Horticultural Reviews

VOLUME 41

WILEY Blackwell

HORTICULTURAL REVIEWS
Volume 41

Horticultural Reviews is sponsored by:

American Society for Horticultural Science
International Society for Horticultural Science

Editorial Board, Volume 41

HORTICULTURAL REVIEWS

Volume 41

edited by
Jules Janick
Purdue University

WILEY Blackwell

A John Wiley & Sons, Inc., Publication

Published by John Wiley & Sons, Inc., Hoboken, New Jersey

Published simultaneously in Canada

Wiley-Blackwell is an imprint of John Wiley & Sons, Inc., formed by the merger of Wiley's global Scientific, Technical, and Medical business with Blackwell Publishing.

***Library of Congress Cataloging-in-Publication Data*:**

ISBN 978-1-118-70737-1 (cloth)

ISSN 0163-7851

Printed in the United States of America

10 9 8 7 6 5 4 3 2 1

Contents

Contributors

Diego Barranco, Department of Agronomy, University of Cordoba, D.P. 14071, Córdoba, Spain

Ignasi Batlle, IRTA Mas de Bover, Ctra. Reus-El Morell, E-43120 Constantí, Tarragona, Spain

T. K. Behera, Division of Vegetable Science, Indian Agricultural Research Institute, New Delhi 110012, India

L. K. Bharathi, Central Horticultural Experiment Station, Bhubaneswar 751019, Odisha, India

Sergio Castro-García, Department of Agricultural Engineering, University of Córdoba, D.P. 14071, Córdoba, Spain

John R. Clark, Department of Horticulture, University of Arkansas, Fayetteville, Arkansas 72701, USA

David J. Connor, Department of Plant Production, Polytechnic University of Madrid, D.P. 28040, Madrid, Spain

Paul J. R. Cronjé, Citrus Research International, Department of Horticultural Science, Stellenbosch University, Stellenbosch 7602, South Africa

María Gómez del Campo, Department of Plant Production, Polytechnic University of Madrid, D.P. 28040, Madrid, Spain

Marcos Egea-Cortines, Genetics, Institute of Plant Biotechnology, Department of Agricultural Science and Technology, Escuela Técnica Superior de Ingeniería Agronómica, Technical University of Cartagena, 30203 Cartagena, Spain

D. Michael Glenn, USDA-ARS-Appalachian Fruit Research Station, 2217 Wiltshire Road, Kearneysville, West Virginia 25430, USA

Irwin Goldman, Department of Horticulture, University of Wisconsin-Madison, Madison, Wisconsin 53706, USA

K. Joseph John, National Bureau of Plant Genetic Resources, KAU (P.O.), Thrissur 680656, Kerala, India

Soo-Hyung Kim, Center for Urban Horticulture, School of Environmental and Forest Sciences, College of the Environment, University of Washington, 3501 NE 41st Street, Seattle, Washington 98195-4115, USA

Peter Läderach, International Center for Tropical Agriculture (CIAT), Managua, Nicaragua

Sandra Landahl, Plant Science Laboratory, Cranfield University, Bedfordshire MK43 0AL, UK

Lembe Samukelo Magwaza, Postharvest Technology Research Laboratory, South African Research Chair in Postharvest Technology, Stellenbosch University, Stellenbosch 7602, South Africa

Bart M. Nicolaï, BIOSYST-MeBioS, Katholieke Universiteit Leuven, Willem de Croylaan 42, 3001, Heverlee Belgium

Umezuruike Linus Opara, Postharvest Technology Research Laboratory, South African Research Chair in Postharvest Technology, Stellenbosch University, Stellenbosch 7602, South Africa

Sunil Pareek, Department of Horticulture, Rajasthan College of Agriculture, Maharana Pratap University of Agriculture and Technology, Udaipur 313001, Rajasthan, India

Cameron Peace, Department of Horticulture, Washington State University, Pullman, Washington 99164, USA

Luis Rallo, Department of Agronomy, University of Cordoba, D.P. 14071, Córdoba, Spain

Pilar Rallo, Department of Agroforestry Sciences, University of Sevilla, D.P. 41013, Sevilla, Spain

Julian Ramirez-Villegas, Decision and Policy Analysis (DAPA), International Center for Tropical Agriculture (CIAT), School of Earth and Environment, University of Leeds, Leeds, UK; CGIAR Research Program on Climate Change, Agriculture and Food Security (CCAFS), Km 17, Recta Cali-Palmira, Apartado Aéreo 6713, Cali, Colombia

Agusti Romero, IRTA Mas de Bover, Ctra. Reus-El Morell, E-43120 Constantí, Tarragona, Spain

Fabiola Ruiz-Ramon, Genetics, Institute of Plant Biotechnology, Department of Agricultural Science and Technology, Escuela Técnica Superior de Ingeniería Agrónoma, Technical University of Cartagena, 30203 Cartagena, Spain

