxylic acid synthase (ACS), and the additional copy was named *CsACS1G* (Trebitsh et al. 1997). The proximal promoter (−410 bp upstream sequence) and open reading frame (ORF) of *CsACS1* and *CsACS1G* are almost the same. Difference between them is the distal promoter sequence. The sequence from *CsACS1G* is homologous to a cucumber *BCAT* gene (Knopf and Trebitsh 2006; Mibus and Tatlioglu 2004). Bioinformatics analysis found that there is a 30 kb DNA fragment repeat (including *CsACS1*, *BCAT*, and an annotated *MYB* gene) in the genomic region where the *F* gene is located. The first 30 kb fragment repeats twice forming a tandem repeat structure, and the 'junction point' is *CsACS1G* (Zhang et al. 2015).

Another species in *Cucumis* besides cucumber, melon (*Cucumis melo*), helps to identify another gynoecy locus. In 1939, a recessive locus (*g*) controlling gynoecious melon was reported (Rosa 1928). Martin et al. determined that the gene is *CmWIP1*, which encodes a C2H2 zinc-finger-type transcription factor (Martin et al. 2009). A transposon insertion at 1.3 kb downstream of the gene inhibited the expression of *CmWIP1*, leading to the initiation of the pistil primordium. In contrast, the expression of *CmWIP1* causes carpel abortion and produces male flower. *CmWIP1* can indirectly inhibit *CmACS7*, which is the andromonoecy-controlling gene described later. Using CRISPR/Cas9 technology, the editing cucumber *WIP1* homologous mutant (*Cswip1*) line also showed gynoecy (Hu et al. 2017). All these studies have confirmed that WIP1 is a conserved gynoecy regulator in *Cucumis*.

It is clarified that the genes controlling gynoecy here function in all pistil-bearing flowers, including female and bisexual flowers. The gynoecy-controlling genes can induce (or release) the differentiation of the pistils in both of female and hermaphroditic flowers.

11.3 Mutant Genes Causing Hermaphroditic Plant

In cucumber, female flower produces long fruit, which is in line with current consumption habits. Therefore, monoecy and gynoecy are naturally accepted as the most common sex type in cucumber production, and as a result, dominate the existing germplasm. In contrast, melon breeders prefer bisexual flowers and the andromonoecious lines (Boualem et al. 2008). Bisexual flower usually makes pollination easier, and the fruit is much rounder than the female flower (Li et al. 2009), both of which are quality traits in melon consumption. Interestingly, melon bisexual flower usually has higher fertilization and seed-setting ability than that in cucumber, which may be the result of domestication in long-term commercial breeding.

In *Cucumis*, the gene that controls the appearance of hermaphroditic plant is conserved. To date, two natural *andromonoecious* alleles (named *m* and *m-1*) have been identified in cucumber, both of which are mutated in *CsACS2* (Boualem et al. 2015; Li et al. 2009; Tan et al. 2015). Like *CsACS1G*, *CsACS2* also encodes an ACS. The mutation of the *m* allele is a single-nucleotide transversion and results in a change in a conserved active site residue. In addition, the *m-1* allele is derived from a 14-bp deletion in the third exon of *CsACS2*, which results in a truncated protein. Mutations cause severe and total loss of ACS enzyme activity in the plants with the *m* and *m-1* allele, respectively. In melon, Rosa first identified andromonoecy as a recessive trait in 1928 (Rosa 1928), and the recessive gene was named *a* before 2015. After the androecy-controlling gene (*a*) was cloned in 2015, the name of controlling gene changed to *m* to avoid confusion (Boualem et al. 2015). Boualem et al. found that a mutation in *CmACS7* was associated with andromonoecious melon, which shared 98% identity on the level of deduced amino acid with cucumber *CsACS2* (Boualem et al. 2008).

Pleiotropy is an additional feature of the andromonoecy-controlling gene. Generally, bisexual flowers produce rounder fruits (Fig. 11.1A). In cucumber, the spherical fruit phenotype was thought to co-segregate with the *m* allele based on a genetic analysis using a large population with a total of 5,500 individuals (Li et al. 2009). Recently, Xin et al. found that *CsACS2* functions in fruit elongation through a ubiquitination pathway (Xin et al. 2019), which supplies a connection between sex determination and fruit development. *CsACS2* transcript only accumulates in pistil-bearing flowers, including female (functional isoform) and hermaphroditic flower (non-functional isoform). The functional isoform (encoded by wild-type *CsACS2*) arrests the development of the staminate primordia and produces female flowers. On the contrary, non-functional isoform loses this function, allowing the staminate grow continually after pistillate primordium initiation, and then produces bisexual flowers (Boualem et al. 2008; Li et al. 2012). Therefore, together with mutation studies, expression regulation is also important to *CsACS2* function.

11.4 Mutant Genes Causing Androecy

Flower sex expression pattern in main vine and lateral branches is different in common cucumber varieties. Compared with main vine, branches have higher feminization potential, and pistil-bearing flowers usually dominate the first several nodes. Strictly speaking, a variety that does not have any pistil-bearing flowers (female or bisexual flowers) in main vine and lateral branches is considered as androecy; if not, it is defined as monoecy (growing female flowers) or andromonoecy (growing bisexual flowers). An androecious plant has no commercial value in production, and the existing varieties are all mutants. In 1969, Kubicki identified a recessive *a* gene enhances the androecy nature in cucumber (Kubicki 1969d). *A* gene is hypostatic to *F*, and the plants of *ffaa* genotype are completely masculine. The *a* gene was cloned in a rare androecious variety 'EREZ', and the wild-type allele is *CsACS11*, also encoding an ACS, which is the third sex-controlling *ACS* gene in cucumber sex determination. Like the *m* gene, the mutant isoform in 'EREZ' had no enzymatic activity (Boualem et al. 2015).

In addition to the natural *a* locus, using EMS-induced mutation analysis, Chen et al. discovered a second cucumber androecious variety, which is mutated in *CsACO2* gene. The wild-type gene encodes a 1-aminocyclopropane-1-carboxylic acid oxidase (ACO), and a single-nucleotide change in the mutant gene leads to loss of enzymatic activity (Chen et al. 2016).

11.5 Other Sex-Related Loci in Cucumber

Using traditional and accidental mutations, genetic analyses have identified many loci controlling sex determination. In some monoecious plants, an *In-F* gene was found to function in increasing the proportion of female flowers. Kubicki reported that after treated by GA, a plant with both *F* and *In-F* genes had no male flowers (Kubicki 1969b). In a same work, Kubicki also studied the gene acr^1 in monoecious lines, which caused plants to produce female flowers in consecutive nodes (Kubicki 1969b). In subgynoecious lines, two independent studies (Bu et al. 2016; Win et al. 2019) found that there is a similar major *QTL* site on chromosome *3* (*sg3.1*), which enhance the degree of femaleness. The relationship among *In-F*, acr^1 and this major *QTL* needs further study. In addition, through artificial mutagenesis, Kubicki identified two loci *h* and *gy* (Kubicki 1980). Different from *m*, the *h* gene controls hermaphroditic flower with normal ovary as the female flower, including the pollination ability and morphology of mature stage. However, after analyzing the existing data, we are not clear the connection and difference between the *h* and the *m-1* allele. The *gy* gene, appearing to enhance femaleness, is genetically linked to the *F* gene. Its role in cucumber was similar to that of melon *g* gene. However, cucumber *CsWIP1* is located on chromosome *4*, and the *F* gene is located on chromosome *6*,

which shows that *gy* and *g* may be different in cucumber. In 1969, Kubicki reported a trimonoecious variety, which bears male, bisexual, and female flowers simultaneously, and he named the recessive mutation *tr* (Kubicki 1969c). Interestingly, the morphological structures of bisexual flowers in *m* and *tr* are different. In the trimonoecious plant, bisexual flowers have superior ovaries (hypogynous), which may be thought as a developmental modification of staminate flowers. While, as normal female flowers, bisexual flowers in andromonoecious plants are epigynous (with inferior ovaries). Unfortunately, the cucumber materials possessing these reported loci (*In-F*, acr^1, *h*, *gy*, *tr*) are not universal. We will pay close attention to the in-depth studies on these genes in the future.

11.6 Relationship Between Ethylene and Sex Determination

Ethylene controls the transition from male to female flowers, and it occupies a dominant position in regulating cucumbers' sex determination (Atsmon and Tabbak 1979; Kamachi et al. 1997; Owens et al. 1980; Takahashi and Jaffe 1984; Takahashi et al. 1982; Trebitsh et al. 1997). In the past few decades, ethylene (or its biosynthesis-related agent) has been used to induce female flowers (Rudich et al. 1969; Tsao 1988; Yin and Quinn 1995), while the agents that interfere ethylene synthesis or perception are used to increase maleness in cucumber (Byers et al. 1972; Owens et al. 1980).

Different responses of staminate and pistillate primordia to ethylene content have been used to explain the phenomenon of selective arrest that occurred in sex determination. Yin and Quinn found that stamen or carpel primordia with different degrees of sensitivity can independently respond to different ethylene concentrations (Yin and Quinn 1995). Compared with the initiation of carpel development, a higher ethylene threshold for inhibiting stamen, coupled with the period of increased ethylene production after carpel formation, would prevent the inhibition of stamen before carpel formation, thereby leading to the appearance of unisexual female flowers (Switzenberg et al. 2014). Study showed that the perception of ethylene in stamen primordia rather than carpel primordium is the key to produce a carpel bud (Little et al. 2007; Switzenberg et al. 2014). In a female bud, ethylene may induce organ-specifically DNA damage in the original primordial anthers. The organ-specific ethylene sensing may need to downregulate *CsETR1* (encodes an ethylene receptor) and upregulate *CsCaN* (encodes a calcium-dependent nuclease) (Gu et al. 2011; Wang et al. 2010).

The biosynthesis of ethylene is the result of enzymatic activation of ACS and ACO, which catalyze S-adenosine-L-methyl (SAM) into ACC, and ACC into ethylene, respectively (Adams and Yang 1979; Yang and Hoffman 1984). After synthesis, ethylene is sensed by receptors (ETRs) located on endoplasmic reticulum. Without ethylene, the receptors activate CTR1 (Constitutive Triple-Response 1), which inactivates EIN2 (Ethylene Insensitive 2), and then inhibits the ethylene response. Ethylene binding to the receptor turns off the phosphorylation activity of CTR1 and activates EIN2. Then, EIN2 enters nucleus and stabilizes EIN3/EIL (Ethylene Insensitive 3 / EIN3-LIKE) transcription factors. These transcription factors can activate the ethylene-targeted genes, including *ERF* (encodes ethylene response factor). Finally, these ERFs initiate expression of downstream ethylene-responsive genes (Klee and Giovannoni 2011; Liu et al. 2015; Zhang et al. 2015).

By now, in cucumber, except for *CsWIP1*, other sex-controlling genes, including *CsACS1G*, *CsACS2*, *CsACS11*, and *CsACO2*, are all participants in ethylene biosynthesis. Because ethylene is directly involved in sex determination of *Cucumis* plants, it is no surprise that identifying these sex-related 'ethylene synthases'. However, since most of all the genes exhibit the same biochemical function, their expression regulation must be precise. In addition, the physiological phenomenon reported by Wang et al. confirmed that high concentration of ethylene was harmful to cucumber young tissue (Wang et al. 2010). We know that the process of

sex determination occurs in the developmental floral bud, which also means that there should be a set of precise expression regulation for these genes encoding ethylene synthases to avoid injury. Therefore, special temporal and spatial coordinated expression of these *ACS* and *ACO* genes in sex determination should be crucial to cucumber sex determination.

11.7 Expression Pattern of the Sex-Related Genes

In 2014, a physiological study conducted by Switzenberg et al. showed that the key reason for determining whether carpel or stamen development is the timing and concentration of exogenous ethylene treatment (Switzenberg et al. 2014). Flower bud development shows that sex determination occurs between Stage 5 and Stage 6 (Fig. 11.2); so, all the genes should play a role before, or at least, no later than these periods. Among the genes, *CsACS1G*, *CsACS2*, and *CsACS11* are only expressed in pistil-bearing flower, while *CsWIP1* is mainly expressed in male flower. The transcription of *CsACO2* is not gender specific. In gynoecious or hermaphrodite cucumber, *CsACS1G* is autonomously expressed (or doesn't need to be induced) in early flower bud and precedes all other genes (Knopf and Trebitsh 2006; Li et al. 2012; Zhang et al. 2021). Later than *CsACS1G*, the accumulation of *CsACS2* transcripts below the pistil primordium starts from Stage 5 and then continually accumulates in the developing central region of the ovary (Saito et al. 2007). Expression signals of *CsACS11* are first detected at Stage 4 under the carpel primordium and continue at least to Stage 8 (Boualem et al. 2015). Chen et al. reported that *CsACO2* expresses below the position of future carpel primordium from Stage 2 to Stage 4 and maintains a relatively low level in the carpel and stamen after Stage 6 (Chen et al. 2016). In male flower, *CsWIP1* is expressed from Stage 4 to Stage 6 (Chen et al. 2016). We summarize the gene expression pattern in Fig. 11.2. The interaction of these genes can be inferred from the expression sequence, time, and duration.

Ethylene treatment also regulates sex-controlling gene expression. Treatment with appropriate concentration of exogenous ethylene upregulates *CsACS1*, *CsACS2*, and *CsACS11* and decreases *CsWIP1* transcription (Li et al. 2012; Tao et al. 2018; Yamasaki et al. 2001). The endogenous ethylene produced by the first expressed gene(s) may also regulate other genes, which can explain the putative interaction between (or among) them. At present, a hypothesis has been proposed: ethylene mediates interactions between different sex-controlling genes, in which *CsACS2*, *CsACS11,* and *CsWIP1* are involved. The clones of CsERF110 and CsERF31, which directly activate *CsACS11* and *CsACS2* expression, respectively, provide evidences for this hypothesis (Pan et al. 2018; Tao et al. 2018).

In addition, auxin, brassinosteroids (BRs), and GA also involve in cucumber sex determination. All these hormones may play roles in directly or indirectly affecting ethylene synthesis or signal transduction (Papadopoulou and Grumet 2005; Rudich et al. 1972; Trebitsh et al. 1987; Yin and Quinn 1995). Trebitsh et al. found that auxin can upregulate *ACS* genes and induce the formation of female flowers (Trebitsh et al. 1987). BRs promote the production of ethylene and indirectly involve in the process, in which *CsPSTK1* (encodes a serine/threonine kinase) may function (Pawełkowicz et al. 2019a). Zhang et al. found that the synthesis of ethylene is inhibited by exogenous GA, and *CsGAMYB1* and *CsGAIP* may play roles. *CsGAMYB1* is a homolog of *GAMYB*, which is a positive regulator in GA signaling. *CsGAIP* is a homolog of *DELLA*, encoding a negative regulator in GA signaling (Zhang et al. 2014a, b).

