Roberto Fritsche-Neto
Aluízio Borém *Editors*

Plant Breeding for Biotic Stress Resistance

Springer

Plant Breeding for Biotic Stress Resistance

Roberto Fritsche-Neto
Aluízio Borém
Editors

Plant Breeding for Biotic Stress Resistance

Springer

Editors
Roberto Fritsche-Neto
Departamento de Fitotecnia
University of Viçosa
Viçosa, Minas Gerais
Brazil

Aluízio Borém
Departamento de Fitotecnia
Universidade Federal de Viçosa
Viçosa, Minas Gerais
Brazil

ISBN 978-3-642-33086-5 ISBN 978-3-642-33087-2 (eBook)
DOI 10.1007/978-3-642-33087-2
Springer Heidelberg New York Dordrecht London

Library of Congress Control Number: 2012948467

© Springer-Verlag Berlin Heidelberg 2012
This work is subject to copyright. All rights are reserved by the Publisher, whether the whole or part of the material is concerned, specifically the rights of translation, reprinting, reuse of illustrations, recitation, broadcasting, reproduction on microfilms or in any other physical way, and transmission or information storage and retrieval, electronic adaptation, computer software, or by similar or dissimilar methodology now known or hereafter developed. Exempted from this legal reservation are brief excerpts in connection with reviews or scholarly analysis or material supplied specifically for the purpose of being entered and executed on a computer system, for exclusive use by the purchaser of the work. Duplication of this publication or parts thereof is permitted only under the provisions of the Copyright Law of the Publisher's location, in its current version, and permission for use must always be obtained from Springer. Permissions for use may be obtained through RightsLink at the Copyright Clearance Center. Violations are liable to prosecution under the respective Copyright Law.
The use of general descriptive names, registered names, trademarks, service marks, etc. in this publication does not imply, even in the absence of a specific statement, that such names are exempt from the relevant protective laws and regulations and therefore free for general use.
While the advice and information in this book are believed to be true and accurate at the date of publication, neither the authors nor the editors nor the publisher can accept any legal responsibility for any errors or omissions that may be made. The publisher makes no warranty, express or implied, with respect to the material contained herein.

Printed on acid-free paper

Springer is part of Springer Science+Business Media (www.springer.com)

Preface

Experience shows that biotic stresses occur with different levels of intensity in all agricultural areas around the world. The occurrence of insects, weeds, and diseases caused by fungus, bacterium, or virus may not be relevant in a specific year but they usually cause yields reduction in most of the years. Global Warming has shifted the paradigm of biotic stresses in most growing areas, especially in topical countries, bringing intense discussion on the scientific forums.

This book was written with the idea of grouping, into a single publication, the most recent advances and discoveries applied to breeding for biotic stresses, covering all major classes of biotic challenges to agriculture and food production. So it presents the state of the art in plant stresses caused by all microorganisms, weeds, and insects and how to breed biotic stresses to overcome them.

Complementing another publication by Springer *Plant Breeding for Abiotic Stress Tolerance*, launched by the same organizers, this book was written by knowledgeable authors with expertise in each of the biotic stresses targeting scientists and students interested in learning how to breed for biotic stress scenarios, allowing the reader to develop a greater understanding of the basic mechanisms of resistance to biotic stresses and develop resistant cultivars.

Roberto Fritsche-Neto
Aluízio Borém

Contents

1 Challenges for Plant Breeding to Develop Biotic-Resistant Cultivars 1
Aluízio Borém and Roberto Fritsche-Neto

2 Breeding for Fungus Resistance 13
Arione da S. Pereira, Cesar Bauer Gomes, Caroline Marques Castro and Giovani Olegario da Silva

3 Breeding for Resistance to Bacterial Diseases 37
Carlos A. Lopes and Leonardo S. Boiteux

4 Breeding for Resistance to Viral Diseases 57
Leonardo S. Boiteux, Maria Esther de Noronha Fonseca, Jairo Vidal Vieira and Rita de Cássia Pereira-Carvalho

5 Breeding for Nematode Resistance 81
Éder Matsuo, Paulo Afonso Ferreira, Tuneo Sediyama, Silamar Ferraz, Aluízio Borém and Roberto Fritsche-Neto

6 Breeding for Resistance to Insect Pests 103
Alexandre Augusto de Morais and José Baldin Pinheiro

7 Breeding for Resistance to Insect Pests of Stored Grain. 127
Alexandre Augusto de Morais and José Baldin Pinheiro

8 Breeding for Weed Management 137
Roberto Fritsche-Neto, Júlio César DoVale, Lino Roberto Ferreira, Francisco Affonso Ferreira and Antônio Alberto da Silva

Index 165

Chapter 1
Challenges for Plant Breeding to Develop Biotic-Resistant Cultivars

Aluízio Borém and Roberto Fritsche-Neto

Abstract The fast world population growth and the increase in the *per capita* income, especially in the emerging nations referred to as BRIC countries (Brazil, Russia, India, China, and South Africa) has created huge pressure for the expansion of the agricultural growing area and the crop yields to meet the rising demand. Additionally, climate change has brought new challenges to agriculture to produce food, feed, fiber, and biofuels. To cope with these new challenges, plant breeding programs have to adopt new strategies to develop cultivars adapted to the new scenario. Experience shows that biotic stresses occur with different intensity in all agricultural areas around the world. The occurrence of insects, weeds, and diseases caused by fungus, bacterium, or virus may not be relevant in a specific year but they usually cause yields reduction in most of the years. The global warming has also shifted the paradigm of biotic stresses in most agricultural areas, especially in the tropical countries, bringing intense discussion on the scientific forums. This book has a collection of the most recent advances and discoveries applied to breeding for abiotic stresses, addressing epidemiological concepts, genetic resources, breeding methods, and molecular approaches geared to the development of resistant cultivars to biotic stresses. Written in an easy to understand style, and describing the breeding for biotic resistance step-by-step the reader will find this book as an excellent source of reference.

Keywords Climate change · Global warming · New diseases · Breeding for resistance · Biotechnology

A. Borém (✉) · R. Fritsche-Neto
Faculty Member at Federal University of Viçosa, Viçosa, Brazil
e-mail: borem@ufv.br

R. Fritsche-Neto
e-mail: roberto.neto@ufv.br

R. Fritsche-Neto and A. Borém (eds.), *Plant Breeding for Biotic Stress Resistance*,
DOI: 10.1007/978-3-642-33087-2_1, © Springer-Verlag Berlin Heidelberg 2012

1.1 Introduction

In 1798, Thomas Malthus shocked the world with a catastrophic forecast: the human population growth was outstripping food, with predictions of famine and high rates of mortality in starving countries. Mr. Malthus lived in a period when the human population reached the first billion people. Since human evolved as a species, about 200,000 years ago, and for the primitive eras the human population increased slowly and gradually. It was only after the birth of agriculture, about 10,000 years ago that the human population growth increased more rapidly. After the Second World War the agriculture improved at a fast rate, making food supply abundant and population growth experience a new era of explosion.

Currently, the world population is over 7 billion people and the World Population Clock keeps registering the continuous increase in number of inhabitants (http://www.apolo11.com/populacao.php). Now the Malthusian specter is not banished and the question "would there be enough food for all?" is very up-to-date (Van Dam and Seidell 2007; Haddad et al. 2010; Haddad and Frankenberger 2003).

The population growth has also brought about the occupation of part of the agricultural areas with developments, roads, ports, power plants, and other facilities, making this a new Geological Era called Anthropocene, once the human activities are the major force molding the planet (Alexandratos 2006; Wright 2010). The challenges to provide food, feed fiber, and bioenergy to meet the world's demand are requiring agricultural efficiency as never though before. On top of all that, global warming brings additional worries to agriculture.

In spite of Malthus predictions, the Anthropocene Era has been so far of relative food abundance, in particular in the developed countries. However, the demand for food has been increasing very rapidly, especially in the so-called BRIC countries (Brazil, Russia, India, China, and South Africa), where the living conditions are improving fast (FAO 2009, 2010; Fig. 1.1). For example, until very recently in Brazil, for many poor-resource people, lunch was basically rice, common bean, and cassava. Meat used to be a scarcer item in the diet of a significant part of the population. Today, most of the economic classes C and D eat food from animal origin, such as eggs, yogurt, cheese, and meat (Demeke et al. 2008; Barrett 2002; Bruinsma 2003). This improvement has been shared by millions of people, who have climbed the social ladder. Evidence is the rise of the economical classes in the last decade. According the World Bank, 50 million Brazilians have moved above the poverty line. The same tendency has been observed in most emerging countries, where the vegetarian diet, based on cereals and grains has been transformed into a much richer and varied diet, including food from livestock.

Food from livestock uses much more natural resources and causes a much higher pressure on the environment. For example, 1 hectare of soybean yields about 3 tons of grains. The same area with pasture produces only 46 kg of beef. Thus, it is easier to understand the huge pressure on agriculture nowadays. To cope with that pressure several new technologies have been brought to farm's field along of the last decades: precision farming, mechanization, irrigation, pesticides,

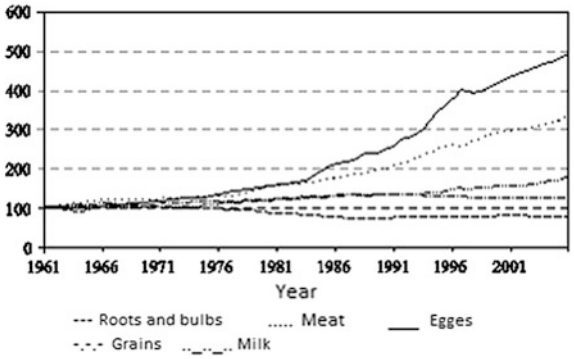

Fig. 1.1 *Per capita* consumption of five staple foods from 1961 to 2001. *Source* Agroanalysis (2011)

Fig. 1.2 Timeline, in human history related to food production

and many others. However, the biggest gain in crop yield came from plant breeding, developing new improved cultivars.

The time line on Fig. 1.2 shows some of the most relevant advances, discoveries, and events in human history related to food production. For example, the discovery of America in 1942 expanded the human diet to corn, potato, common beans, tomato, and many other species. In 1953, Watson and Crick discovered the chemical structure of DNA opening the door for the new era of genetics. With a better understanding of the genetics, plant breeding became more efficient in developing higher yielding- and more stress-resistant cultivars (Borém and Almeida 2011). For example, the Green Revolution was accomplished by Norman Borlaug in the 1960s by the introduction of the dwarf gene into new cultivars, prompting a significant yield increase in rice and wheat and other species production in countries that were facing starvation during the second half of the twentieth century. As a result of plant breeding, especially in the 1960s and 1970s, the productivity of the major crops increased faster than human population (Clay 2011).

Modern agriculture, using improved cultivars and good crop management resulted in increased yield in all crops (Green et al. 2005). These genetic gains were more relevant after the World War II, with the more ample adoption of improved cultivars. With the success of modern agriculture producing food in abundance and of high quality, the food prices dropped systematically along the last decades of the last century (Fuchs et al. 2009). The efficiency of agriculture

was accomplished by a huge investment in research (Vencovsky and Ramalho 2006; Duvick et al. 2004). However, its efficiency in producing food in abundance brought a toll on it, with society taking it for granted, and agriculture research funds shrunken. This is especially a concern with the new challenges ahead for agriculture considering population growth and global warming.

Although the population that goes hungry every night has declined over the last 50 years, there is evidence that famine may surge in the medium and long run. Currently, 925 million people do not have access to the United Nations WHO macronutrients (carbohydrates, proteins and lipids) intake recommendations (FAO 2001, 2002; FAO Global Perspective Studies Unit 2007; WHO/FAO 2003). About another billion people have the so-called occult hunger, that is, bad nutrition, with hypovitaminosis and deficiency of minerals (Evans 1998; Borém and Rios 2011). Furthermore, about another billion inhabitants are consuming food in excess, with risks of overweight, obesity, and diabetes, showing the problem of food distribution in the world (Lopes et al. 2010; Sim et al. 2007).

Nowadays, agriculture has different and huge challenges, which will be even bigger in the coming decades due to the population increase. Population is expected to stabilize around 2050, when, according to the United Nations FAO it will reach 9.1 billion people. It is surprising that even before you finish reading this sentence, there will be three new inhabitants in the world, that is, 1 person every 3 s, 259.000 everyday, and over 7 million per month. This is shocking if compared that it took several thousand years for the world to reach 2 billion people and just in the next 25 years another 2 billion will be brought to the earth. Moreover, people are living longer and also migrating to urban areas (United Nations 2009; USDA 2009). Presently, half of the world's population lives in towns and cities, but by 2050 more than 70 % of population will live in urban areas. At that time there will be 26 megalopolises with over 10 million inhabitants (Clay 2011; Beddington 2010).

The challenges for agriculture in the coming years, due to increase in population size and climate change, are often debated in governmental forums (UK Government 2011). One of the hottest issues is if food production will meet the world demand. On Table 1.1 are the current food production and the estimated needs for 2025. Besides the larger population and the improved economic situation of people, especially in the emerging countries, the competition between land for food and for bioenergy will bring additional stress to food production. Energy demand should increase about 45 % in the next 25 years, and certain areas presently allocated for food production will be allocated for energy crops (Beddington 2010). It must also be realized that about 70 % of soils fit for agriculture have been already chosen for other uses (Clay 2011). According to this author, all agricultural food systems must double its yields by 2050. Otherwise, the World demand will not be met and the Malthusians predictions may come about at this time.

Table 1.1 Present production and estimated demand for food and fiber (in millions of tons)

Product	Production in 2005	Estimated demand in 2025	Additional needed production
Grains	2.219	3.140	921
Oil crops	595	751	156
Perennial crops	243	322	70
Annual crops	352	438	86
Coffee	8	10	2
Fiber	28	36	8
Wood	3.402	4.148	746

Source Adapted de Assad and Pelegrino (2007)

Table 1.2 Maximum grain yield (ton.ha^{-1}) recorded or estimated for different species

Specie	Yield	Source
Rice	10,5 (15,9)[a]	Boyer (1987)
Corn	23,2	Duvick and Cassman (1999)
Wheat	14,1	Tollenaar and Lee (2002)
Sorghum	20,1	Ort and Long (2003)
Soybean	7,3 (22,5)[b]	Ort and Long (2003)

[a] Estimated yield in the function of solar radiation during the growing season (Peng et al. 1999)
[b] Estimated yield in the function of the photosynthetic efficiency (Specht et al. 1999)

1.2 Potential Yield of Crop Species

Before discussing about potential yield it is necessary to address the meaning of biological efficiency, a variable concept according to its end use. For example, the production of food, feed, and fiber depend on the conversion solar energy. So yield should refer to the unit of the product per unit of absorbed solar energy. Crop scientists and economists, among other professionals, like to refer to it on the area unit, that is, amount of the product (kg or ton) per area unit (acre, hectare). The efficiency is, therefore, a relative measurement and it varies according to the environment, with the cultivar, and especially with its variations. Thus, a proper biological index could help plant breeders in obtaining cultivars with better efficiency in using natural available resources.

Potential yield can be defined as the yield obtained when the cultivar is grown with no environmental restrictions, that is, no biotic or abiotic stresses. In this condition, soil nutrients and water are not limiting factors and pests or weeds are effectively controlled (Evans and Fisher 1999). In general, it is difficult, or even impossible, to meet of those criteria to obtain the potential yield. There are reports in the literature about maximum productivity for many crop species. It can also be found in the literature yield potential in function of solar radiation. Those estimates are far beyond the maximum reported yield in commercial field conditions (Table 1.2). A crop yield potential is much larger than the biologic efficiency per area, that is, yield in ton.ha^{-1}, an index commonly used by crop scientists and

farmers. Therefore, the genetic yield potential is much larger than yield recorded in any conditions. That fact occurs due to several environmental influences and management practices that negatively affect the crop performance, called stresses. If any of those factors reduces the biological efficiency it will reflect on the economical yield.

1.3 Biotic Stresses in Agriculture

The plant breeding success, especially in the last century (Denardi and Camilo 1998; Paterniani 1990), was due to selection for individuals with resistance or tolerance to stresses, instead of selection for higher yield potential, and most plant breeders expect this strategy will continue to be the focus of the breeding programs in the future (Tollenaar and Lee 2002).

The biotic stresses are one of the major causes of yield reduction on farmer's fields in most crops. Frequently, one can find reports of losses of up to 100 % of the yield. The most outstanding case of biotic stress in food insecurity occurred in Europe in 1845, especially in Ireland and England, when about 80 % of potato fields were lost due to *Phytophthora infestans*, etiological agent for potato blight. Due to this disaster more than 2 million people died hungry and many other migrated to other regions. Another case of an economic catastrophe was with the Corn Leaf Blight in the corn fields in the 1970s, when most farmers had significant losses due to susceptibility to *Helminthosporium maydis*.

Recently, some studies have shown that Soybean Asian Rust, in Brazil, was responsible for 37–67 % of yield reduction (Kumudini et al. 2008). This disease, from 2006 to 2011, caused US $4 billion income loss for soybean growers in Brazil. In Asia, the soybean losses due to this disease reached up to 80 % (Miles et al. 2003). Should a resistant cultivar be available, a large amount of fungicides with economical and environmental negative effects would not be need to be deployed in the soybean fields.

The spectrum of biotic stresses that may cause crop yield losses is large and diverse. For example, for common beans, Vieira (1983) reports over 45 virus, bacteria, fungi, and nematodes that may reduce crop yield in different regions and situations. Plant breeders have accomplished important success in developing biotic stress-resistant cultivars over the years. The introgression of disease resistance alleles has a stabilizing crop production from season to season. Protected from pests, cultivars can show most of its yield potential.

To complicate, most of the plant pathogens can present pathogenic races or biotypes. This poses an additional hurdle to breeding programs, once a new cultivar resistant to a specific pathogen race may be susceptible to others races. Therefore, when a pathogen race has a mutation and a new race emerges plant breeders have to initiate a new breeding effort to develop a resistant cultivar in an endless battle against the pathogen. Another problem is the shift of prevalent

Table 1.3 Estimated reduction of common bean yield due to diseases

Pathogen	Yield reduction (%)	Reference
Colletotrichum lindemuthianum	55	Vieira (1964)
Meloidogyne sp.	67	Freire and Ferraz (1977)
Phaeoisariopsis griseola	1–41	Santos Filho et al. (1978)
Uromycesphaseoli var. *typical*	21–42	Nasser (1976)
Bean golden mosaic virus	43–73	Vieira (1964)
Bean golden mosaic virus	100	Vieira (1964)

existing pathogenic races in a region, since they may also reduce the life span of a resistant cultivar that after a few growing seasons become susceptible.

Table 1.3 presents estimates of yield reduction in common bean, caused by several pathogens. Those estimates show the economic importance of diseases in food production, especially when large growing areas are considered. Thus, the development of disease-resistant cultivars has been a priority in many breeding programs.

It must also be recognized that the number of insects that causes yield reduction is large, including those that attack the crops during the growing season, feeding on leaves, pods, fruits, and roots. An additional class of insects that causes losses feeding on the harvested crop, like borer, weevils among others exists and causes significant food loss.

Historical evidences show that biotic stresses occur, in high or low intensity, in just all agricultural areas around the world. In some areas, the stresses caused by pests and weeds may not be relevant in a specific year, but they bounce back in the following years. Additionally, climate change is bringing new pests and weeds to relevance in crop production, especially in the tropical regions.

The predictions by the Intergovernmental Panel on Climate Change (IPCC 2007) gave birth to several speculations of what one could expect in the coming decades. What drew more concern from society was food production and food security globally, as well as the agribusiness economic losses (Assad and Pelegrino 2007). Several simulations showed apprehensive scenarios and many governments are concerned about their food security (Lobell et al. 2008; Buntgen et al. 2011).

Overall, global warming should bring larger incidence of insects, diseases, and weeds on farms around the world. Some biotic stresses that have been considered secondary in many crops until now will assume major relevance with climate change. An example is Angular Leaf Spot (*Pseudocercospora griseola*), which were considered a secondary disease of minor importance in most common bean growing areas in Brazil. But in the last years that changed and this disease is now one of the major biotic stresses for this crop.

Breeding efforts for developing insect-resistant cultivars have not been as effective as for disease resistance. However, some insect-resistant cultivars have been developed over the years, as against to *Empoasca kraemer*, *Diabrotica speciosa* and for post-harvest insects, as against to *Acanthoscelides obtectus* and *Zabrotes subfasciatus*. With the arrival of biotechnology, the development of insect-resistant cultivars has been one of the most active areas of research and with good success. For example, Bt cultivars resistant to Lepidoptera are a great success

around the world and have contributed to the reduction of insecticide use in cotton, corn, soybean, and other species. Those resistant cultivars are a good example of achievement in breeding for biotic stress resistance.

It should also be realized that for a good crop, weeds must be controlled. Weeds can be defined as any species growing where it causes losses to the crop grown in that area.

Most large farmers use chemical weed control around the world, since it is efficient and has a competitive cost, when compared to other weed control methods. However, some farmers, especially in developing countries, have a short sight and are focused on immediate profit, using the same herbicide season after season. As it is well known, the use of a same herbicide on an area for several years will result in the selection of weed-resistant biotypes (Powles and Shaner 2001). As a consequence, the population of herbicide-resistant weeds has grown worldwide, becoming an agricultural problem for many farmers. One of the possible contributions of plant breeding to weed management is through allelopathy, the ability of a plant to produce chemical substances that affect other plants in a favorable or on an unfavorable manner, when released on the environment (Wu et al. 1998). The objective of most of those breeding programs target inhibiting weed germination or affect its growth.

Many breeding programs using biotechnology are developing cultivars tolerant to herbicides, such as soybean, corn, cotton, and colza tolerant to glyphosate; corn tolerant to imazaquin; and rice and soybean tolerant to ammonium glufosinate. The possibility to grow corn, cotton, sugarcane, and soybean free of weeds has been very attractive to most farmers around the world, especially due to its economic benefits.

The contribution of plant breeding throughout history in helping agriculture to produce food, feed, fiber, and fuel is very well documented in the scientific literature (Vencovsky and Ramalho 2006; Duvick et al. 2004). However, what will happen in the coming decades with the new challenging scenario will demand from breeders new and more efficient strategies to help agriculture solve the main challenge to humanity—food security (Costa 1974). The objective of this book was to collect and bring to its readers the most recent scientific achievements and the state of the art in breeding for biotic stresses, guiding breeders on their decisions and priority taking in their programs. In the following chapters, the reader will find the most relevant information to breed for fungus, bacterium, virus, nematode, and insect resistance and for weed management. In the other book *Plant Breeding for Abiotic Stress Tolerance* (Fritsche-Neto and Borém 2011); analogous aspects to abiotic stresses are addressed.

1.4 Perspectives

The United Nations estimates that around 2050 the world population will stabilize nearby 9.1 billion people. To make it even worse about 70 % of that population will be living in urban areas. At that time there will be 2–3 billion people with *per capita* income three times higher than presently, consuming twice as much as

today. Consequently, it is clear that the food demand will continue to increase strongly in the coming decades.

Furthermore, there will be the negative effects of global warming/climate change. In this new setting the biotic stresses on crops will exacerbate. Consequently, the current knowledge about insect, disease, and weed management will be defied, requiring from plant scientists and especially from plant breeders new strategies, deep commitment, and hard- and interdisciplinary-work to develop biotic stress-resistant cultivars.

In the coming chapters, knowledgeable experts present the most recent advances in breeding methodologies, plant germplasm, and molecular biology applied to develop cultivars for different situations of biotic stresses.

References

Alexandratos N (2006) World agriculture: towards 2030/50, interim report. An FAO perspective. FAO, Rome

Assad ED, Pelegrino GO (2007) O clima e a potência ambiental. Agroanalysis 27(9):E3–E5

Barrett CB (2002) Food security and food assistance programs. In: Gardner B, Rausser G (eds) Handbook of agricultural economics. Elsevier Science, Amsterdam

Beddington I (2010) Food security: contributions from science to a new and greener revolution. Phil Trans R Soc 365:61–71

Borém A, Almeida GD (2011) Plantas geneticamente modificadas: desafios e oportunidades para regiões tropicais. Suprema, Visconde de Rio Branco, p 390p

Borém A, Rios SA (2011) Milho biofortificado. Suprema, Visconde de Rio Branco, p 211p

Boyer EL (1987) College: The Undergraduate Experience in America. New York: Harper & Row

Bruinsma J (2003) World agriculture: towards 2015/2030. Summary report. FAO, Rome

Buntgen U et al (2011) 2500 years of European climate variability and human susceptibility. Sci Express 331:578–582. 13 Jan 2011, p 1–4

Clay J (2011) Freeze the footprint of food. Nature 475(7356): 287–289 (online)

Costa CP (1974) Cenoura Nacional, em germoplasma para as condições de dias curtos nas regiões tropicais e sub-tropicais. Relatório científico do Departamento de Genética. ESALQ/USP. No 8, p 50–53

Demeke M, Pangrazio G, Maetz M (2008) Country responses to the food security crisis: nature and preliminary implications of the policies pursued. FAO, Rome

Denardi F, Camilo AP (1998) Estratégia do Melhoramento Genético da Macieira na EPAGRI Santa Catarina SA. Anais do II Simpósio sobre atualizações em Genética e Melhoramento de Plantas. UFLA, 1998, p 123–132

Duvick DN, Cassman KG (1999) Post-green revolution trends in yield potential of temperature maize in the North-Central United States. Crop Sci 39:1622–1630

Duvick DN, Smith JSC, Cooper M (2004) Long term selection in a commercial hybrid maize breeding program. In: Janick I (ed) Plant breeding reviews. Part. 2. vol 24, pp 109–152

Evans L (1998) Feeding the ten billion: plants and population growth. Cambridge University Press, Cambridge

Evans LT, Fisher RA (1999) Yield potential: its definition measurement and significance. Crop Sci 39:1544–1551

FAO (2001) State of food insecurity 2001. FAO, Rome

FAO (2002) World agriculture: towards 2015/2030. FAO, Rome

FAO (2009) How to Feed the World in 2050. FAO, Rome

FAO (2010) The state of food insecurity in the world 2010. FAO, Rome
FAO Global Perspective Studies Unit (2007) State of food and agriculture 2007. FAO, Roma
Freire FCO, Ferraz S (1977) Nematóides associados ao feijoeiro, na Zona da Mata, Minas Gerais, e efeitos do parasitismo de Meloidogyne incognita e M. javanica sobre o cultivar "Rico 23". Revista Ceres 24:141–149
Fritsche-Neto R, Borém A (2011) Melhoramento de plantas para condições de estresses abióticos. Suprema, Visconde do Rio Branco, p 248
Fuchs D, Kalfagianni J, Artenson M (2009) Retail power, private standards, and sustainability in the global food system. In: Clapp J, Fuchs D, (eds) Corporate power in global agrifood governance. MIT Press, Cambridge
Green R, Cornell S, Scharlemann J, Balmford A (2005) The future of farming and conservation: response. Science 308:1257
Haddad L, Frankenberger T (2003) Integrating relief and development to accelerate reductions in food insecurity in shock-prone areas. Occasional Paper No. 2. USAID/Office of Food for Peace, Washington
Haddad L, Lindstrom J, Pinto Y (2010) The sorry state of M&E in agriculture: can people centered approaches help? IDS Bulletin 41.6. IDS, Brighton
IPCC (2007) Climate change—the physical sciences basis: summary for policymakers. Intergovernmental Panel on Climate Change, Genebra 18
Kumudini S, Godoy CV, Board JE, Omielan J, Tollenaar M (2008) Mechanisms involved in soybean rust-induced yield reduction. Crop Sci 48:2334–2342
Lobell D, Burke MB, Tebaldi C, Mastrawdrea MD, Falcon WP, Naylor RL (2008) Prioritizing climate chance adaptation needs for food security in 2030. Science 319:607–610
Lopes AS, Daher E, Bastos ARR, Guilherme LRG (2010) Suprimento e extensão das reservas de nutrientes no Brasil. In: Prochnow LI, Casarin V, Stipp SR (Orgs) Boas práticas para uso eficiente de fertilizantes. 1 edn. vol 2. IPNI, Piracicaba, p 283–307
Miles MR, Frederick RD, Hartman GL (2003) Soybean rust: is the U.S. soybean crop at risk? Available at http://www.apsnet.org/online/feature/rust/.Acessadoem12/07/2011
NASSER LCB (1976) Efeito da ferrugem em diferentes estádios de desenvolvimento do feijão e dispersão de esporos de Uromyces phaseoli var. typica (Arth.). Viçosa: UFV, p 79
Ort DR, Long SP (2003) Converting solar energy into crop production. In: Chrispeels MJ, Sadava DE (eds) Plants genes and crop biotechnology. 2nd edn. Jones and Bartlett Publishers International, Londres, p 240–269
Paterniani E (1990) Breeding in the tropics critical. Rev Plant Sci 9(2):125–154
Peng S, Cassman KG, Virmani SS, Sheehy I, Khush GS (1999) Yield potential trends of tropical rice since the release of 1 R8 and the challenge of increasing rice yield potential. Crop Sci 39:1552–1559
Powles SB, Shaner DL (2001) Hebicide resistance and world grains. CRC-Press, Boca Raton 43p
Santos Filho HP, Ferraz S, Sediyama CS (1978) Influência da época de inoculação de Phaeoisariopsis griseola Sacc. sobre três cultivares de feijoeiro. Fitopatologia Brasileira 3:175–180
Sim S, Barry M, Clift R, Cowell SJ (2007) The relative importance of transport in determining an appropriate sustainability strategy for food sourcing. Int J LCA 12:422–431
Specht JE, Hume DJ, Kumudini SV (1999) Soybean yield potential. A genetic and physiological perspective. Crop Sci 39:1560–1570
Tollenaar M, Lee EA (2002) Yield potential, yield stability and stress tolerance in maize. Field Crops Res 75:161–169
UK Government (2011) Foresight project on global food and farming futures: trends in food demand and production. p 39
United Nations (2009) The millennium development goals report. United Nations, New York
USDA (2009) USDA agricultural projections to 2018. USDA, Washington
Van Dam R, Seidell JC (2007) Carbohydrate intake and obesity. Eur J Clin Nutr 61:S75–S99
Vencovsky R, Ramalho MAP (2006) Contribuições do melhoramento genético no Brasil. In: Paterniani E (Org) Ciência, agricultura e sociedade. 1 edn. EMBRAPA, Brasília, pp 41–74

WHO/FAO (2003) Diet, nutrition and the prevention of chronic diseases. WHO, Geneva
Vieira C (1983) Doenças e pragas do feijoeiro. UFV, Imp. univ., Viçosa, pp 85–96
Vieira C (1964) Melhoramento do feijoeiro (Phaseolus vulgaris L.) no Estado de Minas Gerais. Experientiae 4:1–68
Wright J (2010) Feeding nine billion in a low emissions economy—simple, though not easy. A Review for the Overseas Development Institute, London
Wu H, Pratley J, Lemerle D, Haig T, Verbeek B (1998) Differential allelopathic potential among wheat accessions to annual ryegrass. In: Michalk DL, Pratley JE (ed) 9th Australian Agronomy Conference, Charles Sturt University, Anais WaggaWagga, pp 567–571

Chapter 2
Breeding for Fungus Resistance

Arione da S. Pereira, Cesar Bauer Gomes, Caroline Marques Castro and Giovani Olegario da Silva

Abstract Late blight, caused by the oomycete *Phytophthora infestans*, is a serious disease in potato and tomato crops throughout the world. It cuts yields by destroying leaves and rotting tubers during growth, development, and storage. Under favorable weather conditions, late blight is capable of destroying a potato crop in a matter of days. The development of potato varieties resistant and acceptable in the market could offer a number of advantages on the control of the disease. This chapter discusses breeding for resistance to *P. infestans*, but the majority of examples and strategies mentioned in relation to this pathogen can be applied to breeding for resistance to fungi in general. First, the aspects of plant physiological responses, pathogen-host interaction, vertical x horizontal resistance, co-evolution of the pathogen, and the host wild species are reviewed. Then, the germplasm and genetic variability, inheritance of resistance, trait relationships, stress induction and intensity, and duration of the disease, strategy and selection methods, and biotechnology applied to the breeding for fungus resistance are discussed. Also, considerations about the effects of the possible climate change on plant responses to the disease are made. Finally, closing remarks of the chapter are presented.