Paul Sandefur, Department of Horticulture, University of Arkansas, Fayetteville, Arkansas 72701, USA

Ockert P. J. Stander, Department of Horticultural Science, Stellenbosch University, Stellenbosch 7602, South Africa

A. K. Sureja, Indian Agricultural Research Institute, New Delhi 110012, India

Leon A. Terry, Plant Science Laboratory, Cranfield University, Bedfordshire MK43 0AL, UK

Karen I. Theron, Department of Horticultural Science, Stellenbosch University, Stellenbosch 7602, South Africa

Joan Tous, C/Sant Antoni, 44, E-43480 Vila-seca, Tarragona, Spain

Todd C. Wehner, Department of Horticultural Science, North Carolina State University, Raleigh, North Carolina 27695-7609, USA

Julia Weiss, Genetics, Institute of Plant Biotechnology, Department of Agricultural Science and Technology, Escuela Técnica Superior de Ingeniería Agrónoma, Technical University of Cartagena, 30203 Cartagena, Spain

Elhadi M. Yahia, Faculty of Natural Sciences, Autonomous University of Queretaro, Avenida de las Ciencias s/n, Juriquilla, 76230 Queretaro, Mexico

Philipp W. Simon

Dedication: Philipp W. Simon

This volume is dedicated to Dr. Philipp Simon, plant breeder and geneticist, in recognition of his outstanding contributions to horticulture and vegetable crops. Dr. Simon, a leading world authority in carrot and garlic improvement, is a role model for what can be accomplished in vegetable breeding.

Philipp Simon was born and raised in Door County, Wisconsin, in 1950. He attended Carroll College in Waukesha, Wisconsin, where he graduated with a B.S. in Biology in 1972. While a college student, he read books on the subject of plant-based medicine and this influenced him to consider a career in biology and plant science. He enrolled at the University of Wisconsin-Madison and completed his M.S. in Genetics in 1975, working with Professor Stanley Peloquin. Simon's dissertation work focused on pollen vigor as a function of 2*n* gamete formation in that crop and the influence of the paternal parent on the origin of callus in anther culture of *Solanum* hybrids. Simon completed his Ph.D. in Genetics in 1977 and assumed the role of Research Geneticist and Adjunct Professor at Madison in 1978. He was promoted to Assistant Professor in 1980, Associate Professor in 1985, and Professor in 1990. *S*imon is presently the Research Leader for the Vegetable and Cranberry Research Unit of the U.S. Department of Agriculture-Agricultural Research Service and a breeder of carrot, garlic, and other vegetable crops. For more than 30 years, Simon has been a primary contributor to both national efforts in carrot and garlic improvement as well as local efforts at teaching, graduate student training, and mentoring in the fields of plant breeding and plant genetics. Simon's contributions in these areas have shaped the development of these crops globally and had many positive downstream effects on consumers. The genesis of Simon's interest in crop improvement for nutritional quality is a focus on consumer-driven traits in plants, though over the decades his work has served the seed industry as well as farmers and consumers.

I. CARROT

The U.S. carrot crop has a farm value of $530 million annually, making it one of the most valuable U.S. vegetable crops. To date, as with many important food crops in the United States, the great majority of carrot breeding activity is in the private sector. The carrot seed industry is represented by approximately two dozen seed companies, many of whom have a global reach. Throughout this period, the USDA program run by Simon has been a critical contributor to technologies for the inbred-hybrid industry programs, new sources of germplasm for carrot breeding, and analysis of important carrot quality traits. Carrot breeding programs exist in several European countries, as well as in China, Korea, and Brazil, and these have also benefited from germplasm resources and data developed by the USDA program. During a career spanning more than 30 years, Simon's primary foci in carrot have stressed determination of inheritance patterns of sugar, volatile terpenoid, carotene, and anthocyanin accumulation; development of genetic markers, maps, and genomic tools; description of transposable elements; and development of elite genetic stocks.

Knowledge of the flavor genetics of carrot is quite extensive and is attributable largely to the efforts of Philipp Simon and coworkers. Simon's first papers as a faculty member at UW-Madison included studies of the genetic and environmental components of carrot culinary and nutritive value and investigations of sensory and objective parameters of carrot flavor. His work has led to fairly routine procedures for sensory analysis and has helped breeders develop carrot germplasm with improved flavor. Among many discoveries, Simon determined that genetic variation exists for raw carrot flavor, that volatile flavor chemicals are quantitatively inherited, and that genetic variation for total volatile terpenoid levels and sugars account for most of the observed variation in sweetness and flavor preference of raw carrots. Many of the papers published from Simon's group during this period focused on the impact of the horticultural environment on carrot flavor. Research by Simon and students demonstrated patterns of inheritance for sugars stored in carrot roots. Together with student Roger Freeman, Simon found that the balance of reducing sugars to sucrose is controlled by the *Rs* (Reducing sugar) locus, which was discovered and characterized as a naturally occurring "knockout mutant" conditioned by a 2.5 kb insertion in the soluble invertase isozyme II gene.