11.8 Gene Interaction Conferring Sex Determination

Based on biochemical and physiological studies, genetic analyses helped to propose a systematic phenotype-genotype model and up–downstream interaction relationship for the sex-controlling genes in cucumber (Galun 1961; Kubicki 1969a,

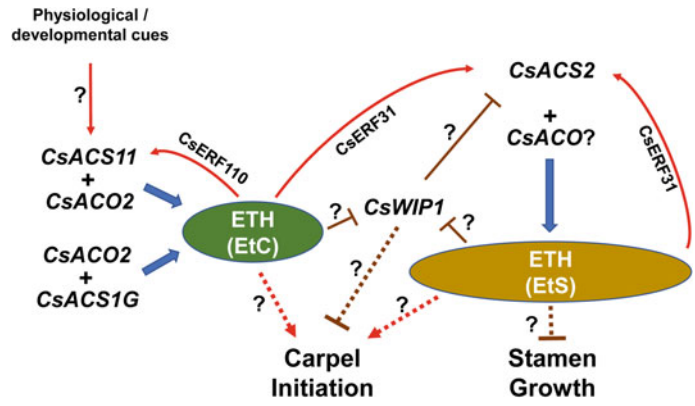

Fig. 11.3 An interaction model of the major genes in cucumber sex determination. Transcriptions of *CsACS1G* and *CsACO2* has no genotype specificity. Red arrow shows directly positive regulation, and 'T' indicates negative regulation. Dashed lines indicate indirect effects. This figure is modified from Li et al. (2019), and has been authorized

b, d; Poole and Grimball 1939; Robinson et al. 1976). We integrate these results to explain the cucumber sex determination (Fig. 11.3).

Based on the suggestion that stamen and carpel have different sensitivity to ethylene content (Switzenberg et al. 2014), two ethylene thresholds are defined for carpel initiation (EtC, Ethylene level for Carpel initiation) and stamen suppression (EtS, Ethylene level for Stamen arrest), and it is believed that EtS is higher than EtC. Therefore, the genotype–phenotype model is considered as:

(1) *F* (*CsACS1G*) is autonomously expressed, and the ethylene produced can reach EtC (but lower than EtS), causing the pistil primordium initiation. The EtC can induce the expression of *M* gene (*CsACS2*), which synthesizes higher (or longer term) ethylene accumulation to reach EtS, then inhibits the staminate primordia development. As a result, the interaction of *F* and *M* (*FFMM*) produces continuous female flowers, resulting in gynoecy.

(2) In the plant with *FFmm* genotype, the realization of EtC is through the autonomous expression of *CsACS1G*, resulting in normal pistil development. However, the mutant *m* gene causes the inactivation of ACS, and there is not enough ethylene to reach EtS, which does not prevent the stamen development. Consequently, the phenotype of the *FFmm* is a plant bearing all bisexual flowers, resulting in hermaphrodite.

(3) With the genotype of homozygous recessive *ff*, sex type of the plant depends on the expression pattern of the *A* gene (*CsACS11*). When *CsACS11* expression is active, the ethylene content will be higher than EtC, but not reach to EtS, which induces pistil differentiation, activates the expression of *CsACS2*, arrests staminate growth, and finally produces female flowers. When *CsACS11* is silent, there is not enough ethylene, then the pistillate primordium cannot initiate, and the stamens keep developing, leading to production of male flowers. Integrating these two conditions in the same plant, the genotype *ffMMAA* produces monoecy.

(4) Same as the phenomenon in hermaphrodite, if the *m* locus is homozygous recessive (*mm*), the stamens of the original female flower are not arrested, and the genotype *ffmmAA* produces andromonoecy;

(5) In a plant with both mutant *F* and *A* genes (*ffaa*), no gene expression can produce higher levels of ethylene than EtC. Therefore, both of *CsACS2* and the pistillate primordium cannot be induced. As a result, only stamens can grow to mature and produce an androecious plant.

Because *CsWIP1* and *CsACO2* mutants were not reported using in previous genetic analyses, the classical phenotype–genotype model does not contain *CsWIP1* and *CsACO2* genes. Martin et al. proposed that *CmWIP1* has a negative regulatory effect on *CmACS7* (*CsACS2* ortholog in melon) expression (Martin et al. 2009). In cucumber, ethylene treatment could decrease *CsWIP1* expression, suggesting that EtC may induce *CsACS2* by suppressing *CsWIP1*. In a plant with mutant *Cswip1*, the uninhibited expression of *CsACS2* directly produces EtS, which is sufficient to initiate the pistillate primordium and inhibit the staminate primordia growth (Hu et al. 2017), thereby producing gynoecy (Fig. 11.3). In addition, the higher content of ethylene (EtS) is also capable of suppressing *CsWIP1* expression, and subsequently keeps continuous activation expression of *CsACS2*, which is supposed a positive feedback regulation by Li et al. (2012). Moreover, in melon, it has been proposed that *CmWIP1* may directly inhibit the initiation of pistillate primordium through an unknown pathway (Boualem et al. 2015). Recently, an S2 bZIP transcription factor, CmbZIP48, was found to physically interact with CmWIP1, and the co-expression and interaction of CmWIP1 and CmbZIP48 were suggested to trigger pistillate primordium abortion in melon (Eleblu et al. 2019).

On the other side, *CsACO2* is believed to (at least) cooperate with *CsACS1G* and *CsACS11* to accomplish ethylene synthesis (Zhang et al. 2021). The *Csaco2* mutant disrupted the formation of EtC, leading to the phenotype of androecy. In the future, cucumber *Cswip1Csaco2* double mutant is needed to develop to determine the relationship between *CsACS2* and *CsACO2*.

Tao et al. found that the expression of *CsACS11* could be induced by ethylene (Tao et al. 2018). However, in a monoecious line, there is no *CsACS1G* to produce ethylene autonomously. Therefore, in future studies, physiological or developmental cues that induce *CsACS11* are needed to study, which may help to answer the question that 'what decides whether *ACS11* is on or off in particular flowers', which was proposed by Ma and Pannell (2016).

11.9 Other Genes Related to Sex Type

There are many transcriptomic, epigenomic, and metabolomic studies related to sex type reported in the last few years (Lai et al. 2017, 2018a, b; Miao et al. 2011; Pawełkowicz et al. 2012, 2019a; Song et al. 2018; Wang et al. 2018, 2019). The genes and cues found in these studies involved photoperiod, temperature, hormone synthesis and signal transduction, blue/red light, lipid and sugar metabolism, and cell cycle, etc. To date, we are not clear whether they are causes or results of the sex determination. Therefore, we cannot summarize the exact position of these genes in the model of sex determination.

11.9.1 Future Prospects

In cucumber, sex type and yield have a high correlation, which is attracting increasing attention from the breeding community and researchers. Here, we have established a regulation model of sex determination based on ethylene core. However, the direct regulatory factors and molecular details are still unclear. Exploring and studying more sex mutations and using reverse genetics are effective methods to identify the next gene that controls sex determination. Moreover, because the key developmental stage of sex determination is identified, some precise methods, such as single-cell RNA sequencing combining laser microdissection may clarify the detailed genetic pathway involved in this process. It is hoped that the proposes and suggestions made here will help to reveal the mechanisms of cucumber sex determination in the future.

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Classical Genetics and Traditional Breeding

12

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Abstract

Cucumber is one of the most important vegetable crops grown worldwide, both under open fields and protected conditions. It is originated in India with a secondary centre of diversity in China and the Near East. It is a cross-pollinated crop with no inbreeding depression and is used as a model crop for studying the various genetic and molecular pathways because of its breeding behaviour and smaller genome size. The distribution of the different *Cucumis* species in the primary, secondary and tertiary gene pool is determined based on cross-compatibility, genetic, phylogenetic and molecular evidence. The cultivated cucumbers are generally monoecious in nature, however, a wide diversity in sex forms is recorded in this species. Gynoecious with only female flower is the most important sex form used commercially in hybrid seed production in cucumber. Significant advancement has been made in understanding the genetics of the flowering traits like sex expression and modification. Studies on inheritance and nature of heritability for important vegetative, fruit and yield-related traits, biotic and abiotic stresses have been reported widely by different research groups. The understanding in the genetics of the important qualitative and quantitative traits facilitated the genomics study in economically important traits. In spite of being highly cross-pollinated in nature, it has very low or negligible inbreeding depression. Different breeding methods are adopted in cucumber based on its genetic architecture and breeding behaviour. Among the popular breeding methods, population improvement, pedigree method and backcross breeding have been adopted widely in the successful development of elite lines with a wide variety of traits. Because of higher yield and better adaptability and resilience, F_1 hybrids are highly popular in cucumber. The development of gynoecious lines is instrumental in developing F_1 hybrids with higher productivity. Protected cultivation in cucumber is largely facilitated by breeding gynoecious parthenocarpic lines. A large diversity is available within the genus *Cucumis* in different parts of Asia and Africa. There is a need to evaluate the entire gene pool for important biotic and abiotic stresses to meet future challenges. Extensive genetic studies need to be conducted for all the traits related to the yield and adaptability of the cucumber genotypes using the available germplasm available with different gene banks and natural diversity.

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12.1 Introduction

The genus *Cucumis* belongs to the gourd family *Cucurbitaceae* and comprises two economically important cucurbit crops, cucumber and melon, grown worldwide. This gourd family consists of around 130 genera and 1,000 species containing several nutritionally important cucurbitaceous vegetables, like *Citrullus lanatus*, *Citrullus amarus, Momordica charantia, Cucurbita pepo, Lagenaria siceraria, Benincasa hispida, Luffa* and *Trichoanthes* species (Renner and Schaefer 2016; Chomicki et al. 2019). With the annexing of Asian and Australian species, so far approximately 66 species have been documented in the genus *Cucumis* (Sebastian et al. 2010; Chomicki et al. 2019; Cheng et al. 2020). Biologically and economically important species, cucumber (*Cucumis sativus*) (20 = 2x = 14) with genome size 367 Mb, is extensively cultivated worldwide including the Indian sub-continent (Zhu et al. 2016). The geographic origin and region of domestication has always been a fundamental question and matter of debate amongst the conflicts regarding the evolution of crop plants. Recent phylogeographic, phylogenetic, molecular and genomic evidences established the Asiatic origin and domestication of cucumber somewhere in the Indo-Gangetic plains with the coexistence of its feral form, *Cucumis sativus* var. *hardwickii* (Sebastian et al. 2010; Qi et al. 2013; Chomicki et al. 2019). These cultivated and feral species of cucumber represent the primary gene pool, while *Cucumis hystrix* (2n = 2x = 24), the wild close relative of cucumber, is considered in the secondary gene pool (Delannay et al. 2010; Li et al. 2011a). Hence, a narrow genetic base is the most important impediment in the genetic improvement programme of *Cucumis.* Cucumber has been regarded as a model for the understanding of various biological processes and organelle genetics as its three genomes exhibit distinct modes of transmission like paternal mitochondrial transmission, maternal and biparental transmission for chloroplast and nuclear genes, respectively (Calderon et al. 2012; Weng 2016). It is the first horticultural crop in which the full draft genome was made publically available (Huang et al. 2009). Relatively smaller diploid genome size, short and annual life cycle, less amount of repetitive DNA percentage and enriched diversity of sex forms offer significant opportunities for classical genetic studies and genome research in this crop.

Cucumber is the fourth most important vegetable crop cultivated worldwide with China being the largest producer followed by India. With the acute pentagonal leaf laminae, the cucumber plants are stiffy hairy with bright yellow flowers borne on leaf axils. Monoecious is the predominant sex form in cucumber, and both pistillate and hermaphrodite flowers have inferior ovary (Paris et al. 2011). The economic part of cucumber, tender immature fruits, usually become ready for harvesting at 5–10 days post anthesis and are consumed as salads, cooked vegetables or in processed form (pickles). Since ancient times, cucumber holds a medicinal status in Indian traditional medicine. The abundant quantity of water content, low calories, presence of triterpenoid cucurbitacins (A, B, C, D, E, I), β-carotene and other phytochemicals impart antioxidant, anticancer, antidiabetic, lipid-lowering and ethnomedicinal properties in cucumber (Mukherjee et al. 2013). The fruits are used as cooling agents and seeds for the treatment of skin-related disorders since antiquity. The genetic and genomic resources in cucumber are limited and the development of high yielding varieties with excellent quality has always been the focus of cucumber breeders worldwide. The presence of parthenocarpy and gynoecious traits in cucumber has tremendous potential for the development of suitable hybrids for low-cost greenhouse cultivation (Wu et al. 2016). Traditionally, simple selection-based breeding methods have been proved instrumental for the genetic improvement

of cucumber. Now, the advent of new technologies accompanied by advance genotyping and phenotyping facilities, next-generation sequencing approaches, genomic selection and other molecular tools has facilitated the genetic and genomic research in cucumber, facilitating the conventional breeding approaches like never before.

12.2 Genetic Resources and Gene Pool

Most of the available sources of evidence indicated India as the primary centre of origin of cucumber and the highest amount of diversity in terms of plant growth, branching, fruit shape, size, colour and texture, maturity duration, bitterness and variation flowering is available in this region (Sebastian et al. 2010). A large diversity in feral form *C. s.* var. *hardwickii* with extreme bitterness, smaller fruit size and multiple lateral branching is also recorded in different parts of India. China and the Near East regions are considered as the secondary centres of diversity for cucumber (Meglic et al. 1996; Staub et al. 1997a). Besides the two cultivated species in *C. sativus* (cucumber) and *C. melo* (melon), several other wild species are present within the genus *Cucumis.* Prominent wild species are *C. hystrix*, *C. callosus*, *C. metuliferus*, *C. muriculatus*, *C. agrestis* and *C. s.* var. *hardwickii*. In the different parts of Africa, Asia and India, more than 50 *Cucumis* species have been identified with very wide diversity for different traits by several workers (Lv et al. 2012; Kacar et al. 2012; Weng 2010; Zhang et al. 2012; Qi et al. 2013). The gene pool concept and classification of different species of *Cucumis* into different gene pools was proposed by Bates et al. (1995), den Nijs and Custers (1990), and Raamsdonk et al. (1989). Generally, it is agreed that the species *C. s.* var. *sativus* and var. *hardwickii* belong to the primary gene pool and *C. hystrix* belongs to the secondary gene pool which is partially cross-compatible with cucumber (Chen and Kirkbride 2000; Chen et al. 2004). The tertiary gene pool consists of distantly related species from other genera or sub-genera (e.g., *Cucumis melo* L. and *Cucurbita* L.), with no cross-compatibility with cultivated cucumber (Chung et al. 2006, Staub et al. 1997a, b). The *C. s.* var. *hardwickii* and *C. hystrix* have been used widely for the introgression of economically important traits from these species. The species from the tertiary gene pool were also used in broadening the genetic base of cucumber using technology like in vitro embryo rescue and somatic hybridization.

12.3 Classical Genetics in Cucumber

During the past 10–15 decades classical genetics has played a central role in enriching our understanding of numerous aspects of biology and expediting crop breeding. The Mendelian principles illuminating heredity were first documented in crop plants and in line with these findings the direct beneficiary was the agriculture sector. The concept of 'gene' was developed in the twentieth century and this acquaintance of gene concept escalated plant breeding for many decades (Vollmann and Buerstmayr 2016). Thereafter, the advent of molecular markers, revamping of molecular genetics and genomics brought significant changes in the theory of gene and consequently accelerated crop breeding. The advancements in genomics have helped in understanding functional, regulatory genetic and epigenetic mechanisms. In addition to genomic selection, the dissection of QTLs controlling complex traits is eye-catching in the Mendelian context as it reveals the loci behaving in Mendelian fashion. Classical genetics have elevated our knowledge of the genetic architecture of plant growth and development in cucumber and stimulated the breeding for qualitative and quantitative traits both in the public and private sectors. Unravelling the genetics of plant architecture traits help breeders to imply appropriate breeding procedures for the genetic improvement

of crop and economically important traits. Cucumber is predominantly monoecious in nature and has become a model for plant growth and physiology, sex expression and organelle genome genetics. The application of Mendelian genetics in cucumber has led to the discovery of a number of genes governing different traits.