A. da S. Pereira (✉) · C. B. Gomes · C. M. Castro
Embrapa Clima Temperado, C. Postal 403Pelotas, RS 96010-971, Brazil
e-mail: arione.pereira@cpact.embrapa.br

C. B. Gomes
e-mail: cesar.bauer@cpact.embrapa.br

C. M. Castro
e-mail: caroline.castro@cpact.embrapa.br

G. O. da Silva
Embrapa Hortaliças/SPM, C. Postal 317Canoinhas, SC 89460-000, Brazil
e-mail: olegario@cnph.embrapa.br

R. Fritsche-Neto and A. Borém (eds.), *Plant Breeding for Biotic Stress Resistance*,
DOI: 10.1007/978-3-642-33087-2_2, © Springer-Verlag Berlin Heidelberg 2012

Keywords Plant breeding · Disease resistance · Oomycete resistance · *Phytophthora infestans* · Potato improvement

2.1 Introduction

The Fungi kingdom is a large group of eukaryotic organisms whose members, fungi, include microorganisms such as yeast, mold, and mildew, as well as the more familiar mushrooms.

Fungi are classified into a separate kingdom from plants, animals, and bacteria. One big difference is that the cell walls of fungi contain chitin, in contrast to plant cell walls that contain cellulose. These and other differences show that fungi form a unique group of interrelated organisms, named the Eumycota (true fungi or Eumycetes), which share a common ancestor (a monophyletic group). Organisms in this kingdom are distinct from oomycetes and myxomycetes (now classified as Mixogastria), which are structurally similar.

Oomycetes are characterized as microorganisms with a similar morphology to fungi, but in terms of taxonomy, the species is related to the Stramenopila kingdom, phylum Oomycota, class Oomycetes, order Pythiales, family Pythiaceae. They differ from true fungi insofar as their cell walls contain cellulose and they have diploid mycelium in part of their life cycle, biflagellate spores, differentiated DNA sequences, and other characteristics (Alexopoulos et al. 1996; Kroon et al. 2004).

It is estimated that 70 % of major plant diseases are caused by fungi, oomycetes, and myxomycetes—microscopic organisms that produce enormous quantities of spores rapidly propagated by wind, water, insects, and animals. An infected plant can release up to 100 million spores, causing secondary infections as it quickly degrades plant cells and simultaneously produces toxins that interfere with plant biological operation. These pathogens are extremely difficult to eliminate because they can remain dormant in the soil, in decomposing vegetation, or on a healthy plant, waiting for perfect climatic conditions to continue contamination.

This chapter discusses plant breeding for resistance to oomycetes, based on the example of the pathogen *Phytophthora infestans*, the agent that causes late blight in potatoes. The majority of examples and strategies mentioned in relation to this pathogen can be applied to breeding for resistance to fungi in general.

Late blight, caused by the oomycete *P. infestans* (Mont.) de Bary, is still a serious disease in potato and tomato crops throughout the world. It cuts yields by destroying leaves and rotting tubers during growth, development, and storage (Hooker 1981). It is capable of completing an asexual cycle, from infection to the production of sporangia, in less than 5 days, and the sporangia can be washed off the leaves and fall onto the soil where their spores subsequently infect tubers (Fry and Goodwin 1997). Under favorable weather conditions, with high relative humidity and at temperatures ranging from 15 °C to around 21 °C (Henfling 1987;

Fig. 2.1 Crop devastated by late blight. Photo: Arione da Silva Pereira

Turkensteen 1996), late blight is capable of destroying a potato crop in a matter of days (Fig. 2.1), resulting in total loss of the crop unless control measures are implemented correctly (Mizubuti and Fry 2006).

In the management of this disease, the main forms of control are based on the use of fungicides and resistant cultivars (Reis et al. 1999). The most widely used practice for controlling late blight in potatoes is the application of fungicides (Sherwood et al. 2001). A number of treatments are required to effectively control the disease. In Brazil, it is usual to begin spraying preventive fungicides for late blight as soon as the first leaves on the potato plant begin to expand, and the fungicide treatment is continued at intervals of 3–5 days until the end of the plant development cycle (Nazareno et al. 1999). There are reports of up to 30 fungicide spray applications during a single productive cycle (Nazareno et al. 1995). According to the International Potato Center (CIP 1996), annual spending worldwide on fungicides for protecting the potato crop stands at around US$ 1 billion. In addition to the cost, the strategy of using fungicides also presents a risk to human health and the environment. Even so, crop losses due to late blight are estimated at 15 % of total global annual potato yield. For small producers, the losses are usually higher, since they cannot afford to buy fungicides and get technical assistance.

Varietal resistance is considered potentially more effective and environmentally sustainable for cutting losses caused by late blight. However, the majority of potato cultivars are very susceptible to the disease, and resistant varieties do not usually produce commercially viable tubers. Therefore, the availability of late blight-resistant cultivars acceptable in the market could offer a number of

advantages, including a considerable drop in potato production costs, increased productivity in areas where fewer inputs are used, more environmentally sustainable production and lower farm worker exposure to fungicides, as well as improving the image of the potato crop and enhancing food safety.

2.2 Plant Physiological Responses

Plant resistance generally depends on the activation of defenses against infection by the pathogen. When the plant's defense responses block the development of the pathogen, the plant–pathogen interaction is referred to as incompatible. The genes of the pathogen that mediate recognition by and activation of the host's defense responses, causing incompatible interactions, are called avirulence genes (Bisognin et al. 2005). The incompatible interactions are generally associated with a hypersensitive response in the host and a high degree of specificity between the pathogen and host genotypes. *P. infestans* and potato cultivars interact in accordance with the genebygene model (Lee et al. 2001).

Phytophthora infestans is a heterothallic species, which means that it requires the interaction of two different thalli, denoted compatibility groups A1 and A2, to reproduce sexually (Luz et al. 2001). This happens when two individuals from the two groups interact to form gametangia that cross to produce an oospore (Fig. 2.2), a resistant sexual spore that survives in the soil and in crop residues. According to Reis et al. (2003), the two compatibility groups are present in Brazil, where isolates of group A1 were reported mainly in tomatoes, and isolates of group A2 exclusively attack potatoes. Santana (2006) subsequently confirmed the predominance of A2 isolates in the potato crops of Southern Brazil, except in the State of Rio Grande do Sul, where isolates of both A1 and A2 were found in similar proportions, suggesting the occurrence of recombinant populations of the pathogen. Recently, during a study of the isolates of *P. infestans* in potato collected in the southwest and south of Brazil over the periods 1998–2000, 2003–2005, and 2008–2010, Oliveira (2010) detected a change in the pathogen population associated with potato over the years. Populations of A1 and A2 of *P. infestans* were detected in a proportion of 1:3 (A1:A2) in the States of Minas Gerais and São Paulo. These discoveries stepped up the need for Brazilian potato breeding programs to develop and select clones with high levels of durable, quantitative resistance (Gomes et al. 2009).

2.3 Pathogen–Host Interaction

The mechanisms controlling potato susceptibility to late blight are complex and leaves and tubers can use different biochemical processes. Furthermore, there may be structural differences in the canopy, anatomical variations in the leaves, and differing race maturation rates (Kirk et al. 2001).

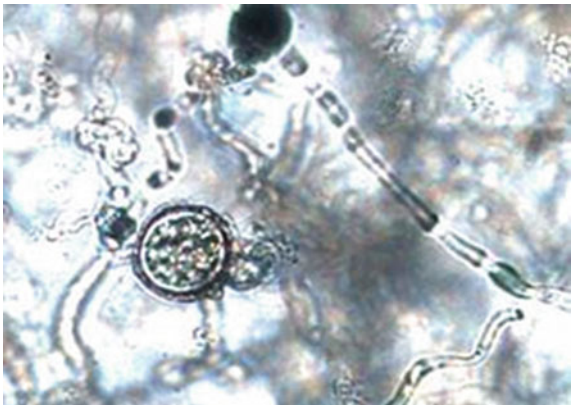

Fig. 2.2 Oospore formed on agar in the presence of the two *P. infestans* compatibility types, A1 and A2. Photo: Flávio Martins Santana

Incompatibility interactions between pathogen and host are generally associated with a hypersensitivity response in the host and a high degree of specificity between pathogen and host genotypes. Hypersensitive genotypes are usually characterized by a rapid necrotic response in the cells attacked, resulting from the reaction to the penetration of oomycete hyphae and causing rapid cell death at the location attacked. This type of resistance is under the direct control of a set of major genes (*R* genes) triggered by a distinct pathogen race. A total of 11 *R* genes, all from *Solanum demissum*, have been characterized in potato (Colton et al. 2006). These 11 *R* genes suggest the presence of 11 factors corresponding to virulence or avirulence in *P. infestans*. The interaction between *P. infestans* and potato cultivars corresponds to the gene-by-gene model (Lee et al. 2001), a fact confirmed by genetic analysis of both host and pathogen (Song et al. 2003).

2.4 Vertical and Horizontal Resistance

Two kinds of resistance to *P. infestans* have been identified in potato (Umaerus and Umaerus 1994; Wastie 1991). Vertical resistance (qualitative, specific resistance, or hypersensitivity) is usually monogenic and effective only for a subset of pathogen races. Horizontal resistance (quantitative, non-specific, or general resistance) is partial or polygenic and thought to be effective against all races of the pathogen. Therefore, potato cultivars with vertical resistance could favor rapid development of an epidemic in the presence of new virulent races of the pathogen in the field, in contrast to cultivars with horizontal resistance, where development of the disease is slower and pathogen resistance more durable (Colon et al. 1995; Turkensteen 1993).

Vertical resistance is controlled by a set of major genes (*R* genes), triggered by a distinct race of the pathogen. However, the monogenic resistance controlled by these genes in potato is easily overcome when new races of *P. infestans* arise (Stewart et al. 2003). Many of the eleven *R* genes have been introduced into

commercial cultivars (Ross 1986), but compatible races of *P. infestans* developed rapidly for all of them. This rapid neutralization of qualitative or vertical resistance due to changes in the pathogen population (Wastie 1991) encouraged researchers to concentrate their efforts to fortify late blight-resistant potatoes on producing cultivars without these *R* genes. Analyzing the complexity of the isolates of *P. infestans* from all the states in Southern Brazil, it was verified that the majority of isolates exhibited complex racial characteristics, expressing almost all the *Avr* genes known for *P. infestans,* compared to a group of eight isolates that did not express at least five of these *Avr* genes (Santana 2006). However, of the eight isolates with the strongest virulence factors in this study, seven were from Rio Grande do Sul, suggesting that new populations of high genetic complexity were occurring. Furthermore, according to the same author, the avirulence genes less frequently observed were *Avr*4 and *Avr*3, which is in line with the observations of Reis (2001), who verified that these genes were being supplanted at a higher frequency in the isolates evaluated.

Horizontal resistance is the degree of resistance exhibited by a plant to all races of a pathogen such as *P. infestans* (Bradshaw 2009), in other words, resistance not involving *R* genes. This kind of resistance has been considered fundamental in protecting new cultivars against late blight, since it is effective against all pathogen variants and is therefore more stable and durable (Landeo et al. 2000). Various specific resistance components help reduce the severity of the disease, varying from one species to another (Colon et al. 1995) and within the same species (Cañizares and Forbes 1995). Infection efficiency, latency, and lesion growth rate are important in *S. microdontum,* whereas infection efficiency, lesion growth rate, and sporulation capacity are important in *S. tuberosum*. Although they can be overcome, qualitative resistance genes can also contribute to quantitative resistance (Stewart and Bradshaw 2001).

The efficiency of quantitative resistance against different pathogen populations has been demonstrated in Europe and North America (Turkensteen 1993; Inglis et al. 1996). In both cases, the authors showed that the resistance rankings of many established cultivars has remained constant over time, although pathogen populations have changed (Fry and Goodwin 1997). Durable resistance has been observed in a number of cultivars from Mexico exposed to a sexual population of the pathogen for over 40 years in Mexico itself (Grunwald et al. 2002), and stability has been verified in a study conducted over an extensive range of environments throughout the world (Forbes et al. 2005).

2.5 Co-evolution

It is widely acknowledged that the oomycete, *P. infestans,* originated and co-evolved in wild *Solanum* species, which produce tubers and are native to the central plateau of Mexico, where the pathogen exhibits greater genetic variability (Niederhauser 1991).

Up to the end of the 1980s, the two compatibility groups of *P. infestans* were found only on the Mexican central plateau (Gomez-Alpizar et al. 2007). In other regions of the world, the US-1 clonal strain was predominant in the oomycete population, and isolates belonged to compatibility group A1 (Goodwin et al. 1994), which is unique and therefore reproduced only asexually.

During the 1980s, isolates of compatibility group A2 were observed in Europe (Hohl and Iselin 1984) and various new alleles were detected (Drenth et al. 1993). But by the end of this decade, new genotypes had also been detected in North America (Deahl et al. 1991), and they consistently exhibited differentiated responses to the fungicide metalaxyl, with a simultaneous increase in the diversity, incidence, and severity of the disease in the majority of producer regions in the United States and Canada (Goodwin et al. 1994). New populations were found not just in Europe and North America but also in Africa, Asia, and South America, including Brazil (Forbes and Landeo 2006; Reis et al. 2003; Santana 2006), suggesting that distribution was global.

The simultaneous occurrence of isolates of both compatibility groups (A1 and A2) favors sexual reproduction of *P. infestans* and the occurrence of recombinants which can exhibit characteristics for greater adaptability, such as greater aggressivity and virulence, as well as fungicide resistance, making late blight more difficult to control (Goodwin 1997). In fact, new pathogen variants more aggressive than those found so far have been observed in North America and Europe (Gavino et al. 2000).

The diversity of the pathogen population in Europe and the characteristics of isolates collected in the field in the United States are evidence of sexual reproduction (Drenth 1994; Flier et al. 2003). In Brazil, populations of *P. infestans* have been showing a differentiated genetic pattern over the last ten years. According to a survey conducted by Reis (2001) at the beginning of this century, Brazilian populations are predominantly characterized as BR-1 in potato and US-1 in tomato, maintaining a clonal structure with no crossings of BR-1 (A2) and US-1 (A1). In a later survey conducted in Southern Brazil by Santana (2006), observations showed that in the states of Paraná and Santa Catarina populations in tomato continued to be clonal (US-1), whereas in potato, in addition to typical BR1 (A2) populations in Santa Catarina and Paraná, the majority of isolates in Rio Grande do Sul and one isolate in Paraná exhibited a PE-3 pattern, with the majority in compatibility group A1. In a more recent study in Brazil (Oliveira 2010), in addition to the patterns most often associated with tomato and potato populations, the occurrence of variations of A2 isolate with a diverse mtDNA pattern was also reported, lending weight to the hypothesis that new strains are occurring in potato crops. However, so far there is no proof of natural hybridization between the two compatibility groups.

The rapid, global resurgence of *P. infestans,* combined with the replacement of the established late blight by a new, genetically more variable populations in many parts of the world, show just how adaptable this pathogen is. Sequenced genome analysis of *P. infestans* has revealed a large intergenic region consisting of repetitive sequences that flank the effectors (Haas et al. 2009). According to these

authors, this is perhaps how *P. infestans* is able to adapt rapidly to new forms of resistance by evolving new effectors. A further concern relating to the possibility of sexual reproduction is the formation of oospores as a persistent source for inoculating the soil to survive periods in which the host plants are absent. This ability to survive absence of the host, combined with new and more aggressive races, presents an enormous threat to potato crops throughout the world.

2.6 Germplasm and Genetic Variability

If we are to make progress in developing new cultivars, access to exploitable genetic variability in the germplasm of the species is fundamental. The process of genetic improvement is highly dependent on the amplitude of the genetic base available (Queiroz and Lopes 2007). Breeding programs for the main crop species make use of wild germplasm to identify sources of resistance to biotic and abiotic stress (Nass et al. 2007).

Among cultivated plants, there is probably no other species that has a richer wild parentage tha, the potato. Some 196 wild potato species have been identified between latitudes 38°N and 41°S, from the southwest of the United States to central Argentina and Chile, (Spooner and Hijmans 2001). This wide diversity of habitats over which the potato is distributed provides an extensive source genetic resources for incorporating resistance to biotic and abiotic stress.

The wild germplasm of *Solanum* represents an enormous pool of genetic diversity for disease resistance genes. On evaluating 90 wild species of the genus *Solanum* for resistance to *P. infestans*, *Globodera pallida* and the PVY, PLRV, PVM, and PVS potato viruses, Ruiz et al. (1998) observed that over 70 % of the species examined expressed resistance to one or more of these diseases. Wild potato species were also evaluated for resistance to *Fusarium sambucinum*. Nine species evaluated (*S. boliviense*, *S. gourlayi*, *S. microdontum*, *S. sancta-rosae*, *S. kurtzianum*, *S. fendleri*, *S. gandarillasii*, *S. oplocense* , and *S. vidaurrei*) showed resistance to the fungus (Lynch et al. 2003).

However, because *P. infestans* is so important to the potato crop, more studies have been conducted on this pathogen. Since the Great Potato Famine caused by late blight in Europe in the 1840s, the species *Solanum demissum* has provided an extensive source of resistance to *P. infestans* for breeding programs. However, the resistance conferred by *S. demissum,* based on specific race resistance genes *R1-R11,* is characterized by the fact that it is easily overcome by new races of the pathogen. As races of *P. infestans* gradually neutralized the resistance of *S. demissum*, other species of the genus *Solanum* were studied. A wide range of wild species has been identified as potential sources of a considerable number of *R* genes that could be more durable than the genes of *S. demissum*. *R* genes have been discovered in *S. berthaultii* (*RPi-ber*), mapped on chromosome X (Ewing et al. 2000; Rauscher et al. 2006), in *S. pinnatisectum* (*Rpi1*) mapped on chromosome VII (Kuhl et al. 2001), in *S. mochiquense* (*Rpimoc1*) mapped on chromosome IX (Smilde et al. 2005), and in *S. phureja* (*Rpi-phu1*) mapped on chromosome IX (Sliwka et al. 2006).

Genes for resistance to *P. infestans* have also been identified in *S. paucissectum* (Villamon et al. 2005) and *S. stoloniferum* (Foster et al. 2009). In *S. microdontum,* a more effective *Quantitative Trait Locus*(QTL) has been identified (Bisognin et al. 2005), and a number of resistance genes have been identified in *S. bulbocastanum*. Two alleles have been found on chromosome VIII at a single locus: *RB* (Song et al. 2003) and *Rpi-blb1* (van der Vossen et al. 2003). On chromosome VI, *Rpiblb2* (van der Vossen et al. 2005) has been identified and on chromosome IV, *Rpi-blb3* (Park et al. 2005). Among the numerous wild species evaluated, greater resistance to *P. infestans* has been found in accessions introduced into Mexico as opposed to those introduced into South America (Douches et al. 2001). Although new races of the pathogen are known to rapidly overcome resistance conferred by race-specific resistance genes *R1-R11*, many potato cultivars that contain the *R* genes of *S. demissum* maintain a higher level of field resistance than genotypes lacking these genes (Gebhardt et al. 2004; Stewart et al. 2003; Trognitz and Trognitz 2007), highlighting the importance of this germplasm.

2.7 Inheritance

Breeding crop potato *Solanum tuberosum* ssp. *tuberosum* (2n = 4x = 48) for resistance to late blight, begun after the Great Potato Famine in Europe of the 1840s, was based on hexaploid source *S. demissum* (2n = 6x = 72), as well as other tuberous *Solanum* species. The vertical resistance controlled by the *R* genes (*R*1–*R*11) was effective against late blight until the development of the corresponding avirulence gene in *P. infestans*. *R* genes confer a hypersensitive response, preventing the late blight pathogen from infecting the plant, until a pathogen with no avirulence genes or a mutated avirulence gene arises so that it is not recognized when the spores germinate in the cells of the 'resistant' variety, which then becomes susceptible to late blight (Colton et al. 2006).

To study the inheritance of quantitative traits and identify superior parents for breeding, progeny evaluation has been proposed (Bradshaw and Mackay 1994). Quantitative, non-specific resistance to late blight in potato is characterized by ongoing phenotypic variation and complex polygenic inheritance, which makes breeding for this trait fairly difficult (Umaerus 1970). Relatively high levels of field resistance to late blight and high estimated heritability (broad- and narrow-sense) were verified in a diploid population of clonal potato families, considered to have no *R* genes and derived from *S. phureja* × *S. stenotomum* (Haynes and Christ 1999).

The uncertainty over the number of genes involved (Simko 2002) and the impossibility or inconclusive results of evaluating a non-specific race (Johnson 1979) has frustrated attempts to breed for this type of resistance to late blight.

Resistance to late blight based on *R* genes can be differentiated from quantitative resistance by studying the combining capacity of plantlets evaluated in the greenhouse. *R* genes tend to increase the specific combining capacity, and general

combining capacity increases for parents with quantitative resistance when compatible virulence genes are present in the population of *P. infestans* (Bradshaw et al. 1995). A significant correlation was observed between the average resistance of the parents and general combining capacity, but was not significant for the resistance response between leaf and tuber (Stewart et al. 1992).

2.8 Trait Relationships

Resistance to *P. infestans* often shows an undesirable link with the late development cycle of potato plants (Umaerus and Umaerus 1994). A positive correlation has been reported between the level of late blight resistance in the field and late maturity and photoperiod sensitivity in potato (Colon et al. 1995; van der Vossen et al. 2005). The presence of separate loci for the two traits seems improbable, since potato breeders have unsuccessfully tried for decades to combine late blight resistance with early leaf maturity (Muskens and Allefs 2002). Molecular marker-assisted studies have also confirmed the link between the two traits, revealing that all the loci for the type of plant development cycle are coincident with loci for resistance to late blight (Collins et al. 1999; Ewing et al. 2000).

Although the evidence supports a physiological link between quantitative resistance and the type of plant development cycle, the presence of genes with pleiotropic effects, or even genes with different functions strictly linked to the same loci cannot be ruled out (Visker et al. 2003). In this sense, there is some indication that selection for late blight resistance without affecting the type of development cycle may be possible, probably because of the presence of QTLs for resistance not linked to QTLs for the type of plant development cycle (Visker et al. 2004).

2.9 Stress Induction: Phenological Stage

The most reliable and effective methods for selecting germplasm resistant to late blight are generally those that involve natural infection or inoculation under field conditions (Fig. 2.3). This requires a location where late blight epidemics occur constantly in successive years, caused by a population of *P. infestans* representative of the areas in which future cultivars will be planted. Selection for high levels of field resistance to the currently prevalent complex race of *P. infestans*, taking account the scores for late blight of one of the parents and sibling clones, is probably the most effective kind of selection (Solomon-Blackburn et al. 2007).

Tests under controlled conditions (in the laboratory or greenhouse) that afford the advantages of speed and accuracy in assessing resistance, and in particular the effects of partial resistance, in a large number of plantlets at the beginning of the selection process are extremely useful and save time. However, these methods need to be efficient at predicting future clone reactions under field conditions.

Fig. 2.3 Severity of *P. infestans* in advanced potato clones. Photo: Arione da Silva Pereira

Similar in vitro tests conducted in other pathosystems are being used to assess potato genotype resistance to *Alternaria solani* (Bussey and Stevenson 1991), and *Heliconia* spp. resistance to *Pestalotiopsis pauciseta* (Serra and Coelho 2007), apple scab disease (*Ventura inaequalis*) (Ivanicka et al. 1996), and other pathogens.

The resistance response in the leaf can be influenced by many variables, such as plant age, spore concentration, inoculation method, leaf position, and nutritional status of the plants (Stewart 1990; Fry and Apple 1986; Dorrance and Inglis 1998). Plants at the floral budding stage or at the onset of blossoming consistently express resistance in various tests (Stewart 1990). Plantlet tests can be useful for eliminating material susceptible to a population if there is a correlation between greenhouse tests and field tests (Caligari et al. 1984). Tests on particular leaflets and leaf disks can provide a practical and efficient way of assessing resistance levels, identifying the virulence locus in specific isolates of *P. infestans* and measuring horizontal resistance components. However, this method requires great care to ensure that plants and leaves are of the same age and at the same growth stage as plants normally attacked in the field. And even then, it is common for results to be influenced by environmental conditions, requiring a large number of experiments and replications, so this procedure cannot fully replace field trials (Dorrance and Inglis 1998).

Potato resistance to late blight on the leaves does not always correlate with tuber resistance. This means that specific assays are required under controlled conditions to select potato genotypes with resistant tubers (Dorrance and Inglis 1998; Wastie et al. 1987).

The presence of *R* genes makes it difficult to breed for horizontal resistance, hampering the observation of this kind of resistance. One alternative is to base selection on races of *P. infestans* that are not affected by the *R* genes present in the target population (Stewart et al. 2003), or to breed using genotypes with no *R* genes and introduce them later to take advantage of any possible benefit (Turkensteen 1993).

Because of its quantitative nature, general potato resistance to late blight cannot be assessed as easily as specific resistance. Reliable phenotypic assessment of general resistance is important for breeding programs, but is especially crucial in genetic analysis, such as the detection and mapping of quantitative trait loci (QTL) (Leonards-Schippers et al. 1994).

2.10 Intensity and Duration

Since late potato blight is a polycyclic disease, a decisive factor in its progression rate is increased severity (disease percentage) in the plant affected. The main objective of producing cultivars with quantitative resistance is therefore to reduce this rate.

Host resistance is theoretically one of the most effective tactics for controlling late blight. Differences in the intensity of the blight serve to show how effective resistant cultivars are. In a study conducted in Mexico comparing late blight intensity in a susceptible cultivar (Alpha) and in a resistant cultivar (Norteña), 40 days after emergence disease severity was observed to be 100 % in Alpha and 4 % in Norteña (Grunwald et al. 2000). However, under the conditions in the south of Brazil, plants of the susceptible Agata cultivar and the resistant BRS Clara cultivar (Fig. 2.4) at 50 days after emergence and 20 days after inoculation with *P. infestans* exhibited respective severity levels of 55 % and less than 1 % (Gomes et al. 2009).

Late blight severity is measured by assessing the percentage of the disease in the lesioned tissue (comparing green and non-green portions) from the time at which the first symptoms arise during the cropping period. To assess the response of a given potato genotype to late blight (polycyclic disease), the recommended parameter is the area under the disease progress curve (AUDPC). The AUDPC is calculated on the basis of the estimated percentage leaf area affected, recorded at different times during the epidemic and expressed in cumulative percentage/days. Therefore, the higher the AUDPC value, the more susceptible the genotype, by comparison with a susceptible and a resistant control, respectively, related to a high (susceptible) and a low (resistant) value for this variable (CIP 2010).

2.11 Strategy and Selection Methods

In view of the complexity involved in breeding for resistance to late blight in potatoes, it is essential to design strategies to facilitate the development of cultivars with durable resistance. However, first and foremost they must present

Fig. 2.4 Potato crop attacked by late blight. On the *left*, resistant cultivar BRS Clara and on the *right*, susceptible cultivar Agata. Photo: Odone Bertoncini

acceptable agronomic and commercial characteristics, especially in regard to the appearance of the tubers for the market in fresh vegetables and tuber quality for industrial processing.