These discoveries helped direct carrot breeders to focus on terpenoids for off-flavors and harsh flavors and on sugars for sweetness. Carrot germplasm released by Simon's program has improved sweet and

mild flavor and higher nutritional value than releases from prior decades. For example, inbred lines B9304 and B2566 are sweet, mild, and succulent (Simon et al. 1987). Germplasm developed by Simon is being widely used by commercial vegetable seed companies, and therefore it is a constituent component to fresh market carrots consumed in the U.S.

Simon and his collaborators and students have spent considerable effort developing a carrot genetic linkage map. To date, this map includes some 500 molecular markers and a number of phenotypic markers for nematode resistance, root pigments, and sugar type. Specific AFLP markers linked to important or interesting phenotypic genes have been converted to more easily evaluated codominant PCR-based markers. These maps have become fundamental tools for carrot geneticists and breeders. Seed companies use markers developed in the Simon laboratory to select for two difficult-to-score traits such as nematode resistance (*Mj-1*) and sugar type (*Rs*). Recently, Simon and colleague David Spooner have employed some of these markers to begin work clarifying the taxonomy of the genus *Daucus*.

Simon also developed molecular markers to identify and differentiate among male fertile carrots and the two major forms of male sterile cytoplasm conditioned by the mitochondrial genome. Molecular markers for the nuclear genome were then used to identify inbred parents and predict their hybrid patterns. A plastid marker was unexpectedly discovered by Simon within *Daucus carota* and used to confirm strict maternal inheritance of this organelle. A transposable element was also unexpectedly discovered, and has been used to develop molecular markers for general mapping and genome assessment. The molecular markers developed by Simon have accelerated the selection process of carrot breeding so that differentiation of male sterile and male fertile plants can be accomplished early in plant growth. This allows removal of undesired male fertile plants long before they flower.

Carrot contains high levels of certain carotenoids such as beta-carotene and alpha-carotene. These molecules are cleaved during digestion and turned into retinol, which is also known as vitamin A. The carotenoid molecules are also called provitamin A carotenoids for this reason. The situation for carrot root pigmentation is fortuitous, as higher levels of provitamin A carotenoids lead to both improved vitamin A delivery and deeper orange colors, which are also preferred by consumers. The appearance of orange carotenoids in carrot roots became widespread in the 17th century. Prior to this period, carrot roots were predominately purple and yellow, where the purple pigmentation

was due to anthocyanin and the yellow to xanthophylls, which are oxygenated carotenoids.

High carotene carrot germplasm released by Simon's program has been an important contributor to improved provitamin A levels of U.S. carrots over the past 40 years. Estimates suggest that these levels have increased by >40% since the 1970s. In addition, improved carotene levels in carrot have stimulated interest in carrot production as a source of provitamin A carotenoids in vitamin A-deficient areas of the world. Simon has worked in Haiti and other countries where vitamin A deficiencies are an important public health problem leading to childhood blindness. He and his colleagues developed the carrot cultivar 'BETA III' to help alleviate vitamin A deficiency, which was tested in 44 developing countries. Carrot trials have also been established in Philippines, India, Guatemala, Nepal, and Haiti. To date, the average carotene content of U.S. carrots is 130 ppm and per capita U.S. carrot consumption is 5.4 kg per annum.

Simon, working with nematologists, identified a major dominant gene, *Mj-1*, which conditions resistance to Javanese root-knot nematode, *Meloidogyne javanica*, a major pest in California carrot-producing regions. The *Mj-1* resistance gene also imparts resistance to *M. incognita*, another major nematode pest in carrot-producing regions. The nematode resistance revealed by this research may have significant impact in the major carrot-producing regions to reduce the need for nematicide application, which is expensive and poses significant environmental risks. Genetic resistance is being actively incorporated into new carrot breeding lines by seed companies using marker-facilitated selection and is appearing in advanced hybrids. Simon's work also demonstrated a genetic component to *Alternaria* leaf blight resistance and initiated germplasm development for carrot breeders. Simon and colleagues developed a method for screening bacterial soft rot resistance that has been used with some success in Europe, where it is a significant storage disease.

Simon's research has demonstrated relatively simple patterns of inheritance for certain aspects of carrot root carotene and anthocyanin accumulation. He has also shown a pattern of clustered quantitative trait loci conditioning the major provitamin A carotenes and lycopene. Knowledge of carrot pigment genetics is being used to improve commercial carrot germplasm for nutritional quality and to develop unique colors (including purple, red, yellow, and white) by several seed companies. Simon's program also released the first "new" carrot root color (purple) for modern use in 1992, with the release of a purple-rooted inbred line.