12.3.1 Genetics of Flowering and Sex Expression

In the angiosperms, the flowering transition representing a pivotal transition from vegetative to the reproductive stage is probably the most significant transitional development in the life cycle of higher plants. This biological process is governed by both endogenous and exogenous factors (Cho et al. 2017). Determination of sex form in angiosperms is of fundamental biological significance for fertilization, fruit development and seed production. It leads to the formation of unisexual flowers which promotes out-breeding and increase genetic diversity. The majority of the angiosperms are hermaphrodite (~90%), however, 4–5% of the plant species are monoecious and the rest of the species exhibit dioecy or intermediate sex forms (Charlesworth 2002; Devani et al. 2017). The majority of the species in the *Cucurbitaceae* including cucumber have diverse sex morphotypes (Chen et al. 2016; Pawełkowicz et al. 2019a). A number of intermediate flower types such as gynomonoecious, andromonoecious and trimonoecious have evolved from a common ancestor having hermaphrodite flowers (Pawełkowicz et al. 2019b). Apart from the common monoecious sex form in cucumber, the other types such as gynoecious, androecious, andromonoecious, subgynoecious and trimonoecious have also been reported in naturally distributed genotypes or mutants (Li et al. 2019). Monogenic control is the simplest form of sex type inheritance, while the complex systems are controlled by multiple loci and sex chromosomes. The gene expression studies revealed that in the female flower buds of cucumber the cell division is maintained at a high level in the area containing arrested stamen primordia (ACASP) (Yamasaki et al. 2017). In the petals and stamens of the staminate flower buds, epidermal cell density does not differ significantly, while in the arrested stamens of pistillate flowers buds, epidermal cell density remains quite higher than petals. Thus, despite higher cell division activity, the cell growth is hampered and consequently programmed cell death (PCD) occurs in the cucumber female flower buds (Yamasaki et al. 2017). The genetics of sex expression in cucumber is mainly governed by four loci, *M, F, A, Gy* and their interplays (Robinson et al. 1976; Pan et al. 2018; Li et al. 2019; Pawełkowicz et al. 2019b; Li et al. 2020). Hence, the genetic constitution of different sex types in cucumber is *MMffAA* (monoecious), *MMFFAA or MMFFaa* (gynoecious), *MMFfAA or MMFfaa* (subgynoecious), *mmffAA* (andromonoecious), *mmFFAA, or mmFFaa* (bisexual or hermaphrodite) and *MMffaa or mmffaa* (androecious). Numerous environmental, epigenetic, other genes or QTLs and hormonal factors affecting sex transition have also been well studied (Li et al. 2019; Pawełkowicz et al. 2019b; Li et al. 2020). The various genes and their encoded proteins with function determining sex expression in cucumber have been comprehensively reviewed by Pawełkowicz et al. (2019b). The molecular characterization of *F* locus determined that an additional copy of ACC (1-aminocyclopropane-1-carboxylic acid) synthase gene '$CsACS_1G$' exists in the gynoecious cucumber lines (Mibus and Tatlioglu 2004; Pawełkowicz et al. 2019b). Likewise, the *M* locus represents *CsACS2* gene (Li et al. 2009). The recessive '*gy*' allelic form is responsible for more stable female sex expression and '*h*' allele (*andromonoecious*-2) controls the bisexual flower development (Pawełkowicz et al. 2019b). '*In-F*' and '*Tr*' are liable for escalating the action of '*F*' gene and trimonoecious sex form, respectively. Ethylene, the gaseous plant hormone, has been credited to control the flower sex in cucumber throughout many decades and has the practical

utility in female flower production in cucurbits (Wang et al. 2010; Pan et al. 2018; Xin et al. 2019). The ethylene biosynthesis involves the conversion of methionine to SAM (S-adenosylmethionine), SAM into *ACC* (1-aminocyclopropane-1-carboxylate) by *ACC* synthase, and eventually, ACC oxidase causes the oxidation of ACC to ethylene (Chen et al. 2016; Pan et al. 2018; Xin et al. 2019). The subgynoecious trait in cucumber which exclusively leads to the formation of female flower sex at later stages is controlled by one pair of recessive '*mod-F2*' gene and one pair of incompletely dominant '*Mod-F1*' gene (Chen et al. 2011). Pati et al. (2015) revealed monogenic dominant control of gynoecious sex expression in the cucumber gynoecious line GBS-1, while a dominant gene was reported to control the parthenocarpy in the cucumber inbred lines PPC-2 and GPC-1 (Jat et al. 2019).

12.3.2 Genetics of Important Vegetative, Fruit and Yield-Related Traits

Improvement of cucumber for economically important traits requires information about genetics and the nature of inheritance of these traits. Understanding the genetic makeup for various vegetative traits enables the plant breeder to develop desirable plant types with optimum growth parameters to maintain the balance of source-sink relationship. The knowledge of the genetics of various fruit quality-related traits such as skin colour, fruit length, fruit diameter, fruit weight, shape index, bitterness, glossiness, warty fruits, green flesh colour, orange/yellow fruit flesh colour and peduncle length is indispensable for improvement programme in cucumber (Yuan et al. 2008; Song et al. 2016a). The traits like fruit weight, fruit length and the number of fruits per plant have a direct correlation with fruit yield in cucumber and the traits like the number of days to anthesis. The first flower node is related to precocity.

12.3.2.1 Vegetative Traits

Multiple lateral branching is one of the most important vegetative traits directly associated with higher yield in cucumber. Increased yield due to multiple lateral branching is mainly because of the increased number of fruits per plant. Number of lateral branches was found to be positively correlated (r = 0.58 to 0.42) with the number of fruit per plant in a processing cucumber population in two locations over two years (Fazio 2001). Similarly, very high and significant positive correlation between MLB and fruit yield was reported by several workers in a diverse population of cucumber (Cramer and Wehner 1998a, b; Cramer and Wehner 1999). Inheritance study of multiple lateral branching concluded that this trait is quantitatively inherited (at least four genes; Wehner 1989) with additive genetic variance and a narrow sense heritability (h^2) of 0.00 to 0.61 in different population. The hair-like structures called trichomes are widely present on different plant organs including vegetative parts such as stems, leaves and tendrils. The occurrence of trichomes acts as the first line of defence in plants against different insect pests, pathogens, transpiration, UV irradiation and adverse temperature (Cui et al. 2016). Numerous spontaneous cucumber glabrous mutants have been reported and well-characterized (Table 12.1). The first such mutant in cucumber was *cucumber glabrous-1* or *mict* (*micro-trichome*) (Li et al. 2015). Similarly, several types of compact plant mutants have been illustrated in cucumber providing compact plant architecture. The specific helical coiling organs called tendrils are also an important vegetative trait in vine plants like cucurbits. The knowledge of genetics, cloning and expression pattern of gene network crucial for tendril development is essential to elucidate tendril organogenesis and utilization in cucumber breeding (Chen et al. 2017). In this regard, various tendril-less mutants have been identified in cucumber. The leaf colour mutants mainly resulted from the inactivation of chloroplast genes. Further, leaf variegation is a frequently observed genetic phenomenon in

Table 12.1 Genetics of quantitative and qualitative traits in cucumber

Trait	Inheritance	Remarks	Reference(s)
Fruit traits			
Tuberculate fruit	Single dominant	Confers warty fruits	Walters et al. (2001), Wang et al. (2020)
Fruit epidermal feature, tubercule size	Single recessive	Control the size of fruit tubercules formation	Yang et al. (2019)
Soft spines	Single recessive	Receptor kinase gene regulating multicellular trichomes	Guo et al. (2018)
Number of spines	Single recessive	Recessive to Ns, confers numerous spines	Zhang et al. (2016)
Fruit spine density	Single recessive	Confers high density of fruit spines	Zhang et al. (2016)
Colour of spines	Single dominant	Confers black colour spines	Li et al. (2013)
Immature fruit colour	Single recessive	White skin colour in immature fruits	Liu et al. (2015)
Yellow-green peel mutant	Single recessive	Confers yellow-green immature fruit colour. It is recessive to dark green and epistatic to light green colour	Hao et al. (2018)
Light green peel	Single recessive	Light green skin colour	Zhou et al. (2015)
Fruit size	Quantitative	Round fruit shape	Pan et al. (2017)
Mango fruit mutation	Single recessive	Exhibits extensive morphological differences in leaves, flowers, fruit, and seeds. Multiple effects on flower growth, female and male sterility	Niu et al. (2018)
Carpel number variation	Simply inherited recessive	Three carpels are incompletely dominant to five	Li et al. (2016)
Fruit length	Quantitative	Determines mature fruit length in cucumber	Wei et al. (2016)
Ovary length	Quantitative	*ovl3.1 and ovl3.2* are major effect QTLs; determines ovary length in cucumber	Wei et al. (2016)
Fruit peduncle length	Quantitative, one major additive gene and additive-dominant polygenes	Provided basis for breeding of long fruit peduncle trait	Song et al. (2016)
Vegetative traits			
Trichome	Single recessive	Trichomes on the hypocotyl	Li et al. (2015)
Trichome	Single recessive	Glabrous stem, petioles and leaves whereas the surface of the fruits, sepals, fruit peduncles and sparse and fine hairs on flower pedicels	Yang et al. (2011)
Trichome	Single recessive	Glabrous phenotype on all aerial organs	Pan et al. (2015)
Trichome less mutant	Single recessive	Absence of trichomes on all aerial organs	Zhao et al. (2015)

(continued)

Table 12.1 (continued)

Trait	Inheritance	Remarks	Reference(s)
Plant architecture	Single recessive	Dwarf plant habit, reduced internode length	Li et al. (2011b), Wang et al. (2017)
Plant architecture	Single recessive	Interact with *bushy* gene to provide dwarf plant habit	Li et al. (2011b)
Tendril less mutant	Single recessive	Absence of tendrils, less trichomes, reduced vine length	Chen et al. (2017)
Tendril less mutant	Single recessive	Spontaneous tendrilless mutant formed branches instead of tendrils	Chen et al. (2017)
Variegated leaf	Single recessive	Confers yellow, white or green sectors in the young leaf throughout the life cycle, contains defective chloroplast	Cao et al. (2018)
Virescent leaf	Single recessive	Displays light yellow cotyledon and true leaf	Miao et al. (2016)
Virescent leaf	Single recessive	Light-sensitive virescent-yellow leaf mutant turns green under low light conditions	Song et al. (2018a)
Curly leaf mutant	Single semi-dominant and single dominant	Upward rolled leaf phenotype, curly petals	Rong et al. (2019)
Round leaf	Single recessive	Determine round leaf shape with smooth margin	Zhang et al. (2018)
Fruit quality and flesh color			
Bitterness	Single recessive	Non-bitter foliage and fruits	Andeweg and DeBruyn (1959), Zhang et al. (2013)
Bitterness	Single dominant	Extremely bitter fruits	Zhang et al. (2013)
Fragrance	Single recessive	Pandan-like fragrance in cucumber	Yundaeng et al. (2015)
β-carotene	Single recessive	Orange colour endocarp	Bo et al. (2012)
Flesh thickness	Quantitative	Control the flesh thickness in cucumber	Xu et al. (2015)
Green flesh colour	Quantitative	Major effect QTL for flesh colour and flesh extract colour	Bo et al. (2019)

tropical and subtropical plants mainly. In cucumber, the EMS-induced variegated leaf mutant reflecting green-yellow-white variegation phenotype has been reported (Cao et al. 2018). Leaf virescent is another mutant that causes light yellow cotyledons or true leaf. In cucumber also different virescent leaf colour mutants are reported, which are mainly under the genetic control of recessive nuclear genes (Table 12.1).

12.3.2.2 Fruit-Related Traits

As compared to other members of *Cucurbitaceae,* the cucumber fruits are recognized by a distinguished 'wart' character having economic importance (Xu et al. 2015). Both warty and non-warty types of peel are present in cucumber, where the warty type is predominant in Chinese cultivated types and the majority of American and European types are having non-warty-type

traits. The warty type of fruit peel trait is dominant to the non-warty type (Table 12.1) (Walters et al. 2001; Wang et al. 2007). The cucumber fruits are characterized by the presence of tubercles, thick cuticles and trichomes (large trichomes are called spines) (Yang et al. 2019). Trichomes are significant and specific traits that emerged from the epidermal cells of almost all the land plants. The soft spine trait in cucumber, governed by '*ts*' gene, could regulate the breeding of cucumber fruits with tender spines (Guo et al. 2018). The inheritance of genes governing different epidermal features and other fruit quality traits in cucumber is presented in Table 12.1. The number of fruit spines is also an important fruit quality trait in cucumber and has been reported to be governed by different genes such as *s, s-2, s-3, ss* and *ns* (Zhang et al. 2016). The black colour spines on the cucumber fruit surface are specialized trichomes and this trait is dominant to white spines (Li et al. 2013; Liu et al. 2019). The *B* locus determining black spine colour has been characterized as an *R2R3-MYB* transcription factor, *CsMYB60,* which regulates the flavonols and proanthocyanidins pigment in black spines (Liu et al. 2019). Likewise, the immature cucumber fruit skin colour is a pivotal agronomic trait influencing consumer choice apart from dull or glossy and mottled or uniform skin. Different skin colours in cucumber like yellow-green, dark green, white and light green are under genetic control (Table 12.1) (Liu et al. 2015). The MutMap and genotyping analysis determined the *CsMYB36* transcription factor conferring yellow-green peel mutant in cucumber (Hao et al. 2018). Cucumber fruit size is a quantitative trait measured by fruit length (L) and diameter (D) or length/diameter (L/D) ratio (Pan et al. 2017). The fruit size variation in cucumber is controlled by several QTLs such as *FS3.2, FS3.3* and *FS2.1, FS2.2* that are involved in fruit elongation and radial growth, respectively (Wang et al. 2014; Pan et al. 2017). To some extent, the ovary shape is also a decisive factor in determining fruit shape (Wei et al. 2016). Fruit shape is a highly heritable character and is largely influenced by cell division and environmental factors. In this context, Zhang et al. (2019) reported five interacting QTLs (*FS1.1, FS1.2, FS2.1, FS3.1* and *FS6.1*) related to fruit shape in cucumber. Here *FS3.1* is accountable for elongated fruit shape and *FS6.1* for enhancing fruit diameter. Carpel number is another important fruit quality trait in cucumber influencing internal quality, fruit shape and size (Li et al. 2016). The commercially available different cucumber types in markets have generally three carpel numbers; however, the deviation from three numbers is also present which can vary from 2 to 7. The fine genetic mapping revealed that *CsCLV3* is the candidate gene for '*cn*' controlling the carpel number variation in cucumber (Li et al. 2016). The fruit length in cucumber is reported to be controlled by QTLs and is affected by agronomic and environmental conditions (Wei et al. 2016). In a recent study, Wei et al. (2016) reported 8 QTLs for immature and mature cucumber fruit length (Table 12.1). Flesh thickness is also an important trait in cucumber having a central role in yield trait. The flesh thickness in cucumber is a polygenic trait (Xu et al. 2015).