The particular case of late blight presents the challenge of breeding for a quantitative trait, since resistance is linked to late maturity and photoperiod sensitivity (van der Vossen et al. 2005), making it even more difficult to design an adequate strategy.

Despite the logical use of quantitative resistance in breeding strategies, it can be seen that the cultivars released with this kind of resistance have made a very limited contribution. There are many socioeconomic factors that result in a reluctance to adopt and use a new potato cultivar, especially market forces (Walker et al. 2003). In Brazil, traits related to disease resistance do not generally carry much weight among the key factors in the success of potato cultivars, and even on the organic potato market, tubers still have to look good (Pereira 2011).

In the short term, it is probably not practicable to produce a commercially successful potato cultivar with adequate levels of durable resistance so that fungicides are no longer necessary. Even so, breeding program selection for a high level of quantitative resistance is still worth aiming for, since it could lead to reductions in fungicide use if integrated disease control is deployed. Furthermore, it is possible to argue that the best strategy for breeding in durable resistance is to combine new *R* genes with high levels of field resistance, which is a more realistic proposition in practice (Solomon-Blackburn et al. 2007). The residual effects of *R* genes overcome by *P. infestans* are considered desirable in combination with

high levels of field resistance (Steward et al. Stewart et al. 2003). With this in mind, it has been suggested that we should prospect for and use new broad-spectrum resistance *R* genes for introgression or rapid transfer by genetic engineering to new or existing potato cultivars (van der Vossen et al. 2005).

Another proposed strategy is pyramidizing different resistance genes taken from *Solunum* wild relatives in clones and cultivars modified to offer broad-spectrum genetic resistance. Pyramidizing genes from different sources (and in the present case, affording different levels of resistance) could result in higher level or more durable resistance to late blight (Tan et al. 2010).

Identifying characteristics of interest in wild potato germplasm and introgressing it into cultivated material requires a lot of time and effort. Many sources of late blight resistance are not adapted to cropping conditions in many regions of the world due to late maturity and other tuber characteristics (Bisognin et al. 2002). However, there is no doubt regarding how useful these species and primitive cultivars could be in breeding for resistance to *P. infestans*. However, in addition to blight resistance and adaptation traits, improvement of this germplasm must also include the traits of higher economic value required in cultivars. The material to be used as the parent in blocks for crossing population generations for developing new cultivars must be capable of producing progenies with superior commercial traits, in addition to late blight resistance.

In the strategy adopted for the Embrapa potato breeding program, before being used as parents, clones bred for resistance to late blight are put through progeny trials. Crossed with parents known to have good general combining capacity in respect of traits of economic importance, the clones are observed to find out whether they have the potential to generate superior populations. The strategy for developing new cultivars resistant to late blight includes crossing cultivars with some resistance and acceptable agronomic characteristics. These were the very same crossings from which the BRS Clara cultivar was recently selected as resistant to late blight, with medium maturity and possessing the main commercial characteristics required for the fresh tubers market. The type or types of resistance of this cultivar is yet to be elucidated.

2.12 Biotechnology

Over the last few years, biotechnology has had a huge impact on world agriculture. Since its inception in the 1980s with the development of molecular marker techniques, considerable progress has been made in mapping resistance genes in plants, which has led in a short time to a move in genetic resistance research away from studies based exclusively on the phenotype and toward studies based on genotype resistance (Simko et al. 2007).

As an example in potatoes, QTL for resistance to late blight have been mapped in a large number of experimental diploid populations of potato, and also in tetraploid populations. These QTLs have been mapped on almost all potato

chromosomes, and those with the greatest effects are located on chromosome V, in a region flanked by RFLP markers GP21 and GP179. This region also contains a more effective QTL related to plant maturity (Collins et al. 1999; Bormann et al. 2004), a problem already discussed in this chapter. However, QTLs for late blight resistance detected on other chromosomes are not linked to the QTLs related to maturity, and it is possible to use molecular marker-assisted selection for late blight resistance, independent of selection for cycle traits (Bormann et al. 2004). One example is the *RPI-phu1*gene, mapped on potato chromosome IX and not significantly correlated with the duration of the vegetative cycle (Sliwka et al. 2006).

In addition to QTLs that confer resistance, known as quantitative resistance loci (QRL), some 40 dominant genes that confer qualitative resistance (*R* genes) have been located in the potato genome. These *R* genes are thought to be capable of detecting the gene-specific avirulence in the pathogen initiating the transduction of signals for activating defense mechanisms (Hammond-Kosack and Jones 1997). Eight resistance genes have already been cloned in potato. Molecular characterization has grouped all these genes as *R* and/or *R* homolog genes. Most of these homologs seem to code for proteins similar to functional *R* genes. Studies are under way to further elucidate the functions of these homologs in the plant–pathogen interaction. One of the questions that is still awaiting an answer in regard to resistance genes is whether their evolutionary rate is directly linked to their gene specificity, i.e., whether broad-spectrum resistance genes such as *Rpi-blb1* and *Rpi-blb2*, evolve more slowly than race-specific genes *R1-R11* (Simko et al. 2007).

With the advances made in the field of genomics, the number of *R* genes and QRLs mapped, isolated, and sequenced in potato is growing rapidly. An online database was recently made available for exploiting resistance genes in tuberous species of the genus *Solanum*. The database, known as Sol*R*gene, contains information on *R* genes in potato and wild relatives, providing an easily accessible and useful resource for researching and implementing resistance to diseases affecting the potato (Vleeshouwers et al. 2011a; Vleeshouwers et al. 2011b). These advances have been helping to develop new potato cultivars resistant to *P. infestans*, using both molecular marker-assisted selection, developing markers that flank the region containing the gene of interest, accelerated screening, and selection of germplasm of interest (Gebhardt et al. 2004), as well as genetic transformation.

Due to its high capacity for in vitro regeneration, the potato is considered as a model species for methods such as somatic hybridization and *Agrobacterium*-mediated transformation (Steve 2007). Ongoing acceleration in the discovery of wild potato genes that confer resistance to late blight has raised queries concerning how these genes can be rapidly and efficiently incorporated into cultivated germplasm, since we are dealing with different species and reproductive barriers may be present in the germplasm in question. Plant transformation therefore affords an efficient method for transferring these genes (Maniruzzaman et al. 2010).

Recently, the *RB* gene isolated in *S. bulbocastanum* and conferring broad-spectrum resistance to *P. infestans* was introgressed into four potato cultivars in the United States: ‘Katahdin’, ‘Superior’, ‘Dark Red Norland’, and ‘Russet Burbank’.

In preliminary experiments, the transgenic lines exhibited high levels of resistance *P. infestans* after inoculation under greenhouse conditions. In another study on the same cultivars conducted by Halterman et al. (2008), leaf resistance was confirmed but no increase in tuber resistance was verified. The scientific approaches for obtaining genetically modified transgenic and/or cisgenic cultivars (cisgenic modification involves using resistant genes found in the gene pool of the cultivated species) have proved fairly promising for developing blight-resistant cultivars. Details were recently published of the development of a transgenic cultivar resistant to late blight, cv. Fortuna (Biotechnologie.de 2011). Blight resistance was conferred by pyramidizing two genes, *blb1* and *blb2*, from the wild species, *S. bulbocastanum*.

As a result of advances in genomics, the development of genetically modified plants able to tolerate fungal diseases is now a reality. Examples can be seen in a number of plant species, such as soybeans resistant to rust (Pionner 2011), grapes resistant to powdery mildew, and gray rot (Francesco and Watanabe 2008) and tomatoes resistant to a number of fungal and bacterial diseases (Lin et al. 2004).

2.13 Climate Change

Climate change is expected to result in rising temperatures, affecting plant responses to diseases and the pathogenicity of agents, as well as host–pathogen interactions (Coakley et al. 1999; Lopes et al. 2008; Ghini et al. 2011). Alterations in responses will vary according to the host and pathogen involved. It has been shown that a rise in temperature alters the genetic resistance of many crops. In wheat, the resistance conferred by allele *Sr6* on *Puccinia graminis* was high when tested at a temperature of 20 °C, but nonexistent at a temperature of 25 °C (Mayama et al. 1975). In coffee, development of urediniospores of *Hemileia vastatrix* in susceptible genotype leaves was stunted when it was inoculated with the pathogen and subjected to a high controlled temperature (Ribeiro et al. 1978). Although this does not apply across the board, it is generally the case that problems with pests and diseases increase as the temperature rises (Haverkort and Verhagen 2008), since this allows an increase in the number of pathogen multiplication cycles (Ghini et al. 2011). It is therefore important to consider the possible impacts of climate change on existing national diseases, as well as the increased risk of the introduction of new causal agents (Mafia et al. 2011).

In Brazil, possible climate change with temperature increases of 1.2–3.2 °C between 2010 and 2060 will cause a drop in potato yield between 20 and 30 % in traditional potato cropping regions (Hijmans et al. 2003) and will affect interactions among pathogens, and between pathogens and potato plants.

The projected variation in the intensity of *P. infestans* in the two main cropping periods predicts equal intensity in the winter and a drop during the rainy season in Brazil (Lopes et al. 2008). Late blight is favored by temperatures between 8 °C and 18 °C for production of zoospores that spread the disease at a faster rate. However, temperatures between 18 °C and 24 °C favor the direct germination of

sporangia, which also spread the disease and can remain viable at temperatures close to 30 °C.

Global warming will probably lower the possibility of severe late blight epidemics. However, in view of the high physiological plasticity of the oomycete and its consequent adaptation to higher temperatures, late blight will remain an important disease for the potato crop. As a way of softening the projected negative impacts of climate change on potato yield, the development of heat-tolerant, blight-resistant cultivars should be a priority in genetic improvement programs (Lopes et al. 2011; Hijmans et al. 2003).

2.14 Conclusions

The global resurgence of a more aggressive and genetically more variable *P. infestans*, together with society's demand for potato production systems that are less dependent on chemical inputs, make the development of cultivars with a high level of stable and durable resistance to potato late blight an even more pressing matter. Since cultivars with vertical resistance (qualitative, specific) have not retained their resistance in the field, the strategy with the greatest potential for developing cultivars is probably based on horizontal resistance (quantitative, general). A cultivar with this kind of resistance would be combined with strategic resistance management in a system of integrated disease management (IDM).

Sources of quantitative late blight resistance have been found in many wild species of *Solanum*. However, if breeding for a quantitative trait is difficult, the situation is further aggravated if this trait exhibits an undesirable combination with another important trait. In this sense, the development of potato cultivars with stable and durable resistance to late blight has to meet the challenge presented by the link between resistance and late maturity/photoperiod sensitivity. The presence of loci linked to late blight resistance and late plant development has been the rule. But resistance QTLs not linked to late maturity have been detected, suggesting that there is a possibility of combining blight resistance and early maturity.

Another challenge that has impeded the general acceptance of blight-resistant cultivars by potato producers relates to the commercial characteristics of the tubers. Although traits related to pest and disease resistance are very important in a new cultivar, they are not important enough to outweigh other key factors involved in the success of a cultivar. Even in cultivars for organic production systems, consumers demand tubers that look good.

New scientific approaches have generated increasing expectations of obtaining blight-resistant cultivars, including pyramidizing the major marker-assisted genes and producing genetically modified cultivars, such as the blight-resistant variants of cultivars widely accepted on the market and with low potential consumer rejection, for use as genetic sources.

Breeding for resistance to other fungi will depend on the particular features of each pathogen.

References

Alexopoulos CJ, Mims CW, Blackwell M (1996) Introduce Mycology. Wiley, New York

Biotechnologie.de (2011) Business—BASF applies for approval for another biotech potato. http://www.biotechnologie.de/BIO/Navigation/EN/root,did=140524.html. Accessed 29 Nov 2011

Bisognin DA, Douches DS, Jastrzebski K, Kirk WW (2002) Half-sib progeny evaluation and selection of potatoes resistant to the US8 genotype of *Phytophthora infestans* from crosses between resistant and susceptible clones. Euphytica 125:129–138

Bisognin DA, Douches DS, Buszka L, Bryan G, Wang D (2005) Mapping late blight resistance in *Solanum microdontum* bitter. Crop Sci 45:340–345

Bormann CA, Rickert AM, Castillo Ruiz RA, Paal J, Lubeck J, Strahwald J, Buhr K, Gebhardt C (2004) Tagging quantitative trait loci for maturity-corrected late blight resistance in tetraploid potato with PCR-based candidate gene markers. Mol Plant Microbe Interact 17:1126–1138

Bradshaw JE (2009) Breeding for field resistance to late blight of potato at SCRI. Acta Hortic 834:87–100

Bradshaw JE, Stewart HE, Wastie RL, Dale MFB, Phillips MS (1995) Use of seedling progeny tests for genetical studies as part of a potato (*Solanum tuberosum* subsp. *tuberosum*) breeding programme. Theor Appl Genet 90:899–905

Bradshaw JE, Mackay GR (1994) Breeding strategies for clonally propagated potatoes. In: Bradshaw JE, Mackay GR (eds) Potato genetics. CAB International, Wallingford

Burton WG (1981) Challenges for stress physiology in potato. Am Potato J 58:3–14

Bussey MJ, Stevenson WR (1991) A leaf disk assay for detecting resistance to early blight caused by *Alternaria solani* in juvenile potato plants. Plant Dis 75:385–390

Caligari PDS, Mackay GR, Stewart HE, Wastie RL (1984) A seedling progeny test for resistance to potato foliage blight [*Phytophthora infestans* (Mont.) de Bary]. Potato Res 27:43–50

Cañizares CA, Forbes GA (1995) Foliage resistance to *Phytophthora infestans* (Mont.) de Bary in the Ecuadorian national collection of *Solanum phureja* ssp. *phureja* Juz. and Buk. Potato Res 38:3–10

Cip—Centro Internacional de la Papa (1996) Research needed to halt rapidly spreading late blight strains. In: The international potato center annual report. CIP, Lima

Cip—Centro Internacional de la Papa (2010) Procedimientos para pruebas de evaluaciones estándar de clones avanzados de papa. Guia para cooperadores internacionais. CIP, Lima

Coakley SM, Scher MH, Chakraborty S (1999) Climate change and plant disease management. Annu Rev Phytopathol 37:399–426

Collins A, Milbourne D, Ramsay L, Meyer R, Chatot-Balandras C, Oberhagemann P, De Jong W, Gedhardt C, Bonnel E, Waugh R (1999) QTL for field resistance to late blight in potato are strongly correlated with maturity and vigour. Mol Breed 5:387–398

Colon LT, Budding DJ, Keizer LCP, Pieters MMJ (1995) Components of resistance to late blight (*Phytophthora infestans*) in eight American *Solanum* species. Eur J Plant Pathol 101:441–456

Colton LM, Groza HI, Wielgus SM, Jiang JM (2006) Marker-assisted selection for the broad-spectrum potato late blight resistance conferred by gene *RB* derived from a wild potato species. Crop Sci 46:589–594

Deahl KL, Goth RW, Young RJ, Sinden SL, Gallegly ME (1991) Occurrence of the A2 mating type of *Phytophthora infestans in* potato fields in the United States and Canada. Am Potato J 68:717–726

Dorrance AE, Inglis DA (1998) Assessment of laboratory methods for evaluating potato tubers for resistance to late blight. Plant Dis 82:442–446

Douches DS, Bamberg JB, Kirk W, Jastrzebski K, Niemira BA, Coombs J, Bisognin DA, Felcher KJ (2001) Evaluation of wild *Solanum* species for resistance to the US-8 genotype of *Phytophthora infestans* utilizing a finescreening technique. Am J Potato Res 78:159–165

Drenth A (1994) Molecular genetic evidence for a new sexually reproducing population of *Phytophthora infestans* in Europe. Dissertation, Wageningen Agricultural University, The Netherlands

Drenth A, Goodwin SB, Fry WE, Davidse LC (1993) Genotypic diversity of *Phytophthora infestans* in the Netherlands revealed by DNA polymorphisms. Phytopathology 83:1087–1092

Ewing EE, Simko I, Smart CD, Bonierbale MW, Mizubuti ESG, May GD, Fry WE (2000) Genetic mapping from field tests of qualitative and quantitative resistance to *Phytophthora infestans* in a population derived from *Solanum tuberosum* and *Solanum berthaultii*. Mol Breed 6:25–36

Flier WG, van den Bosch GBM, Turkensteen LJ (2003) Stability of partial resistance in potato cultivars exposed to aggressive strains of *Phytophthora infestans*. Plant Pathol 52:326–337

Forbes GA, Chacon G, Kirk HG, Huarte M, Damme MV, Distel S, Capezio S, Mackay G, Stewart H, Lowe R, Duncan J, Mayton H, Fry WE, Andrivon D, Ellisseche D, Pelle R, Platt H, Mackenzie G, Tarn R, Colon LT, Budding DJ, Lozoya-Saldana H, Hernandez-Vilchis A (2005) Stability of resistance to *Phytophthora infestans* in potato: an international evaluation. Plant Pathol 54:364–372

Forbes GA, Landeo JA (2006) Late blight. In: Gopal J, Ksm P (eds) Handbook of potato production, improvement, and postharvest management. Haworth Press, Binghamton

Foster SJ, Park TH, Pel M, Brigneti G, Sliwka J, Jagger L, van der Vossen EAG, Jones JDG (2009) *Rpi-vnt1.1*, a Tm-2(2) homolog from *Solanum venturii*, confers resistance to potato late blight. Mol Plant Microbe Interact 22:589–600

Francesco L, Watanabe M (2008) Vintage genetic engineering. Nat Biotechnol 26:261–263

Fry WE, Goodwin SB (1997) Re-emergence of potato and tomato late blight in the United States. Plant Dis 81:1349–1357

Fry WE, Apple AE (1986) Disease management implications of age-related changes in susceptibility of potato foliage to *Phytophthora infestans*. Am Potato J 63:47–55

Gavino PD, Smart CD, Sandrock RW, Miller JS, Hamm PB, Lee TY, Davis RM, Fry WE (2000) Implications of sexual reproduction for *Phytophthora infestans* in the United States: generation of an aggressive lineage. Plant Dis 84:731–735

Gebhardt C, Ballvora A, Walkemeier B, Oberhagemann P, Schuler K (2004) Assessing genetic potential in germplasm collections of crop plants by markertrait association: a case study for potatoes with quantitative variation of resistance to late blight and maturity type. Mol Breed 13:93–102

Gomes CB, Pereira A da S, Stocker CM, Bosenbecker VK (2009) Reação de genótipos de batata à requeima (*Phytophthora infestans*). Embrapa Clima Temperado, Pelotas

Gomez-Alpizar L, Carbone I, Ristaino JB (2007) An Andean origin of *Phytophthora infestans* inferred from mitochondrial and nuclear gene genealogies. Proc Natl Acad Sci U S A 104:3306–3311

Goodwin SB, Cohen BA, Fry WE (1994) Panglobal distribution of a single clonal lineage of the Irish potato famine fungus. Proc Natl Acad Sci U S A 91:11591–11595

Goodwin SB (1997) The population genetics of Phytophthora. Phytopathology 87:462–473

Grunwald NJ, Rubio-Covarrubias OA, Fry WE (2000) Potato late-blight management in the Toluca Valley: forecasts and resistant cultivars. Plant Dis 84:410–416

Grunwald NJ, Cadena Hinojosa MA, Rubio Covarrubias OA, Rivera Peña A, Niederhauser JA, Fry WE (2002) Potato cultivars from the Mexican national program: sources and durability of resistance against late blight. Phytopathology 92:688–693

Ghini R, Hamada E, Bettiol W (2011) Impacto das mudanças climáticas sobre as doenças de plantas. In: Ghini R, Hamada E, Bettiol W (eds) Impactos das mudanças climáticas sobre doenças de importantes culturas no Brasil. Embrapa Meio Ambiente, Jaguariuna

Halterman D, Kramer LC, Wielgus S, Jiang J (2008) Performance of transgenic potato containing the late blight resistance gene RB. Plant Dis 92:339–343

Haas BJ, Kamoun S, Zody MC et al (2009) Genome sequence and analysis of the Irish potato famine pathogen *Phytophthora infestans*. Nature 461:393–398

Hammond-Kosack KE, Jones JDG (1997) Plant disease resistance genes. Annu Rev Plant Physiol Plant Mol Biol 48:575–607

Haverkort AJ, Verhagen A (2008) Climate change and its repercussions for the potato supply chain. Potato Res 51:223–237

Haynes KG, Christ BJ (1999) Heritability of resistance to foliar late blight in a diploid *Solanum phureja* x *S. stenotomum* hybrid potato population. Plant Breed 118:431–434

Henfling JW (1987) Late blight of potato: *Phytophthora infestans*. CIP, Lima

Hijmans RJ, Condori B, Carrillo R (2003) The effect of climate change on global potato production. Am J Potato Res 80:271–279

Hohl HR, Iselin K (1984) Strains of *Phytophthora infestans* from Switzerland with A2 mating type behaviour. Trans Br Mycol Soc 83:529–530

Hooker WJ (1981) Compendium of potato diseases. American Phytopathology Society, Saint Paul

Inglis DA, Johnson DA, Legard DE, Fry WE, Harem PB (1996) Relative resistances of potato clones in response to new and old populations of *Phytophthora infestans*. Plant Dis 80:575–578

Ivanicka J, Kellerhals M, Theiler R (1996) Evaluation of scab [*Venturia inaequalis* (Cooke) Wint] on shoots and detached leaves from in vitro greenhouse grown plants of the apple (*Malus domestica* Mill) cultivars Golden Delicious and Florina. Gartenbauwissenschaft 61:242–248

Johnson R (1979) The concept of durable resistance. Phytopathology 69:198–199

Kroon LPNM, Bakker FT, van den Bosch GBM, Bonants PJM, Flier WG (2004) Phylogenetic analysis of *Phytophthora* species based on mitochondrial and nuclear DNA sequences. Fungal Genet Biol 41:766–782

Kuhl JC, Hanneman RE Jr, Havey MJ (2001) Characterization and mapping of *Rpil*, a late blight resistance locus from diploid (1EBN) Mexican *Solanum pinnatisectum*. Mol Genet Genomics 265:977–985

Kirk WW, Felcher KJ, Douches DS, Coombs J, Stein JM, Baker KM, Hammerschmidt R (2001) Effect of host plant resistance and reduced rates and frequencies of fungicide application to control potato late blight. Plant Dis 85:1113–1118

Landeo JA, Gastelo M, Roncal E, Mendoza A (2000) Phenotypic stability for horizontal resistance to potato late blight in population B. Am J Potato Res 77:406

Lee T, Robold A, Testa A, Klooster JW, Govers F (2001) Mapping of avirulence genes in *Phytophthora infestans* with amplified fragment length polymorphism markers selected by bulked segregant analysis. Genetics 157:949–956

Leonards-Schippers C, Gieffers W, Schaefer-Pregl R, Ritter E, Knapp SJ, Salamini F, Gebhardt C (1994) Quantitative resistance to *Phytophthora infestans* in potato: a case study for QTL mapping in an allogamous plant species. Genetics 137:68–77

Lin WC, Lu CF, Wu JW, Cheng ML, Lin YM, Yang NS, Black L, Green SK, Wang JF, Cheng CP (2004) Transgenic tomato plants expressing the *Arabidopsis* NPR1 gene display enhanced resistance to a spectrum of fungal and bacterial diseases. Transgenic Res 13:567–581

Lopes CA, Reis A, Shimoyama NY (2008) Impacto potencial das mudanças climáticas sobre as doenças da batata no Brasil. In: Ghini R, Hamada E (eds) Mudanças climáticas. Impactos sobre doenças de plantas no Brasil. Embrapa Informação Tecnológica, Brasilia

Lopes CA, Silva GO, Cruz EM, Assad E, Pereira AS (2011) Uma análise do efeito do aquecimento global na produção de batata no Brasil. Hort Bras 29:7–15

Lozoya-Saldana H, Belmar-Diaz C, Bradeen JM, Helgeson JP (2005) Characterization of *Phytophthora infestans* isolates infecting transgenic and somatic hybrid potatoes resistant to the pathogen in the Toluca Valley, Mexico. Am J Potato Res 82:79

Luz EDMN, Santos AF dos, Matsuoka K, Bezerra JL (2001) Doenças causadas por *Phytophthora* no Brasil. Livraria e Editora Rural, Campinas

Lynch DR, Kawchuk LM, Chen Q, Kokko M (2003) Resistance to *Fusarium sambucinum* in wild and cultivated *Solanum* species. Am J Potato Res 80:353–358

Mafia RG, Alfenas AC, Loos RA (2011) Impacto potencial das mudanças climáticas sobre doenças na eucaliptocultura no Brasil. In: Ghini R, Hamada E, Bettiol W (eds) Impactos das mudanças climáticas sobre doenças de importantes culturas no Brasil. Embrapa Meio Ambiente, Jaguariuna

Maniruzzaman; Haque, ME; Habib MA, Haque MM, Chakrabarti SK (2010) *Agrobacterium* mediated RB gene transformation into Indian potato variety (Kufribahar) for late blight resistant. Thai J Agric Sci 43:183–188

Mizubuti ESG, Fry WE (2006) Potato late blight. In: Cooke BM, Jones DG, Kaye B (eds) The epidemiology of plant disease. Springer, Dordrecht

Mayama S, Daly JM, Rehfeld DW, Daly C (1975) Hypersensitive response of near-isogenic wheat carrying the temperature-sensitive *Sr6* allele for resistance to stem rust. Physiol Plant Pathol 7:35–47

Muskens MWM, Allefs JJHM (2002) Breeding for late blight resistance; views from practice. In: Wenzel G, Wulfert I (eds) Potatoes today and tomorrow. Abstracts of papers and posters of the 15th triennial conference of the EAPR. European Association for Potato Research, Bonn, p 85

Naess SK, Bradeen JM, Wielgus SM, Haberlach GT, Mcgrath JM, Helgeson JP (2000) Resistance to late blight in *Solanum bulbocastanum* is mapped to chromosome 8. Theor Appl Genet 101:697–704

Nass LL, Nishikawa MA, Fávero AP, Lopes MA (2007) Pré-melhoramento de germoplasma vegetal. In: Nass L (ed) Recursos genéticos vegetais. Embrapa Recursos Genéticos e Biotecnologia, Brasilia

Nazareno NRX, Brisola AD, Zandoná JC (1995) Uso de agroquímicos na cultura da batata em Curitiba e Guarapuava. IAPAR, Londrina

Nazareno NRX, Scotti CA, Mafioletti RL, Boschetto N (1999) Controle da requeima da batata através do monitoramento das variáveis climáticas. Fitopatol Bras 24:170–174

Niederhauser JS (1991) *Phytophthora infestans*: the Mexican connection. In: Lucas JA, Shattock RC, Shaw DS, Cooke LR (eds) *Phytophthora*. Cambridge University Press, Cambridge

Oberhagemann P, Chatot-Balandras C, Schafer-Pregl R, Wegener D, Palomino C, Salamini F, Bonnel E, Gebhardt C (1999) A genetic analysis of quantitative resistance to late blight in potato: towards marker-assisted selection. Mol Breed 5:399–415

Oliveira SAS (2010) Genetic structure, aggressiveness and fungicide sensitivy of *Phytophthora infestans* populations from the south and southest regions of Brazil. Dissertation, Universidade Federal de Viçosa

Park T-H, Gros J, Sikkema A, Vleeshouwers VGAA, Muskens M, Allefs S, Jacobsen E, Visser RGF, van der Vossen EAG (2005) The late blight resistance locus Rpi-blb3 from *Solanum bulbocastanum* belongs to a major late blight R gene cluster on chromosome 4 of potato. Mol Plant Microbe Interact 18:722–729

Pereira da AS (2011) Avanços no melhoramento genético na batata visando à obtenção de materiais resistentes a fatores bióticos. In: Nava DE, Gomes CB, Barros DR (eds) Workshop manejo fitossanitário na cultura da batata. Embrapa Clima Temperado, Pelotas

Pionner (2011) Asian soybean rust resistance. http://www.pioneer.com/pv_obj_cache/pv_obj_id_51053F0033096677BD283A113E1E529FC2BA0100/filename/AsianRust.pdf. Accessed 6 Dec 2011

Queiroz MA, Lopes MA (2007) Importância dos recursos genéticos vegetais para o agronegócio. In: Nass L (ed) Recursos genéticos vegetais. Embrapa Recursos Genéticos e Biotecnologia, Brasilia

Rauscher GM, Smart CD, Simko I, Bonierbale M, Mayton H, Greenland A, Fry WE (2006) Characterization and mapping of RPi-ber, a novel potato late blight resistance gene from *Solanum berthaultii*. Theor Appl Genet 112:674–687

Reis EM, Medeiros CA, Casa RT, Mendez C (1999) Previsão de doenças de plantas: sistemas para a requeima e para a pinta preta da batateira. Summa Phytopatol 25:60–65

Reis A (2001) Caracterização das populações de *Phytophthora infestans* das regiões Sul e Sudeste do Brasil. Dissertation, Universidade Federal de Viçosa

Reis A, Smart CD, Fry WE, Maffia LA, Mizubuti ESG (2003) Characterization of isolates of *Phytophthora infestans* from Southern and Southeastern regions of Brazil from 1998 to 2001. Plant Dis 87:896–900

Ribeiro IJA, Mônaco LC, Tissel Filho O, Sugimori MH (1978) Efeito de alta temperatura no desenvolvimento de *Hemileia vastatrix* em cafeeiro. Bragantia 37:11–16

Ross H (1986) Potato breeding—problems and perspectives. Verlag Paul Parey, Berlin