Interestingly, the first domesticated carrot roots were purple, and the crop remained purple-rooted for many centuries. Purple pigmentation of roots continued in parts of Asia and the Middle East but was largely lost in Europe and North America until very recently. To date, purple root pigmentation has made a comeback in carrot. Working with graduate student John Navazio, Simon also incorporated genes for orange fruit flesh color into U.S. pickling cucumbers and released the first U.S. orange cucumber.

II. GARLIC

Garlic is very important in the United States and worldwide. To date, global production of garlic exceeds 3 million tonnes with a value of $50 million to U.S. growers. Garlic production has been known for at least 5,000 years but, remarkably, routine seed production has never been reported for this crop. It is unclear if garlic has simply lost the ability to produce seed through genetic drift over millennia. Therefore, in spite of its long history, little is known about the genetic variation for this important world crop. No reports of true seed production in garlic can be found prior to 1950, and very little information has accumulated since that time. Working with a graduate student Margaret Pooler, Simon developed the first true seed production system for garlic in the United States and transferred this technology to the garlic industry so that garlic breeding and routine seed production is feasible for the first time. This work initially made use of controlled environment production in combination with certain garlic clones. To data, millions of garlic seed have been produced. Thus, for the first time in history garlic has been transformed from a strictly asexually propagated crop to one where classical plant breeding is now possible. A similar effort to develop garlic seed production was also independently undertaken in Japan. The availability of true garlic seed provided the basis for establishing the first genetic linkage map for garlic. Part of the successes of these projects resulted from the observation that bulbils in garlic inflorescences compete with developing seed, so routine bulbil removal was performed in early generations of garlic selected for seed production. The recognition and utilization of garlic's broad genetic base was an important component of the success of true seed production, since it was germplasm from close to the center of diversity for garlic in Central Asia, that contributed most significantly to the success in producing garlic seed.

III. TEACHING, TRAINING, AND MENTORING

For many years, Philipp Simon provided lectures on transposable elements in Stan Peloquin's legendary course on plant ctyogenetics. Simon's insight into the work of Barbara McClintock and the breakage–fusion–bridge cycle was a highlight of these lectures. One of the best aspects of Simon's formal teaching is his ability to help students understand the many levels of genetic organization from the most basic, fundamental cellular level to the organismal level. Being a plant breeder helps. Like his mentor Peloquin, Simon also has contributed to the teaching of fundamental genetics in Biocore 301, the first semester of the four-semester honors biology sequence at the University of Wisconsin-Madison. In a series of approximately 15 lectures, Simon takes the students from Mendelian heredity to population genetics and also runs some of the laboratories on cytogenetics. During the course of his career, Philipp Simon has trained 23 Ph.D. students, 2 M.S. students, 14 post-doctoral researchers, and 8 visiting scientists. He has also contributed significantly to the graduate research of 8 graduate students who received their degrees at other institutions but completed a portion of their research in his laboratory.

IV. GERMPLASM AND INTERNATIONAL ACTIVITIES

Simon's evaluation of molecular marker variation in germplasm collections of carrot and garlic demonstrated an unexpectedly high level of genetic diversity in carrot relative to other outcrossing diploid crop plants, and also higher diversity in garlic than expected for a strictly clonally propagated crop. These studies were the first evaluations of germplasm variation in these crops. This knowledge has been applied by carrot breeders in broadening the germplasm base of cultivated carrot breeding stocks and by garlic breeders in selecting all of the garlic stocks used for garlic seed production in the United States. For both crops, there was generally poor correlation between morphological traits, geographic origin, and molecular diversity. A wild relative of garlic, *A. longicuspis*, clustered together with no clear separation from garlic, suggesting these species are not genetically or specifically distinct. The molecular variation observed confirmed broad diversity in garlic.

Philipp Simon has provided leadership for the Vegetable and Cranberry Research Unit of the USDA-ARS at Madison, as well as for vegetable researchers and the vegetable industry. Since 1986, Simon has arranged germplasm and cultivar evaluation trials that are attended by

vegetable growers and seed industry representatives, including two popular annual trials in Bakersfield, California. He has been an active trainer of research apprentices, interns, graduate students, postdoctoral research associates, and visiting scientists from around the world. Simon has served as a cooperating scientist to Pakistan in a project focused on diseases of bulb vegetables, to India in a project focused on carrot breeding, to Brazil in a project on *in vitro* improvement of garlic, and to Bangladesh in a project on garlic and onion improvement. Simon's carrot and onion quality program at Madison serves as a model for establishment of similar vegetable quality laboratories in U.S. industry and other countries. Researchers from 36 U.S. companies and from India, Nepal, Bangladesh, Pakistan, China, Japan, Korea, Turkey, Syria, Poland, Germany, The Netherlands, France, Italy, Greece, England, Norway, Guatemala, Canada, Australia, Argentina, New Zealand, Brazil, Mexico, England, Denmark, and Nigeria were informally trained or otherwise assisted by Simon in their laboratory planning and programming. He has also initiated and codeveloped the RoBuST database to support Apiaceae and Alliaceae research and education.