12.3.2.3 Quality, Flesh Colour and Miscellaneous Traits

Cucurbitacins are the important components of cucumber fruit and foliage as they release toxins as defensive agents against insect pests and herbivores (Zhang et al. 2013). Breeders mostly look for fruits with low bitterness which is also caused by cucurbitacins. Numerous genes have been identified and well-characterized for controlling this trait (Table 12.1). Thus bitterness in cucurbits has complex genetics and is also affected by environmental factors. In cucumber, a non-bitter line was identified in an American cultivar Long Green where the non-bitterness is governed by a monogenic recessive gene (Table 12.1) (Andeweg and DeBruyn 1959). Similarly, other genes governing bitterness or non-bitterness in cucumber were identified like *bi-2, bi-3, Bt-1, Bt-2* and their inheritance is in a single-locus fashion. The *bi-1* is epistatically recessive to the *bi-3* allele and cause non-bitter foliage and fruits as well.

β-carotene is one of the most important carotenoids having antioxidant and anticancer

properties. Cucumber itself has less quantity of β-carotene 22–48 lg/100 g FW (Kandlakunta et al. 2008). However, a botanical variety of cucumber, Xishuangbanna gourd (XIS; *Cucumis sativus* L. var *xishuangbannanesis* Qi et Yuan), found in the Xishuangbanna area of China is a good source of β-carotene (Bo et al. 2012). The mature fruits of this gourd have orange colour endocarp/mesocarp with β-carotene content of about 700 µg/100 g FW. It is cross-compatible with cultivated cucumber and could be used for the breeding of cucumber with high β-carotene content. The orange flesh colour is under the genetic control of recessive genes as demonstrated by several broad-based crosses of XIS gourd x commercial hybrids (Navazio and Simon 2001). Cuevas et al. (2010) reported that the quantity of mesocarp β-carotene content is governed by two recessive genes while in the endocarp it is controlled by a single recessive gene.

12.4 Genetics of Biotic and Abiotic Stress Resistance

Cucumber is the most important cucurbit vegetable cultivated worldwide and suffers from several biotic and abiotic stresses. The production and productivity of this crop are reduced significantly because of numerous biotic and abiotic stresses (Table 12.2). Different bacterial, fungal and viral diseases cause huge yield loss in cucumber. Breeding strategies have been focused on host plant resistance against different types of viral, fungal and bacterial pathogens in cucumber. The resistance to diseases is mainly accompanied by *R* genes (Harris et al. 2013). A series of defence signalling cascades are activated by *R* gene-mediated recognition of invasive pathogen effectors, which results in systematic acquired resistance (SAR) in crop plants. The major fungal pathogens affecting cucumber are powdery mildew, downy mildew, *Alternaria* leaf spot, anthracnose, scab, gummy stem blight, damping off and fusarium wilt. The bacterial pathogens affecting cucumber fruit yield and quality includes bacterial wilt, angular leaf spot and bacterial fruit blotch. Likewise, the viral diseases also cause huge loss in cucumber and include cucumber mosaic virus, squash mosaic virus, Zucchini yellow mosaic virus, cucumber green mottle mosaic virus, cucurbit aphid-borne yellows virus, tomato leaf curl New Delhi begomovirus and tomato yellow leaf curl virus. For disease resistance breeding, the understanding of the genetics of resistance is one of the basic requirements. The unravelling of classical and molecular genetics of disease resistance is the most challenging and practically relevant job in crop plants (Keller et al. 2000; Dong et al. 2019; Chen et al. 2020). Varying results of inheritance of disease resistance for different fungal, bacterial and viral pathogens have been reported in varying sources of cucumber (Table 12.2). Inconsistent reports are available regarding resistance to biotic stresses in cucumber such as dominant, recessive, epistatic and quantitative. Different types of inheritance could be due to the use of different approaches to measure the resistance, source of resistance and environmental conditions. Powdery mildew (PM) and downy mildew (DM) are two major devastating fungal diseases in *Cucumis* species. The linkage analysis has revealed that downy mildew [*Pseudoperonospora cubensis* (Berk. and Curt.) Rostov] and powdery mildew (*Sphaerotheca fuliginea* Poll.) genes are either tightly linked loci or have the same chromosome location in cucumber (Olczak-Woltman et al. 2011). The identification of linked molecular markers has facilitated markers-assisted breeding for resistance to various diseases in cucumber. The resistance to downy mildew is mainly governed by recessive genes; however, in the breeding line GY14A of cucumber, polygenic resistance to downy mildew was reported (Olczak-Woltman et al. 2011). Likewise, recently many reports have reported quantitative resistance to downy mildew in cucumber (Zhang et al. 2013; Wang et al. 2016; Li et al. 2018; Wang et al. 2018b). Similarly, inheritance of resistance to powdery mildew in cucumber has been investigated with inconsistent results. The genetics of resistance to powdery mildew in cucumber is quite complex involving multiple

Table 12.2 Genetics of biotic and abiotic stress resistance in cucumber

Biotic stress	Genetics	Source	Reference (s)
Fusarium Wilt (*Fusarium oxysporum* f. sp. *cucumerinum*) race 1	Single dominant	SMR18	Vakalounakis (1993)
Scab (*Cladosporium cucumerinum*)	Single dominant	SMR18	Vakalounakis (1993)
Fusarium Wilt (*Fusarium oxysporum* f. sp. *cucumerinum*)	Polygenic	Rijiecheng	Dong et al. (2019)
Downy mildew (*Pseudoperonospora cubensis*)	Three recessive genes	Aojihai	Shimizu et al. (1963), Olczak-Woltman et al. (2011)
Downy mildew (*Pseudoperonospora cubensis*)	Single recessive	Poinsett	Fanourakis and Simon (1987), Olczak-Woltman et al. (2011)
Downy mildew (*Pseudoperonospora cubensis*)	Quantitative	K8	Zhang et al. (2013)
Downy mildew (*Pseudoperonospora cubensis*)	Quantitative	PI 197088	Li et al. (2018)
Downy mildew (*Pseudoperonospora cubensis*)	Quantitative	W17120	Wang et al. (2016)
Powdery mildew (*Erysiphe cichoracearum* and *Sphaerotheca fuliginea*)	Two recessive genes	Natsufushinari	De Ruiter et al. (2008), Chen et al. (2020)
Powdery mildew	Single recessive	PI 200815, PI 200818	De Ruiter et al. (2008), Chen et al. (2020)
Powdery mildew	Single recessive	Wisconsin SMR 18	De Ruiter (2008), Chen et al. (2020)
Powdery mildew	Quantitative	PI 197088	Wang et al. (2018b)
Powdery mildew	Quantitative recessive	WI 2757	He et al. (2013)
Powdery mildew	Quantitative recessive	NCG122	Liu et al. (2017b)
Gummy stem blight (*Didymella bryoniae*)	Quantitative and mainly governed by three pairs of additive epistatic major genes	PI 183967	Zhang et al. (2017)
Cucumber vein yellowing virus	Monogenic, incompletely dominant	CE0749	Pujol et al. (2019)
Scab (*Cladosporium cucumerinum*)	Single dominant	9110Gt	Zhang et al. (2010)
Target leaf spot (*Corynespora cassiicola*)	Single recessive	D31	Wen et al. (2015)
Cucumber mosaic virus	Quantitative	02245	Shi et al. (2018)

(continued)

Table 12.2 (continued)

Biotic stress	Genetics	Source	Reference (s)
Angular leaf spot (*Pseudomonas syringae* pv. *lachrymans*)	Polygenic recessive	Gy14	Slomnicka et al. (2018)
Low temperature totlrance	Quantitative	CG104 tolerance inbred line	Dong et al. (2019)
Waterlogging tolerance	Quantitative	PW0832	Yeboah et al. (2008)
Salt tolerance	Quantitative	CG104	Liu et al. (2021)
High temperature tolerance	Quantitative	02245	Dong et al (2020)
Low temperature germination ability	Quantitative	Coolgreen	Yagcioglu et al. (2019)
Low temperature tolerance	Quantitative	65G	Song et al. (2018b)

genes and metabolic pathways (Chen et al. 2020). The classical genetics revealed multiple recessive genes for powdery mildew resistance in Puerto Rico 37, while 1–2 major and 1–2 minor genes in PI 197,087 (Barnes and Epps 1956; Chen et al. 2020). In the line PI 2,008,151 the resistance to PM was controlled by one recessive gene, while in Natsufushinari two recessive genes were reported. Then, a major recessive gene (*s*), one major dominant gene (*R*) and a dominant suppressor gene (*I*) were reported to control the resistance to PM in two varieties P1212233 and P123514 (Shanmugasundaram et al. 1971, 1972). Further, numerous genetic loci carrying resistance to powdery mildew were identified in cucumber (Table 12.2). Scab (*Cladosporium cucumerinum*) is another most prevalent disease of cucumber throughout the world. The monogenic dominant resistance to scab has been reported in cucumber (Zhang et al. 2010). Target leaf spot (TLS) (*Corynespora cassiicola*) caused by a phytopathogenic fungus having a wide host range of > 530 plant species severely affects cucumber (Wen et al. 2015). The nature of genetics of resistance to TLS in cucumber is complex and quantitative, dominant, recessive inheritance has been reported. In a study, the single dominant gene '*Cca*' was reported to control the resistance in Royal Sluis 72502 cucumber (Abul-Hayja et al. 1978). Gummy stem blight (GSB) (*Didymella bryoniae*) is another major disease of cucurbits causing significant yield loss of up to 80% (Zhang et al. 2017). The genetics of resistance to GSB is complex and is polygenic in nature. The bacterial pathogen (*Pseudomonas syringae* pv. *lachrymans*) causes angular leaf spot (ALS) in cucumber and the polygenic inheritance of resistance has been reported in cucumber (Olczak-Woltman et al. 2009). Recently, Slomnicka et al. (2018) reported recessive *psl* locus governing resistance to ALS including QTLs.

An ipomovirus, cucumber vein yellowing virus (CVYV) transmitted by whitefly was first reported in the Mediterranean basin (Pujol et al. 2019). Its effective control relies on breeding disease resistance cucumber cultivars, for which the knowledge of genetics and availability of molecular markers is crucial. Recently, the monogenic and incompletely dominant mode of inheritance of resistance to CVYV was reported in cucumber (Pujol et al. 2019). CMV (cucumber mosaic virus) having a wide host range was first reported in cucumber in 1934 (Shi et al. 2018). The varying reports of the genetics of resistance to CMV such as dominant, recessive, polygenic and monogenic are available (Shi et al. 2018). Wasuwat and Walkers (1961) reported monogenic dominant resistance to CMV in Wisconsin SMR 12, while in another study the resistance was under the control of three independent dominant genes (Kooistra 1969). Resistance to

cucumber mosaic virus (CMV) in *C. sativus* var. *hardwickii* is reported by Munshi et al. (2008) and they have reported single recessive gene controlling resistance.

Environmental stresses like drought, heat and salinity are major abiotic stresses affecting crop yield and quality of most horticultural crops. The promising results for combating abiotic stresses based on conventional breeding approaches have not been met due to their complex quantitative nature. The understanding of complex quantitative genetics and identification of genes/QTLs are prerequisites to curb the adverse effects of abiotic constraints on crop plants. Among the various stresses, salinity stress has several adverse effects on horticultural crops including cucurbits (Sharma et al. 2016; Elsheery et al. 2020). The production of cucumber is significantly reduced by salinity. In a study, the role of epistatic and additive gene effects was demonstrated for salinity tolerance in cucumber salt-tolerant line 11411S (Kere et al. 2013). Drought is another major abiotic stress affecting cucumber, and tolerance to drought is a complex quantitative trait regulated by different mechanisms (Wang et al. 2018a). In the heavy rainfall area, cucumber production is constrained by waterlogging. The genetics of waterlogging tolerance is dependent on various morphological and physiological traits which are quantitative in nature (Yeboah et al. 2008; Xu et al. 2017). The waterlogging tolerance in cucumber line PW0832 is having a moderately high narrow-sense heritability, enabling the use of PW0832 for breeding tolerant cultivars in cucumber (Yeboah et al. 2008).

Heat stress, another major abiotic stress in cucumber, leads to drastic yield loss by disrupting the expression of proteins mainly associated with photosynthesis (Xu et al. 2018). Hence, the heat-tolerant genotypes have high photosynthetic activity relative to heat-sensitive genotypes when exposed to high-temperature stress. The cucumber is a thermophilic cucurbit with an optimum temperature of 25–30 °C for growth and development and temperature above 35 °C leads to heat stress in cucumber with wilting of stem and leaves in a short time span at 50 °C (Yu et al. 2018). Calmodulin has been reported to play a role in heat stress in crop plants. In this context, Yu et al. (2018) reported that overexpression of *CsCaM3,* isolated from inbred line '02–8', resulted in high-temperature stress tolerance in cucumber. Recently, Wang et al. (2019) reported that heat stress resistance in cucumber inbred line 'L-9' is under the genetic control of a single recessive gene. Different stress-responsive gene families such as heat shock proteins and tonoplast sugar transporters (TST) playing a role in plant growth and development have been identified. The identification and characterization of genes, QTLs, involved in resistance to different abiotic stresses could promulgate the development of abiotic stress-tolerant cultivars in cucumber.

12.5 Traditional Breeding

Crop domestication and human civilization are co-evolutionary processes connected with plant breeding. The crop breeding approaches have tremendous potential and have been used successfully in agriculture for the past five to ten decades to enhance the yield and genetic gain of crop plants. Plant breeding can be demonstrated as a consequent accumulation of favourable alleles in the elite genotypes and resulting new phenotypes. The rediscovery of Mendel's laws urged the use of genetic principles in plant breeding. As most of the commercial traits are under the genetic control of polygenic loci, quantitative genetics became an integral part of plant breeding. Plant breeding involves the creation, selection and fixation of superior phenotypes for the generation of superior crop genotypes. The selection of desirable types among the different variants is the core of plant breeding. In the crop plants including cucurbits, the course of domestication led to the deliberate selection of high yield-related traits, robust plant architecture and ease of harvesting. The domestication of cucumber from its feral form *Cucumis sativus* var. *hardwickii* was accomplished about 3000 years ago (Qi et al. 2013). There are six major groups of cucumber based upon fruit

character variation and geographic origin. The small size fruits having numerous small spines are French cornichons and are mainly used for pickling. The European greenhouse types are having long and smooth fruits suitable for salad purpose. Other types having short fruits, protruding warts and large spines are American pickling cucumbers. While fruits having medium to long sizes with thick skin, protruding warts bearing large spines are American slicing cucumbers. The other types are medium-short Middle Eastern cucumbers having tiny spines and are wartless, thus used for pickling and slicing as well. In contrast, the Far Eastern cucumbers are quite long in size bearing protruding warts and spines. Generally, the short-fruited types are used for pickling purpose, while long-fruited types are consumed as fresh and cooking, or in Orient. In addition, another two groups have been reported, one from Southwestern China and another from Nepal and high elevations of the Indian region. The genetic improvement of cucumber is a complex process that entails the crossing of elite germplasms followed by refinement of populations and isolation of desirable inbred lines for commercial hybrid development.