Ruiz de Galarreta JI, Carrasco A, Salazar A, Barrena I, Iturritxa E, Marquinez R, Legorburu FJ, Ritter E (1998) Wild *Solanum* species as resistance sources against different pathogens of potato. Potato Res 41:57–68

Santana FM (2006) Distribuição e caracterização de isolados de *Phytophthora infestans* (Mont.) de Bary associados à batata (*Solanum tuberosum* L.) na região Sul do Brasil. Dissertation, Universidade Federal de Pelotas

Serra IMRS, Coelho RSB (2007) Mancha de Pestalotiopsis em helicônia: caracterização da doença e potenciais fontes de resistência. Fitopatol Bras 32:44–49

Sherwood SG, Cole DC, Paredes M (2001) Reduction of risks associated with fungicides: technically easy, socially complex. In: Fernández-Northcote EN (ed) Complementing resistance to late blight (*Phytophthora infestans*) in the Andes. Global initiative on late blight. International Potato Center, Lima

Simko I (2002) Comparative analysis of quantitative trait loci for foliage resistance to *Phytophthora infestans* in tuberbearing *Solanum* species. Am J Potato Res 79:125–132

Simko I, Jansky SH, Stephenson S, Spooner DM (2007) Genetics of resistance to pests and disease. In: Vreugdenhil R, Bradshaw J, Gebhardt C, Govers F, MacKerron DKL, Taylor MA, Ross HA (eds) Potato biology and biotechnology: advances and perspectives. Elsevier, Oxford

Sliwka J, Jakuczun H, Lebecka R, Marczewski W, Gebhardt C, Zimnoch-Guzowska E (2006) The novel, major locus Rpi-phu1 for late blight resistance maps to potato chromosome IX and is not correlated with long vegetation period. Theor Appl Genet 113:685–695

Smilde WD, Brigneti G, Jagger L, Perkins S, Jones JDG (2005) *Solanum mochiquense* chromosome IX carries a novel late blight resistance gene Rpimoc1. Theor Appl Genet 110:252–258

Solomon-Blackburn RM, Stewart HE, Bradshaw JE (2007) Distinguishing major-gene from field resistance to late blight (*Phytophthora infestans*) of potato (*Solanum tuberosum*) and selecting for high levels of field resistance. Theor Appl Genet 115:141–149

Song J, Bradeen JM, Naess SK (2003) Gene RB cloned from *Solanum bulbocastanum* confers broad spectrum resistance to potato late blight. Proc Natl Acad Sci U S A 100:9128–9133

Spooner DM, Hijmans RJ (2001) Potato systematics and germplasm collecting 1989–2000. Am J Potato Res 78(237–268):395

Steve M (2007) Developments in transgenic biology and the genetic engineering of useful traits. In: Vreugdenhil R, Bradshaw J, Gebhardt C, Govers F, MacKerron DKL, Taylor MA, Ross HA (eds) Potato biology and biotechnology: advances and perspectives. Elsevier, Oxford

Stewart HE (1990) Effect of plant age and inoculum concentration on expression of major gene resistance to *Phytophthora infestans* in detached potato leaflets. Mycol Res 94:823–826

Stewart HE, Wastie RL, Bradshaw JE, Brown J (1992) Inheritance of resistance to late blight in foliage and tubers of progenies from parents differing in resistance. Potato Res 35:313–319

Stewart HE, Bradshaw JE (2001) Assessment of the field resistance of potato genotypes with major gene resistance to late blight [*Phytophthora infestans* (Mont.) de Bary] using inoculum comprised of two complementary races of the fungus. Potato Res 44:41–52

Stewart HE, Bradshaw JE, Pande B (2003) The effect of the presence of *R*-genes for resistance to late blight (*Phytophthora infestans*) of potato (*Solanum tuberosum*) on the underlying level of field resistance. Plant Pathol 52:193–198

Tan MYA, Hutten RCB, Visser RGF, van Eck HJ (2010) The effect of pyramiding *Phytophthora infestans* resistance genes *RPi-mcd1* and *RPi-ber* in potato. Theor Appl Genet 121:117–125

Trognitz BR, Trognitz FCH (2007) Occurrence of the *R1* allele conferring resistance to late blight in potato *R*-gene differentials and commercial cultivars. Plant Pathol 56:150–155

Turkensteen LJ (1993) Durable resistance of potatoes against *Phytophthora infestans*. In: Jacobs TH, Parlevliet JE (eds) Durability of disease resistance. Kluwer Academic Publishers, Dordrecht

Turkensteen LJ (19960 Fungal diseases. In: Asscheman E, Bokx JA, Brinkman H, Bus CB, van Delft M, Hotsma PH, Meijers CP, Mulder A, Scholte Y, Turkensteen LJ, Wustman R, van der Zaag DE (eds). Potato diseases, pests and defects. NIVAA, Holland

Umaerus V (1970) Studies on field resistance to *Phytophthora infestans*. 5. Mechanisms of resistance and applications to potato breeding. Z Pflanzenzuchtg 63:1–23

Umaerus V, Umaerus M (1994) Inheritance of resistance to late blight. In: Bradshaw JE, Mackay GR (eds) Potato genetics. CAB International, Wallingford

van der Vossen E, Sikkema A, Hekkert BL, Gros J, Stevens P, Muskens M, Wouters D, Pereira A, Stiekema W, Allefs S (2003) An ancient R gene from the wild potato species *Solanum bulbocastanum* confers broad-spectrum resistance to *Phytophthora infestans* in cultivated potato and tomato. Plant J 36:867–882

van der Vossen EAG, Gros J, Sikkema A, Muskens M, Wouters D, Wolters P, Pereira A, Allefs S (2005) The *Rpi-blb2* gene from *Solanum bulbocastanum* is an *Mi-1* gene homolog conferring broad-spectrum late blight resistance in potato. Plant J 44:208–222

Villamon FG, Spooner DM, Orrillo M, Mihovilovich E, Pérez W, Bonierbale M (2005) Late blight resistance linkages in a novel cross of the wild potato species *Solanum paucissectum* (series *Piurana*). Theor Appl Genet 111:1201–1214

Visker MHPW, Keizer LCP, van Eck HJ, Jacobsen E, Colon LT, Struik PC (2003) Can the QTL for late blight resistance on potato chromosome 5 be attributed to foliage maturity type? Theor Appl Genet 106:317–325

Visker MHPW, van Raaij HMG, Keizer LCP, Struik PC, Colon LT (2004) Correlation between late blight resistance and foliage maturity type in potato. Euphytica 137:311–323

Vleeshouwers VGAA, Raffaele R, Vossen J, Champouret N, Oliva R, Segretin ME, Rietman H, Cano LM, Lokossou A, Kessel G, Pel MA, Kamoun S (2011a) Understanding and exploiting late blight resistance in the age of effectors. Annu Rev Phytopathol 49:507–531

Vleeshouwers VGAA, Finkers R, Budding D, Visser M, Jacobs MMJ, Berloo R, Pel M, Champouret N, Bakker E, Krenek P, Rietman H, Huigen D, Hoekstra R, Goverse A, Vosman B, Jacobsen E, Visser R (2011b) Sol*R*gene: an online database to explore disease resistance genes in tuberbearing *Solanum* species. BMC Plant Biol 11:116–123

Walker TS, Bi YP, Li JH, Gaur PC, Grande E (2003) Potato genetic improvement in developing countries and CIP's role in varietal change. In: Evenson R, Gollin D (eds) Crop variety improvement and its effect on productivity: the impact of international agricultural research. CABI Publishing, Cambridge

Wastie RL, Caligari PDS, Stewart HE, Mackay GR (1987) A glasshouse progeny test for resistance to tuber blight (*Phytophthora insfestans*). Potato Res 30:533–538

Wastie RL (1991) Breeding for resistance. In: Ingram DS, Williams PH (eds) *Phytophthora infestans:* The cause of late blight of potato. Advances in plant pathology, 7. Academic Press, San Diego

Chapter 3
Breeding for Resistance to Bacterial Diseases

Carlos A. Lopes and Leonardo S. Boiteux

Abstract The control of bacterial diseases in plants is difficult and usually requires the combination of several complementary management measures. In this context, genetic resistance is considered to be an effective low-cost strategy that could easily be adopted by farmers, who acquire this built-in control technology within the seeds of a resistant cultivar. To be effective, breeding for disease resistance requires deep knowledge of processes involving the interactions among the plant, the pathogen, and the environment. The development of bacterial resistant cultivars is a complex task, which comprises multidisciplinary actions involving the complexity of the plant and the diversity of the pathogen as well as an appropriate interaction with the productive chain. In this chapter, we provide an overview of the advances and perspectives of breeding plants for bacterial disease resistance in distinct pathosystems involving field and vegetable crops.

Keywords Plant breeding · Plant diseases · Quantitative genetics · Biotechnology

3.1 Introduction

The control of bacterial diseases in plants is difficult, especially after the establishment of the epidemics. This difficulty reflects the diversity of the inoculum sources, the rapid multiplication of the pathogen following infection, the

C. A. Lopes (✉) · L. S. Boiteux
Embrapa Hortaliças, Brasilia, Brazil
e-mail: carlos.lopes@embrapa.br

L. S. Boiteux
e-mail: boiteux@cnph.embrapa.br

R. Fritsche-Neto and A. Borém (eds.), *Plant Breeding for Biotic Stress Resistance*,
DOI: 10.1007/978-3-642-33087-2_3, © Springer-Verlag Berlin Heidelberg 2012

emergence of variants capable of overcoming or neutralizing certain control strategies, the low level of cultivar resistance, and the low availability of registered chemical or biological products. An effective control strategy requires the integration of preventive measures, among them the use of cultivars with genetic resistance to bacterial diseases, which are of low cost, have small environmental impact, and are of ease adoption by farmers.

3.2 Pathogen–Host Interaction

The infection process of phytopathogenic bacteria requires the genetic activation of recognition, contact establishment, host colonization, and infection. These complex events have been gradually elucidated through the use of modern molecular biology and genetic techniques. For example, the recognition of the host by bacteria initially occurs upon the perception and recognition of chemical signals, especially those of the phenylpropanoid metabolic pathway, such as plant flavonoids. For bacteria, particularly *Agrobacterium tumefaciens*, *Pseudomonas syringae*, and *Xanthomonas campestris*, the enzyme histidine kinase is the sensor molecule that allows for the pathogen association with the host plants. There are also signals that allow bacteria to communicate with one another, triggering processes that are generally dependent on the size of the population, which is a phenomenon known as quorum sensing. In this process, bacteria regulate gene expression by the concentration of the diffusible signaling molecules produced by them. In gram-negative bacteria, the most well-known signaling molecules are the N-acyl homoserine lactones (AHLs). After recognition, quorum sensing is essential for the colonization of the host, conjugative transfer of plasmids, regulation of the type III secretion system (T3SS), and the production of extracellular polysaccharides (EPS) (Kado 2010).

Virulence, which is the ability of a pathogen to induce disease to a plant, varies among isolates. For phytopathogenic bacteria, virulence depends on several factors, including population (quorum sensing), the ability to invade and colonize the host, and withstand plant defense mechanisms. Phytopathogenic bacteria use T3SS to colonize their hosts, extract essential nutrients, and cause disease (Kado 2010). Different T3SS secrete a set of distinct virulence factors (effectors). These proteins are crucial elements for pathogenesis, as they mediate the breakdown or suppression of components of the plant cell defense network. For example, in *Ralstonia solanacearum*, the synthesis of the major virulence factor EPS is regulated through the quorum sensing system, which is abundantly produced at high bacterial cell densities or when the pathogen invades the xylem vessels of host plants (Milling et al. 2011). If recognized by the host cellular defense system. The effector might be subsequently converted into an avirulence factor (*avr* gene) according to the gene-for-gene model.

3.3 Plant Breeding for Disease Resistance

Plant breeding has made an unquestionable contribution to society and agriculture. Recent evidences of climate change indicate the need for development of cultivars that are genetically resistant to pathogens well-adapted to high temperatures, such as *Pectobacterium* spp., *Dickeya* spp., and *Ralstonia solanacearum*. Furthermore, there is a growing concern related to the serious threat of genetic erosion due to these environmental changes. For example, it has been predicted that 16–22 % of the 108 wild potato species of the genus *Solanum* employed in breeding programs are threatened with extinction by 2055 (Info-Resources Focus 2008).

The control of plant diseases, including those of bacterial origin, requires integrated measures, among which genetic resistance has been considered. Plant breeding programs are generally designed for long-term objectives and should, therefore, be well-planned with a clear focus, achievable targets, and a broad view of the agribusiness chain. Although disease resistance has received high priority in many breeding programs, it is only one of the many desired characteristics that a newly developed cultivar must have. In practical terms, a disease-resistant cultivar must also have high yield and quality as the most important characteristics for many species. Russel (1978) suggested that plant breeders should primarily be concerned with avoiding the extreme susceptibility of cultivars to diseases. Therefore, selections performed in the field under natural pathogen pressure tend to increase the chances of obtaining cultivars with resistance to a greater number of diseases, even those that have not been a direct target, which is a phenomenon known as non-intentional selection (Heiser 1988).

Having defined that disease resistance is a priority for breeding programs, the following considerations should be addressed before initiating the selection procedures:

1. **Is the target disease really important?** The importance of a given disease is not the only reason to initiate a resistance breeding program. Many plant diseases are able to cause damage to crops and result in a loss of productivity or quality. The main rationale to justify a breeding for resistance program must be that these diseases cannot be controlled in an effective manner using alternative control methods.
2. **Is the pathogen sufficiently known?** It is crucial to understand the variability of the pathogen to determine the type of resistance that should be incorporated into elite germplasm. A phytopathologist should be consulted to evaluate the pathogen variability and to define the appropriate inoculation methodology, which would allow a clear distinction between resistant and susceptible genotypes.
3. **Are there sources of resistance?** Typically, this information can be obtained from the literature. When these sources exist, the development of resistant cultivars might also be challenged by the increasing difficulty of germplasm exchange. In Brazil, a license to collect specimens is required from the Brazilian Institute of Environment and of Renewable Natural Resources (Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis—IBAMA)

and the Council for the Management of the Genetic Heritage (Conselho de Gestão do Patrimônio Genético—CGEN).

4. **What type of resistance should be sought?** In case of pathogens with little variation, qualitative resistance (monogenic/oligogenic) is preferred, due to the ease of introgression and incorporation. Quantitative resistance (polygenic) has been recommended for highly variable pathogens, which, despite complex introgression into genotypes of commercial interest, protects the plant against all the variants of the pathogen.
5. **What is the most appropriate breeding method?** The reproductive system of the plant species determines the breeding method. Thus, different methods are required for either autogamous, allogamous or vegetatively propagated plant species as well as for their distinct ploidy levels. The selection of resistance traits that display high heritability and simple genetic control, mainly of the additive type, might be achieved through the individual performance or, rather, the performance *per se* of inbred lines or populations. When the characteristic has low heritability or displays inheritance that is conditioned by genes with non-additive effects, selection should be based on progeny testing and hybrid performance. Notably, if there is a significant maternal effect, then there will be a difference in the selection of the female parent for a particular crossing.

3.4 Sources of Disease Resistance

The sources of disease resistance, including the bacterial diseases, have been identified mainly in wild species. Therefore, genetic breeding programs with a wide genetic base would take the advantage of having genes of interest available when needed. According to Rick (1986), traditional breeding programs aiming to develop pure inbred lines with high yield potential and good commercial characteristics promotes the narrowing of the genetic base. Fortunately, the genetic resources of the centers of origin and diversity provide an extraordinary source of useful alleles, especially with regard to disease resistance. For the tomato alone, the germplasm collection contains more than 60,000 accessions in gene banks worldwide (Ross 1998). In Brazil, more than 4,000 accessions of tomato are maintained at Embrapa, Agronomic Institute of Campinas (Instituto Agronômico de Campinas), and Federal University of Viçosa (Robertson and Labate 2007).

Plant breeders usually have access to an abundance of academic literature on the reaction of a plant species germplasm to bacterial diseases. However, particularly for the case of quantitative resistance, it is common to find conflicting informations, and it is not always easy to identify the most reliable data. These conflicts do not reflect an ethical problem, as discrepancies can result from different data interpretation and from environmental effects. Most discrepancies do reflect weaknesses in the experimental methodology and the selection systems used. This chapter will discuss how important is the choice of the right selection

Table 3.1 Differences between qualitative and quantitative resistance

Characteristics	Qualitative resistance	Quantitative resistance
Reaction to pathogen variants	Specific	Non-specific
Environmental stability	Stable	Unstable
Inheritance	Monogenic/oligogenic	Polygenic
Level of resistance	High	Moderate
Epidemiologically based concept	Vertical	Horizontal

methodology in order to allow for a clear distinction between resistant and susceptible genotypes. The appropriate methodology will enhance experimental precision, which allows for the differentiation of more subtle expression levels of quantitative resistance. An effective selection of disease resistant plants requires breeders to have a clear understanding of the pathosystem being studied. Therefore, the knowlegde about the variability of the pathogen (virulence and aggressiveness), the variability of the host plant as a function of the type and inheritance of resistance, and the effects of the environmental conditions on the manifestation of the phenotypic expression of the disease is desirable.

3.5 Qualitative Resistance Versus Quantitative Resistance

The development of disease resistant cultivars requires knowledge of the types of resistance and the genetic mechanisms that regulate the expression of this trait. There are two categories of resistance: complete resistance, which is controlled by one or a few genes, and incomplete or partial resistance, which is conditioned by several genes that individually contribute with smaller effects. Several names are used to describe these types of resistance, such as vertical versus horizontal, complete versus incomplete, monogenic versus polygenic, and qualitative versus quantitative (Table 3.1). For standardization, the terms **qualitative** and **quantitative** will be used herein.

Qualitative resistance confers a high degree of resistance and is easily incorporated. It is generally achieved through backcrossing (Borém and Miranda 2009). A major limitation of qualitative resistance is its low durability, especially in pathosystems in which the pathogen has high evolutionary potential, such as bacteria. In addition, this type of resistance is not always available, even among wild host species. Bacterial pathogens with high evolutionary rates can overcome specific recognition mechanisms, while non-specific mechanisms ensure the stability of the resistance when faced with new pathogen variants.

The quantitative resistance in the vast majority of the pathosystems studied to date does not confer a degree of resistance as high as qualitative resistance, although it offers durable and effective protection against several variants of the pathogen.

3.6 Hypersensitivity Reaction and Qualitative Resistance

Certain bacteria, such as *R. solanacearum* and *A. tumefaciens*, are capable of infecting wide range of hosts. Other bacteria display specificity, such as *Erwinia amylovora*, which attacks apples, pears, and other rosaceous plants, and *P. syringae* pv. *tomato*, which only attacks tomato. The contact between the bacteria and a host plant might result in a **compatible** interaction, where the bacterial pathogen rapidly multiply in the intercellular spaces of the host, leading to the expression of disease symptoms, or an **incompatible** interaction, in which the pathogen fail to multiply and the plant does not display disease symptoms. The incompatible reaction is normally followed by rapid cellular collapse, which delimits the affected area and impedes the spread of the bacteria to neighboring tissues. This phenomenon is called hypersensitivity reaction (HR), which is closely associated with the qualitative resistance to bacterial diseases. HR occurs when the bacterial pathogen injects effector proteins into the host plant using a secretion system, such as T3SS. In response to the effector proteins, resistant plants undergo biochemical reactions, resulting in the almost immediate interruption of the infection process, thus preventing the bacteria from invading other parts of the plant. Recognition of the pathogen by a resistant plant requires the presence of *hrp* (*hypersensitive reaction and pathogenicity*) genes in the bacterial pathogen and a pair of corresponding genes in in both the bacterial pathogen and the host, which are the Avr (avirulence) and R (resistance) genes, respectively.

3.7 Molecular Markers

The development of dense genetic maps and the use of several molecular markers in linkage disequilibrium with genomic regions encompassing bacterial resistance factors allowed for the localization and isolation of plant genes and the establishment of marker-assisted selection (MAS) programs. Genes for bacterial resistance have been mapped, and many have already been isolated and characterized. In addition, projects for sequencing the complete genome of various bacteria and host plants are either underway or have been completed. The information obtained with those projects has been used to generate new diagnosis systems and phylogenetic tools for identification of pathogens as well as new molecular marker systems associated with resistant phenotypes. Moreover, this data bank has served as an important instrument in MAS programs to speed up the incorporation of various resistance factors in a single genotype.

Regions of the host plant genome containing genes for the expression of particular quantitative characteristics, including resistance to diseases, are called quantitative trait loci or QTL (Young 1996). The genes associated with QTL might be located on different chromosomes and contribute differently to the expression of the phenotype. An efficient molecular marker system facilitates the selection of resistant genotypes

in a large number of seedlings in less time and without the risk of introducing the pathogen into areas where it is still not present (preemptive breeding). These marker systems might significantly accelerate the gains of breeding programs. The combination of MAS and field selection has been one of the most efficient breeding strategies. One example is the development of tomato line with simultaneous resistance to the bacterial spot caused by the T1 strain of *X. campestris* pv. *vesicatoria* and bacterial speck (Yang and Francis 2007). In fact, experience with several crops has demonstrated that the ideal strategy includes the combination of molecular and traditional breeding methods.

However, MAS can be an unreliable method for monitoring bacterial resistance traits when the markers are not in strong linkage disequilibrium with the genes of interest. In this case, there is the risk that the markers and the target phenotype will be separated through recombination. The use of functional markers corresponding to the gene that controls a given phenotype is ideal. Examples of functional markers available for bacterial resistance are the *Pto*/*Prf* locus, which controls resistance to bacterial speck in tomatoes, and the *Bs-2* and *Bs-3* genes, which control resistance to bacterial spot in species of the genus *Capsicum* (Römer et al. 2010).

3.8 Cell Biology and Transgenics

The sources of resistance to bacterial diseases have typically been studied in wild species. However, the introgression from wild species or taxonomically correlated genera might be problematic when there is a barrier to sexual isolation or unilateral incompatibility in the crossing. One of the main barriers in interspecific crosses is the incompatibility of the endosperm, which results in the abortion of the embryo. These barriers might be overcome using in vitro culture techniques, which have been employed in different plant species.

The advances in plant transformation techniques, especially the phytopathogenic bacteria *A. tumefaciens*, also using allowed for the development of new transgenic cultivars with resistance to bacterial diseases. The main strategies using transgenics for bacterial resistance have been the expression of different peptides with antimicrobial action and the use of other gene products that are effective against leaf and fruit pathogens with the objective of preventing seed transmission (Oard and Enright 2006).

The mobilization of genes within the same species or related taxa via genetic engineering has also been employed for resistance to bacterial diseases, e.g., the transfer of the *Bs-2* gene for resistance to bacterial spot from the genus *Capsicum* to the tomato, in which the sources of resistance are polygenic and difficult to incorporate (Tai et al. 1999).

3.9 Methodologies to Identify Sources of Resistance

In the search for sources or in the evaluation of resistance to bacterial diseases, it is imperative that the selection of false resistant materials must be avoided. Also, the method should be precise to detect intermediate levels of resistance, which are often relevant in case of quantitative resistance. Thus, the following precautions are recommended:

(1) Developing a statistical design to obtain robust responses in relation to the evaluated treatments. It is crucial to define the minimum plot size and the number of replicates. High coefficients of variation, which lead to confusing interpretations, are frequently observed when few plants are used in the experimental plot because escapes are common for diseases especially the ones caused by soil pathogens.
(2) Ensuring that the inoculated plants are uniform. Different physiological ages among plants of different genotypes might mask differences in their phenotypic expression of resistance. For example, older plants are usually more resistant than young ones. This observation is more relevant in the case of quantitative resistance.
(3) Selecting an isolate that is representative of the pathogen. For a variable pathogen, it is necessary that the plant accessions under evaluation be challenged with more than one isolate.
(4) Using appropriate inoculum concentration. This concentration, which normally varies from 10^6 to 10^8 colony forming unit (cfu)/ml, depends primarily on the virulence of the isolate and the inoculation method.
(5) Using an appropriate inoculation method, according to the plant age and inoculum concentration, which facilitates the differentiation of resistant and susceptible genotypes. The method should approximate the natural infection mode of the pathogen (Box 1, Fig. 3.1, and Table 3.2).
(6) Considering that different genetic factors might regulate the resistance in different organs of the plant. Illustrative examples are the different reactions in the leaves and pods of beans against common bacterial blight and different genetic control of local and systemic infections of bacterial canker in tomato.
(7) Using susceptible controls to verify the success of the inoculation and resistant controls to assess whether the inoculation method is excessively drastic.
(8) Using a grading scale for clear differentiation among levels of resistance. Quantitative scales should be preferentially employed in order to facilitate statistical analysis (Fig. 3.2).
(9) Performing appropriate statistical analyses. Clustering tests that discriminate resistant and susceptible individuals are typically used. When the number of evaluated genotypes is large (more than 30), a mean separation test (Tukey or Duncan) is not recommended; rather, a test that distinguishes the genotypes from the controls (Dunnett) or forms groups according to the ranges in the levels of susceptibility and resistance (Scott-Knott) is preferred. A statistician should be always consulted. A simplified example of the screening steps is presented in Fig. 3.3.

Box 1—Methods used for bacterial inoculation in plants (Fig. 3.1)

Drastic methods: injuring the stem with an infected toothpick, injecting bacteria directly into the vessels, injuring the leaves with needles or awls, cutting the leaves with contaminated scissors.
Intermediate methods: dipping damaged roots in an inoculum suspension, spraying a bacterial suspension on leaves previously wounded with carborundum, dipping leaves in an inoculum suspension, embedding seeds in an inoculum suspension.
Light methods: spraying the undamaged organs, dipping the undamaged roots, pouring the inoculum suspension over the plant stem, exposing the plants to natural infections.

3.10 Advances Obtained in Genetic Breeding Involving Bacterial Diseases

In this section, we will present pathosystems that involve bacterial diseases in economically important crops. In these examples, we attempt to show the importance of the disease and the advancements that have been achieved in breeding programs, taking into account the variability of the respective pathogens, which are mostly composed by a complex of variants, strains, and species.

(1) ***Acidovorax avenae*** **subsp.** ***citrulli*** **(AAC) in cucurbits**—Bacterial blotch (BB), or bacteriosis, is devastating to watermelon and melon crops grown in hot and humid environments. The pathogen is seed transmitted in an efficient manner. After being established in a field, it is difficult to control the disease, even with repeated applications of copper-based chemicals. Genetic resistance might be a control option in the case of accidental planting of infected seeds or dissemination of the pathogen via contaminated aerosols from neighboring farms. Currently, there are no cultivars of melon or watermelon with adequate levels of resistance to BB mostly because it has been difficult to identify useful sources of resistance to the disease (Wechter et al. 2011).

Burdman et al. (2005) identified two major groups of AAC: one associated with cucurbits other than watermelon, and another group associated with watermelon. In Brazil, BB is important in melon cultivated in the Northeast Region, where Oliveira et al. (2007) observed the physiological, biochemical, and pathogenic differences among melon isolates of AAC. All of the AAC isolates infected melon and watermelon, although specificity was demonstrated among isolates from the same host species, which caused the most severe symptoms. The susceptibility of watermelon to melon isolates is concerning, as BB had not yet been reported in watermelon in that region, even at production areas where both crops are grown.

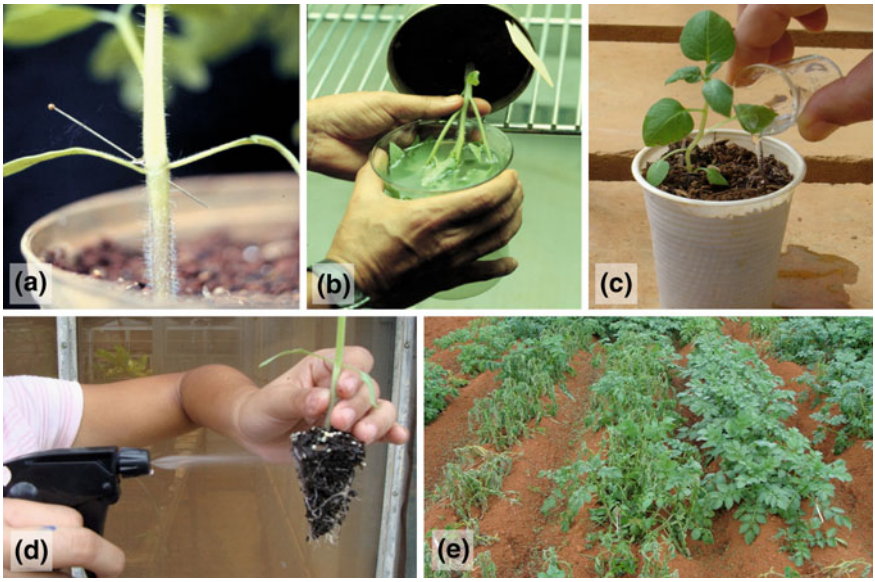

Fig. 3.1 Some inoculation methods used to select plants resistant to bacterial diseases: **a** Insertion of a pin into plant stem after applying a drop of the inoculum suspension. **b** Dipping the leaves in an inoculum suspension. **c** Pouring a inoculum suspension at the base of the plant to inoculate the roots. **d** Directly spraying the roots with inoculum suspension. **e** Planting in infested soil for natural inoculation

Table 3.2 Examples of the combination of inoculum concentration, inoculation method, plant age in the effectiveness of discriminating between resistant and susceptible genotypes for quantitative resistance

	Seedling		Adult plant	
Inoculum concentration (cfu/ml)	Drastic method	Light method	Drastic method	Light method
10^6	++ Some escape	+ Escape	++ Possible escape	+ Escape
10^7	+++	++ Possible escape	++ Possible escape	+ Escape
10^8	+ Break in resistance	+++	+++	++ Possible escape

Relative effectiveness of the methodology: (+) slightly effective; (++) moderately effective; (+++) highly effective

The lack of reports of an epidemic of the disease in watermelon, however, reinforces the idea that there are distinct groups of isolates.