Walking into the second floor entrance of the Plant Science building on the campus of the University of Wisconsin-Madison, one will encounter a hallway lined with tables that are heaped with carrots. Students, visiting scientists, and postdoctoral associates stand in front of the tables, wearing lab coats and wielding knives, trimming and cutting carrot roots, and making selections for breeding and seed production. Stacked next to the table are cardboard boxes bearing California postmarks and the unmistakable scent of carrot volatiles. Hanging from the ceiling are posters covering a wide range of research topics—from nematode resistance to Mediterranean germplasm collections to carrot gene-sequencing projects. Black and white photographs of plant chromosomes and unique cytogenetic features cover surfaces in the laboratory, and cabinets abound overflowing with theses, papers, articles, and notebooks. This is the Simon laboratory, one of the world's foremost destinations for the study of carrot and garlic genetics and breeding. Simon's contributions to improving these crops have been influential during the past 30 years, and his commitment to student and scientist training has improved the outlook for vegetable breeding globally.

V. HONORS AND AWARDS

Simon was named USDA, ARS Senior Scientist of the Year, Midwest Area in 2001 and was awarded the USDA Secretary's Honor Award for

Superior Service in 2002. He was elected Fellow of American Society for Horticultural Science in 2002 and named the American Society for Horticultural Science Outstanding Researcher in 2003. Simon was awarded an Honorary Doctorate from the Agricultural University of Krakow, Poland, 2003.

Philipp Simon is dedicated to both his career and his family. He and his wife Sandy have two grown children and have lived in Madison for many years. Through his work in germplasm collection and breeding, Philipp has had the opportunity to travel the world, and he considers traveling one of his hobbies. He is an avid follower of politics and reads broadly on a number of subjects. He is widely known as a kind and thoughtful person who has contributed much while remaining modest; a rare and highly desirable quality in a colleague.

I. L. GOLDMAN

Department of Horticulture
University of Wisconsin-Madison
Madison, Wisconsin 53706, USA

1

Circadian Regulation of Horticultural Traits: Integration of Environmental Signals

Marcos Egea-Cortines, Fabiola Ruiz-Ramon, and Julia Weiss
Genetics, Institute of Plant Biotechnology
Department of Agricultural Science and Technology
Escuela Técnica Superior de Ingeniería Agronómica
Technical University of Cartagena
30203 Cartagena, Spain

ABSTRACT

Plants, animals, and fungi have evolved to contain an internal physiological clock that responds to external stimulus such as the light/dark cycles created by the rotation of the Earth. This pacer is known as the circadian clock. It is composed of a complex set of genes that is conserved in higher plants. Originally thought to be a mere coordinator of basic processes, research has shown that the clock plays a key role in aspects as important as flowering time, productivity, tuberization, and dormancy. Its functions are all related to the seasonal development in many crops. But the circadian clock intimately controls other biological processes such as adaptation to cold, pathogen resistance, stomatal movement, and scent production. Most of the knowledge about the plant circadian clock has been established by research on *Arabidopsis* but the apparent conservation of the circadian clock components in cereals, trees, and floriculture crops means that the circadian clock may influence many agriculturally relevant traits such as flowering, dormancy, productivity, or fruit and flower aromas.

KEYWORDS: cold acclimatization; dormancy; flowering time; gibberellins; plant growth; productivity; scent production; tuberization

Horticultural Reviews, Volume 41, First Edition. Edited by Jules Janick.

I. INTRODUCTION

Plants are sessile organisms that have to cope with environmental fluctuations such as sharp changes in light and temperature on a daily basis. As a result, developmental programs in plants are partly controlled by environmental cues. How the main environmental signals are integrated into a default program of growth and development has been elucidated in many plants by a mixture of field experiments, breeding, genetics, and physiological studies. Today important evidence suggests that most if not all responses of phytoplankton (Prezelin 1992), cyanobacteria (Sandh et al. 2009), mosses (Imaizumi et al. 2002), and higher plants (Koornneef and Peeters 1997) to the environment are somewhat controlled by the circadian clock (de Montaigu et al. 2010). The circadian clock is formed by a set of genes whose main function appears to be the coordination of environmental cues and physiological responses (see below). Initial observations of rhythms in plants started with the rhythmic movement of leaves, reported already in 1726 (see McClung 2006 for a historical perspective of research on circadian rhythms in plants). Although early molecular experiments were performed in pea and wheat (Kloppstech 1985; Nagy et al. 1988), much of our knowledge has been accumulated in the plant

model *Arabidopsis thaliana*. Given the importance of the circadian clock as a general controller of plant growth, development, and response to stress, we expect to see an increase of knowledge transferred to horticultural crops. *Arabidopsis* might be used further to identify clock genes and how they function, but proof/application of the concept requires the identification of genes from the circadian clock causing modifications in horticultural traits such as flowering time, abiotic stress resistance, productivity, or volatile production. Furthermore, differences with *Arabidopsis* might explain crop singularities helping to improve cultural practices and breeding.