12.5.1 Breeding Behaviour, Objectives and Breeding Methods

12.5.1.1 Breeding Behaviour

It is imperative to understand the typical breeding behaviour of the cucumber before undertaking any breeding programme as this crop is different from most of the typical cross-pollinated crops. Cucumber is entomophilous in nature and the bee-like *Apis florea*, *Apis dorsata* and *Apis mellifera*, *Nomioides* sp., *Helictine* sp. are the major pollinating agents. Few beetles like *Conpophilus* sp. and moths like *Planidia* sp. *Pygargonia* sp. are reported to be acting as a pollinator in certain areas. However, the different species of honeybees are the main agents to effect pollination in most of the cucumber-growing areas. The cucurbits group of vegetables is significantly different from other classical cross-pollinated vegetable crops like cabbage, onion, carrot etc. in terms of their breeding behaviour and system. Although it is out-crossing in nature, however, the extent of the inbreeding depression is significantly low. Therefore, it is possible to practice single plant selection even from F_2 population to derive superior genotypes. In heterozygous crops like cucumber with low or insignificant inbreeding, depression is explained by a homozygous balance in the crops. This is mainly because of higher planting distance and growing of small population over time. All the genes showing deleterious effects under homozygous condition are eliminated in the process of evolution. Therefore, selfing in these crops is not affected through inbreeding depression. Therefore, different modified breeding methods of both self-pollinated and cross-pollinated crops are practised in cucumber based on the breeding objectives.

12.5.1.2 Breeding Objectives

The development of high-yielding cultivars with good fruit quality is the primary objective of cucumber breeders. Breeding cultivars with earliness character and high female to male flower ratio is another important aim of cucumber breeders. The uniform maturity, long-lasting ability, desirable fruit shape as influenced by market and consumers are also a major focus of the cucumber breeding programme. The major breeding objectives practised worldwide may be summarized as follows:

i. Lowest node number at which first pistillate flower appear which gives an indication of early maturity.
ii. High female to male flower ratio.
iii. Attractive light green/green fruits with smooth fruit surface without prominent spines and prickles, crispy with tender flesh.
iv. Uniformly long cylindrical fruits without a neck.
v. Fruits free from carpel separation showing hollow spots.
vi. Non-bitter fruits.

vii. Minimum number of seeds at marketable maturity.
viii. Resistance to important diseases and pests like downy mildew, powdery mildew, leaf spot, Fusarium wilt, leaf curl and mosaic viruses, fruit fly, aphid and mites.
ix. Capacity for high mineral utilization by the plants to produce higher yields.
x. Bunching fruit habit producing multiple pistillate flowers on individual nodes for harvesting finger size fruits to suit whole fruit canning for export especially in the case of gherkin.
xi. Gynoecious, parthenocarpic with multiple pistillate behaviours for protected condition.

The genetic improvement for different quantitative and qualitative traits ranging from plant architecture, fruit quality, flesh colour, prolonged shelf life, β-carotene and processing quality to biotic stress resistance is another important focus of cucumber breeders. The use of biotic stress-resistant hybrids/varieties is the most cost-effective, simple and eco-friendly means of combating various crop plant diseases. Hence, in the early genetic improvement programmes of cucumber, disease resistance breeding has been the major objective of breeders. Determining sex expression and development of stable gynoecious/parthenocarpic lines in cucumber for greenhouse cultivation is important for higher yield potential. Nowadays, in the era of climate change, abiotic stress resistance is also one of the components of cucumber genetic improvement programmes. Despite being cross-pollinated crop, self-compatibility is predominant in cucumber due to more homozygote balance and thus, it does not reflect significant inbreeding depression.

12.5.1.3 Breeding Methods

Owing to insignificant inbreeding depression in cucumber, the individual plant selection is successfully practised. The major breeding approaches in cucumber include mainly the introduction, pureline selection, mass selection, recurrent selection, pedigree method followed by hybridization, single seed descent method, backcrossing and heterosis breeding (Sitterly 1972; Wehner 1988; Zijlstra et al. 1995; Delannay et al. 2010; Jat et al. 2019). Breeding methods are determined by the objective for improvement and traits under consideration. Breeding objectives are mainly driven by the market demand and the methods are dependent on the targeted product to be developed. Most of the breeding methods have a few sequential steps like population development and improvement, development of desirable lines, identification of parental combination for hybrid development, identification for parents for stress tolerance and evaluation under multiple locations to realize the actual potential of a developed genotype. Different methods for improvement are generally adopted in parallel to achieve the breeding objectives. Population improvement is practised to develop the base population with desirable traits like earliness, higher yield, fruit quality as one component. At the same time, the pedigree method followed by hybridization is also adopted if we want to combine some of the desirable traits for two different parents into a single genotype. Another component of the same breeding programme could be back-crossing for introgression of a particular trait like disease resistance to any other major gene for its introgression in widely cultivated variety/parent of hybrid deficient for that trait (Staub and Grumet 1993). With the advent of molecular markers and next-generation sequencing technology, the functional genomics has become an integrated component to accelerate the efficiency of the classical improvement programme. The major breeding methods adopted for the improvement of different traits of cucumber are as follows.

i. Recurrent selection: This is the most popular breeding method for the improvement of quantitative traits in different cross-pollinated crops. Because of the large plant size and nature of our crossing, this method is not used frequently in cucumber improvement. Under the condition of the limited number of researchers involved and resources, this method may not be the most suitable as it requires handling a large number of population over a period of time. However, this is the most effective method to

improve complex traits like fruit yield. This method requires initial genetic resources with large diversity for traits like fruit size, fresh colour, biotic stress resistance for substantial gain through this method (Wehner and Cramer 1996). The typical selection cycle for recurrent selection (mass, full and half-sibs) in cucumber is 2–3 years because of its plant size and generation cycle (Wehner 1989).

A population with a broad genetic base can be developed by inter-crossing 2–4 elite and distantly related hybrids. Manual inter-crossing among at least 20 elites, diverse genotypes with 2–3 generations followed by pollination with insects for 2 or more generations allying selection pressure thereafter is very effective in creating wide-based population. Simple recurrent selection is recommended for a set of highly heritable traits. Reciprocal recurrent selection (RRS) can be practised to develop two populations with less heritable traits like yield-associated components (Cramer and Wehner 1998a, Cramer and Wehner 1999). RRS is effective in developing two different populations for their use in hybrid development although this method is cost and labour-extensive. Identification of a method for large-scale evaluation is the prerequisite for population improvement (Wehner 1989). In the typical cross-pollinated-based breeding models, recurrent selection methods with at least 200 individuals (or progenies of individuals) per population are evaluated and the selected 20 are inter-crossed to create the next cycle of selection. Once a unique population is developed, the elite population can then be released as commercial cultivars or lines are developed for their use in hybrid evaluation and production (Wehner 1998a, 1998b).

ii. Line development:

a. *Pedigree selection:* This is more common and widely used in the development of improved lines for commercial cultivation as a variety, or development of parents for hybrid development in cucumber. Two widely adopted genotypes which are complementary to each other in terms of traits were used for crossing in developing F_1 and F_2 progenies. One parent may have traits yield, early maturity and good fruit quality while other parents could have acceptable yield, earliness, disease resistance and devoid of fruit quality. F_2 progenies are developed by selfing or sibbing of the F_1 plant(s) and selected F_2 plants are used in developing F_2 progenies. From the F_3 families, in general, the best plant is selected for further progeny advancement. Family row selection is practiced for quantitative traits in F_4 and the number of plants per family is selected for developing the next generation. The F_6 families are generally uniform and behave like inbred without much segregation within the family. The selection typically involves the use of eight-plant plots for traits such as early flowering, number of pistillate flowers, and fruit number and quality. In terms of number of plant families selected are 54 F_2 plants, 36 F_3 plants, 24 F_4 families and 18 F_5 lines. Improvement of traits of low heritability in cucumber (e.g., yield and quality components) that are associated with QTLs having complicated negative associations and epistatic effects may benefit from the application of phenotypic and marker-assisted selection strategies (Behera et al. 2010).
b. *Single-seed descent*: This is a modified form of the pedigree method used in the rapid development of inbreds taking the advantage of greenhouse and off-season nursery without selection till the advanced generation (F_6 onwards). This method is more effective for the improvement of quantitative traits like yield, earliness and may not be the best method for the improvement of traits like disease resistance. However, the selection for several quantitative traits can be performed through the removal of plants/families with undesirable traits. This method can be practised only when there is a facility for rapid generation advancement.
c. *Backcrossing*: This method is used for the transfer of one or more traits controlled by major genes with qualitative inheritance (resistance to downy mildew, determinate growth habit, nematode resistance) into an inbred which is superior for yield and quality

but lacks one or more major trait. Generally, six generations of backcrossing are needed for the introgression of a major gene with a nuclear genome of the recurrent parent. However, the approach differs based on whether the trait is controlled dominant or recessive gene.

For the transfer of a trait controlled by a recessive gene, the recurrent parent is crossed with the donor parent, and the F_1 is backcrossed to the recurrent parent. In one scheme, the F_1 is self-pollinated to produce the F_2, which segregates for the trait of interest. Individuals from the F_2 that possess the trait are backcrossed to the recurrent parent to produce the BC_1. The BC_1 generation is then self-pollinated to produce the BC_1F_1, which is evaluated for the trait. Individuals possessing the trait of interest are selected and backcrossed to the recurrent parent. This process is repeated until the BC_6 generation where the best individuals are self-pollinated and selected for the trait to produce the improved inbred. For the transfer of a trait controlled by a dominant gene, the recurrent parent is crossed with the donor parent, and the F1 is subsequently backcrossed to the recurrent parent. The BC_1 generation is then evaluated, and individuals possessing the trait are backcrossed to the recurrent parent. This process is repeated until the BC6 generation where the best individuals are self-pollinated and selected for homozygous expression of the trait using progeny testing.

Backcross breeding has played a significant role in cucumber genetic improvement. During 1995, the inter-specific cross between primary gene pool and secondary gene pool species, *Cucumis sativus* and *Cucumis hystrix,* respectively was attempted (Chen et al. 1997). The progeny of this cross was male and female sterile, thus subsequent chromosome doubling resulted in fertile amphidiploids species. The self-pollination for subsequent generations led to the development of new species *Cucumis hystivus* (2n = 4x = 38). It has practical implications in the incorporation of gummy stem blight resistance in commercial cultivars as *Cucumis hystrix* is a carrier of resistant genes to this particular disease. The marker-assisted backcross breeding and selection in inter-specific derived population may be useful for enhancing the cucumber genetic diversity. In this context, Behera et al. (2011) reported introgression backcrossing employing *C. hystivus* for increasing genetic variability in cucumber. Breeding for major diseases and the development of resistant cultivars has been successfully achieved in cucumber. Numerous wild species of *Cucumis* such as *Cucumis africanus, Cucumis anguria, Cucumis ficifolius* and *Cucumis myriocarpus* can be used in backcross breeding programmes as they are the carrier of disease-resistant genes.

The first breeding effort for downy mildew resistance in cucumber started in the USA during the twentieth century in 1939, when Chinese Long and Puerto Rico No. 37, the moderately resistant cultivars were crossed with the commercial cultivars (Barnes 1961; Holdsworth et al. 2014). Subsequently, many resistant cultivars were released by the public sector such as 'Marketmore', Marketmore 97, Platinum, 'Salt and Pepper' and 'Poinsett' series in the pedigrees of numerous fresh market cultivars. The monogenic or oligogenic resistance derived from 'Chinese Long' or PI 197087 was contained in most of the released cultivars. Recently, Holdsworth et al. (2014) by employing the pedigree method of selection followed by hybridization developed the downy mildew resistant line 'DMR-NY264'. 'Kaohsuing No 3', a heat and moisture tolerant cucumber cultivar, was developed in Taiwan by adopting the Pedigree method of selection following the bulk population method and then subsequently five generations of selfing (Liu et al. 2017a). The saturated and high-density genetic linkage map has been developed in cucumber (Behera et al. 2011; Zhu et al. 2016). The selection efficiency during population improvement can be enhanced through marker-assisted selection (MAS). Japanese Long Green, Straight 8 and Poinsett are important introductions of cucumber in India.

iii. Heterosis breeding: When a good number of improved lines are available with a breeder, heterosis breeding by making all possible combinations is the next logical step to exploit

heterosis for yield and other related traits. If the number of lines is very large, it may not be possible to make evaluate all possible combinations as the number of combinations even for 30 parents will be [30 x (30–1)]/2 = 435 without including the reciprocals. Therefore, it may not be possible to make all possible combinations and we have to select the parents based on their traits and they should be complementary to each other. In cucumber, a large number of publicly developed inbreds/open-pollinated lines have been recommended for commercial cultivation and they are improved with several desirable traits. However, the F_1 hybrids provide an avenue for proprietary protection of commercial inbred lines, and private sector stakeholders are mainly involved in the development of hybrids mainly because of the scope for propriety protection.

The phenomenal success in heterosis breeding in cucumber is mainly due to manipulation of sex expression to the desired direction. Most of the F_1 hybrids are gynoecious x gynoecious or gynoecious x monoecious, though monoecious x monoecious hybrids are also available in some particular segments. The genetic control of sex mechanism in cucumber, especially of the gynoecious sex form, has made it possible to exploit heterosis in cucumber. Gynoecy condition where all the flowering nodes in the main, secondary and tertiary branches bear pistillate flowers in the leaf axils is important for sex form, which has made phenomenal exploitation of hybrid vigour possible in cucumber. Hybrids in cucumber are important not only for yield and earliness but also for external attributes of uniformity of size and shape, especially in slicing cucumber. Most of the F_1 hybrids have attractive colour, flesh texture and other quality traits and multiple resistances to diseases due to the fact that dominant genes control resistance to some important diseases. Different methods can be adopted for the production of hybrid seed of a cucumber. Since flowers of cucumber are very small and a large amount of seed can be obtained from a single cross bagging of female flowers of monoecious line, hand pollination can be effectively practised for seed production.

The commercial production of gynoecious cucumber seed was made possible only when it was discovered that gynoecious inbreds could self-reproduce if a growth regulator is applied to induce male flower formation (Robinson 1999). Peterson and Anhder (1960) for the first time discovered the effect of gibberellic acid (1500–2000 ppm) on the promotion of male flower formation in cucumber. However, due to erratic male flower induction by the use of gibberellic acid, application of silver compound such as silver nitrate (250–400 ppm) is done to induce male flowers. Silver ions inhibit ethylene action and thus promote male flower formation in gynoecious cucumber plants. However, due to phytotoxic effects such as burning of plants, silver thiosulphate (400 ppm) is now widely used by seed producers for the maintenance of gynoecious cucumber lines. It induces male flowering of cucumber plants over a longer period and is less phytotoxic compared to silver nitrate. When the temperature exceeds beyond 30 °C the stability of gynoecious sex expression is affected. Unfortunately, the temperate gynoecious lines are unstable for gynoecy under high temperature and long photoperiodic conditions because of their thermospecific response for gynoecious stability. That is why the gynoecy in cucumber did not receive much attention in tropical countries.