(2) ***Xanthomonas axonopodis* pv. *passiflorae* (XAP) in passion fruit**—Passion fruit bacteriosis is one of the major diseases that occur under conditions of high temperature and humidity. The disease initially appears on the tender parts of the

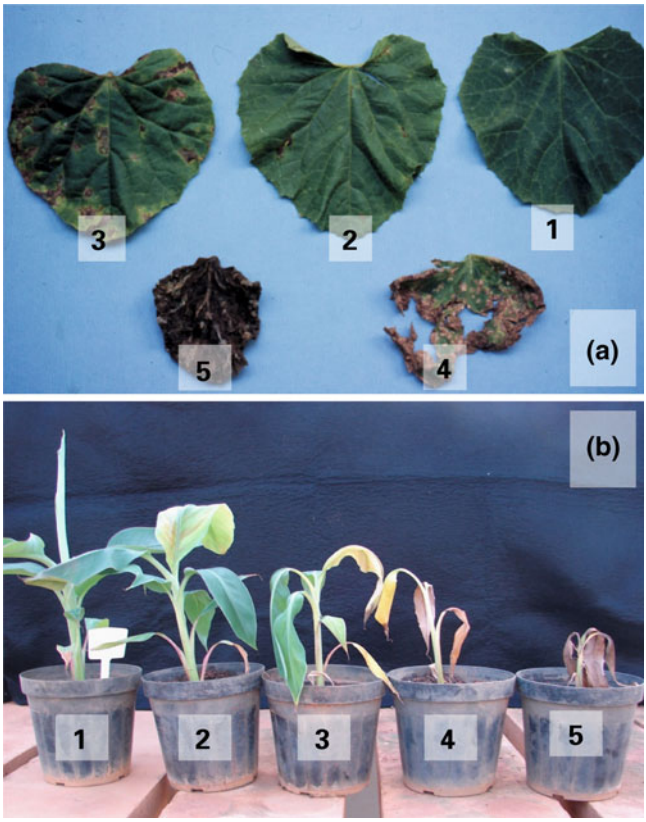

Fig. 3.2 Severity scale scores, varying from 1 (without symptoms) to 5 (total necrosis or death). **a** Melon blotch (*Acidovorax avenae* subsp. *citrulli*). **b** Moko disease in banana (*Ralstonia solanacearum* race 2)

plant, primarily on the leaves, which exhibit dark green wet spots that later dry out. The bacteria invade the leaf veins and sistemically spreads, causing die-back and premature leaf drop. The lesions on fruits are oily and vary from green to brown. Although some genotypes of *Passiflora edulis* (cultivated species) are less attacked than others, a satisfatory level of resistance for adequate control has not been described for this species. Several accessions of different *Passiflora* species have been evaluated for disease resistance (Junqueira 2010). Several *P. caerulea* accessions have been used as sources of resistance genes in breeding programs for passion fruit. QTL of additive effects associated with resistance in the fruits and leaves were identified in segregating populations of *P. alata* and *P. edulis* f. *flavicarpa,* (Braga 2011).

Nakatani et al. (2009) observed a high degree of polymorphism among the XAP isolates from different Brazilian States. The observed variability is explained by the coevolution of the pathogen and host and the fact that Brazil is an important diversity center for *Passiflora*. Generally, centers of plant diversity are also centers

Fig. 3.3 Description of the screening steps for resistance to *Ralstonia solanacearum*. **a** Production of uniform and vigorous seedlings. **b** Wounding the lower third of the roots. **c** Dipping the wounded roots in the inoculum suspension. **d** Transplantation of the inoculated accessions and cultivation in an environment favorable for disease development (bacterial wilt). **e** Evaluation of the disease and selection of resistant genotypes.

of pathogen diversity (Leppik 1970). This genetic variability explains, at least in part, the contradicting results found in the literature. Therefore, Braga (2011) recommends an evaluation of the resistance in various locations within an ecological region and among ecologically distinct regions.

(3) ***Pseudomonas syringae* pv. *tomato* (PST) in tomato**—Bacterial speck is a destructive disease occurring tomatoes grown in mild and humid climates. This disease induces circular lesions with a yellowish halo on the leaves, which is a symptom that might be confused with bacterial spot, and dark specks on the petiole, peduncle, and fruits. This disease is less common in processing tomatoes due to the employment of cultivars with qualitative resistance, which provides the plants with an immune-like response to this disease. A complex locus with a dominant effect (*Pto/Prf*) controls resistance to the disease. The *Pto* gene encodes a kinase and was the first resistance gene identified in tomato (Martin et al. 1993). The *Prf* gene is physically associated with *Pto* and provides complete resistance (Salmeron et al. 1996).

Molecular marker-assisted selection (MAS) has been used to monitor the incorporation of the *Pto*/*Prf*/*Fen* gene cluster in tomato (Yang and Francis 2007). The *Fen* gene controls sensitivity (formation of local lesions) in response to the application of the insecticide Fenthion (Salmeron et al. 1996). Due to its lack of complexity and close linkage with *Pto/Prf*, this reaction has been employed as a phenotypic marker in the selection of resistant plants and, in certain situations, eliminating the use of MAS.

Although qualitative resistance (monogenic/oligogenic) is typically ephemeral, the resistance conferred by the *Pto/Prf* locus in Brazil has not been "broken" by PST isolates after its employment for years and several locations. The stability of the resistance can be explained the "guard model", in which an R protein from the host (Prf) does not directly interact with the Avr protein of the pathogen but rather guards or monitors the status of another host protein (Pto), which is the target of the gene product Avr (Bent and Mackey 2007).

(4) **The *Xanthomonas* complex in *Capsicum***—Bacterial spot (BS) is common in bell peppers and hot peppers of the genus *Capsicum* during warm and rainy periods or in sprinkler-irrigated crops. In the leaves, which easily fall from the diseased plant, the pathogen causes dark wet spots of various shapes and sizes. The manifestation of this disease in fruits, although less common, is characterized by crater-shaped lesions that are initially whitish and subsequently turn brown.

BS is caused by four distinct taxa within the genus *Xanthomonas*, which were previously considered to belong to a single species (*X. axonopodis* pv. *vesicatoria*). However, more recently they were classified as new species: *X. euvesicatoria*, *X. vesicatoria*, *X. gardneri*, and *X. perforans*. Among these, *X. euvesicatoria* (XEV) has been most frequently found in *Capsicum*. Sources of HR-type resistance were identified in *C. annuum*, *C. pubescens*, and *C. chacoense* for XEV, all of which exhibit monogenic and dominant inheritance. These genes were named as *Bs-1*, *Bs-2*, and *Bs-3*, respectively. These genes have been isolated, and MAS methods have been established (Römer et al. 2010). However, the variability of the cognate effector genes (*avrBs1*, *avrBs2*, and *avrBs3*) have led to the breakdown of the three resistance genes in inbred lines when they were incorporated as single/individual genes.

Analysis of the spectrum of virulence in a collection of XEV isolates revealed the presence of at least 11 strains (P0–P10) in *Capsicum* (Vallejos et al. 2010). The P6 strain has been identified as a virulent strain, even for *Capsicum* genotypes that contain a pyramid of the three dominant resistance genes. Sources of resistance to the P6 strain were identified in *C. pubescens* and in lines derived from *C. annuum*. Studies concerning genetic inheritance and mapping indicate genetic control through two independent and recessive genes (*bs-5* and *bs-6*), which, in combination, regulate complete resistance to isolates of XEV P6 strain (Vallejos et al. 2010).

(5) **The *Xanthomonas* complex in tomato**—Symptoms of bacterial spot (BS) in tomato are similar to those induced in *Capsicum*, but they leave a "blight" aspect on the oldest leaves without causing premature leaf fall. Four different species of *Xanthomonas* and five strains (T1–T5) were identified infecting tomato based on molecular taxonomy studies (Jones et al. 1998) and different responses to infection using a series of differential host accessions (Wang et al. 2011). This variability is described in terms of species and strains, with some correlation between the two. *Xanthomonas euvesicatoria* corresponds to the T1 strain; the isolates of the T2 strain might correspond to two species: *X. vesicatoria* and *X. gardneri*; and the isolates of the T3, T4, and T5 strains are classified within the species *X. perforans*. HR-like resistance to isolates of the T3 strain was identified in some *S. lycopersicum*

accessions that were susceptible to the T4 strain for which *S. pennellii* is a good source of resistance. The large variability among and within species of the *Xanthomonas* complex is the major challenge for controlling the disease.

A source of quantitative resistance (devoid of HR) that is effective against a broad spectrum of strains was identified in one accession of *S. lycopersicum* var. *cerasiforme*. Three QTLs (*Rx-1*, *Rx-2*, and *Rx-3*), which control resistance to isolates of *X. euvesicatoria* (T1 strain), were identified in the breeding line 'Hawaii-7998' (Wang et al. 2011). The QTL *Rx-3*, which is also effective against isolates of *X. perforans* (T3 strain), is in repulsion phase linkage with the *Pto*/*Prf*/*Fen* gene cluster, which controls resistance to bacterial speck (PST), thereby hindering the combination of resistance to the two diseases (Yang and Francis 2007). The introgressed locus of *S. pimpinellifolium Rx-4* also confers resistance to isolates of *X. perforans* (T3 strain) (Wang et al. 2011).

Although high correlation levels were observed between the bacterial spot severity in greenhouse and in the field, Mello et al. (1997) showed that the high resistance of some genotypes observed in the field would not have been predicted based on greenhouse trials. Therefore, field validation of genotypes selected as resistant in tests under controlled conditions (screenhouses, greenhouses, growth chambers) is highly recommended. In summary, selection based on tests only performed in a controlled environment might result in the premature elimination of genotypes with quantitative resistance that might eventually be exhibited in adult plants.

(6) ***Clavibacter michiganensis* subsp. *michiganensis* (CMM) in tomato**—Bacterial canker (BC) occurs sporadically and causes significant losses when it occurs under field conditions. This disease induces various symptoms, such as plant wilt, necrosis of the leaf edges, stem cankers, spots on the fruits, and vascular browning depending upon the infection is local or systemic. The success of breeding for resistance to BC has been limited due to the lack of studies characterizing the diversity of isolates of the pathogen, which makes difficult the identification of broad spectrum resistance sources. There is also a lack of standardization of the inoculation methods, which leads to conflicting interpretations among studies. For example, there is evidence that genotypes resistant to localized infection (leaf and fruit damage) are not resistant to the systemic phase of infection (plant wilt) (Yang and Francis 2007).

Kronka (2004) and Kabelka et al. (2002) identified sources of partial resistance to BC in different *Solanum* species, and several of these resistance factors have been incorporated into commercial genotypes with the help of molecular markers. Five QTLs for BC resistance were located in *S. peruvianum* accessions (Vanheusden et al. 1999), and two QTLs with major effects were identified in *S. habrochaites* (Kabelka et al. 2002).

(7) ***Ralstonia solanacearum* (RS) in potato**—Bacterial wilt of potato (BWP) is a major disease, especially during the summer season. This disease causes typical wilt symptoms, as it affects the transportation of water from the roots to the leaves, resulting from the plugging of the xylem vessels, which become brown during

infection. Affected tubers have a dark vascular ring, with exudation of bacterial ooze. The tubers easily rot due to the invasion of other microorganisms.

Potato cultivars with a high degree of resistance to BWP are not yet available. A genetically complex (polygenic) partial resistance was identified, which involves genes with large and minor effects (Tung et al. 1990). Therefore, resistance does not provide sufficient protection as an exclusive control measure. In addition, it has been a difficult task for the potato breeding programs to combine resistance with other commercially important characteristics under polygenic control, such as tuber skin type and color, tuber shape and size, and yield. This difficulty is due, in part, to the complexity of genetic combination in tetraploid species (such as *S. tuberosum*) along with the wild origin of resistance factors.

The main genes for resistance come from wild potato species, such as *S. phureja*, *S. raphanifolium*, *S. sparsipilum, S. microdontum*, *S. commersonii*, and *S. stenotomum* (Fock et al. 2005). Due to the ease of crossing, the resistance of *S. phureja* has been most commonly used. In Peru, the cultivars Molinera and Caxamarca (derived from *S. phureja*) were developed specifically for resistance to BW and played an important role in controlling the disease in the Andean region, where the race 3 of this pathogen has been identified. However, these sources of resistance did not display an adequate response in tropical climates, where there is higher pathogen variability (Lopes 2005). When evaluated in Brasilia, DF, these accessions were not suitable for cultivation, due to their low adaptability (poor appearance of the tubers and low yield) and low levels of resistance. These results support the hypothesis of Tung et al. (1990) that resistance to BW manifests as a consequence of the adaptability of a genotype to a particular region. In Brazil, among the cultivars of economic importance, 'Achat' exhibits stable, broad spectrum field resistance to BW (Lopes 2005). However, disease resistance was not sufficiently relevant to avoid its replacement by susceptible cultivars that had a better tuber appearance, such as 'Monalisa', 'Cupido', and 'Agata'. The clone MB03 (derived from *S. phureja*) selected at Embrapa Hortaliças (Lopes et al. 2004) exhibits a high degree of resistance to BWP and has been used as the primary source of resistance in the breeding program for the potato at Embrapa. In an attempt to identify alternative sources of resistance, the genotypes of *S. commersonii* were evaluated in Uruguay (Siri et al. 2009) and in Italy (Carputo et al. 2009) with promising results. The sequencing of the potato genome, which was recently concluded, should accelerate the chromosomal location and characterization of the resistance factors, which is essential for the development of clones and cultivars resistant to BWP.

(8) ***Ralstonia solanacearum* (RS) in tomato**—Bacterial wilt (BWT) causes significant losses in tomatoes and is therefore considered as a limiting factor in this crop in locations with high temperature and humidity, such as the Northern Region of Brazil. This disease causes plant wilt during the warmest hours of the day, and vascular browning, which can be observed when peeling the base of the stem of the wilted plant. There are no commercial tomato cultivars with a satisfactory degree of resistance, and the control of the disease depends on the adoption of integrated preventive measures. Under protected cultivation, control has been

implemented using hybrid rootstocks resistant to BWT, which are already available on the market.

The breeding line 'Hawaii 7997' was identified as the best source of resistance in terms of general combining ability (Hanson et al. 1998), while 'Hawaii 7996' displayed stability and large spectrum of action against different isolates (Scott et al. 2005). Because RS is a highly variable pathogen, the development of stable resistance is essential. Therefore, 'Hawaii 7996' has been used in the majority of breeding programs and inheritance studies. The presence of distinct strains, races and phylotypes that define regional clusters reinforces the need to evaluate resistance against local isolates.

Four *Bwr* QTLs were identified in 'Hawaii 7996', and the locus with the major effect is located on chromosome 6 (*Bwr-6*). The QTLs *Bwr-4* and *Bwr-8* were detected only under conditions of high temperature (Carmeille et al. 2006). In the breeding standpoint, the presence of the QTL *Bwr-6* is a challenge, due to its repulsion phase linkage with small fruit size and susceptibility to other important tropical pathogens, such as root-knot nematodes (*Meloidogyne* spp.) and begomovirus.

(9) ***Xanthomonas axonopodis* pv. *phaseoli* (XAP) in beans (*Phaseolus vulgaris*)**—Common bacterial blight (CBB) caused by isolates of XAP is a frequent bean disease, especially in crops subject to high temperatures and humidity. This disease causes wet spots and necrosis on the leaves in varying sizes and shapes that are associated with a thin yellowish halo. In green pods, the lesions are round, initially wet, and subsequently acquire a reddish color. Cultivars with adequate resistance levels have been developed, yet resistance to the disease is not always accompanied by high seed yield and commercial quality. Different sources of resistance have been reported in *P. vulgaris*, *P. acutifolius*, and *P. coccineus* (Michaels et al. 2006; Miklas et al. 2006; Park et al. 2007; Silva et al. 2009; Shi et al. 2011).

Liu et al. (2008) and Tar'an et al. (2001) reported molecular markers linked to QTLs associated with resistance to CBB in beans. These authors, however, pointed out that certain QTLs were identified in studies conducted under greenhouse with a small plant population. Caution should be taken when using these markers for analysis, and their validity should be assessed under different experimental conditions. Schuster et al. (1983), Maringoni et al. (1993), and several other studies have demonstrated the independence of the resistant reaction to CBB in leaves, pods, and seeds. Therefore, artificial inoculations should consider the possibility of different reactions among these organs, preferably using field evaluations with natural infections.

(10) ***Xanthomonas axonopodis* pv. *manihotis* (XAM) in cassava**—Bacterial blight of cassava (BBC) is one of the most important diseases of cassava (*Manihot esculenta*) in Brazil and in other countries with a tropical climate, especially in certain regions of Africa. This disease induces wilting, leaf spots, and die-back with necrosis of the vascular system and exudation of bacterial ooze. BBC is difficult to control and requires integrated measures, including genetic resistance.

Resistance to BBC resulting from the introgression of genes from *M. glaziovii*, is polygenic and has additive genetic control. Six regions of the genome were identified controlling field resistance to this disease (Jorge et al. 2001). However, different QTLs were identified in different years, which might reflect changes in the genetic and pathogenic structure of the pathogen populations. In fact, from the point of view of the pathogen *x* host interaction, there is evidence of pathotype *x* cultivar specificity based on variability studies. For example, the isolates from South America displayed higher variability than did the isolates from Africa (Wydra et al. 2004). Similarly, Nery-Silva et al. (2007) showed that an isolate obtained in the area of Uberlândia, MG was the most virulent for cultivated cassava , whereas an isolate from the area of Lavras, MG, was the most virulent for wild cassava. The cultivars Vassoura, Amarela, Vermelha, Castelinho, and the clone CPAC88-11 displayed the highest levels of resistance.

References

Bent AF, Mackey D (2007) Elicitors, effectors, and R genes: the new paradigm and a lifetime supply of questions. Annu Rev Phytopathol 45:399–436

Borém A, Miranda GV (2009) Melhoramento de plantas. 5a. edição. Viçosa: Editora UFV. p 529

Braga MF (2011) Mapeamento de QTL (Quantitative Trait Loci) associados à resistência do maracujá doce à bacteriose. ESALQ (Tese de Doutorado), Piracicaba

Burdman S, Kots N, Kritzman G, Kopelowitz J (2005) Molecular, physiological, and host-range characterization of *Acidovorax avenae* subsp. *citrulli* isolates from watermelon and melon in Israel. Plant Dis 89:1339–1347

Carmeille A, Caranta C, Dintinger J, Prior P, Luisetti J, Besse P (2006) Identification of QTLs for *Ralstonia solanacearum* race 3-phylotype II resistance in tomato. Theor Appl Genet 113: 110–121

Carputo D, Aversano R, Barone A, Di Matteo A, Iorizzo M, Sigillo L, Zoina A, Frusciante L (2009) Resistance to *Ralstonia solanacearum* of sexual hybrids between *Solanum commersonii* and *S. tuberosum*. Am J Potato Res 86:196–202

Fock I, Luisetti J, Collonnier C, Vedel F, Ducreux G, Kodja H, Sihachakr D (2005) *Solanum phureja* and *S. stenotomum* are sources of resistance to *Ralstonia solanacaearum* for somatic hybrids of potato. In: Allen C, Prior P, Hayward AC (eds) Bacterial wilt disease and the *Ralstonia solanacearum* species complex. APS, St. Paul, pp 253–259

Hanson PM, Licardo O, Hanudin JF, Wang JF, Chen JT (1998) Diallel analysis of bacterial wilt resistance in tomato derived from different sources. Plant Dis 82:74–78

Heiser CB (1988) Aspects of unconscious selection and the evolution of domesticated plants. Euphytica 37:77–81

INFO (2008) Resources Focus. 2008, 10 de novembro. Potatoes and Climate Change. Disponível em http://www.inforesources.ch/pdf/focus08_1_e.pdf

Jones JB, Stall RE, Bouzar H (1998) Diversity among Xanthomonads pathogenic on pepper and tomato. Annu Rev Phytopathol 36:41–58

Jorge V, Fregene M, Vélez CM, Duque MC, Tohme J, Verdier V (2001) QTL analysis of field resistance to *Xanthomonas axonopodis* pv. *manihotis* in cassava. Theor Appl Genet 102: 564–571

Junqueira KP (2010) Resistência genética e métodos alternativos de controle da bacteriose do maracujazeiro causada por *Xanthomonas axonopodis* pv. *passiflorae*: Brasília: UnB (Tese de Doutorado)

Kabelka E, Franchino B, Francis DM (2002) Two loci from *Lycopersicon hirsutum* LA407 confer resistance to strains of *Clavibacter michiganensis* subsp. *michiganensis*. Phytopathology 92:504–510

Kado CI (2010) Plant Bacteriology. APS Press, St. Paul, p 336

Kronka AZ (2004) Cancro bacteriano do tomateiro: Metodologia de inoculação, reação de genótipos do hospedeiro e eficiência de químicos sobre o controle: Piracicaba: ESALQ, 2004. p 79. (Tese de Doutorado)

Leppik EE (1970) Gene centers of plants as sources of disease resistance. Annu Rev Phytopathol 8:323–344.

Liu S, Yu K, Park SJ (2008) Development of STS markers and QTL validation for common bacterial blight resistance in common bean. Plant Breeding 127:62–68

Lopes CA (2005) Murchadeira da batata. Associação Brasileira da Batata, Itapetininga, p 66

Lopes CA, Quezado Duval AM, Buso JA (2004) 'MB 03': clone de batata resistente à murcha bacteriana. Boletim de Pesquisa e Desenvolvimento. Embrapa Hortaliças. Brasília, DF. 2004. p 14

Maringoni AC, Fregonese LH, Tofoli JG, Kurozawa C (1993) Reação foliar e da vagem de feijoeiro a *Xanthomonas campestris* pv. *phaseoli* e transmissão da bactéria pelas sementes. Fitopatologia Brasileira 18:412–415

Martin GB, Brommonschenkel SH, Chunwongse J, Frary A, Ganal MW, Spivey R, Wu T, Earle ED, Tanksley SD (1993) Map-based cloning of a protein kinase gene conferring disease resistance in tomato. Science 262:1432–1436

Mello SCM, Lopes CA, Takatsu A, Giordano LB (1997) Resistência de genótipos de tomateiro à mancha bacteriana, em campo e em casa de vegetação. Fitopatologia Brasileira 22:496–501

Michaels TE, Smith TH, Larsen J, Beattie AD, Pauls KP (2006) OAC Rex common bean. Can J Plant Sci 86:733–736

Miklas PN, Kelly JD, Beebe SE, Blair MW (2006) Common bean breeding for resistance against biotic and abiotic stresses: from classical to MAS breeding. Euphytica 147:105–131

Milling A, Babujee L, Allen C (2011) *Ralstonia solanacearum* extracellular polysaccharide is a specific elicitor of defense responses in wilt-resistant tomato plants. PLoS ONE 6:e1583

Nakatani AK, Lopes R, Camargo LE (2009) Variabilidade genética de *Xanthomonas axonopodis* pv. *passiflorae*. Summa Phytopathologica 35:116–120

Nery-Silva FA, Fernandes JJ, Juliatti FC, Melo B (2007) Reação de germoplasma de mandioca a *Xanthomonas axonopodis* pv. *manihots*. Semina: Ciências Agrárias 28:3–10

Oard S, Enright F (2006) Expression of the antimicrobial peptides in plants to control phytopathogenic bacteria and fungi. Plant Cell Rep 25:561–572

Oliveira JC, Silveira EB, Mariano RM, Cardoso E, Viana IO (2007) Caracterização de isolados de *Acidovorax avenae* subsp. c*itrulli*. Fitopatologia Brasileira 32:480–487

Park SJ, Yu K, Liu S, Rupert T (2007) Release of common bean germplasm line HR67. BIC Annu Rep 50:221–222

Rick CM (1986) Germplasm resources in the wild tomato species. Acta Horticulturae 190:39–47

Robertson LD, Labate JA (2007) Genetic resources of tomato (Lycopersicon esculentum Mill.) and wild relatives. In: Razdan MK, Mattoo AK (eds) Genetic Improvement of Solanaceous Crops. Tomato. Vol 2. Science Publishers, Enfield, NH, pp 25–75 (638p)

Römer P, Jordan T, Lahaye T (2010) Identification and application of a DNA-based marker that is diagnostic for the pepper (*Capsicum annuum*) bacterial spot resistance gene *Bs3*. Plant Breeding 129:737–740

Ross RJ (1998) Review paper: global genetic resources of vegetables. Pl Var Seeds 11:39–60

Russel GE (1978) Plant Breeding for Pest and Disease Resistance. Butterworths, London, p 486

Salmeron JM, Oldroyd GED, Rommens CMT, Scofield SR, Kim H-C, Lavelle DT, Dahlbeck D, Staskawicz BJ (1996) Tomato *Prf* is member of the leucine-rich repeat class of plant disease resistance genes and lies embedded within the *Pto* kinase gene cluster. Cell 86:123–133

Schuster ML, Coyne DP, Behre T, Leyna H (1983) Sources of *Phaseolus* species resistance and leaf and pod differential reaction to common blight. HortScience 18:901–903

Scott JW, Wang JF, Hanson PM (2005) Breeding tomatoes for resistance to bacterial wilt, a global view. Acta Horticulturae (ISHS) 695:161–172

Shi C, Navabi A, Yu K (2011) Association mapping of common bacterial blight resistance QTL in Ontario bean breeding populations. BMC Plant Biol 11:1–11

Silva A, Santos I, Balbinot AL, Matei G, Oliveira PH (2009) Reação de genótipos de feijão ao crestamento bacteriano comum, avaliado por dois métodos de inoculação. Ciência e Agrotecnologia 33:2019–2024

Siri MI, Galván GA, Quirici L, Silvera E, Villanueva P, Ferreira F, Franco Fraguas L, Pianzzola MJ (2009) Molecular marker diversity and bacterial wilt resistance in wild *Solanum commersonii* accessions from Uruguay. Euphytica 165:371–382

Tai TH, Dahlbeck D, Clark ET, Gajiwala P, Pasion R, Whalen MC, Stall RE, Staskawicz BJ (1999) Expression of the *Bs2* pepper gene confers resistance to bacterial spot disease in tomato. Proc Natl Acad Sci 96:14153–14158

Tar'an B, Michaels TE, Pauls KP (2001) Mapping genetic factors affecting the reaction to *Xanthomonas axonopodis* pv. *phaseoli* in *Phaseolus vulgaris* L. under field conditions. Genome 44:1046–1056

Tung PX, Rasco ET, Zaag PV, Schmiediche P (1990) Resistance to *Pseudomonas solanacearum* in the potato: I. Effects of sources of resistance and adaptation. Euphytica 45:203–210

Vallejos CE, Jones V, Stall RE, Jones JB, Minsavage GV, Schultz DC, Rodrigues R, Olsen LE, Mazourek M (2010) Characterization of two recessive genes controlling resistance to all races of bacterial spot in peppers. Theor Appl Genet 121:37–46

VanHeusden AW, Koornneef M, Voorrips RE, Bruggenmann W, Pet G, VrielinkvanGinkel R, Chen X, Lindhout P (1999) Three QTLs from *Lycopersicon peruvianum* confer a high level of resistance to *Clavibacter michiganensis* ssp. *michiganensis*. Theor Appl Genet 99:1068–1074

Wang H, Hutton SF, Robbins MD, Sim SC, Scott JW, Yang WC, Jones JB, Francis DM (2011) Molecular mapping of hypersensitive resistance from tomato 'Hawaii 7981' to *Xanthomonas perforans* race T3. Phytopathology 101:1217–1223

Wechter WP, Levi A, Ling KS, Kousik C, Block CC (2011) Identification of resistance to *Acidovorax avenae* subsp. *citrulli* among melon (*Cucumis* spp.) plant introductions. HortScience 46:207–212

Wydra K, Zinsou V, Jorge V, Verdier V (2004) Identification of pathotypes of *Xanthomonas axonopodis* pv. *manihotis* in Africa and detection of specific quantitative trait loci (QTL) for resistance to cassava bacterial blight. Phytopathology 94:1084–1093

Yang W, Francis DM (2007) Genetics and breeding for resistance to bacterial diseases in tomato: prospects for marker-assisted selection. In: Razdan MK, Mattoo AK (eds) Genetic improvement of solanaceous crops. Tomato, vol 2, Tomato. Science Publishers, Enfield p 638, 379–419

Young ND (1996) QTL mapping and quantitative disease resistance in plants. Annu Rev Phytopathol 34:479–501

Chapter 4
Breeding for Resistance to Viral Diseases

Leonardo S. Boiteux, Maria Esther de Noronha Fonseca, Jairo Vidal Vieira and Rita de Cássia Pereira-Carvalho

Abstract Genetic resistance to viruses and/or their vectors is the most practical and efficient method of disease control. Therefore, significant research effort has been devoted to this field. In fact, great achievements in breeding plants for virus resistance have been made using classical and molecular breeding approaches. The biggest obstacle for these breeding programs is to identify and incorporate, on a large scale, multiple resistance factors in elite genetic materials and anticipate potential problems with emerging viral diseases in association with the challenges that climate change is bringing to food production. The development of dense genetic maps, with molecular markers in strong linkage disequilibrium with virus resistance alleles, has enabled the establishment of marker-assisted selection of superior genotypes and the isolation and cloning of many virus resistance genes. Such studies have been facilitated in some of the species for which complete genome sequences are now available. Moreover, the knowledge about viral genes and genomes has been used in the development of resistant transgenic plants and they have been effective in different pathosystems; paving the way towards a new collection of anti-viral biotech breeding strategies.