Circadian regulation is often considered plant specific, but rhythmic regulation of biological processes also occurs in cyanobacteria, fungi, and animals. It is extensively studied in the field of chronobiology. Two extensive reviews on the historical perspective of the circadian clock in plants have been published recently (McClung 2006, 2011). Harmer (2009) reviewed clock structure in *Arabidopsis*, while Yakir et al. (2007) and de Montaigu et al. (2010) reviewed the current view on circadian outputs controlling plant growth, flowering time, and cold response. The object of the current review is to provide an overview of the clock structure. We cover with some detail the environmental inputs that set the clock, a process called entrainment. We include examples of the knowledge of clock and related topics in plants of horticultural interest.

As many biological processes show rhythmic patterns, a detailed terminology describing a rhythm and its changes has developed over the years, which helps to identify changes in this phenomenon. An important component of the language used in chronobiology and data analysis tools originated in the field of signal processing in electrical engineering where wave-like signals are analyzed. Thus, it has remained a common language to a large extent, and new concepts related to biological aspects have enriched it, making it quite elaborate. Although not all the terminology has been used in the current review, we have compiled a table with a comprehensive list of terms used in chronobiology, for educational purposes and to ease reading further literature (Table 1.1). It is just good practice that data gathering, terminology, and measurements are standard as it allows proper data analysis, sharing of data, and classification of the different responses. Fig. 1.1 presents examples that indicate how changes in circadian regulation are observed.

II. GENERAL STRUCTURE OF THE PLANT CIRCADIAN CLOCK

A. *Arabidopsis*

Two physical signals, light and temperature, are constantly changing as a result of Earth axial rotation providing night and day as well as the

Table 1.1. Terminology used in chronobiology.

Term	Definition and/or description
Acclimation	Physiological changes occurring within the lifetime of an organism that reduce the strain caused by experimentally induced changes in particular climatic factors such as ambient temperature and/or photoperiod. The acclimation period is critical to obtaining reliable experimental data.
Acrophase	Peak of a mathematical curve fit to data. Refers to the time when a process has its maximum, starting from a point defined by the scientist, for example, dawn. It may be expressed in (negative) degrees as the lag from the acrophase reference ($360^{\circ}C = 1$ period) or in calendar time units (hours, minutes, etc.).
Aliasing	Detection of a false period that is longer than the underlying true period as a result of sampling taken wide apart.
Amplitude	Distance from rhythmic mean to the peak or to the trough of a mathematical model (e.g., cosine) used to approximate a rhythm. A process without rhythm will have amplitude of zero.
CC	Constant environmental conditions. In chronobiology, CC indicates lack of environmental synchronizers, that is, constant light, constant temperature.
Circadian	Roughly 24 h, describing rhythms with about a 24 h cycle length whether they are synchronized with a 24 h periodic surrounding or not.
Circadian time	Time that spans the circadian period in relation to the light/dark regimen under synchronized conditions.
Circannual	A rhythm with a period of about 1 year (± 2 months), synchronized with or desynchronized from the calendar year.
Circaseptan	A rhythm with a period of about 7 (± 3) days, which may or may not be synchronized with the calendar week.
Circatrigintan	A rhythm with a period of about 30 (± 5) days.
Cosinor procedure	A mathematical–statistical method of describing a rhythm by determining by least squares technique the cosine curve best fitting to the data and exploring the presence of a rhythm by examining the null hypothesis for amplitude in an *F*-test. If a rhythm can be described by this procedure, the cosinor yields a rhythm-adjusted mean (MESOR), an amplitude as measure of the extent of the rhythm, and an acrophase as indication of its timing with variance estimates for each of the three parameters.
Damping	Decrease in amplitude of a rhythm over time.
DD	Continuous dark conditions.
Endogenous rhythm	A biological rhythm that persists in the absence of external cues and is probably genetic.
Entrainment	Coupling of the period and phase of a biological rhythm (e.g., circadian) with another cycle (e.g., 24 h solar day). Entrainment signals (synchronizers) are light and temperature. Probably other components such as sugars play a role.
Free-running	Desynchronization of the period of a biological rhythm from the period of a known environmental synchronizer. Status of a rhythm under constant conditions (absence of synchronizers).