Efforts have been directed during the recent past towards developing gynoecious sex forms under our tropical and indigenously adapted background and two lines, DC-102 and DC-103 with stable gynoecious sex even at temperatures around 40 °C have been developed at IARI. These are being tested further to exploit in heterosis breeding programme at Division of Vegetable Science, ICAR-Indian Agricultural Research Institute, New Delhi. Hybrids of cucumber are produced mainly by crossing gynoecious lines with monoecious lines. Though other systems of producing gynoecious hybrid seeds such as gynoecious x gynoecious have been proposed, gynoecious x monoecious hybrids are still the most widely grown (Robinson 1999). Most of the commercial hybrids based on gynoecious cucumber lines are a blend of

gynoecious hybrid and monoecious seed. In addition, homozygous gynoecious hybrid seed has been produced by crossing two gynoecious lines after one has been treated with a growth regulator to induce male flowers (Robinson 1999). The gynoecious cultivars or hybrids should be protected from pollination, because due to fertilization, their fruits may become misshapen. Exogenous application growth regulators, viz., MH (100–200 ppm) or Ethrel (150–200 ppm) for temporary suppression of male flower in the female line of monoecious plants has also been commercially adopted for producing F_1 hybrids. The North Indian conditions permit a very short growing period for cucumbers and maximum yield realization could not be obtained under open fields. So protected cultivation technology with proper training, pruning and nutritional support can be exploited for off-season breeding of cucumber. Greenhouse production technology has shown a four-fold realization of yield in commercial hybrid Pusa Sanyog during the off-season as compared with cultivation under open conditions.

In India, the first report of heterosis breeding in cucumber was demonstrated during 1970s with the release of the first gynoecious x monoecious hybrid 'Pusa Sanyog' by IARI, Regional Station, Katrain, Kullu Valley, Himachal Pradesh. Then a monoecious x monoecious hybrid, Pant Sankar Kheera 1 was released by GBPUAT, Pantnagar. Recently, one gynoecious x monoecious F1 hybrid, Pusa Cucumber Hybrid-18 has been developed by the Division of Vegetable Science, ICAR-Indian Agricultural Research Institute, New Delhi. Sex expression has played a significant role in heterosis breeding in cucumber. The development of gynoecious hybrid cultivars in cucumber is achieved by crossing gynoecious and monoecious parents. The growing of gynoecious and parthenocarpic hybrids under protected cultivation has enhanced the productivity manyfold. Generally, the blending of gynoecious and monoecious types (10%) is maintained in many commercial seed-lots of cucumber for making sufficient pollen available in the pollination of complete gynoecious x gynoecious hybrids.

12.5.2 Breeding for Protected Cultivation

Cucumber is one of the leading vegetable crops grown under protected cultivation worldwide. Development and availability of a particular plant type are required for its successful cultivation under protected condition. Generally, indeterminate plant type with the constant internode length throughout the length of the vine is suitable under such condition. The growth habit is important in breeding programmes as it increases yield and availability period to a greater extent. Ideotype breeding along with the incorporation of useful genes for the parthenocarpic character can be utilized on a large scale in poly-houses. Parthenocarpic, gynoecious cucumber genotypes are suitable for protected cultivation as these varieties develop fruits automatically without any pollination. Breeding effort should be concentrated on important fruit characteristics such as shape, colour, spine type (coarse or fine), spine colour (white or black), skin thickness and surface warts, high TSS, crispness, enhanced shelf life, resistance to biotic and abiotic stress, highly responsive to fertilizer, photo and thermo insensitiveness. The emphasis, however, is to develop parthenocarpic, gynoecious F_1 hybrids with wider adaptability. The development of suitable ideotype, novel genes for biotic and abiotic stress resistance is the need of the hour for breeding cucumber varieties/F_1 hybrids suitable for protected cultivation. The increased farm income under protected cultivation has made the cultivation of cucumber more popular worldwide. Further, plant breeding efforts have permitted to solve specific problems of varieties suitable for protected cultivation as well as general problems which also benefit the open field cultivation. Ideotype breeding along with the incorporation of useful genes in crops like parthenocarpic can be utilized on a large scale in poly-houses. Gene'*pc*' responsible for parthenocarpy, '*F*' responsible for short inter-nodal length can be utilized through pure line and backcross breeding methods to establish them in plant population in parthenocarpic cucumber.

Breeding parthenocarpic cucumber hybrids for field production is more difficult than for greenhouse production. For the greenhouse hybrids, fruit set at the first five lower nodes is not important since fruit that is set at the lower nodes are removed. For outdoor production, it is essential that fruit set starts as early as possible at the lower nodes. Thus, for the development of outdoor parthenocarpic cucumber hybrids, it is necessary to utilize the parental lines with a high percentage of parthenocarpy and less dependent on the environment.

Keeping in view the above facts, the concept of poly-house vegetable breeding programme was developed at Pantnagar during 2002 in cucumber and tomato. Some of the good genotypes were isolated and pure line and hybrid breeding programmes were adopted for improvement of these genotypes. After three years of multi-location testing at tarai, midhills and higher hills under poly-house condition, two genotypes of parthenocarpic cucumber, namely Pant Parthenocarpic Cucumber-2 and Pant Pant Parthenocarpic Cucumber-3, were identified and released by Uttarkhand State Variety Release Committee during May 2011 for commercial cultivation at farmers' poly-house. The programme on the development of parthenocarpic gynoecious varieties for protected cultivation was undertaken during 2010 at the Division of Vegetable Science ICAR-IARI, New Delhi. In the winter of 2014–15, 17 breeding lines which were advanced to F_8 and showing true gynoecious and parthenocarpic behaviour were evaluated under low-cost poly-house. The line DPaC-6 was observed as most promising as it expressed 25.0% and 16.7% higher yield than check Pant Parthenocarpic Cucumber-2 and Asma, respectively. Though its yield (122.5 t/ha) was less than the best check F_1 hybrid Kion (127.0 t/ha) it was statistically at par. The yield obtained by DPaC–6 (122.5 t/ha) can be considered quite high since it was obtained during the off-season (winter season) under low-cost poly-house without using any energy. On the basis of its consistent superior performance, DpaC-6 was identified by IARI Variety Identification Committee in 2016. The inheritance studies of fruit skin colour and parthenocarpy were also conducted by crossing DPaC-6 and monoecious cucumber variety PusaUday. The F_1 progeny showed intermediate colour between dark green DPaC-6 and light green Pusa Uday. The F_1 progeny showed true gynoecious parthenocarpic behaviour as its fruits were seedless and developed without pollination which suggested that the gynoecious parthenocarpic trait is governed by a single dominant gene. The F_1 progenies were advanced to F_2 and also simultaneously backcrossed with parthenocarpic line DPaC-6 to confirm the monogenic dominant nature of parthenocarpy.

12.6 Conclusion

Tremendous progress has been made in unravelling genetic architecture and basic understanding of cucumber genetic improvement through classical genetics and traditional breeding in the past many decades. Classical genetics has helped in understanding the heritability of different quantitative and qualitative traits in cucumber. Much progress has been made in elucidating the genetics of disease resistance, taxonomy and phylogenetic relationships. Numerous genes controlling various monogenic, oligogenic and polygenic traits have been identified, and subsequently, the breeding programmes for the genetic improvement of cucumber have been initiated. Based on the traditional breeding for the past ten decades, different commercial hybrids/cultivars have been released in cucumber. However, the improvement in quantitative traits is limited by traditional breeding; hence in this context, the advent of molecular breeding and genomics have played a significant role in cucumber. The integration of traditional breeding programmes with advanced molecular tools will certainly accelerate the cucumber genetic improvement in the era of climate change.

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13 Biotechnological Innovations in Cucumber (*Cucumis sativus* L.) Development—Current Scenario and Future Perspectives

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Abstract

Transgenic plants are genetically engineered crops having desirable traits, which are stable and often pass to the progeny. The wild varieties grown in the natural environment are prone to attack by various biotic and abiotic factors which result in yield reduction, qualitatively and quantitatively. Genetic engineering provides a plausible way to combat such stresses by reorganizing biochemical photosynthetic pathways. Cucumber (*Cucumis sativus* L.) is one of the most popular economically important crops which is widely cultivated throughout the world. Apart from its economical uses, cucumber is also used as a model plant to study sex determination and cell trafficking system in plant vascular biology. The availability of assembled draft genome sequences for cucumber provides the treasured basis for the transformation of desired traits by the identification of candidate genes. Various techniques are employed for *Agrobacterium*-mediated and other gene transfer methods for cucumber. Tissue culture techniques using different explants for complete plantlet regeneration are discussed in this chapter. This chapter also summarizes the current status of cucumber transgenic with respect to phenotypic stability of transformed trait and its inheritance to the progeny. The availability of transgenic plants with respect to new and improved variety is likely to be an important factor influencing the continued development of transgenic technology, its subsequent field trials, and commercial availability. However, various social and ethical factors have been an obstacle in the process of transgenic plant development.

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13.1 Introduction

The cucumber (*Cucumis sativus* L.) is one kind of major cucurbitaceous fruit, bred all over the world (He et al. 2008). Cucumber (*Cucumis sativus* L.) is a major fruit utilized as an economically important edible food and is widely cultivated throughout the globe (Huang et al. 2009; Wang et al. 2013). As a fresh market culinary crop, the economic importance of cucumber varies differently along different geographical regions. It is categorized as a popular

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vegetable in southern Asia, America, and most of the European countries (Nishibayashi et al. 1996b; Esquinas-Alcazar et al. 1983).

13.1.1 Distribution and Importance of Cucumber

Cucumber is a member of the family *Cucurbitaceae*. Family *Cucurbitaceae* comprises annual or perennial herbs, creepers, and climbers which are native to temperate and tropical regions. The family houses 98 genera and about 975 species of food and ornamental plants. Important members of the family are cucumbers, gourds, melons, squashes, and pumpkins that make up the family *Cucurbitaceae* economically important. The *Cucumis* genus comprises over 30 different species. *Cucumis* is an important genus of the *Cucurbitaceae* family comprising mostly creepers which include the cucumber (*Cucumis sativus*), muskmelons (*Cucumis melo*), and Indian gherkin (*Cucumis anguria*) (Sebastian et al. 2010). This crop is mostly produced for the fresh market throughout the world where China, as a market leader in terms of production, being the highest producer among all cucumber-producing countries. Cucumber also holds the position to be among the top ten vegetables produced throughout the world (Sebastian et al. 2010).

13.1.2 Genome Organization and Inheritance

Cucumber possesses a small haploid genome having a size of 367 Mbp with a basic number of x = 7 (Arumuganathan and Earle 1991). Three different modes of transmission of chromosomes are employed by cucumber, which makes it a unique model to study the inheritance traits in filial generations. Chromosomes are transmitted maternally for chloroplasts, paternally for mitochondria, and biparentally for nuclear DNA (Havey 1997; Havey et al. 1998). Indeed, this mitochondrial genome of cucumber happens to be the largest genome among all eukaryotic organisms (Plader et al. 2007).

Due to these properties cucumber has received much attentiveness for being a model crop for vascular biology and inheritance pattern studies. The precise genome sequence for cucumber was decoded by Huang et al. (2009), and further efforts are now made to develop a detailed genetic and cytogenetic map for this crop (Ren et al. 2009). More details for cucumber genome annotation, genetic maps, and QTLs are being constantly appended in the International Cucurbit Genomics Initiative (http://icugi.org) which has now been completely upgraded to (http://cucurbitgenomics.org). This newly appended database has a genome sequence available for 15 members of the *Cucurbitaceae* family. Furthermore, the database also provides EST and expression analysis data which includes the transcriptomic data for cucumber and other related species.

13.1.3 Importance of Cucumber Transformation

Though its geographical distribution being so varied, still the crop faces significant challenges against several biotic factors, namely diseases caused due to infestation of pests and microorganisms, especially Cucumber mosaic virus, Green mottle mosaic virus, leaf fungal diseases, angular leaf spot, fusarium wilt and cucurbit scab, nematode infestation, anthracnose, leaf blotch which causes a huge loss in the net attainable yield (Bacci et al. 2006; He et al. 2008). Apart from this, the yield is further compromised by other environmental vagaries mostly in the form of abiotic factors that include extreme temperature regime and prolonged water deficit and salinity stress (Chojak-Koźniewska et al. 2018). A lot of efforts are being made for developing an improved variety that shows a better yield and significant tolerance against environmental extremes. However, it is still a challenge to improve yield and tolerance of cucumber by conventional breeding owing to its narrow genetic base, having a genetic variability of only 3–8% (Plader et al. 2007). Improvement via conventional breeding-related strategies is

further exacerbated because of long crop breeding time and extreme incompatibility toward related species (Kho et al. 1980). Although several defence and pathogenesis-related genes and their alleles already exist in nature, however, it is still a challenge to breed an improved variety just by conventional breeding. Therefore, it is imperative to make use of recombinant DNA technology for transferring the elite genes directly to the host genome in a relatively short span. Also, transformation in cucumber is not only aimed to provide resistance against biotic or abiotic stresses but it also applies to generate new improved varieties with respect to reduce bitterness, fruit size, sweetness, and to some extent to increase the content of secondary metabolites. The genome sequence of cucumber has also provided the list of important genes that may further help to augment the process (Huang et al. 2009). The transformation efficiency and stabilization of transgene construct in plants are largely governed by the availability of regeneration protocols from diverse explants. A major barrier limiting transformation is the recalcitrant nature of the crop, the requirement of sterile culture for plant regeneration, and the non-efficient transformation process (Niazian et al. 2017). All these factors impede the generation of novel transformants carrying agronomically important traits and limit other reverse genetics-related studies.

The chapter mostly focuses on methods and factors that are used for enhancing the transformation processes and also highlights the current status and future perspective of transgenic cucumbers possessing elite traits for disease resistance and stress tolerance and their commercial utility.

13.2 Methods Used for Cucumber Transformation

Genetic improvement of cucumber using biotechnological tools requires an efficient and reproducible method of plant regeneration which ascertains an efficient and stable basis for its genetic transformation. Several groups have defined a number of diverse protocols, however, every protocol suffers from some of the other lacunae. Moreover, other extraneous factors, namely genotypes, explant source, seedling age, growth regulator, media composition, and environment, also play a key role in influencing the reproducibility of regeneration and transformation (Punja et al. 1990; Sarmento et al. 1992; Nishibayashi et al. 1996b; Vasudevan et al. 2007; Wang et al. 2013). The problem of sterility barrier and crossing incompatibility seems an acute barrier especially in cucumber, (*C. sativus* L.) where the successful crossing is only reported in *C. sativus* and closely related *C. hardwickii,* but very few reports of successful crossing exist in other species (Trulson et al. 1986). To overcome this concern various biotechnological interventions have been exclusively utilized for developing an improved cucumber genotype. Among them, the most utilized are tissue culture-based techniques, namely micropropagation, in vitro regeneration using various explants, *Agrobacterium*-mediated transformation, and direct gene transfer for incorporating one or more traits of interest.

13.2.1 Tissue Culture-Based Regeneration: Direct and Indirect Regeneration

Two different methodologies are employed for plantlet regeneration through tissue culture, namely indirect regeneration having distinguishable callus phase and direct regeneration that is free from intermittent callus phase. The direct regeneration procedure holds superior standards as having a minimal deviation from the mother explants or having negligible somaclonal variation. Both types can be achieved by using cotyledonary explants (Msikita et al. 1990; Colijn-Hooymans et al. 1994) or leaf-derived callus (Nadolska-Orczyk et al. 1984; Mishra and Bhatnagar 1995) and leaf microexplants (Burza and Malepszy 1995b, Burza et al., 2006) or may also be sometimes protoplast-derived (Burza and Malepszy 1995a, Burza et al., 2006). Various groups have reported cotyledonary explants for

direct shoot organogenesis and achieved encouraging results in cucumber (He et al. 2006; Rajagopalan and Perl-Treves 2005; Tabei et al. 1998).