L. S. Boiteux (✉) · M. E. de Noronha Fonseca · J. V. Vieira
Embrapa Hortaliças, Brasília, DF, Brazil
e-mail: boiteux@cnph.embrapa.br

M. E. de Noronha Fonseca
e-mail: mesther@cnph.embrapa.br

J. V. Vieira
e-mail: jairo@cnph.embrapa.br

R. de Cássia Pereira-Carvalho
University of Brasília, Brasília, Brazil
e-mail: rcpcarvalho@unb.br

R. Fritsche-Neto and A. Borém (eds.), *Plant Breeding for Biotic Stress Resistance*,
DOI: 10.1007/978-3-642-33087-2_4, © Springer-Verlag Berlin Heidelberg 2012

Keywords Plant breeding · Plant diseases · Quantitative genetics · Biotechnology · Virus resistance

4.1 Introduction

4.1.1 Characteristics of Viral Diseases and Impacts on Disease Control

Viruses are obligate intracellular parasites that use the machinery of the host cell to complete their lyfe cycle. This intimate virus–host association limits the repertoire of control strategies for viral diseases. In this scenario, genetic resistance to viruses and/or their vectors emerges as the most practical and efficient method of control.

Plant resistance to viral infection exhibits varied forms of phenotypic expression: (i) resistance to plant-to-plant or long-distance transmission (for example, effects on the vector and/or the contamination level of seeds); (ii) inhibition of intracellular multiplication, or true immunity; (iii) resistance to infection via a hypersensitive response; (iv) resistance to viral translocation (cell-to-cell or systemic movement) from initially infected cells often resulting in symptomless, subliminal infections; and (v) tolerance and/or attenuation of symptoms (Cooper and Jones 1983; Fraser 1992; Gómez et al. 2009). The terminology adopted in this chapter is summarized in Table 4.1.

4.1.2 Factors Linked to Virus Emergence and Impacts on Breeding Programs

Different genetic and agroecological factors are linked to the emergence, epidemics, and pandemics of viral diseases, including (i) genetic recombination and transfer of genetic material between viral species/isolates; (ii) synergisms between viral species; (iii) the introduction of new biotypes or species of vectors; (iv) virus mutations for increase in the host range; (v) virus dispersion among continents or within a region; (vi) modifications of agricultural practices; (vii) agricultural activity intensification; (viii) the introduction of new crops in new areas of cultivation; (ix) further intensification of global/regional germplasm movement; (x) the introduction of distinct sets of disease-prone cultivars and hybrids; (xi) the breakdown of genetic resistance factors by new viral variants; (xii) the presence of complexes of pathogens and/or their vectors; (xiii) the presence of alternative hosts in cultivated species, weeds and native flora; and (xiv) integration events of viral gene segments in the nuclear genome of the host plant (Fargette et al. 2006). These examples

Table 4.1 Terminology regarding plant resistance to viruses

Resistance The ability of accessions and/or varieties of a given host plant species to suppress, reduce, or delay the injuries and damages caused by viruses. There are degrees and/or levels of resistance, with immunity as the strongest expression of this trait
Immunity An absolute state of resistance to infection. The virus is not detected after successive inoculations and/or exposure to viruliferous vectors. Viral replication/translocation is not detected
Hypersensitive response Histological, morphological, and biochemical plant modifications induced by a virus, resulting in localization and/or spatial restriction of the virus. Phenotypic expression is usually characterized by local lesions and it is dependent on multiple factors (spatiotemporal, varietal, viral isolate, and environmental)
Tolerance The plant is locally or systematically (with viral replication) infected, but the expression of symptoms is absent or mild
Isolate A viral population sample taken and purified from the host plant or directly from the vector using biological and/or molecular strategies for characterization
Strain Virus variant that shares biological, serological, or molecular characteristics with a type species. It can also be defined by its virulence profile in different hosts and its genomic variability
Pathotype A subdivision of viral variants in terms of their ability to infect given group of hosts (cultivars or related species), including varieties with characterized resistance factors
Resistance breakdown Occurs when a variant of a virus species able to attack/infect a cultivar considered as resistant or that carries a resistance gene/*locus* becomes predominant in the viral population
Gene silencing systems of the host plant Different systems of plant defense against viruses, which involve the silencing of viral genome components that are crucial to the infection process. These mechanisms have been used in strategies aiming to develop transgenic plants with virus resistance
Viral suppressors of gene silencing Some viruses can disable the defense systems and/or induce gene silencing in their hosts, leading to the inactivation of resistance mechanisms and/or facilitating the process of infection. These virus counter-defense mechanisms include the presence of RNA silencing suppressors and the adoption of silencing-tolerant RNA conformations

illustrate the events that can lead to the emergence of new plant–virus–vector–environment interactions.

4.1.3 Viral Genera and Species of Greatest Economic Significance in Plants

A select group of ten viruses has been identified as the most relevant worldwide from scientific and economic standpoints (Scholthof et al. 2011), including the following, in order of significance: *Tobacco mosaic virus* (*Tobamovirus* genus); *Tomato spotted wilt virus* (*Tospovirus*); *Tomato yellow leaf curl virus* (*Begomovirus*); *Cucumber mosaic virus* (*Cucumovirus*); *Potato virus Y* (*Potyvirus*); *Cauliflower mosaic virus* (*Caulimovirus*); *African cassava mosaic virus* (*Begomovirus*); *Plum pox virus* (*Potyvirus*); *Brome mosaic virus* (*Bromovirus*); and *Potato virus X*

(*Potexvirus*). The following additional viral species were highlighted: the *Citrus tristeza virus* (*Closterovirus*); *Barley yellow dwarf virus* (*Luteovirus*); *Potato leafroll virus* (*Polerovirus*); and *Tomato bushy stunt virus* (*Tombusvirus*). Vegetables are surely among the cultivated species most affected by diseases of viral etiology. In this chapter, we discuss the advances in the development of resistant varieties in this group of hosts against viral species classified within the most significant genera of the tropics, including *Begomovirus*, *Tospovirus*, *Potyvirus*, *Polerovirus*, *Tobamovirus*, and *Cucumovirus*.

4.2 Major Viral Genera Affecting Horticultural Crops

Potyvirus—(*Potyviridae* family): is one of the most important genera of plant viruses. The particles are elongated and flexuous (680–900 nm in length and 11–13 nm in diameter). Virus can be transmitted by aphids (non-circulative relationship), pollen, grafting, and seeds. Plants infected by species of this genus show mosaic-like symptoms, blistering, and leaf deformations. A peculiar feature of the *Potyviridae* family members with diagnostic value is the formation of scroll-shaped cytoplasmic inclusion bodies. Host plants are found in different botanical families, including *Solanaceae*, *Fabaceae*, *Cucurbitaceae*, *Amaranthaceae*, *Chenopodiaceae*, and *Passifloraceae*. Potato virus Y (PVY) is the type species, capable of infecting several economically significant *Solanaceae* members, including potatoes, tomatoes, and sweet peppers. The viral genome is approximately 10 kb in length and consists of a single positive-sense single-stranded RNA (ssRNA+) molecule with a single open reading frame (ORF), a protein attached to the 5′ end (Vpg), and a poly-A tail at the 3′ end. Genomic RNA translation results in a 350 kDa polyprotein, which, upon cleavage, yields 8–10 products. Most proteins are multifunctional. The viral protein HC-Pro (helper component protease), for example, may function as a post-transcriptional gene silencing suppressor and protease, cleaving its own carboxyl-terminus. HC-Pro associates with CP (coat protein) to enable vector transmission, which is also related to short-distance and possibly long-distance viral movement. Another key Potyvirus protein is VPg, which associates with the eukaryotic translation initiation factor 4E (eIF4E) at the ribosomes of eukaryotic cells and thus enables the initiation of the genomic RNA translation process.

Tospovirus—(*Bunyaviridae* family): it has as its type species the *Tomato spotted wilt virus* (TSWV), which is the causal agent of the "spotted wilt" disease. The Tospovirus was initially considered monotypic, but molecular and biological characterization studies of isolates have shown a large number of species within the genus. *Tomato chlorotic spot virus* (TCSV), *Groundnut ringspot virus* (GRSV), and *Chrysanthemum stem necrosis virus* (CSNV) are the predominant species in South America (Dianese et al. 2010). Tospovirus species infect more than 1,000 monocotyledonous and dicotyledonous plants belonging to more than 82 botanical families. The tospoviruses have a spherical particle and a tripartite genome

consisting of a negative sense ssRNA large (L) and two smaller (ambisense) RNAs known as medium (M) and small (S), respectively. This tripartite genome organization is a source of viral variability that can generate new isolates capable of overcoming the resistance genes of the host plant. One of the viral proteins (NS_S) acts as a host silencing suppressor. The three RNAs are encapsidated by the N protein, forming ribonucleoproteins (RNPs). The envelope surrounding the RNP complex is composed by membranes derived from the host plant combined with the viral glycoproteins Gn and Gc (Scholthof et al. 2011). The transmitted circulatively/propagatively through thrips vectors (species from the *Thrips* and *Frankliniella* genera). The virus is only acquired during the first and second larval stages of these insects. Symptoms include growth arrest, purple blotching or browning of leaves, spots in concentric chlorotic and necrotic ring pattern, severe leaf deformation, chlorosis, leaf mottling, and intense necrosis.

Begomovirus—(*Geminiviridae* family): known to be transmitted by *Bemisia tabaci* (Order: Hemiptera, Sub-order: Sternorrhyncha and family: *Aleyrodidae*); they infect dicotyledonous plants and display twinned icosahedral particles and a genome consisting of either one (monopartite) or two (bipartite) single-stranded circular DNA molecules. Those two viral components are designated DNA-A and DNA-B, each with approximately 2,600 bases (Scholthof et al. 2011). More than a hundred different species/strains of begomovirus have already been found infecting different hosts in different regions of the world, especially Solanaceae, legumes, cassava, and cotton (Scholthof et al. 2011).

Tobamovirus: species of this genus exhibit particles with cylindrical, elongated, and rigid morphology, approximately 300 nm in length and 18 nm in diameter. The genome consists of a single ssRNA+ approximately 6 kb in size encoding four proteins (Scholthof et al. 2011). The Tobacco mosaic virus (TMV) is the type species of the genus. That genus has been divided into different subgroups. Subgroup 1 includes viruses that infect Solanaceae (for example, TMV and *Tomato mosaic virus*, ToMV). Subgroup 2 is composed of species that infect brassica, and subgroup 3 is composed of viruses of cucurbits. These viruses are transmitted by contact and over long distances by contaminated seeds. They are highly stable and may remain in the environment for long periods.

Polerovirus—Potato leafroll virus (PLRV): is the type species of this genus within the *Luteoviridae* family. The particles are isometric (approximately 25 nm in diameter). Viral transmission is done through aphids, and the virus–vector relationship is circulative and non-propagative. The PLRV genome consists of ssRNA+ approximately 5.8 kb in size and eight open reading frames (ORFs). PLRV replication is confined to the phloem. PLRV has a protein (P0) with a function that involves suppressing post-transcriptional gene silencing, disabling the defenses of the host plant (Taliansky et al. 2003).

Cucumovirus—(*Bromoviridae* family): *Cucumber mosaic virus* (CMV) is the type species of this genus. CMV particles are icosahedral and approximately 29 nm in length. The CMV genome is tripartite, with five genes distributed among three genomic ssRNAs+ and two sub-genomic RNAs (Scholthof et al. 2011). The

CMV isolates are divided into two large groups, I (subdivided into IA and IB) and II, according to the serological properties and sequence variability of the coat protein-encoding gene (Palukaitis and García-Arenal 2003). Typical symptoms are flower and fruit mosaics, mottles, and deformations. CMV diseases are very difficult to control given the numerous host plants (100 botanical families comprising both mono and dicotyledons) and transmission by different aphid species. CMV was initially found in cucumbers, but it can quite frequently induce severe symptoms in pumpkins, Capsicum, melons, tomatoes, carrots, lettuce, spinach, beans, weeds, and ornamental plants.

4.3 Advances in Horticultural Plant Breeding for Resistance to Viral Diseases

Here specific pathosystems and resistance genes used in the control of viral diseases of horticultural crops will be discussed. The sections are organized according to the advances made in resistance against species belonging to the six genera of viruses briefly described above. Resistance to viruses is, in general, a qualitative trait that is little affected by the environment; that is, it has high heritability. Thus, breeding methods used for the purpose of incorporating resistance depends on selection based on individual behavior (for example, mass selection, stratified mass selection, and backcrossing, among others), directly (resistant or susceptible) and, when possible, conducted in early stages, aiming to accelerate the selection gains.

4.4 Breeding for Resistance to Potyvirus Species

4.4.1 Breeding for Resistance to Potyviruses in Capsicum (Hot and Sweet Peppers)

Eight resistance genes have been characterized in *Capsicum* species for PVY, *Pepper yellow mosaic virus* (PepYMV), and at least five other species of Potyvirus. The *pvr-1* genes (from *C. chinense*) and the allelic series *prv*-2 (*pvr2*^{1} and *pvr2*^{2}) from *C. annuum* have been fully characterized and encode eukaryotic translation initiation factor 4E-type proteins (LeGall et al. 2011). A QTL (quantitative trait locus) in *C. frutescens* 'Perennial' cosegregated with the *pvr*^{2} *locus* and was named *pvr-1*^{3}. Recently, it was demonstrated that the *pvr2*^{1}, *pvr2*^{2}, and *pvr2*^{3} alleles and the *pvr-1* gene occupy the same *locus*, and the members of that allelic series were re-named as *pvr-1*, *pvr-1*^{1}, *pvr-1*^{2}, and *pvr-1*^{3} (Wang and Bosland 2006). The recessive *locus prv-3* (which controls resistance to isolates of *Pepper mottle virus* (PepMoV)) comes from the Brazilian variety *C. annuum* 'Avelar'. The

dominant gene *Pvr-4* (effective against most PVY variants) and the recessive gene *prv-5* (controlling resistance to PVY pathotype-0) were identified in *C. annuum* 'CM-334'. Another recessive gene was characterized in *C. frutescens* 'perennial' and termed *prv-6,* which is complementary to the *prv-1 locus*. The dominant gene *Pvr-7* (closely related to *Pvr-4*) was characterized in *C. chinense* and is effective against Potyvirus species of the USA. The recessive gene *prv-8* was also characterized in 'CM-334' against isolates of Spain. The PVY isolates that infect *Capsicum* are divided into three pathotypes according to the resistance-breaking ability (or lack thereof) conferred by recessive alleles of the *pvr-1* series. In Brazil, PVY infection had been considered the main limiting factor for *Capsicum* production since the 1960s. In the early 1990s, a new PVY isolate (termed PVY^{m}) emerged as a threat to sweet pepper crops due to its potyvirus resistance-breaking ability regarding the market-leading variety, 'Magda' (Nagai 1993). The PVY^{m} virulence profile was similar to isolates of PVY pathotype-1,2. However, genome sequence analyses demonstrated that PVY^{m} represented a new species, PepYMV (Inoue-Nagata et al. 2002), which currently prevails in Brazil. Sources of resistance to PepYMV were identified in *C. chinense* 'PI 159236' (recessive inheritance) and 'CM-334' (dominant inheritance), with the latter most likely conditioned by the gene *Pvr-4* (Boiteux et al. 1996).

4.4.2 Breeding for Resistance to Potyviruses in Potato (*Solanum tuberosum L.*)

The PVY variability of interest for potato genetic breeding is represented in five main groups of strains: Y^{C}, Y^{O}, Y^{N}, Y^{Z} and Y^{E}. Strains are defined according to their ability to induce hypersensitive responses (HR) in potato clones with the *Nc*, *Nytbr*, and *Nz* genes. The Y^{C} strain induces HR in plants with the *Nc* gene, Y^{O} in plants with the *Nytbr* gene, and Y^{Z} in those with the *Nz* gene. The isolates of Y^{N} and Y^{E} strains break down the resistance controlled by these three genes. In turn, Y^{E} isolates break down the resistance controlled by *Nc*, *Nytbr*, and *Nz* without inducing necrosis in tobacco. Cultivars with one or more such genes have been developed. The PVY genome components that interact with those genes remain uncharacterized. The recent focus of research on potato PVY variability has been the characterization of recombinant groups of strains including $Y^{N:O}$, Y^{NTN}, and $Y^{N\text{-}Wi}$. In this regard, the *Ryadg* gene/*locus* shows great potential for use in breeding because it confers resistance to all currently known PVY strains in addition to Potato virus X. *Ryadg* gene-linked markers were identified, and marker-assisted selection was developed (Gray et al. 2010).

4.4.3 Breeding for Resistance to Potyviruses in Tomato (*Solanum lycopersicum* L.)

The symptoms of PVY infection in tomato include mosaic and top necrosis. In Brazil, PVY epidemics in tomato were more common before the 1960s in regions with the simultaneous cultivation of Capsicum and tomato (Nagai, 1993). For this reason, the Agronomical Institute of Campinas (Instituto Agronômico de Campinas—IAC) started a breeding program aimed at incorporating resistance to the mosaic caused by PVY isolates. The first cultivar released with resistance to PVY was 'Ângela', resulting from interspecific crossings with *S. pimpinellifolium* (source of the *rt* recessive gene) (Nagai 1993). In tomato, PepYMV can also cause mottling and mosaic. Sources of immunity-like resistance to PepYMV were identified in *S. habrochaites* and *S. peruvianum*. Sources with simultaneous resistance to PepYMV and PVY were also identified in *S. habrochaites*. Resistance to both viruses may be associated with the presence of the *pot-1* gene or an allele of this gene, as *S. habrochaites* is the original source of this resistance factor. That recessive gene, an ortholog of the *pvr*-2 resistance gene, also encodes an eIF4E-like protein (LeGall et al. 2011).

4.4.4 Breeding for Resistance to Potyviruses in Lettuce (*Lactuca sativa* L.)

The *Lettuce mosaic virus* (LMV) is one of the most economically important lettuce pathogens (Pavan et al. 2008). Symptoms include mosaic, stunt, leaf distortion, and leaf area reduction. LMV has a wide range of hosts, especially within the *Asteraceae* family. Sources of resistance to LMV have been identified in *L. sativa* and in wild species including *L. saligna, L. serriola*, *L. virosa*, and *L. perennis* (Lebeda et al. 2009). Currently, the primary LMV-resistance genes used in breeding have been the $mo1^1$ (= *g*) and $mo1^2$ (= *mo*) recessive alleles. The reaction of genetic materials with these two resistance genes has been used in the identification of LMV pathotypes. Pathotype IV is known for breaking the resistance of the $mo1^2$ gene (which is effective against pathotypes I, II and III) and, therefore, is a potential threat. Recently, a new classification of LMV isolates into two subgroups was proposed: LMV-Common (isolates unable to overcome the resistance of the $mo1^1$ and $mo1^2$ genes) and LMV-Most (resistance-breaking isolates for the $mo1^1$ and $mo1^2$ genes). Cosegregation and functional complementation studies indicate that the eIF4E-like proteins encoded by the $mo1^1$ and $mo1^2$ genes/alleles are involved in the resistance response (LeGall et al. 2011). Two dominant LMV-resistance genes were also identified in *L. sativa* (named *Mo2*) and *L. virosa* (*Mo3*) (Lebeda et al. 2009).

4.4.5 Breeding for Resistance to Potyviruses in Muskmelon (*Cucumis melo L.*)

Mosaics are the most common symptoms induced by species of potyvirus in cucurbits. Aphids transmit these viruses that are found worldwide, resulting in both quantitative and qualitative losses. (*Potyviridae*) The three main causal agents are: Papaya ringspot virus (watermelon strain) (PRSV-W), Watermelon mosaic virus (WMV), and Zucchini yellow mosaic virus (ZYMV). Under natural conditions, transmission by different species of aphids is non-circulative and non-persistent, and *Aphis gossypii* and *Myzus persicae* are the most prevalent vectors. Sources of resistance to potyvirus in *C. melo* germplasm are quite limited. However, two sources deserve special mention by displaying multiple resistances: 'TGR-1551' (collected in Zimbabwe) and 'PI 414723' (India). 'TGR-1551' shows two dominant genes of resistance to WMV (*Wmv* gene) and ZYMV (*Zym* gene). 'PI 414723' also has a factor of resistance to PRSV-W, which segregates independently from *Zym* (Anagnostou et al. 2000). Sources of allelic resistance to PRSV-W were identified in 'PI 180280' and 'PI 180283'. The 'WMR 29' line showed resistance to two species of potyvirus (PRSV-W and *Moroccan watermelon mosaic virus*). Sources of resistance to WMV in *C. melo* are scarce. The 'TGR-1551' line is infected by WMV in mechanical inoculation assays, but infected plants exhibit only mild symptoms. A recessive gene controls that resistance. 'TGR-1551' was resistant to WMV and ZYMV in *A. gossypii* transmission assays but not in mechanical inoculations. In this regard, 'TGR-1551' had similar behavior to 'PI 161375', which is resistant to different potyvirus species based on its resistance to the *A. gossypii* vector controlled by the dominant *Vat* gene (Díaz-Pendón et al. 2005). **'**TGR-1551' was also resistant to the Cucurbit yellowing stunting disorder virus transmitted by *B. tabaci* (Díaz-Pendón et al. 2005).

4.4.6 Breeding for Resistance to Potyviruses in Cucumber (*Cucumis sativus L.*)

Searches for sources of resistance to potyviruses and inheritance studies have been conducted in cucumber (Park et al. 2000). Resistance to PRSV-W is controlled by a recessive gene (*prsv-1*) in the 'Surinam' cultivar and by a dominant gene (*Prsv-2*) in the 'TMG1' line. These two genes are, apparently, allelic. Resistance to ZYMV in 'TMG1' and 'Dina' was inherited also as a recessive trait (zym). The 'TMG1' line shows a group of allelic or closely linked genes that control resistance to different viral species including *Moroccan watermelon mosaic virus*, WMV, and ZYMV. Genetic mapping studies aimed at identifying markers and isolating genes related to resistance to potyvirus species in cucumber are in progress (Meyer et al. 2008).

4.4.7 Watermelon [Citrullus lanatus (Thunb.) Matsum. and Nakai] Breeding for Resistance to Potyviruses

Sources of resistance to PRSV-W isolates were identified in accessions of watermelon from Africa and in *C. lanatus* var. *colocynthis* (Vieira et al. 2010). Inheritance studies conducted on 'PI 244017', 'PI 244019', and 'PI 485583' indicated that a recessive gene (*prv*) controls resistance in all accessions. Resistance to WMV in watermelon shows a complex inheritance involving three genes (Guner and Wehner 2008). Sources of resistance to ZYMV were identified in *C. lanatus* var. *lanatus* ('PI 595203') and *C. lanatus* var. *citroides*. High resistance levels were confirmed in 'PI 595203', 'PI 386025', 'PI 386026', and 'PI 494528' (Guner and Wehner 2008). 'PI 482261' showed recessive inheritance (*zym*). *Citrullus lanatus* var. *lanatus* 'PI 595203' showed a wide range of effective resistance against different ZYMV isolates and moderate resistance to WMV. Genetic mapping with 'PI 595203' demonstrated a connection between resistance to ZYMV and mutations on a gene encoding eIF4E-like proteins (LeGall et al. 2011).

4.5 Breeding for Resistance to *Tospovirus* Species

4.5.1 Tomato Breeding for Resistance to Tospovirus

Infection by *Tospovirus* species causes serious damage in tomatoes (Fig. 4.1). Several sources of genetic resistance have been found in *Solanum* (*Lycopersicon* section), especially in *S. peruvianum* (Dianese et al. 2011). Indeed, the primary source used in genetic breeding is the *Sw-5* dominant gene (incorporated from a *S. peruvianum* genotype), which confers broad resistance to different isolates and species of *Tospovirus* (Boiteux and Giordano 1993). The *Sw*-5 gene restricts viral systemic infection, and the inoculated leaves show a hypersensitivity response (Brommonschenkel et al. 2000). That gene belongs to the class of resistance genes termed CC-(NB-ARC)-LRR, with leucine-rich regions (LRR) and conserved nucleotide-binding (NB) sites (Brommonschenkel et al. 2000). Despite its great utility, the *Sw-5* gene has some limitations: (1) resistance expression may be adversely affected under either high inoculum pressure or in regions where there are drastic fluctuations between day and night temperatures; (2) phenotypic expression does not show complete penetrance; and (3) some isolates may break the resistance. For these reasons, research into alternative sources of resistance has been conducted. New sources of broad-spectrum resistance to isolates of TSWV, GRSV, TCSV, and CSNV have been identified (Dianese et al. 2011). In Brazil, there are reports of great epidemics induced by *Tospovirus* infection. One of the most significant epidemics sparked a great crisis that affected the tomato processing industry in the Brazilian Northeast in the early 1990s. Therefore, Embrapa Hortaliças (Brazilian Agricultural Research Corporation for Horticultural Crops) and the Agronomical Institute of

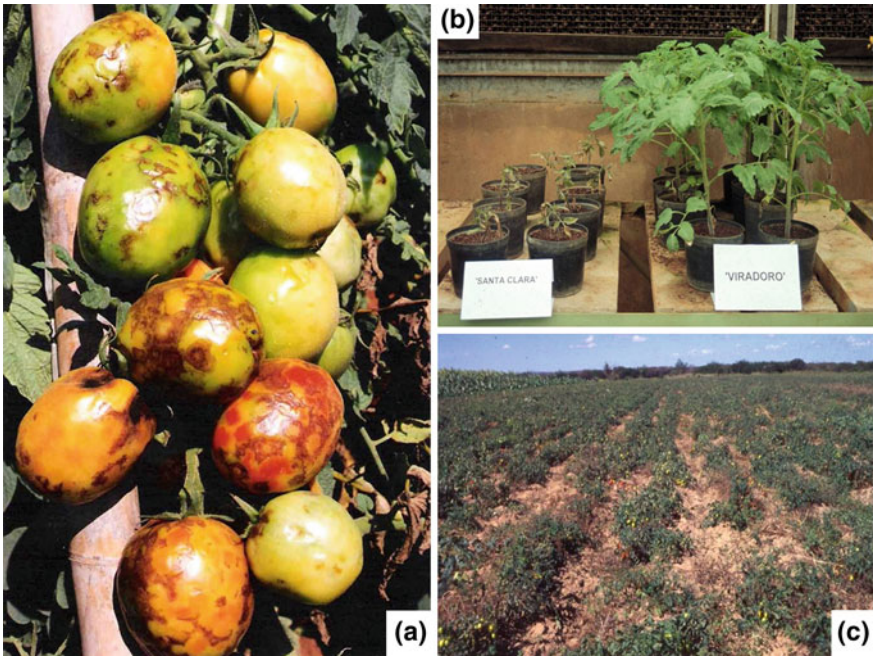

Fig. 4.1 *Tospovirus* in tomato: **a** symptoms in fruits; **b** phenotypic expression of the *Sw-5* gene, present in the Viradoro cultivar and absent in the Santa Clara cultivar; **c** reduction in the plant stand due to a *Tospovirus* attack on tomatoes in Petrolina-PE, Brazil

Pernambuco (Instituto Agronômico de Pernambuco—IPA) established a joint breeding program, which resulted in the release of the 'Viradoro' cultivar, resistant to *Tospovirus* (Giordano et al. 2000) (Fig. 4.1). The *locus* encompassing the *Sw-5* gene was mapped, cloned, and characterized (Brommonschenkel et al. 2000). The genetic data generated enabled the development of a system of codominant and functional molecular markers (Fig. 4.2) that can be used in marker-assisted selection (Dianese et al. 2010). The 'BRS Tospodoro' cultivar was recently released and included resistance to *Tospovirus* among its features (Giordano et al. 2010).

4.5.2 Capsicum Resistance to Tospovirus

The same *Tospovirus* species that attack tomatoes may also infect *Capsicum*, causing serious economic losses (Fig. 4.3). Sources of field resistance, especially *C. chinense* 'PI 159236', have been identified (Boiteux et al. 1993). 'PI 159236' showed a typical hypersensitive response and large local lesions upon mechanical inoculation with TSWV isolates. Inheritance studies indicate that a dominant allele (termed *Tsw*) controls the hypersensitive response against TSWV isolates (Boiteux

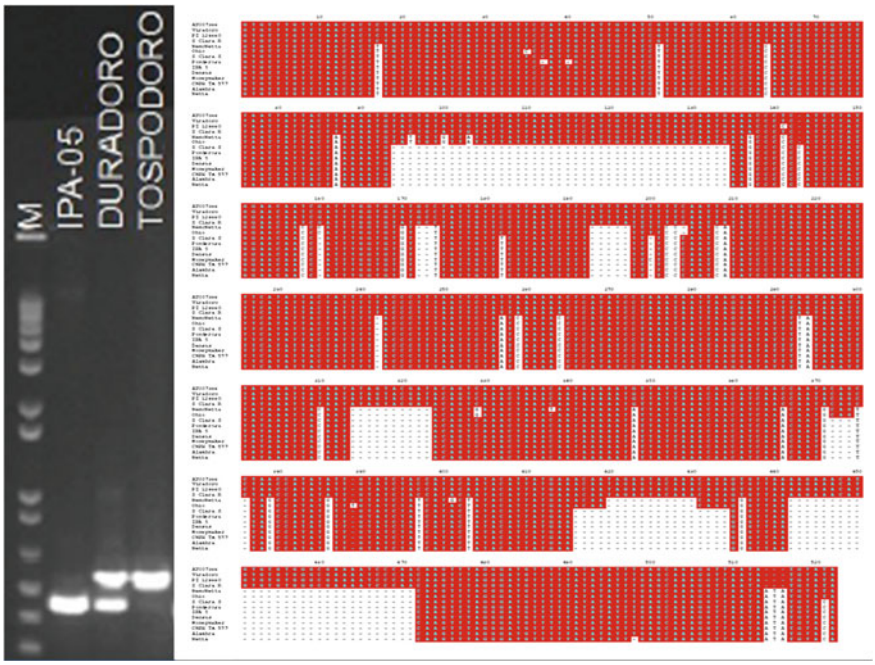

Fig. 4.2 System of functional markers identifying the *Sw-5 locus* (resistance to *Tospovirus*) in tomato: *left* amplicon patterns of 'IPA-5' (susceptible), 'Duradoro' (heterozygote) and 'Tospodoro' (resistant); *right* sequence alignment of a *Sw5b* gene segment including 14 resistant and susceptible genotypes. Nucleotides in *red* are conserved in different genotypes. Areas marked in *white* represent regions with nucleotide polymorphisms and deletions (restricted to susceptible genotypes)

and de Ávila 1994). However, in mechanical inoculation assays, resistance was not effective against GRSV and TCSV isolates (Boiteux 1995). The *Tsw* allele was mapped in *Capsicum*, and closely linked molecular markers were identified (Jahn et al. 2000).