Table 1.1. (*Continued*)

Term	Definition and/or description
Frequency	Number of cycles for a given amount of time. It is the reciprocal of the period.
Gating	Pacing, or limiting a biological event to a certain period.
LD	Light period followed by dark period. Thus, 16 h light:8 h dark. LD might not always be 24 h periods as some experiments test effect of shorter or longer LDs.
Lighting regime	The light–dark cycle (LD), or constant light (LL), or constant dark (DD) conditions used for chronobiologic studies.
LL	Continuous illumination.
Masking	Change of rhythm characteristics (acrophase, amplitude, or Mesor) as a result of changes in environmental conditions.
Mesor	Midline estimating statistic of rhythm. The value midway between the highest and the lowest values of the (cosine) function best fitting to the data. The "M" is equal to the arithmetic mean only for equidistant data covering an integral number of cycles.
Pacemaker	A functional entity capable of self-sustaining oscillations that synchronize other rhythms. It is an internal component or set of components, not an external synchronizer.
Period (t)	Duration of one complete cycle in a rhythmic variation.
Photoperiod	In a light/dark regimen the duration of the light span (e.g., in light/dark = LD 12:12 h, the photoperiod L = 12 h).
Scotoperiod	In a light/dark regimen the duration of the dark span (e.g., light/dark = LD 12:12 h, the scotoperiod D = 12 h).
Synchronizer	Environmental signal or input that entrains a biological rhythm. In the literature several synonyms are used such as entraining agent, time giver, or Zeitgeber.
Trough	The lowest point in a series of measurements obtained as a function of time.
Ultradian rhythm	Peak of a mathematical curve fit to data. Refers to the time when a process has its maximum, starting from a point defined by the scientist, for example, dawn. It may be expressed in (negative) degrees as the lag from the acrophase reference (360° period) or in calendar time units (hours, minutes, etc.).
Zeitgeber	Time giver (German), it does not give time, but is a synchronizer.
Zeitnehmer	Time receiver (German); a molecule or mechanism that serves as input of environmental signals to the clock.

revolution of the tilted Earth around the sun that provides seasonal effects. It is a challenge for organisms to maintain a stable program of morphogenesis when important parameters regularly vary. The current hypothesis is that the circadian clock has evolved as a gene network that has a robust behavior, allowing daily adjustments to environmental changes such as photosynthetic apparatus maintenance or emission of

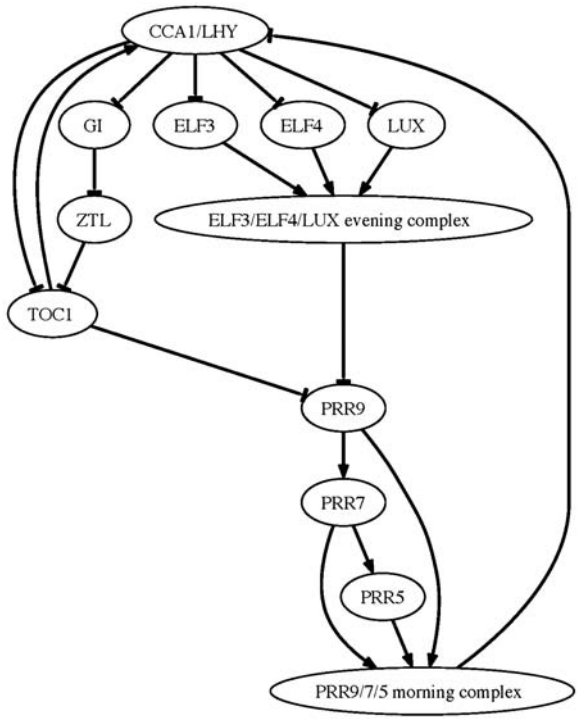

Fig. 1.1. A simplified structure of the current model of the circadian clock in *Arabidopsis*. The current model of the circadian clock comprises three groups of genes that are classified as the morning, midday, and evening loop. The morning loop is formed by three members of the same gene family *PRR9*, *PRR7*, and *PRR5*. These proteins form a complex that inhibits the midday loop formed by the genes *CCA1* and *LHY*. The evening loop is formed by *GI* and *ZTL*, two proteins that inhibit *TOC1* (another member of the *PRR* family), and a complex formed by *ELF3*, *ELF4*, and *LUX*. This evening complex inhibits the morning complex, thus closing the daily circle.

scent matching the time of pollinator activities (Locke et al. 2006; Akman et al. 2010; Thommen et al. 2010). A second task would be to consider long-term morphogenetic changes such as flowering, winter dormancy, and adaptation to cold or heat during the seasons. An endogenous clock should help maintain a constant flux of processes yet must be robust enough—for example, to prevent a short-day plant, would flower after being exposed to random shading on a dark day.