Usually without using any extraneous inducer the time required, from explant inoculation to the time when plants get ready for greenhouse transfer, is around 10 weeks to 6 months. Transformation efficiency obtained by using this regeneration protocol was recorded in the range of 1.5–6.3% for regenerated shoots and varied from 1.4% to 10% in the obtained transgenic plants calculated against the total number of inoculated explants (He et al. 2008). The obtained results can be optimized for other explants like cotyledons, hypocotyls, leaves, and petioles, to further curtail the time spent on cucumber transformation.

13.2.2 Micropropagation

Micropropagation technique entails using synthetic media for in vitro multiplication of cells or organs utilizing a small part as an explant from a superior or elite parent. Several groups have attempted to utilize in vitro micropropagation or mass multiplication of cucumber with variable efficiencies. An efficient procedure for micropropagation of cucumber from seeds is reported by Hisajima et al. (1989). It utilizes a multistepped protocol for shoot induction from seeds, followed by shoot multiplication and lastly root induction which displayed an exceptionally high efficiency. Using this protocol approximately 1,600,000 cucumber shoots, with minimal deviation, were regenerated from a single shoot in a span of one year. Another study performed by Burza and Malepszy (1995b) utilizes the use of 2 −3 mm^2 pieces of leaf micro explants of *C. sativus* and *C. anguria* for shoot regeneration in a short span of five weeks. However, in vitro micropropagation also suffers from a major drawback as the plants regenerated from the mature explants are mostly found to have aberrant morphology and are mostly polyploid/mixoploid at the chromosomal level. Unfortunately, tissue culture-based regeneration likely resulted in callus formation and thus a high rate of somaclonal variations is observed in the progeny. Hence more progressive methods are required that are able to precisely transfer the gene of interest with minimal linkage drag which is the major drawback of conventional breeding. Recombinant DNA technology has paved the path for further development of cucumber, as among various techniques employed, the most utilized is *Agrobacterium*-mediated gene transfer and direct gene transfer methods.

13.2.3 Recombinant DNA Technology for Transgenic Generation

In crop improvement, genetic transformation provides a platform for transferring genes, cloned from prokaryotic or eukaryotic origin to a suitable host, creating new properties in a relatively short span of time without altering the existing traits (Gardner 1993). Yin et al. 2005 reported that mostly all transformation procedures for cucumber improvement programs utilize tissue culture-based methods where gene transformation is mostly performed via *Agrobacterium*-mediated gene transfer (Miao et al., 2009). Although few researchers also report direct gene transfer, namely the biolistic method, which will be described in the latter part of this chapter.

13.2.3.1 Agrobacterium-Mediated Gene Transfer

Agrobacterium is a type of plant-pathogen also known as a natural genetic engineer that infects plants especially the wounded sites and leads to tumorous outgrowth (Xu et al., 1993). *Agrobacterium* is distributed into five species based on their disease-causing ability and the choice of their host range in plants (Otten et al. 1996; Gelvin 2003). However, *A. tumefaciens* and *A. rhizogenes* are the most utilized species for plant transformation. Both differ in their specific properties as the former induces crown gall disease while the latter is known to induce hairy root disease. This variability among various strains of *Agrobacterium* is governed by a

plasmid inherited inside the bacteria, namely Ti (tumor) and Ri (Root) inducing plasmids (Hwang et al. 2017). However, it becomes too labor-intensive to regenerate plantlets from transgenic hairy root cultures, therefore Ri plasmid-based transformation technique holds a limited role in cucumber transformation (Tang and Samuels 2001).

13.2.3.2 Direct Gene Transfer

Another method used for the successful transfer of ectopic genes into cucumber is a direct gene transfer method. This method makes use of microprojectile bombardment and pollen tube pathway for transgene integration into the cucumber genome. Microprojectile or particle bombardment makes use of DNA coated gold or tungsten particles which are accelerated under an inert environment of Helium gas. These accelerated particles are allowed to bombard in a suitable explant to release the plasmid DNA where it gets integrated stably. However, this technique is not often used to transform cucumber since this is prone to integrate multiple copies of the desired gene resulting in gene silencing of the obtained transgenic line. In a related study, Chee et al. (1992) generated transgenic cucumber by bombarding a reporter gene in embryogenic callus suspension cultures and obtained a transformation frequency of 16%. Shockingly, they concluded that among them only one-fourth of plantlets were successfully able to express the reporter gene. Later, it was concluded that multiple copies of transgene were incorporated into the genome which triggered gene silencing hampering the expression of a reporter gene. Multicopy integration and rearrangement were also reported using *uid*A (GUS) and *npt*II (kanamycin/Neomycin) as probes which were confirmed by PCR and Southern blot analysis.

13.2.3.3 Pollen Tube Pathway Method

This method entails directly injecting the exogenous DNA into the reproductive part of cucumber, especially the pollen tube or the ovary. Injecting DNA is directly performed 12 h post-fertilization which results in the successful integration of the exogenous DNA. However, very few reports exist for the successful transformation of cucumber using a pollen tube pathway. According to Li et al. (2000) very high transformation frequency was achieved using cucumber variety "Jingyan" when 100 mg/kg of DNA was directly injected into the ovary 12 h after pollination but contrarily a very low fruit setting ratio and the number of seeds per fruit was obtained, making this technique to be of less utility.

Apart from all these major techniques utilized in gene transfer, several other relatively less utilized techniques are also used. Among them, direct gene transfer using protoplast culture, suspension culture, haploid cell culture, viz., ovary culture, anther culture, and somatic embryo culture have been sparingly utilized for gene transformation. Very few authors have even attempted to generate transgenic cucumber plants via somatic embryogenesis using cotyledonary explants which were mediated through *A. tumefaciens* (Chee 1990; Tabei et al. 1994). The reason for this is the occurrence of a spontaneous mutation that is seen more in somatic embryogenesis as compared to direct organogenesis; therefore, direct organogenesis is supposed to be more suitable for regeneration studies and for the generation of transgenic plants. However, very scanty reports are available to date for direct gene transfer which succeeds in generating transgenic cucumber plants having single-copy trait integration that is stably inherited over several generations.

13.3 Factors Affecting Transformation Efficiency

Reports indicated that using acetosyringone at 50–200 μM concentration during co-cultivation was found helpful in augmenting *Agrobacterium*-mediated transformation frequency (Gelvin 2003). In addition, another chemical namely abscisic acid (ABA) was efficiently able to induce the production of adventitious shoots using cotyledonary explants (Tabei et al. 1998). Several research groups have used ABA treatment for cotyledonary explants and reported

increased organogenesis in various cucumber cultivars (Gal-On et al. 2005; He et al. 2006; Rajagopalan and Perl-Treves 2005; Vengadesan et al. 2005).

Optimization transformation procedure in plants initially requires a reporter gene, mostly GUS, and one selectable marker gene mostly kanamycin or neomycin to be introduced into the plant cell prior to the integration of gene (s) of interest. A reporter gene encodes an enzyme that displays assayable activity by substrate cleavage which can be used as an indicator to monitor the transcriptional activity of a gene for which the gene product is not known or is not easily identifiable so as to get a fair idea that the plant tissue subjected to transformation has really been transformed or not (Gardner 1993). The original gene sequence to be studied is replaced with the reporter gene sequence and fused downstream to a strong promoter. This modified vector is now introduced into *Agrobacterium* cells which will be used to infect the plant tissue followed by co-cultivation screening. After the process is optimized the reporter gene is replaced with the gene of interest and the transformation process is repeated for obtaining viable transformants.

13.4 Applications of Biotechnology for Cucumber Improvement

The continued exponential increase of the human population demands the availability of more food products. On the other hand, limited acreages and various stresses slower down the production of edible plants. Traditional breeding programs can be combined with our recent understanding of genome biology to produce quality crops. Genetic engineering and plant biotechnology for crop improvement can broadly be categorized into three main areas: (1) Combating plants against continued and ever-changing biotic and abiotic stress; (2) increasing crop yield and control of plant growth vegetatively and reproductively; and (3) production of biochemicals and pharmaceuticals in plants.

Recently, the available annotated draft genome of cucumber provides valuable insight for the identification of candidate genes in the cucumber for diverse important agronomic traits. These genes often act in a cascade and provide a better understanding of the activation of metabolic pathways. A complete understanding of functional genomics of the cucumber genome is the superior resource for a scientist to impede the new varieties with valuable and economically marketable agricultural traits. Traditional breeding methods are often obstacles to sexual incompatibility between and within the species. Hence, breeding offers a narrow window for the up-gradation of elite germplasm. Recent advances in genome editing and transgenic technologies have provided a new impulse to cucumber transformation. The goal of cucumber transgenic program is to increase productivity and quality of fruit by identifying and pyramiding the genetic potential of different cultivars and thereby providing resistance against multiple biotic and abiotic stresses. Further, the availability and standardization of efficient tissue culture systems from different explant sources also facilitated rapid developments of novel trait transfer in cucumber. This section reviews the progress in cucumber biotechnology.

13.4.1 Biotic Stress Resistance

Various biotic factors, viz., viruses, fungi, nematodes, and bacteria pose a serious threat to the cultivation and production of cucumber throughout the globe. The only solution to combat such biotic threats was either to use pesticides extensively or to rely on conventional breeding programs. The use of pesticides, nowadays, is very limited owing to their health deteriorating effects on living organisms. On the other hand, conventional breeding is very limited in cucumber due to restricted genetic diversity and sexual incompatibility. Hence transgenic approaches by the introduction of exogenous genes are in application to

overcome limitations of conventional breeding in cucumber development.

13.4.1.1 Virus Resistance

Compared to other biotic infections, viral infections are difficult or nearly impossible to cure by conventional chemical methods. Viruses often change their epitope profiles or they will show visible symptoms only after successful disease establishments in host plants. So far, an efficient chemical method to cure viral diseases of cucumber is unavailable. On the other hand, no useful genetic resource against virus resistance is available in important pickle type and vegetable type cultivar of cucumber. Potyviruses namely Cucumber Mosaic Virus (CMV), Watermelon Mosaic Virus (WMV), and Zucchini Yellow Mosaic Virus (ZYMV) are among the viruses that infect cucumber. Virus resistance strategies are centered to provide resistance against these viruses. Coat protein genes are important candidate genes to provide virus resistance in transformed plants. Transgenic plants expressing virus coat protein genes show delayed symptom appearance or resistance against viruses. Chee and Slightom 1991) were first to transfer CMV-C coat protein (CP) gene into cucumber by *Agrobacterium*-mediated transformation. Field trials showed that transgenic plants did not obtain resistance against CMV infection (Chee and Slightom 1991). Successful establishment of virus resistance through coat protein was first reported by Gonsalves et al. (1992) in a vegetable-type cultivar of cucumber by the transformation of CMV-C CP gene. Out of the total, 36% transgenic plants showed no symptoms against CMV infection under greenhouse condition. Furthermore, transgenic cucumbers showed more vegetative and fruit growth than those of non-transformed plants. Transgenic cucumber expressing CMV-O CP gene was reported by Nishibayashi et al. (1996a). Transformed cucumber plants showed a higher degree of resistance to CMV infection and produce milder symptoms on double inoculation of CMV and ZYMV, which individually produce severe symptoms of necrosis on susceptible varieties. *Agrobacterium*-mediated transfer of WMV CP gene and plant regeneration from cotyledon explant was reported by Wang et al. (2000). Transgenic plants showed a lower incidence of virus disease. A novel approach to tobamovirus resistance by transferring the viral replicase gene was done by Gal-On et al. (2005). Putative 54 KDa Cucumber fruit mottle mosaic tobamovirus (CFMMV) replicase gene was transferred to cotyledonary explant of cucumber by *Agrobacterium*. Transgenic plants showed higher immunity immune to soil-borne CFMMV infection by mechanical and graft inoculation. Transgenic plants also showed resistance to root infection by CFMMV when planted in soil containing a significant amount of CFMMV titer. Another novel strategy of transformation of the pokeweed antiviral protein (PAP) gene was reported by Cao et al. (2011). PAP inhibits the effect of plant viruses that belong to a group of ribosome-inactivating protein I (RIP I). Transgenic T_0 plants harboring the PAP gene from plants of the *Phytolacca* genus did not show any symptoms of CMV infection in the field condition. *Agrobacterium*-mediated genetic transformation of cucumber with GFP-tagged WMV genes, under constitutive expression of CaMV 35S promoter was achieved by Khidr et al. (2012). Integration of viral genes into the host plant genome confers broad spectrum resistance against these viruses (Wilson 1993; Gonsalves et al. 1994). Genome editing provides an elucidative way for substantial immunity against a broad spectrum of viruses. Chandrasekaran et al. (2016) reported the use of CRISPER/Cas9 technology to confer virus resistance. T3 progeny, homozygous for Cas9/subgenomic RNA targets N- and C-terminus of eIF4e (eukaryotic translation initiation factor 4E) gene. Edited plants exhibited a higher level of immunity against Cucumber vein yellowing virus (Ipomovirus), Zucchini yellow mosaic virus (Potyvirus), and Papaya ringspot mosaic virus-W (Potyvirus). In contrast, heterozygous and non-edited wild varieties showed susceptibility to these viruses. All these reports clearly highlight the importance of transgenic technology to raise virus-resistant cucumber variety without compromising botanical and fruiting characteristics.

13.4.1.2 Fungal Resistance

Various fungi are reported to infect cucumber plants in the field. Fungal infection on cucumber is characterized by yellow lesions on the leaf and small venation. Opposite to viral infection, fungal infections are self-transferred and seed-borne too. *Botrytis*, *Phytophthora*, *Fusarium*, and *Alternaria* are among the most common fungal pathogens infecting cucumber. Chitinase are hydrolyzing proteins that degrade chitin. The role of chitinase as an antifungal protein was reported in many plants to confer antifungal properties. Chitinases are pathogenesis-related proteins that show higher-level expression during fungal infection. Cucumber chitinase is acidic in nature. Transformation of cucumber with chitinase genes from tobacco, bean, and petunia was reported by Punja and Raharjo (1996). Expression of transgenes in transgenic plants showed no significant disease resistance when inoculated with *Botrytis*, *Colletotrichum*, *Alternaria*, and *Rhizoctonia* (Raharjo et al. 1996). Chitinase cDNA under the intrinsic expression of CaMV 35S promoter from the rice was transformed by *Agrobacterium*. When transgenic plants were infected with gray mold, *Botrytis cinerea*, 15 out of 20 independent shoots showed resistance compared to non-transgenic plants (Tabei et al. 1998). Enhanced expression and intracellular accumulation of rice chitinase genes have provided resistance against gray mold in transgenic cucumber (Kishimoto et al. 2002). Overexpression of the rice class I chitinase gene also confer resistance to Phytophthora rot (*Phytophthora nicotianae* var. *parasitica*) (Kishimoto et al. 2003). Overexpression of class III chitinase gene (CHI2) in cucumber exhibits revocable resistance against infection to gray mold, as transgenic plants did not exhibit disease symptoms initially but later developed serious disease symptoms (Kishimoto et al. 2004). Transgenic expression of a novel antifungal gene from *Ginko biloba* seed kernels in cucumber showed enhanced resistance against blight disease caused by *Fusarium oxysporum* (Liu et al. 2010). Recently, miRNA-mediated resistance against target leaf spot (TLS), which is caused by *Corynespora cassiicola*, was reported by Wang et al. (2019). Agarobacteria-infiltered novel miRNA constructs silence the indigenous expression of miRNA in cucumber cotyledon and thus improve disease resistance in transgenic cucumber plants.