4.5.3 Lettuce Breeding for Resistance to Tospovirus

Partial resistance to *Tospovirus* in lettuce was initially reported in 'PI 342517' ('Ancora') and 'PI 342444'. Those sources show partially dominant inheritance, and the resistance factors are apparently allelic, as the progeny from this crossing showed similar resistance levels to the two parents (O'Malley and Hartmann 1989). Other sources of resistance were detected in 'Tinto' and the progeny of an interspecific crossing between *L. sativa* and *L. saligna* (Cho et al. 1995). In Brazil, a *Tospovirus*-resistance breeding program in lettuce began in 1987 at the Agronomical Institute of Campinas (Instituto Agronômico de Campinas) (Nagai

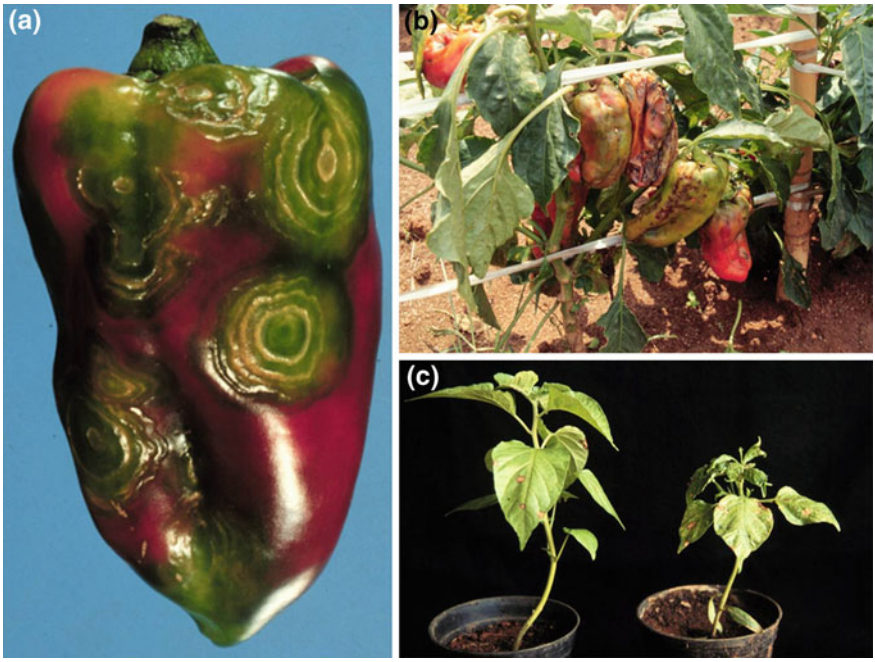

Fig. 4.3 *Tospovirus* in *Capsicum*: **a** symptoms in fruits. **b** symptoms in plants. **c** phenotypic expression (local lesions) of *Capsicum chinense* 'PI 159236' (*Tsw gene*) upon mechanical inoculation with *Tomato spotted wilt virus* (*left*) and *Groundnut ringspot virus* (*right*)

1989). The initial goal was to introduce resistance to the 'Manteiga' varietal group by developing progeny from the 'Regina' *x* 'PI 342517' crossing (Nagai 1989). The 'PI 342444' was also identified as a promising material for cultivation under Brazilian conditions, showing infection levels well below those found in standard controls (Guimarães et al. 2009).

4.6 Breeding for Resistance to *Begomovirus*

4.6.1 Tomato Breeding for Resistance to Begomovirus Species

The chemical control of begomoviruses through insecticide applications aimed at reducing the vector population has been ineffective and very costly in tomatoes for processing and for consumption *in natura*. Production losses above 60 % have been reported in susceptible varieties (Giordano et al. 2005a). In Brazil, the occurrence of *Begomovirus* in tomato can be divided into two phases. In the first phase (between the 1960s and 1970s), reports of begomovirus infection were sporadic and insignificant. In the 1990s, an extremely diverse group of

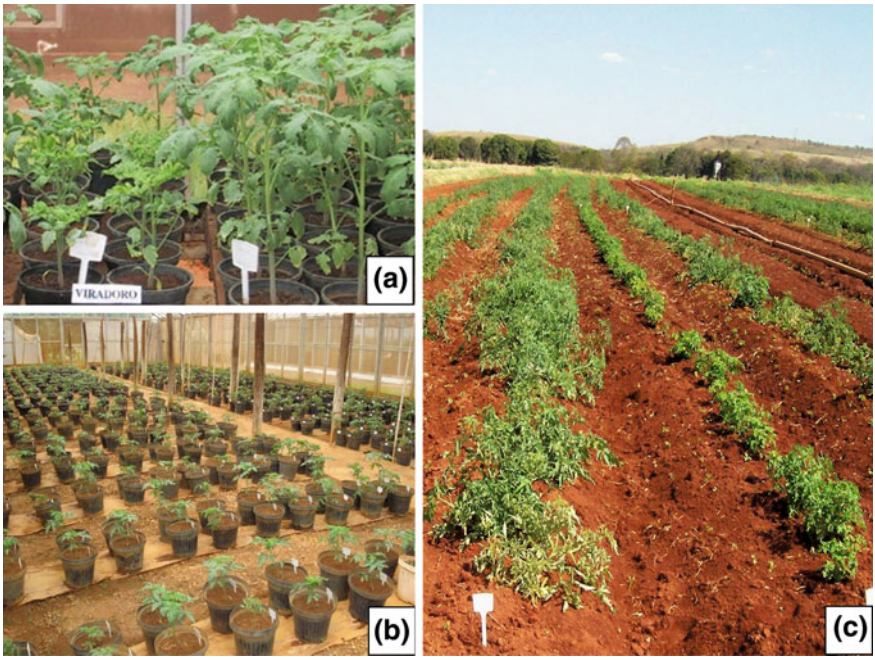

Fig. 4.4 *Begomovirus* in tomato: **a** phenotypic expression of resistance sources with the *Ty-1* gene (*right*) and the susceptible 'Viradoro' control (*left*), exhibiting symptoms of apical chlorosis and stunting. **b** Greenhouse progeny evaluation (inoculation via whitefly, *Bemisia tabaci*). **c** Field evaluation of resistant (*left*) and susceptible (*right*) progeny (natural inoculum)

begomoviruses emerged in Brazil, coinciding with the introduction and dissemination of a new biotype of the whitefly vector: *B. tabaci* biotype B. Typical symptoms of viral infection include leaf vein chlorosis, mosaic, rugosity, stunting, and curling (Fig. 4.4). The polyphagous feeding habits of *B. tabaci* biotype B favored its rapid dissemination, and epidemics of begomovirus disease began to be found in many producing areas in Brazil (Ribeiro et al. 2003). More than ten tomato-infecting *Begomovirus* species have been characterized in Brazil. Many of them show regional and endemic occurrence. This variability of *Begomovirus* species is the primary challenge faced by breeding programs aiming to incorporate broad, stable, and durable resistance.

Therefore, the primary strategies of these breeding programs have been the search for broad-range resistance sources and/or the pyramidization of different resistance genes in elite lines. Several sources of resistance to *Begomovirus* were identified in wild species of the *Solanum* genus. The *Ty*-1 *locus* (introgressed from *S. chilense*) was effective against different isolates of different *Begomovirus* species on different continents (Zamir et al. 1994; Giordano et al. 2005b; Boiteux et al. 2007a). The *Ty*-2 *locus* was introgressed from *S. habrochaites* and showed reasonable efficacy against Brazilian isolates (Boiteux et al. 2007b). The *Ty*-3 gene/*locus* (partially dominant and from *S. chilense* 'LA-2779') confers a high

level of resistance to TYLCV and intermediate resistance to isolates of bipartite-genome species in Florida (Ji et al. 2007). The *tcm*-1 gene (from *S. lycopersicum* 'Tyking') has been effective against Brazilian bipartite (Giordano et al. 2005b) and European monopartite (García-Cano et al. 2008) begomoviruses. 'FLA653' is the recessive *tgr*-1 gene source derived from multiple crossings involving *S. chilense* 'LA-2779' and 'Tyking'.

During the breeding process, various resistance genes have been introgressed into tomatoes from different wild species. Many of those resistance genes have been mapped on chromosome 6, particularly the *Mi* and *Ty*-1 genes (Zamir et al. 1994). In some genetic materials, the *Mi* gene (resistance to some *Meloidogyne* species) and the *Ty-1* gene are in repulsion linkage (De Castro et al. 2007), thus hampering the development of cultivars resistant to both pathogens (Pereira-Carvalho et al. 2010). Another key issue to be considered relates to resistance to the whitefly vector, which can occur through mechanisms of antibiosis, antixenosis, and tolerance. In some tomato species, glandular trichomes are responsible for the secretion and/or accumulation of metabolites, termed allelochemicals. Accessions of *S. pennellii* and *S. pimpinellifolium* showed a lower frequency of plant infection with *Begomovirus* due to *B. tabaci* resistance conditioned by the presence of acyl sugar-accumulating trichomes (type IV).

4.7 Breeding for Resistance to *Tobamovirus* Species

4.7.1 Tomato Breeding for Resistance to Tobamovirus

TMV and ToMV frequently cause latent infections (without symptoms) in tomato, but aggressive strains can induce leaf mosaic, white spots on fruits, curling, leaflet reduction, in addition to a bulbous appearance on leaves and aspermia in fruits (Lanfermeijer et al. 2005). In the field, the transmission of these viruses is mechanical, through direct contact between plants and farmers' hands. Another efficient form of transmission is through contaminated seeds. In tomato, ToMV shows different pathotypes, characterized by the responses of a series of differential hosts with distinct resistance genes: the *Tm* gene (from *S. habrochaites*) and the *Tm-2 and Tm-2-2* genes (= $Tm\text{-}2^a$) from *S. peruvianum* (Lanfermeijer et al. 2005). These genes are incorporated separately or hierarchically in different cultivars and hybrids. The *Tm-2* and *Tm2-2* genes have already been cloned, and their products differ in only four amino acids. Marker-assisted selection may be used for these genes.

4.7.2 Capsicum *Breeding for Resistance to Tobamovirus*

Four *Tobamovirus* resistance genes were identified in the germplasm of *Capsicum*. The L^1 gene is derived from *C. annuum*, L^2 from *C. frutescens*, L^3 from *C. chinense,* and L^4 from *C. chacoense*. The *Tobamovirus* isolated from infected sweet pepper plants are classified as pathotypes P0, P1, P1-2, and P1-2-3 according to the reactions found in series of differential cultivars (Tomita et al. 2011). Resistance to the P1-2 pathotypes can be found in several cultivars containing the L^3 gene. The L^4 gene shows the broadest spectrum of resistance and is effective against the different pathotypes of *Tobamovirus* described in *Capsicum* (Tomita et al. 2011).

4.8 Breeding for Resistance to *Polerovirus* Species

4.8.1 Potato Breeding for Resistance to PLRV

PLRV is one of the major potato pathogens in nearly all regions of the world. The symptoms of infection are leaf curling, generalized plant yellowing, and stunting. The host plants of this virus are restricted to Solanaceae, and it may eventually affect the commercial production of tomatoes, in which it can induce symptoms, including plant yellowing from the bottom up. In potatoes, the symptoms can be either primary (from the field) or secondary (from infected tubers). Cultivars immune to PLRV are not yet available (Solomon-Blackburn and Barker 2001). The greatest advance in classical breeding has been the transfer of resistance factors from diploid wild species to cultivated (tetraploid) species. Among the promising sources of resistance, *S. brevidens*, *S. etuberosum*, *S. chacoense*, and *S. raphanifolium* stand out. The resistance of *S. etuberosum* (a diploid species unable to produce tubers) was introgressed into *S. tuberosum* via somatic hybridization. Inheritance studies showed control through a dominant allele identified as Rlr_{etb}. This allele is being incorporated into elite clones, and marker-assisted selection is already in progress (Kelly et al. 2009).

4.9 Breeding for Resistance to *Cucumovirus* Species

4.9.1 Capsicum Breeding for Resistance to Cucumber Mosaic Virus (CMV)

Various sources of resistance to CMV have been identified in *Capsicum*, including *C. annuum* 'Perennial', 'Vania', 'Sapporo-oonaga', and 'Nanbu-oonaga'; 'BG2814-6' and 'LS 1839-2-4' from *C. frutescens;* and *C. baccatum* 'PI 439381-1-3' (Kang

et al. 2010). Most sources show quantitative inheritance (polygenic) with tolerance-like phenotypic expression, hindering incorporation into commercial materials. Recently, a source of immunity-like resistance has been incorporated into the *C. annuum* Korean variety 'Bukang'. Inheritance studies show control through a dominant gene termed *Cmr1* (Cucumber mosaic resistance 1). Genetic mapping works were carried out to locate the *Cmr1* gene, and closely linked SNP (single nucleotide polymorphism) markers were identified (Kang et al. 2010).

4.9.2 Melon Breeding for Resistance to CMV

Sources of resistance to CMV were identified in some accessions including 'PI 161375' and 'TGR-1551'. The latter was resistant to CMV isolates in vector transmission assays but susceptible in mechanical inoculation assays (Díaz et al. 2003).

4.10 Marker-Assisted Selection, Isolation, and Types of Virus Resistance Genes in Plants

The development of dense genetic maps, with markers in strong linkage disequilibrium with virus resistance genes/alleles, has enabled the establishment of marker-assisted selection of superior genotypes, in addition to further-refined genomic mapping studies and the subsequent isolation of those genes. Several disease resistance genes have been isolated using high-resolution genetic/physical mapping strategies (map-based cloning or positional cloning). Such studies have been accelerated/facilitated in some species for which complete genomes are now available. Ideal molecular markers are those termed functional, that is, derived from the very genes conferring resistance to viruses. Indeed, a growing collection of genes (dominant and recessive) controlling resistance to diseases of viral etiology was and has been isolated through positional cloning in different plant species, and this information can be used in the development of functional markers (Tables 4.2 and 4.3). The initial data on the structure of virus resistance genes now enable the development of further efficient and/or simplified methods based on homology with analogous/homologous viral genes (homology-based methods) or the search for candidate genes (Tomita et al. 2011). Cloned genes structurally characterized as dominant (Table 4.2) fit the typical R gene (known as race- or isolate-specific) within the gene-for-gene interaction model. The largest class of dominant genes encodes proteins with nucleotide-binding (NB) and leucine-rich repeat (LRR) domains, which recognize avirulence factors encoded in the viral genome. The C-terminal regions of the LRR domains drive recognition and specificity. The NB domain may be the molecular switch regulating the signal transduction activation leading to the resistant phenotype.

Table 4.2 Dominant virus resistance genes previously characterized in different species of host plants

Virus	Gene symbol	Host	Avirulence factor
Tobacco mosaic virus (TMV) (*Tobamovirus*)	*N*	Tobacco	Replicase/ helicase
Tomato mosaic virus (ToMV)	*Tm-2 and Tm2-2*	Tomato	Movement protein
Tobamovirus	L^1, L^2, L^3 e L^4	*Capsicum*	Coat protein
Potato virus X (PVX) (*Potexvirus*)	*Rx1*	Potato	Coat protein
PVX (*Potexvirus*)	*Rx2*	Potato	Coat protein
Potato virus Y (*Potyvirus*)	*Y-1*	Potato	Uncharacterized
Tomato spotted wilt virus (*Tospovirus*)	*Sw-5*	Tomato	Movement protein
Cucumber mosaic virus (CMV) (*Cucumovirus*)	*RT4-4*	*Phaseolus vulgaris*	Gene 2a
CMV	*RCY1*	*Arabidopsis*	Coat protein
Tobacco etch virus (TEV) (*Potyvirus*)	*RTM1*	*Arabidopsis*	Uncharacterized
TEV	*RTM2*	*Arabidopsis*	Uncharacterized
Turnip crinkle virus (*Carmovirus*)	*HRT*	*Arabidopsis*	Coat protein
Soybean mosaic virus (*Potyvirus*)	*Rsv1*	Soy	Uncharacterized

Adapted from Maule et al. (2007)

Nearly half of the virus resistance genes in plants show recessive inheritance, and all of the recessive factors that are presently cloned (in pathosystems exclusively involving RNA viruses) represent mutations in genes encoding eukaryotic translation initiation factors (Table 4.3). The presence of recessive genes indicates that the resistance phenotype results from the loss of function of a host gene that would confer susceptibility in its wild-type state/version. However, other recessive genes show no mutations in *elF4E* and *elF4G*, indicating that this would not be the only class of genes responsible for monogenic, recessive resistance to viruses in plants. Molecular data from the characterization of these genes has enabled the design of highly specific PCR primers that work as key tools for marker-assisted selection, aimed at the pyramidization of several resistance factors within the same genetic material.

4.11 Transgenic Strategies for Controlling Viral Diseases

Viral genes have been widely used in the development of resistant transgenic plants (pathogen-derived resistance) and have been effective in different pathosystems (Goldbach et al. 2003). The first viral gene expressed in plants for the development of resistance was the TMV coat protein-encoding gene. The pioneering study (led by Dr. Roger Beachy's team) showed the efficiency of this strategy in generating tobacco plants that are resistant to TMV. Constructs with viral genes encoding the

Table 4.3 Recessive virus resistance genes that have already been mapped or characterized in different host plants

Host	Virus	Viral family/ genus	Protein class
Arabidopsis	*Tobacco mosaic virus* (TMV)	*Tobamovirus*	Transmembrane
Arabidopsis	*Cucumber mosaic virus* (CMV)	*Bromoviridae*	elF4E1
Arabidopsis	*Cucumber mosaic virus* (CMV)	*Bromoviridae*	eIF4G
Arabidopsis	*Turnip crinkle virus* (TCV)	*Tombusviridae*	eIF4G
Arabidopsis	*Tobacco etch virus* (TEV)	*Potyviridae*	eIF (iso)4E
Capsicum annuum	*Pepper veinal mottle virus* (PVMV)	*Potyviridae*	eIF4E e eIF(iso)4E
Capsicum annuum	*Potato virus Y* (PVY)	*Potyviridae*	eIF4E
Capsicum annuum	*Tobacco etch virus* (TEV)	*Potyviridae*	eIF4E
Capsicum chinense	*Pepper mottle virus* (PepMoV)	*Potyviridae*	eIF4E
Capsicum chinense	*Potato virus Y* (PVY)	*Potyviridae*	eIF4E
Cucumis melo	*Melon necrotic spot virus* (MNSV)	*Tombusviridae*	eIF4E
Hordeum vulgare	*Barley mild mosaic virus* (BaMMV)	*Potyviridae*	eIF4E
Hordeum vulgare	*Barley yellow mosaic virus* (BaYMV)	*Potyviridae*	eIF4E
Lactuca sativa	*Lettuce mosaic virus* (LMV)	*Potyviridae*	eIF4E
Oryza sativa	*Rice yellow mottle virus* (RYMV)	*Sobemovirus*	eIF(iso)4G
Oryza glaberrima	*Rice yellow mottle virus* (RYMV)	*Sobemovirus*	eIF(iso)4G
Oryza sativa	*Rice dwarf virus* (RDV)	*Reoviridae*	NAC-domain
Oryza sativa[a]	*Rice tungro spherical virus* (RTSV)	*Sequiviridae*	eIF4G
Pisum sativum	*Pea seed borne mosaic virus* (PSbMV)	*Potyviridae*	eIF4E
Pisum sativum	*Bean yellow mosaic virus* (BYMV)	*Potyviridae*	eIF4E
Solanum habrochaites	*Potato virus Y* (PVY)	*Potyviridae*	eIF4E
Solanum habrochaites	*Tobacco etch virus* (TEV)	*Potyviridae*	eIF4E
Brassica rapa[a]	*Turnip mosaic virus* (TuMV)	*Potyviridae*	eIF(iso)4E
Prunus davidiana[a]	*Plum pox virus* (PPV)	*Potyviridae*	eIF(iso)4E
Citrullus lanatus[a]	*Zucchini yellow mosaic virus* (ZYMV)	*Potyviridae*	eIF(iso)4E
Phaseoulus vulgaris[a]	*Bean common mosaic virus* (BCMV)	*Potyviridae*	eIF4E

[a] Mapping indicating cosegregation with genes encoding elongation factors
Source Adapted from Legall et al. (2011)

RNA-dependent RNA polymerase, protease, movement protein, satellite, and defective RNAs in addition to non-coding regions of the viral genome have also been used with varying degrees of success. Initially, viral gene expression was presumed to be necessary for resistance to be effective. However, several results in recent years have shown that post-transcriptional gene silencing (PTGS) may occur, which does not require full gene expression. In the case of plant–virus interactions, infected hosts have been found to accumulate different classes of small interfering RNAs (siRNAs) from pathogen sequences. In this mechanism, invading viral RNAs

(and any other similar RNA) are targeted for degradation by the host cell defense system. This strategy (called RNA interference or RNAi) has been used to develop transgenic plants that are resistant to viruses, including the first transgenic bean resistant to *Bean golden mosaic begomovirus* (Aragão and Faria 2009). However, as mentioned, some viruses manage to suppress the silencing systems, causing a potential decrease in the efficacy of this strategy. Some of these suppressors of post-transcriptional gene silencing have been extensively studied in different viral species (e.g., HC-Pro of *Potyvirus*, NSs in *Tospovirus*, 2b of *Cucumovirus* and P19 of *Tombusvirus*).

The mobilization of genes using genetic engineering techniques, both between different genetic materials from a single species and between related species (termed cisgenesis in the literature), may also be an interesting approach for controlling viral diseases (Jacobsen and Schouten 2007). Resistance genes can be transferred from the same host varieties, from wild species or from phylogenetically more distant taxonomic groups. This strategy can be more effective when the resistance factor is present in related species and/or displays activity against viruses with a broad spectrum of host plants (e.g., *Tobamovirus*, *Cucumovirus* and *Tospovirus*). For example, Picoli et al. (2006) used this strategy to transfer the tomato *Sw-5* gene into the eggplant.

4.12 Final Considerations

Resistance to viruses and/or their vectors is the most feasible disease control strategy. Therefore, significant research effort has been devoted to this field. In fact, great strides have been achieved using classical and molecular breeding. The biggest obstacle for these programs is to identify and incorporate, on a large scale, multiple resistance factors in elite materials and anticipate potential problems with emerging viral diseases, given the challenges that climate change will pose to food production.

References

Anagnostou K, Jahn MM, Perl-Treves R (2000) Inheritance and linkage analysis of resistance to zucchini yellow mosaic virus, watermelon mosaic virus, papaya ringspot virus and powdery mildew resistance in melon. Euphytica 116:265–270

Aragão FJL, Faria JC (2009) First transgenic geminivirus-resistant plant in the field. Nat Biotechnol 27:1086–1088

Boiteux LS (1995) Allelic relationships between genes for resistance to Tomato spotted wilt virus in *Capsicum chinense*. Theor Appl Genet 90:146–149

Boiteux LS, de Ávila AC (1994) Inheritance of a resistance specific to *Tomato spotted wilt tospovirus* in *Capsicum chinense* 'PI 159236'. Euphytica 75:139–142

Boiteux LS, Giordano LB (1993) Genetic basis of resistance against two *Tospovirus* species in tomato (*Lycopersicon esculentum*). Euphytica 71:151–154

Boiteux LS, Nagata T, Dutra WP, Fonseca MEN (1993) Sources of resistance to Tomato spotted wilt virus (TSWV) in cultivated and wild species of *Capsicum*. Euphytica 67:89–94

Boiteux LS, Cupertino FP, Silva C, Dusi AN, Monte DC, van der Vlugt RAA, Fonseca MEN (1996) Resistance to potato virus Y (pathotype 1–2) in Capsicum annuum and Capsicum chinense is controlled by two independent major genes. Euphytica 87:53–58

Boiteux LS, Oliveira VR, Silva CH, Makishima N, Inoue-Nagata AK, Fonseca MEN, Giordano LB (2007a) Reaction of tomato hybrids carrying the *Ty-1* locus to Brazilian bipartite *Begomovirus* species. Horticultura Brasileira 25:20–23

Boiteux LS, Pereira-Carvalho RC, Inoue-Nagata AK, Fonseca MEN, Resende RO, Fernández-Muñoz R (2007b) Reação de acessos de tomateiro portando o gene *Ty-2* (introgredido de *Solanum habrochaites* f. *glabratum*) a um isolado de begomovírus de genoma bipartido. Horticultura Brasileira 25:S97

Brommonschenkel SH, Frary A, Frary A, Tanksley SD (2000) The broad-spectrum *Tospovirus* resistance gene *Sw-5* of tomato is a homolog of the root-knot nematode resistance gene *Mi*. Mol Plant Microbe Interact 13:1130–1138

Cho JJ, Custer DM, Brommonschenkel SH, Tanksley SD (1995) Conventional breeding: Host-plant resistance and the use of molecular markers to develop resistance to Tomato spotted wilt virus in vegetables. Acta Horticulturae (ISHS) 431:367–378

Cooper JI, Jones AT (1983) Responses of plant to viruses: proposals for the use of terms. Phytopathology 73:127–188

De Castro AP, Blanca JM, Díez MJ, Vinãls FN (2007) Identification of a CAPS marker tightly linked to the *Tomato yellow leaf curl* disease resistance gene *Ty-1* in tomato. Eur J Plant Pathol 117:347–356

Dianese EC, Fonseca MEN, Goldbach R, Kormelink R, Inoue-Nagata AK, Resende RO, Boiteux LS (2010) Development of a locus-specific, co-dominant SCAR marker for assisted-selection of the *Sw-5* (*Tospovirus* resistance) gene cluster in a wide range of tomato accessions. Mol Breed 25:133–142

Dianese EC, Fonseca MEN, Inoue-Nagata AK, Resende RO, Boiteux LS (2011) Search in *Solanum* (section *Lycopersicon*) germplasm for new sources of broad-spectrum resistance to four *Tospovirus* species. Euphytica 180:307–319

Díaz JA, Mallor C, Soria C, Camero R, Garzo E, Fereres A, Alvarez JM, Gómez-Guillamón ML, Luis-Arteaga M, Moriones E (2003) Potential sources of resistance for melon to nonpersistently aphid-borne viruses. Plant Dis 87:960–964

Díaz-Pendón JA, Fernández-Muñoz R, Gómes-Guillamón ML, Moriones E (2005) Inheritance of resistance to watermelon mosaic virus in *Cucumis melo* that impairs virus accumulation, symptom expression, and aphid transmission. Phytopathology 95:840–846

Fargette D, Konaté G, Fauquet C, Muller E, Peterschmitt M, Thresh JM (2006) Molecular ecology and emergence of tropical plant viruses. Annu Rev Phytopathol 44:235–260

Fraser RSS (1992) The genetics of resistance to plant viruses. Annu Rev Phytopathol 28:197–200

García-Cano E, Resende RO, Boiteux LS, Giordano LB, Fernández-Muñoz R, Moriones E (2008) Phenotypic expression, stability and inheritance of a recessive resistance to monopartite begomoviruses associated with tomato yellow leaf curl disease in tomato. Phytopathology 98:618–627

Giordano LB, Ávila AC, Charchar JM, Boiteux LS, Ferraz E (2000) Viradoro: a tospovirus-resistant processing tomato cultivar adapted to tropical environments. HortScience 35:1368–1370

Giordano LB, Fonseca MEN, Silva JBC, Inoue-Nagata AK, Boiteux LS (2005a) Efeito da infecção precoce de Begomovírus com genoma bipartido em características de fruto de tomate industrial. Horticultura Brasileira 23:815–818

Giordano LB, Silva-Lobo VL, Santana FM, Fonseca MEN, Boiteux LS (2005b) Inheritance of resistance to the bipartite *Tomato chlorotic mottle begomovirus* derived from *Lycopersicon esculentum* cv. 'Tyking'. Euphytica 143:27–33

Giordano LB, Boiteux LS, Quezado-Duval AM, Fonseca MEN, Resende FV, Reis A, González M, Nascimento WM, Mendonça JL (2010) BRS Tospodoro: a high lycopene processing tomato cultivar adapted to organic crop systems and with multiple resistance to pathogens. Horticultura Brasileira 28:241–245

Goldbach R, Bucher E, Prins M (2003) Resistance mechanisms to plant viruses: an overview. Virus Res 92:207–212