The current proposed structure of the plant circadian clock consists of three interrelated loops of genes that act by mutual activation and repression (Pokhilko et al. 2012) (Fig. 1.2). These feedback loops form an

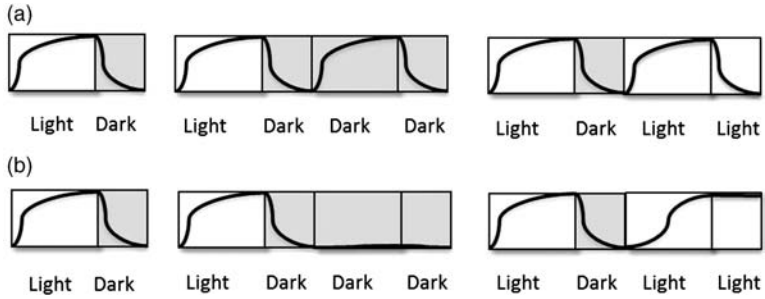

Fig. 1.2. Experimental design to identify processes that are circadian regulated in plants. As circadian experiments are timed usually, time zero is when light are turned on for a period and then off. This gives a pattern of light/dark, in most cases represented as LD. It follows that after a period of LD, the system is challenged with either a continuous light LL or an extended night (continuous dark) or DD. (a) Processes that are circadian regulated will maintain a rhythmic function in continuous dark (DD) and continuous light (LL). (b) A process that is light dependent will typically show a downregulation in continuous dark (DD) and constant high level in continuous light (LL).

oscillator that effectively cycles every day at a certain pace or amplitude (Table 1.1). As in many other biological regulatory processes, at least two levels of interaction occur inside the clock. One is at the transcriptional level, where activation and repression of gene expression play the main role. The second level of interaction is posttranslational changes where proteins form complexes and are selectively degraded or modified by phosphorylation. But the clock in plants also has an additional degree of complexity as several genes involved in clock function code for a photoreceptor that changes conformation and activity as a result of the light input (Jarillo and Pineiro 2006).

There are five *PSEUDORESPONSE REGULATOR* genes in the *Arabidopsis* genome, *PRR9*, *PRR7*, *PRR5*, *PRR3*, and *PRR1*, the latter known as *TIMING OF CAB EXPRESSION 1* (*TOC1*) (Uemura et al. 2010). All of them are components of the plant circadian clock. Assuming the morning as the beginning of a daily cycle, the first genes that show activity in the circadian clock are *PRR5*, *PRR7*, and *PRR9*. These genes act repressing the next loop of the clock in such a way that it causes a delay in its activation (Nakamichi et al. 2010). Two MYB transcription factor paralogs *LATE ELONGATED HYPOCOTYL* (*LHY*) and *CIRCADIAN CLOCK ASSOCIATED 1* (*CCA1*) form the middle loop, as they are expressed during the early part of the day. *CCA1* and *LHY* expression is repressed by *PRR5*, *PRR7*, and *PRR9*, from morning till midnight (Nakamichi et al. 2010), but *CCA1* and *LHY* activate *PRR5*, *PRR7*,

and *PRR9*. This interplay of repressing a function that then activates backwards creates a temporal pacer. A second component of the middle loop is *TOC1*. Recent work has shown that *TOC1* and the rest of the *PRR* family members are DNA-binding proteins (Gendron et al. 2012), indicating that their function in transcriptional control occurs via direct binding to regulatory sequences of target genes. The gene *REVEILLE8*/*LIKE CCA1 LHY 5* is a MYB transcription factor found recently to activate the *TOC1* gene, thus creating an additional connection between the morning and evening loops (Farinas and Mas 2011). The REV8 protein physically interacts with regulatory region of *TOC1* activating histone hyperacetylation. This causes a local loosening of the chromatin increasing the accessibility to the transcriptional machinery.

The evening loop comprises the genes *EARLY FLOWERING 3* and *4* (*ELF3* and *ELF4*), *LUX ARRHYTHMO* (*LUX*), *GIGANTEA* (*GI*), and the protein with photoreceptor capacity *ZEITLUPE* (*ZTL*). A recent work has shown that the ELF3, ELF4, and LUX proteins form a protein complex called the evening complex (Nusinow et al. 2011). The evening complex can bind DNA via LUX (Helfer et al. 2011), and represses its own expression and that of the morning gene *PRR9* (Dixon et al. 2011). This repression of the morning loop by the night loop closes the circle. Two recent papers have shown that *TOC1* is a general transcriptional repressor of the evening genes, that is, during the night, many genes have low transcriptional activity because of *TOC1* (Huang et al. 2012; Pokhilko et al. 2012). Again this mutual activation and repression of the clock genes creates waves of activation and repression that effectively pace the plant cell. The evening part of the clock is not completely understood. A number of components are missing and the way known components interact with each other remains incompletely defined. As a summary, the plant circadian clock has the architecture of several negative feedback loops interconnected with each other. These loops have been defined as morning, midday, and evening loop based on the time of the day when these genes display a maximum peak of expression.

B. Clock Genes in Crops

If we consider circadian regulation, we identify three layers where evolution might show conservation and divergence. One is the presence of conserved genes, orthologous to those found in *Arabidopsis* and other plants. A second more subtle but in this case as important is the conservation of the gene interactions found in other clocks, that is, the network motifs (Alon 2007). Yet a third level is the