13.4.2 Abiotic Stress Resistance

Abiotic stress is a key environmental factor that leads to significant retarded plant growth and a decrease in yield. Abiotic stress can prominently impede plant growth by declining biomass production. Various abiotic stresses, viz., salt, chilling, temperature, pesticides, etc. were reported to hinder plant growth and development. Chilling stress resistance and pesticide residue stress resistance are the two most important reported in transgenic cucumber. Chilling at or below 4 °C induces oxidative damage and produces irreversible injury in cucumber fruit. Oxidative stress accompanied by lipid peroxidation is an earlier response to chilling stress in cucumber. Such damage to the tissue will evoke the cascade of biotic infection, eventually producing fruit with no edible value.

13.4.2.1 Chilling Stress Tolerance

The deficiency of cold-tolerant cucumber germplasm is a hindrance in classical breeding. Genetic transformation techniques providing chilling stress resistance mainly target cis-acting elements and inducible genes, thereby providing chilling tolerance in sensitive crop varieties. Upon exposure to cold stress, the plant immediately activates a cascade of the signaling process to combat negative effects induced by cold stress. Various cis-elements activate the expression of functional genes. Among these elements, C-repeat elements/dehydration-responsive elements (CRT/DRE), where C-repeat binding factors (CBFs) bind, are induced positively by an upstream inducer of CBF expression (ICE) gene, with two homologs, ICE1 and ICE2.

Dehydrins (DHN) proteins from late embryogenesis abundant [Lea] D11 family shows upregulation in chilling stress (Close 1997). Dhn10 gene from *Solanum sogarandinum* fused with 1633 bp promoter region of *Solanum*

sogarandinum glucosyl transferase (GT) gene (pGT::Dhn10). Fusion constructs with all regulatory elements were transferred by *Agrobacterium*, and transgenic lines of cucumber are produced. The prospective role of the Dhn10 gene in cold tolerance was observed in three T1 transgenic cucumber lines. Out of three, one line showed a significant increase in its chilling tolerance with no apparent chilling injury. The rest two lines showed no or significantly decreased freezing tolerance in experimental conditions (Yin et al. 2004a). Similarly, Mróz et al. (2015) transform pGT::Dhn24 fusion from *Solanum sogarandinum*. Transgenic cucumber showed no evidence for increased cold tolerance. The transformation of *cbf1* gene in cucumber cotyledon was reported by Deng et al. (2004). Transgenic cucumber expressing the *Arabidopsis cbf1* gene showed a significantly decreased level of chilling injury symptoms, viz., decrease in membrane injury, increase in the level of SOD and CAT enzymes for scavenging-free radicle oxygen, and increase in proline and relative water content (Gupta et al. 2012). *Arabidopsis thaliana nit2* gene encodes enzyme nitrilase which indole-3-acetonitrile (IAN) to indole-3-acetic acid (IAA), an important auxin. Transgenic cucumber expressing exogenous *nit* gene showed enhanced resistance to various abiotic environmental stresses (Jang et al. 2013).

Fusion of the trehalose-6-phosphate (T-6-P) synthase (TPS) and T-6-P phosphatase (TPP) of *Escherichia coli* is called TPSP (Trehalose 6-phosphate synthase and phosphatase). This novel bifunctional TPSP possesses both activities of phosphatase and synthetase and is reported to accumulate trehalose upon various abiotic stresses in *Escherichia coli* and tobacco (Jang et al. 2003). To evaluate the role of TPSP in providing chilling tolerance to cucumber, Kim et al. (2010) transformed the TPSP gene construct under CaMV 35S promoter and nopaline synthase (nos) and bar gene as marker system. Transformed plants were shown to synthesize and accumulate three times more amount of trehalose than non-transformed plants. Conversely, transformed plants show abnormal growth morphology including stunted growth and sterility. These results clearly suggest that the accumulation of trehalose is toxic to growth in cucumber (Kim et al. 2010).

Signal transduction pathways often initiate a cascade of cross-talks where several molecules are involved. These cross-talks are extremely complex to decipher in plants. One such signaling cascade is the mitogen-activated protein kinase (MAPK) cascade, which shows upregulation in various abiotic stresses. The signaling cascade involves a series of phosphorylation of MAPK to MPAK kinase (MAPKK) to MAPKK kinase (MAPKKK). This signaling cascade leads to various cell responses including cell division, production of reactive oxygen species (ROS) scavengers, and other signaling molecules to combat abiotic stress. Wang et al. (2013) use the affecting MAPK signaling pathway to confer abiotic stress resistance in *Cucumis sativus* L. Genetic construct containing MAPK was transformed to cucumber cotyledons. Successful transformants showed various levels of resistance against abiotic stress (Wang et al. 2013).

G-proteins (guanine nucleotide-binding proteins) are multimeric heterotrimers composed of three subunits: Gα, Gβ, and Gγ. In plants, the Gγ subunit is a trimer composed of three structurally distinct subunits: A, B, and C (Yan et al. 2018). These proteins play important signaling molecules providing cellular response against abiotic stress. Type C subunit of Gγ subunit is transformed in cucumber. Transgenic cucumber constitutively expressing type C subunit of Gγ subunit showed increased accumulation of CBF and anti-oxidative enzymes such as SOD, peroxidase (POD), catalase, and glutathione reductase and glutathione S-transferase (GST). Thus, mediating signal transduction in response to cold stimuli in cucumber without compromising other growth parameters (Bai et al. 2018).

YUC proteins (YUCCA) catalyze the conversion of Indole 3-pyruvic acid to Indole 3-acetic acid (IA), an important phytohormone auxin. Thus, the YUC gene family catalyzes the rate-limiting step in the auxin biosynthesis pathway. Cucumber possesses 10 YUC family genes (CsYUCs), which work in a coordinated manner to maintain auxin concentration in

cucumber. Experimental studies showed that CsYUC8 and CsYUC9 are upregulated under high-temperature stress. CsYUC4 shows down-regulation in response to the lower temperature. CsYUC10b is upregulated under chilling stress and regulated by CsYUC10b and CsYUC11. CsYUC11 provides tolerance against salinity stress (Yan et al. 2016).

13.4.2.2 Pesticide Resistance

Chemical pesticides are widely used to control pests in cucumber plants. These pesticides are recalcitrant to natural degradation and often absorb and form pesticide residue in cucumber fruit. Consumption of such fruit is toxic to human health and the environment. One such pesticide used for cucumber cultivation is Propamocarb (PM). Some of the cucumber varieties are known to accumulate a lower amount of PM in their tissues. In such cultivars, genes namely CsMAPEG (*Cucumis sativus* membrane-associated proteins in eicosanoid and glutathione metabolism) are strongly and constitutively expressed. Transfer of CsMAPEG gene constructs in high pesticide residue abundance cultivar of cucumber results in increased concentration of SOD, POD, and GST accompanied by metabolic degradation of PM in transgenic plants. The results showed the CsMAPEG cascade in PM degradation in cucumber (Zhang et al. 2019).

13.4.3 Yield Improvement

The typical plant breeding programs for crop improvement are tedious and time-consuming. The exponential increase in the human population demands a rapid solution for yield improvement in plants without compromising nutrient content. Plant tissue culture and marker-assisted selection that form the basis of plant biotechnology offer unique opportunities to provide sustainable agricultural practices. Modern genomic editing practices should be used as an aid of classical breeding to increase the yield.

The first successful attempt toward yield increase in cucumber was reported by Salyaev et al. (2002). Uridine diphosphate glucuronosyltransferase (*UDT*) from *Zea mays* and acetyl Co-A binding protein gene (*ACB*) from *Arabidopsis thaliana* are transformed to cucumber. *UDT* encodes the enzyme IAA glucose synthase and thereby overexpression of *UDT* leads to the accumulation of auxin (IAA) in transformed cucumber. *ACB* acetyl Co-A binding protein gene (*ACB*) catalyzes the prime step in fatty acid biosynthesis. Transformed cucumber showed a marked increase in yield compared to non-transformed plants (Salyaev et al. 2002a, b). The role of G-proteins in enhanced seed germination and root growth was reported by Yan et al. (2018). G-protein gene, *CsGPA1*, was transformed to cucumber. Transformed plants showed earlier seed division, root growth, and hypocotyl elongation through cell division and enhanced activity of root apical meristem (RAM) (Yan et al. 2018). Phytohormone, gibberellin (GA) is reported to regulate many functions, especially in fruit development. The role of GA in locule, interior cavities of fruit development is a complex process and is regulated by a series of different genes. (Cong et al. 2008). Overexpression of GA receptor gene *CsGID1a* is shown to control locule formation in transgenic cucumbers (Liu et al. 2016). Another important epidermal feature of cucumber fruit is tubercle formation. Yang et al. (2019) demonstrated that *CsTS1* gene expression positively regulates tubercle formation and increases the economic value of cucumber (Yang et al. 2019). Little Leaf (LL) locus in cucumber is known to control organ size and development. Yang et al. (2018) showed that overexpressing of the LL allele in wild-type cucumber varieties leads to an increase in leaf size, flowers fruits, and seeds (Yang et al. 2018).

13.4.4 Nutrition and Taste Enhancement

Improvement in nutrition and taste enhancement are important traits that are to be addressed by plant biotechnology. There are several reports available for taste development and nutritional

enhancement in cucumber. Thaumatin is a plant protein production in the fruit of the West African perennial plant, *Thaumatococcus daniellii* Bentham. Thaumatin is a natural sweetener and is often used as a taste enhancer in food products. Szwacka et al. (2000) reported transformation of Thaumatin gene is cloned in plant binary vector and express under CaMV 35S constitutive promoter and under neomycin phosphotransferase II (NPT II) marker selection. Transformed cucumber lines show accumulation of a higher level of thaumatin mRNA and protein expression from T_1 progeny (Szwacka et al. 2000, 2002; Yin et al. 2004b). Another important trait transfer to cucumber is an enhancement of carotene content. Carotenoids are important precursor producing chromogenic substances which imparts peculiar color to the plant parts. Also, carotenoids are precursors for abscisic acid (ABA), and especially β-carotene is a precursor for vitamin A. Jang et al. (2016) reported successful stable transformation of to introduce phytoene synthase-2a carotene desaturase (PAC genes) into a cucumber. Transformants overexpressing PAC gene showed enhanced beta-carotene content (Jang et al. 2016).

13.4.5 Improvement in Fruit Quality

Improving the fruit quality for taste, size, shelf life, ripening, nutritional enhancement, etc. are demanding traits for transformation by plant biotechnology. An important trait for fruit quality in cucumber is parthenocarpy or seedless fruit. Seedless fruit is a choice over wild-type seed fruits for processing and ease of consumption. As pollinating agents are not necessary for parthenocarpic fruits, plants can be grown under a suitable controlled enclosed condition like a greenhouse when environmental factors are not suitable for plant growth. The first successful event of transgenic cucumber for parthenocarpy was reported by Yin et al. (2006). *IaaM* gene from *Pseudomonas syringae* pv *savastona,* coding for auxin, indole acetic acid (IAA), under the control of a specific promoter, *DefH9*, from *Antirrhinum majus*, was transformed to cucumber. IAA is expressed specifically in developing ovule, and increased expression of auxin gene promotes parthenocarpy in developing fruit (Mezzetti et al. 2004, Yin et al. 2006). Total fruit yield produced by the transformed cucumber, 70–90% were parthenocarpic (Yin et al. 2004, 2006). Auxin-binding protein 1 (ABP1) from *Arabidopsis thaliana* is transformed in cucumber cotyledon by *Agrobacterium tumefaciens*. Successful transformants showed 31% production of parthenocarpic fruit (Bai et al. 2004). MADS box genes are important genes that regulate inflorescence and fruit development. MADS box genes, *CSMADS06*, are transformed and overexpressed in cucumber varieties S06 and S52. Positive cucumber transformants show inflorescence blooming and shown to set fruits (Lai et al. 2007).

13.4.6 Nutraceuticals and Biopharming

Biopharming is the use of transgenic plants or animals carrying foreign genes coding for medicinally important therapeutic proteins such as hormones, enzymes, antibodies, vaccines, etc. The term "edible vaccine" is popularly in use when vaccine protein is developed as transgenic in the edible part of the plant and can be consumed directly. Apart from, biopharmaceuticals, nutrition enhancers are also the prime target for transgenic plant development. Anti-oxidative and anti-aging enzyme superoxide dismutase (SOD) coding gene, CuZnSOD cDNA (mSOD1) from *Manihot esculenta*, under ascorbate oxidase promoter and phosphinothricin (PPT) resistance as the selectable marker are transformed in cucumber. The transformed cucumbers showed a higher level of SOD expression that was three times higher in fruits of transgenic plants compared to fruits of non-transgenic plants (Lee et al. 2003). *Cucumis sativus* also served as a potential system for the transformation and production of exogenous proteins. Shi et al. (2006) used hairy root culture produced after transformation with *Agrobacterium rhizogenes* carrying binary vector with the gene sequence of EHRH cardenolide

16′-O-glucohydrolase derived from Digitalis lanata. This enzyme catalyzes the deglycosylation of steroid glycosides. Deglycosylation activities of transformed cucumber were measured by HPLC (Shi et al. 2006). GLP-1 (Glucagon-like peptide-1) is a 30-amino acid residue hormone which regulates the secretion of insulin to control blood glucose levels. A synthetic analog of the GLP-1 gene was constructed and transformed into a cucumber. Oral administration of transgenic fusion protein (GLP-T) to diabetic mice reduces blood glucose levels (Lei et al. 2009). These results provide a new strategy to cure diabetes. Production of oral vaccines in cucumber is useful and economically viable. Sindhu et al. (2010) reported the expression of hepatitis B virus surface antigen (HBsAg) in cucumber. HBsAg gene was cloned in plant binary vector pCAMBIA 3300 under constitutive expression of CaMV 35S promoter and PPT gene as a selection marker. Transformed cucumber plants were shown to express HBsAg, as confirmed by Western blot, ELISA, and MALDI-ToF.

The number of transformed traits produced in transgenic plants is often low due to low transgenic frequency and gene expression level. Future efforts need to be directed toward marker-free selection, tissue and organ-specific expression, and modification of promoters.

13.5 Conclusion and Future Prospects

Cucumber (*Cucumis sativus* L.) is an important horticultural crop and is consumed worldwide. The most important efforts in transgenic cucumber are directed toward virus resistance and changes in the ripening process. Other important traits such as fungal resistance, abiotic stress tolerance, and biofortification increase the yield and nutritive value of cucumber. Enhanced expression of SOD, carotene, HBsAg, GLP-1, etc. in cucumber provides prime examples of the successful and stable transformation of transgenes in cucumber. Standardization of *agrobacterium* or biolistic-based transformation methods followed by standard techniques of tissue culture to raise complete plantlets led to an increase in transformation efforts in cucumber. Availability of draft genome sequence of cucumber opened up the door of genome editing and gene silencing by RNAi using dsRNA, siRNA, and artificial miRNA to incorporate desirable traits such as fruit quality, biotic and abiotic stress resistance, and biopharmaceutical production. Construction of chimeric construct provides multiple trait transfer in cucumber. Genetically modified (GM) cucumber has a potential future. The release of GM cucumber as a commercial variety needs a mandatory risk assessment to avoid the risk effects of transgene escape.

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