Gómez P, Rodríguez-Hernández AM, Moury B, Aranda MA (2009) Genetic resistance for the sustainable control of plant virus diseases: breeding, mechanisms and durability. Eur J Plant Pathol 125:1–22

Guimarães AM, Pavan MA, Silva N (2009) Comportamento de progênies F5 de alface às tospoviroses em condições de campo. Unimar Ciências 18:37–40

Gray S, DeBoer S, Lorenzen J, Karasev AV, Whitworth J, Nolte P, Singh R, Boucher A, Xu H (2010) Potato virus Y: an evolving concern for potato crops in the United States and Canada. Plant Dis 94:1384–1397

Guner N, Wehner TC (2008) Overview of Potyvirus resistance in watermelon In: Pitrat M (ed) Cucurbitaceae 2008, Proceedings of the IXth EUCARPIA meeting on genetics and breeding of Cucurbitaceae. INRA, Avignon (France), pp 445–452

Inoue-Nagata AK, Fonseca MEN, Resende RO, Boiteux LS, Monte DC, Dusi AN, de Ávila AC, van der Vlugt RAA (2002) *Pepper yellow mosaic virus*: A new potyvirus in sweet pepper, *Capsicum annuum*. Arch Virol 147:849–855

Jahn M, Paran I, Hoffmann K, Radwanski ER, Livingstone KD, Grube RC, Aftergoot E, Lapidot M, Moyer J (2000) Genetic mapping of the *Tsw* locus for resistance to the *Tospovirus Tomato spotted wilt virus* in *Capsicum* spp. and its relationship to the *Sw-5* gene for resistance to the same pathogen in tomato. Mol Plant Microbe Interact 13:673–682

Ji Y, Schuster DJ, Scott JW (2007) *Ty-3*, a begomovirus resistance locus near the *Tomato yellow leaf curl virus* resistance locus *Ty-1* on chromosome 6 of tomato. Mol Breed 20:271–284

Jacobsen E, Schouten H (2007) Cisgenesis strongly improves introgression breeding and induced translocation breeding of plants. Trends Biotechnol 25:219–223

Kang WH, Hoang NH, Yang HB, Kwon JK, Jo SH, Seo JK, Kim KH, Choi D, Kang BC (2010) Molecular mapping and characterization of a single dominant gene controlling CMV resistance in peppers (*Capsicum annuum* L.). Theor Appl Genet 120:1587–1596

Kelley KB, Whitworth JL, Novy RG (2009) Mapping of the potato leafroll virus resistance gene, Rlr_{etb}, from *Solanum etuberosum* identifies interchromosomal translocations among its E-genome chromosomes 4 and 9 relative to the A-genome of *Solanum* L. sect. *Petota*. Mol Breed 23:489–500

Lanfermeijer FC, Warmink J, Hille J (2005) The products of the broken *Tm-2* and the durable $Tm\text{-}2^2$ resistance genes from tomato differ in four amino acids. J Exp Bot 56:2925–2933

Lebeda A, Dolezalova I, Kristkova E, Kitner M, Petrzelova I, Mieslerova B, Novotna A (2009) Wild *Lactuca* germplasm for breeding: current status, gaps, and challenges. Euphytica 170:15–34

LeGall O, Aranda MA, Caranta C (2011) Plant resistance to viruses mediated by translation initiation factors. In: Caranta C (ed) Recent advances in plant virology. Horizon Scientific Press, Norfolk, UK, pp 177–194

Maule AJ, Caranta C, Boulton MI (2007) Sources of natural resistance to plant viruses: status and prospects. Mol Plant Pathol 8:223–231

Meyer JDF, Deleu W, Garcia-Mas J, Havey MJ (2008) Construction of a fosmid library of cucumber (*Cucumis sativus*) and comparative analyses of the eIF4E and eIF(iso)4E regions from cucumber and melon (*Cucumis melo*). Mol Genet Genomics 279:473–480

Nagai H (1989) PI 342517, uma introdução de alface com resistência ao vírus de vira cabeça. Horticultura Brasileira 7:66

Nagai H (1993) Tomate. In: Furlani AMC, Viégas GP (eds) O Melhoramento Genético de Plantas no Instituto Agronômico. IAC, Campinas-SP, pp 301–313

O'Malley PJ, Hartmann RW (1989) Resistance to tomato spotted wilt virus in lettuce. HortScience 24:360–362

Park YH, Sensoy S, Wye C, Antonise R, Peleman J, Havey MJ (2000) A genetic map of cucumber composed of RAPDs, RFLPs, AFLPs, and loci conditioning resistance to papaya ringspot and zucchini yellow mosaic viruses. Genome 43:1003–1010

Palukaitis P, García-Arenal F (2003) Cucumber mosaic virus. No. 400 in: descriptions of plant viruses. Association of Applied Biologists, Warwick, UK

Pavan MA, Krause-Sakate R, Silva N, Zerbini FM, LeGall O (2008) Virus diseases of lettuce in Brazil. Plant Viruses 2:35–41

Pereira-Carvalho RC, Boiteux LS, Fonseca MEN, Díaz-Pendon JA, Moriones E, Fernández-Muñoz R, Charchar JM, Resende RO (2010) Multiple resistance to *Meloidogyne* spp. and to bipartite and monopartite *Begomovirus* spp. in wild *Solanum* (*Lycopersicon*) accessions. Plant Dis 94:179–185

Picoli EAT, Lima GSA, Lau D, Oliveira JCF, Laia ML, Zerbini FM, Brommonschenkel SH, Fári MG, Otoni WC (2006) Resistance gene Sw-5 of tomato confers resistance to TCSV in *Solanum melongena*. Int J Hortic Sci 12:41–47

Ribeiro SG, Ambrozevícius LP, Ávila AC, Bezerra IC, Calegario RF, Fernandes JJ, Lima MF, Mello RN, Rocha H, Zerbini FM (2003) Distribution and genetic diversity of tomato-infecting begomoviruses in Brazil. Arch Virol 148:281–295

Solomon-Blackburn RM, Barker H (2001) Breeding virus resistant potatoes (Solanum tuberosum): a review of traditional and molecular approaches. Heredity 86:17–35

Scholthof KBG, Adkins S, Czosnek H, Palukaitis P, Jacquot E, Hohn T, Hohn B, Saunders K, Candresse T, Ahlquist P, Hemenway C, Foster GD (2011) Top 10 plant viruses in molecular plant pathology. Mol Plant Pathol 12:938–954.

Taliansky M, Mayo MA, Barker H (2003) *Potato leafroll virus*: a classic pathogen shows some new tricks. Mol Plant Pathol 4:81–89

Tomita R, Sekine KT, Mizumoto H, Sakamoto M, Murai J, Kiba A, Hikichi Y, Suzuki K, Kobayashi K (2011) Genetic basis for the hierarchical interaction between *Tobamovirus* spp. and *L* resistance gene alleles from different pepper species. Mol Plant Microbe Interact 24:108–117

Vieira JV, Ávila AC, Silva GO (2010) Avaliação de genótipos de melancia para resistência ao Papaya ringspot vírus, estirpe melancia. Horticultura Brasileira 28:7–11

Wang D, Bosland PW (2006) The genes of *Capsicum*. HortScience 41:1169–1187

Zamir D, Ekstein-Michelson I, Zakay Y, Navot N, Zeidan M, Sarfatti M, Eshed Y, Harel E, Pleban T, Van-Oss H, Kedar N, Rabinowitch HD, Czosnek H (1994) Mapping and introgression of a tomato yellow leaf curl virus tolerance gene, Ty-1. Theor Appl Genet 88:141–146

Chapter 5
Breeding for Nematode Resistance

Éder Matsuo, Paulo Afonso Ferreira, Tuneo Sediyama, Silamar Ferraz, Aluízio Borém and Roberto Fritsche-Neto

Abstract Nematode control is complex; therefore, the prevention of an initial phytonematode infestation in pathogen-free areas is of fundamental importance. However, after the entry of the nematode into an area, it is virtually impossible to eradicate because it is a soil-dwelling organism. In this respect, the genetic resistance of plants to nematodes is considered one of the most efficient and economically feasible methods to prevent production losses. Thus, breeding for nematode resistance is essential in crop management to obtain a high yield and stable production. This chapter contains discussions related to phytopathology, genetics, and breeding that are of great importance for the development of nematode-resistant cultivars.

Keywords Plant breeding · Soil diseases · Quantitative genetics · Biotechnology

É. Matsuo · P. A. Ferreira · T. Sediyama · S. Ferraz · A. Borém · R. Fritsche-Neto (✉)
Engineer Agronomist, Federal University of Viçosa, Viçosa, Brazil
e-mail: roberto.neto@ufv.br

É. Matsuo
e-mail: matsuoeder@yahoo.com.br

P. A. Ferreira
e-mail: paulo.ferreira@ufv.br

T. Sediyama
e-mail: tuneo@ufv.br

S. Ferraz
e-mail: silamar@ufv.br

A. Borém
e-mail: borem@ufv.br

R. Fritsche-Neto and A. Borém (eds.), *Plant Breeding for Biotic Stress Resistance*,
DOI: 10.1007/978-3-642-33087-2_5, © Springer-Verlag Berlin Heidelberg 2012

5.1 Introduction

Brazil is considered an important agricultural breadbasket of the world and has established itself as a major food-exporting country. Brazilian agribusiness exports in the period from September 2010 to August 2011 totaled US$88.3 billion. The soybean industry accounted for US$21.5 billion and stood out as one of the most exported products. Coffee and fruit juices also showed impressive results; from January to September 2011, the magnitude of coffee exports was US$6.1 billion, and that of orange juice was US$1.7 billion (MAPA 2011).

The expansion of the area cultivated with different crops throughout different regions of the country combined with inadequate crop management and the continued use of disease-susceptible genotypes can give rise to increased numbers of pathogenic organisms. Among these pathogens, nematodes stand out. Nematode control is complex; therefore, the prevention of the initial phytonematode infestation in pathogen-free areas is of fundamental importance. After entry of the nematode into an area, it is virtually impossible to eradicate because it is a soil-dwelling organism. Thus, the genetic resistance of plants to nematodes is considered one of the most efficient and economically feasible methods to prevent production losses (Roberts 2002). However, it is important to have prior knowledge of the nematode population in the cultivation area to choose and plant resistant genotypes (Ferraz et al. 2010).

Thus, breeding for nematode resistance is essential for crop management that is, obtaining a high yield and stable production. This chapter contains discussions related to phytopathology, genetics, and breeding that are of great importance for the development of nematode-resistant cultivars.

5.2 Terminologies

Plant resistance to pathogens is defined as the plant's ability to reduce or inhibit a pathogen attack (Wingard 1953), which may occur simply by a reduction of the injured area, leaf spots, number of lesions, by a modification of phenotypic traits, or by reducing the pathogen population.

In nematology, plant resistance is defined as the plant's ability to inhibit the phytonematode reproduction (Cook and Evans 1987). In studies related to the search and development of nematode-resistant cultivars, resistance is found when a certain plant inhibits the nematode reproduction compared to susceptible genotypes (those that allow nematode reproduction and disease onset) (Trudgill 1991). It is important to note that a certain genotype can only be called resistant or tolerant to a pathogen when its performance is compared with that of another genotype of the same species that is particularly susceptible or intolerant (Sikora et al. 2005). Susceptibility is the opposite of resistance, which is defined as the plant's capacity to allow abundant nematode reproduction (Roberts 2002). Furthermore, the plant's response to the

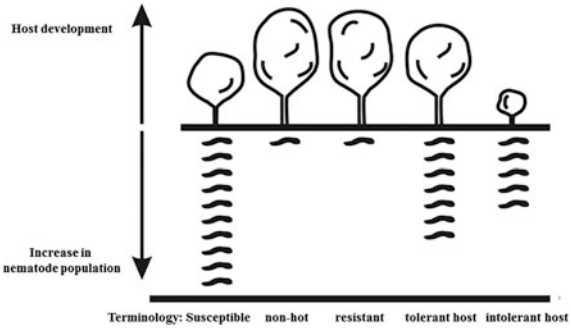

Fig. 5.1 Diagrammatic representation of the terms that describe host development in response to nematodes and their reproduction in plants. Adapted from Roberts (2002)

phytonematode parasitism has been separated from the plant's ability to support its reproduction (Fig. 5.1) because they are non-correlated variables, such that susceptible or resistant plants can be independently considered tolerant or intolerant (Canto-Sáenz 1985; Starr et al. 2002a, b).

Tolerance and intolerance describe the plant response to parasitism, with a tolerant plant exhibiting less suppression in the production than an intolerant plant with similar levels of parasitism (Cook and Evans 1987; Trudgill 1991; Roberts 2002).

Distinctions between resistance, susceptibility, tolerance, and intolerance are not always possible, and minor or moderate levels of resistance are often recognized as tolerance. Similarly, susceptibility is usually treated as intolerance (Roberts 2002).

The relationship between resistance and tolerance has not been well elucidated for most of the resistant genotypes. It is known that such traits may be independently inherited. Therefore, resistant plants may be intolerant and suffer injuries even at low infection levels (Roberts 1990).

There are plants that are immune to, or do not host, nematodes. Immunity is defined as a plant condition that leads to a failure of the reproduction and development of nematodes within the vegetal tissues, which is often by mechanisms that inhibit penetration at the early infection stage. These plants do not suffer the damage that is caused by nematodes (Cook and Evans 1987; Roberts 2002).

Different methodologies and definitions have been adopted in plant breeding due to the lack of standardized terms that are used in analyses of resistance to nematodes. Thus, a division of resistance categories based on nematode reproduction is generally adopted, which is compared to a standard susceptible variety, and can be designated as follows: highly resistant (similar to immunity, when there is no detection of nematode reproduction in the evaluated genotype), resistant, moderately resistant, and susceptible (Fig. 5.2).

In addition to an assessment of resistance, this methodology is also applicable in the assessment of tolerance. However, the exclusive use of phenotypic traits to assess resistance, such as the number of root-knot galls and root lesions, can cause errors in the selection program. For example, when the *Mig-1* and *Mjg-1* genes are expressed in Lima bean plants (*Phaseolus lunatus* L.), they prevent the formation

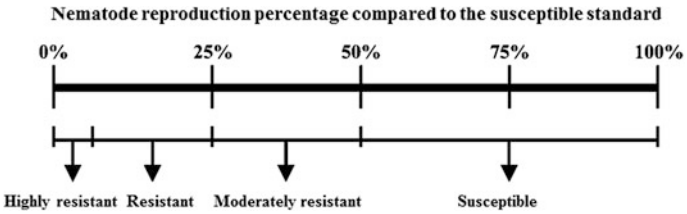

Fig. 5.2 Diagrammatic representation of the host response to nematode reproduction. Adapted from Starr and Mercer (2010)

Table 5.1 Terms that describe the plant response to nematodes

Host efficiency in nematode reproduction	Damage caused by nematodes	
	Statistically significant	Not statistically significant
Efficient	Susceptible	Tolerant
Not efficient	Hypersusceptible	Resistant

Adapted from Canto-Sáenz (1985)

of root-knot galls caused by *Meloidogyne incognita* (Kofoid and White) Chitwood and *M. javanica* (Treub) Chitwood, respectively; however, they do not influence the nematode reproduction in these genotypes (Helms et al. 2004; Roberts et al. 2008). These phenotypic traits are used because they enable a fast identification of resistant individuals, and the evaluation of nematode reproduction by egg count, number of egg masses per root system, number of eggs per gram of root, number of second stage juvenile (J_2), and number of individuals per root system should be used in more advanced stages of the breeding program because they are more accurate parameters (Starr and Mercer 2010).

There are also two parameters that can be used together to evaluate the plant response to nematodes: the reproduction factor (RF) and the damage caused by the nematode (Table 5.1).

The plant response to nematodes is categorized as follows: resistant (when the plant is a bad nematode host, RF < 1.0, and there is no damage); tolerant (the plant is a nematode host, RF > 1.0, and there is no damage); susceptible (the plant is a nematode host and there is damage), and intolerant or hypersusceptible (the plant is a bad nematode host yet there is damage) (Canto-Sáenz 1985).

Some breeding programs are subject to criticism for the use of a methodology that contrasts with other programs because, in general, there are no standardized terminology and procedures. To avoid these problems, a great deal of planning must go into choosing which methodology will be adopted. Furthermore, it is valid to evaluate the most current methodologies that are specific for the pathosystem of interest.

5.3 Plant-Pathogen Interaction

Plants can activate several lines of defense against nematode parasitism. The first line of defense is triggered by recognition of the pathogen-associated molecular patterns (PAMPs) present on potential pathogens, which initiates the plant defense systems. Examples of PAMPs are conserved cell surface structures, such as cuticles, lipids, glycogens, and proteins that are associated with the nematode body wall. PAMPs are recognized by distinct cell surface receptors in plants that activate the immune response. This process is called PAMP-triggered immunity (PTI). This first line of defense confers a resistance to pathogens and is defined as non-host plant resistance.

Pathogens use effector proteins to block PTI, leading to virulence. These effectors are called virulence factors because they make the nematode population virulent. Virulence is defined as the ability of the nematode or other pathogen to reproduce in a host plant that has one or more resistance genes. However, some cultivars evolved to produce specific monitoring proteins, called resistance (R) proteins that recognize the effector proteins (called avirulence factors—*Avr*). Avr proteins in plants mount a second line of defense to pathogens, called effector-triggered immunity (ETI). ETI is stronger and usually displays a hypersensitivity reaction (HR) that features cell death in combination with the pathogen infection (Jones and Dangl 2006; Torres 2010; Williamson and Roberts 2010).

The *Mi* and *Me* genes are examples of resistance genes that activate a rapid cell death in tomato (*Solanum lycopersicon* Mill.) and pepper (*Capsicum annuum* L.) plants, respectively, at the feeding sites of *M. incognita, M. javanica,* and *M. arenaria* (Neal) Chitwood, while the *Mex* gene is a resistance gene to *M. exigua* Goeldi in coffee plants (*Coffea arabica* L.) (Williamson and Roberts 2010).

Genetically, during the parasitism process, the nematode virulence genes (*Avr* genes) can be recognized by the plant resistance genes (*R* genes), which triggers a defense response that will influence the reproduction of phytonematodes. In phytopathology, these genes are typically called *avirulence* genes or *Avr* genes. However, some nematologists refer to them as *parasitism* genes or *Par* genes (Fig. 5.3).

5.4 Plant Physiological Responses

Resistant plants can activate physiological defense responses against nematodes in either the pre-infection and post-infection phases or in both phases, thereby reducing the population of phytonematodes. Plants with responses at the pre-infection phase have chemical and physical barrier mechanisms to pathogens, and these barriers are considered the plants' first line of defense. Some substances produced by certain plant species and released into the soil as root exudates possess repellent or nematicidal effects, which prevent nematodes from penetrating

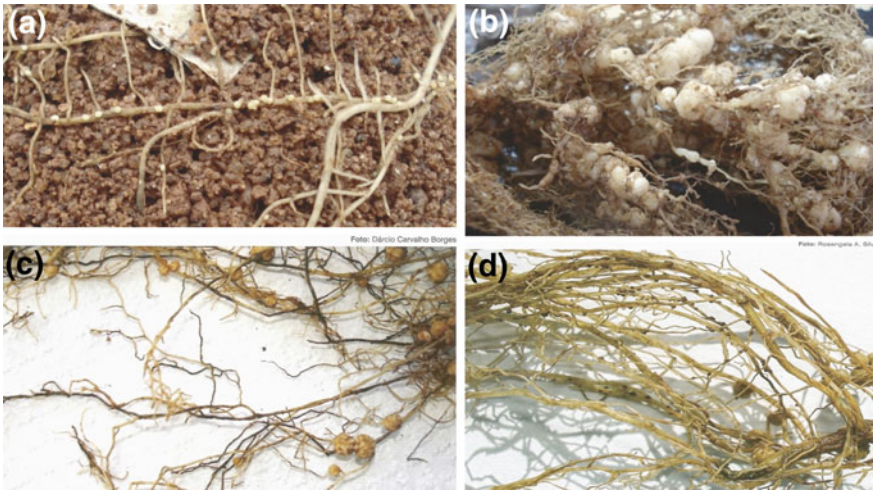

Fig. 5.3 The different types of nematodes that occur in soybean plants. **a** *Heterodera glycines*; **b** *Meloidogyne* spp.; **c** *Pratylenchus brachyurus*; and **d** soybean root with several egg masses of *Rotylenchulus reniformis* (small dark masses in the shape of a half circle, at the root surface). *Source* Photos A and B from Eder Matsuo; C from Dárcio Carvalho Borges, cited by Inomoto and Silva (2011); and D from Rosangela A. Silva, cited by Inomoto and Silva (2011)

the roots and result in an immunity for the plants. This is the case of alpha-terthienyl, nimbidin and pyrocatechol, which are produced by marigold (*Tagetes* spp.), neem (*Azadirachta indica* A. Juss.), and weeping lovegrass (*Eragrostis curvula* Nees), respectively (Huang 1985). Furthermore, during the infection process, nematodes must penetrate the plant cell walls and establish feeding sites with the aid of stylets. During this process, plants that have physical barriers, such as cell walls that are thicker or greater amounts of elements that confer structural resistance to cells, may hinder the penetration of nematodes and the establishment of feeding sites, thereby resulting in less pathogen reproduction.

Even if the nematodes are able to penetrate the plant roots, post-infection resistance mechanisms can inhibit the pathogen development. These mechanisms are turned on after recognition of the nematode by resistance proteins that activate the signaling process of the defense response. Thus, the biochemical changes in the plant tissues begin, preventing, for example, the normal development of feeding sites, which degenerate (Silva 2001). In the roots of resistant plants, few nematodes develop to the adult phase. Additionally, there is the development of more males, and there is a lower fertility rate when the females eventually reproduce (Roberts et al. 1998).

In addition to inhibiting the formation of feeding sites in the roots, the nematodes can also be affected by nematotoxic substances produced by plant tissues, such as phenolic compounds, chlorogenic acid, and terpenoids, after penetration (Huang 1985; Chitwood 2002). Furthermore, some plant species, especially leguminous plants, can rapidly synthesize low molecular weight antimicrobial compounds,

called phytoalexins, in response to nematode penetration (Silva 2001). In soybean plants (*Glycine max* (L.) Merr.) inoculated with *M. Incognita*, the production and accumulation of the phytoalexin glyceollin was higher in the Centennial (resistant) cultivar than in the Pickett 71 (susceptible) cultivar. However, when Centennial was inoculated with *M. Javanica*, to which it is susceptible, there was no significant accumulation of glyceollin (Kaplan et al. 1980a, b). *Meloidogyne incognita* appears to be more sensitive to the action of glyceollin than *M. Javanica* (Huang 1985). The increased activity of certain enzymes, such as catalase, peroxidase, and polyphenol oxidase, is also one of the factors that can inhibit the development of nematodes in plant tissues (Roberts et al. 1998; Silva 2001).

5.5 Classification of the Resistance

Resistance can be classified into the following three categories depending on the number of genes responsible for conferring the trait: (i) monogenic (a single resistance gene, e.g., the *Mi* gene in tomatoes); (ii) oligogenic (two to three resistance genes, e.g., resistance to cyst nematode (*Heterodera glycines* Ichinohe) in some soybean genotypes); and (iii) polygenic (multiple resistance genes involved) (Silva 2001). In turn, the phenotypic effects expressed by the resistance genes may be the result of major or minor genes.

From an epidemiological standpoint, resistance can be divided into vertical (race specific, qualitative, effective against intraspecific pathogen variants including races, pathotypes, or biotypes) or horizontal (race non-specific, quantitative, effective against all pathogen variants) transmission (Vanderplank 1963). Vertical resistance is usually conferred by only one or a few major genes, while horizontal resistance is conferred by minor genes (polygenic), with small additive effects and quantitative inheritance (Silva 2001).

In general, quantitative resistance tends to be more durable under conditions of a greater selection pressure on the phytonematode population (Roberts 2002).

5.6 Coevolution

Resistance can be regarded as a reflection of coevolutionary forces between the host and the parasite. A more specialized relationship between the nematode and plant leads to a higher probability of having specific resistance and parasitism genes (Roberts 2002).

The endoparasitic and sedentary semi-endoparasitic nematodes, such as *Meloidogyne* spp. Goeldi, *Heterodera* spp. Schmidt, *Rotylenchulus* spp. Linford and Oliveira and *Tylenchulus* spp. Cobb, are highly specialized parasites. Resistance to these nematodes can be found at the genus level, against different species within a

genus or even against different variants within a species (races, pathotypes or biotypes).

The group of migratory endoparasitic nematodes, such as *Pratylenchus* spp. Filipjev, *Radopholus* spp. Thorne and *Aphelenchoides* spp. Fischer, and of ectoparasites, such as *Xiphinema* spp. Cobb, are less specialized parasites, as are their food requirements. The selection programs for plants resistant to these nematodes are more difficult to study than sedentary nematodes because there is no strong selective force for the *R* genes, which results in a low frequency of the resistance genes against these phytonematodes in plants. The challenge of releasing cultivars resistant to less specialized nematodes must be overcome by breeders because many of the main nematodes occurring in the tropics belong to the group of migratory endoparasites, which cause cellular destruction without modifying the host, such as *Radopholus* and *Pratylenchus* (Roberts 2002; Luc et al. 2005).

In addition to the type of parasitism exhibited by the nematode, the evolution of the reproduction mode of these phytopathogens can influence breeding programs because the populations can change rapidly in the field from avirulent to virulent, thereby overcoming the resistance. Because all breeding programs expect that the released cultivars have a durable resistance, the different reproduction modes of the nematodes should be considered. For example, *H. glycines* have different field populations (pathotypes and biotypes) because it reproduces by amphimixis (sexual reproduction). However, the populations of a single *Meloidogyne* species do not vary widely because they reproduce by parthenogenesis (asexual reproduction).

Thus, many attributes should be considered in the study of nematode resistance, for example nematode-related traits, such as the time necessary to complete a life cycle, form of reproduction, form of parasitism, genetic and phenotypic variability of populations, and environmental features including high temperature.

5.7 Germplasm and Genetic Variability

The soybean germplasm collections in Brazil are maintained by Embrapa Genetic Resources and Biotechnology (Embrapa Recursos Genéticos e Biotecnologia), Central Agri-business Cooperative for Technological and Economic Development (Cooperativa Central Agropecuária de Desenvolvimento Tecnológico e Econômico Ltda. – Coodetec), The Agri-business Institute of Campinas (Instituto Agronômico de Campinas) Monsoy S.A., University of São Paulo–Luiz de Queiróz College of Agriculture (Universidade de São Paulo–Escola Superior de Agricultura "Luiz de Queiróz", State University of Londrina (Universidade Estadual de Londrina), Federal University of Viçosa (Universidade Federal de Viçosa) (Sediyama et al. 2005), Embrapa Soy (Embrapa Soja), Federal University of Uberland (Universidade Federal de Uberlândia) and other research institutions.

In soybeans, the main phytonematodes are cyst nematodes (*H. glycines*), root-knot nematodes (*M. incognita* and *M. javanica*), root lesion nematodes (*Pratylenchus*

brachyurus (Godfrey) Filipjev and Schuurmans Stekhoven), and reniform nematodes (*Rotylenchulus reniformis* Linford and Oliveira).

The first sources of a soybean resistance to *H. glycines* were the introduction of North American plants or cultivars; however, currently, national cultivars have been used because, besides being resistant, they are adapted to the growing conditions in Brazil (Dias et al. 2009). Matsuo et al. (2011) analyzed soybean genotypes (F_8 generation) resistant to cyst nematodes (SCN), race 3, along with UFVS 2010 (resistance standard), Lee 74, and Emgopa 313 (susceptibility standards). The analyses indicated genetic variability among genotypes and the potential for use in breeding programs for resistance to SCN, race 3.

The main source of resistance used in the development of cultivars that are resistant or moderately resistant to *M. javanica* and *M. incognita* is the North American cultivar Bragg or the national cultivars of the resistant Bragg lineage (Silva 2001; Silva et al. 2001a; Dias et al. 2010). Therefore, the narrow genetic base of soybean to nematodes of the genus *Meloidogyne* is restricted (Silva et al. 2001a).

For *P. brachyurus*, studies with soybean have been conducted to identify genotypes that are resistant or have a good tolerance. Evaluations in greenhouses and in naturally infested areas have indicated that the main cultivars recommended for central Brazil differ in terms of the capacity for nematode reproduction (Ribeiro et al. 2007; Miranda et al. 2011) and tolerance for nematodes (Dias et al. 2008).

With respect to *R. reniformis,* soybean cultures have considerable genetic variability. However, to date, few studies have been able to combine this resistance with other desirable agronomic traits, such as precocity and herbicide resistance (Dias et al. 2010).

For banana crops (*Musa* spp. L.), the main germplasm collection in Brazil is held by Embrapa Cassava and Fruits (Embrapa Mandioca e Fruticultura) (Santos 2011). In the field, genotypes that are relatively tolerant of *R. similis* (Cobb) Thorne were identified by Hartman et al. (2010). Subsequently, Santos (2011) evaluated banana accessions in relation to this phytonematode based on the multiplication rate of the nematodes and the plant growth. It was concluded that 54.5 % of the clones were tolerant to *R. similis.* Moreover, it was noted that the search for resistance depends on the genetic variability of the host plant and on the variability among the nematode populations.

Two of the main citrus (*Citrus* spp. L.) germplasm banks in the world are in Brazil, specifically in the Sylvio Moreira APTA Citrus Center (São Paulo Agency for Agribusiness Technology; Centro APTA Citros "Sylvio Moreira") and in Embrapa Cassava and Tropical Fruits (Oliveira 2006). The genetic variability of the genus *Citrus* is impressive and may be of great use in breeding programs aimed at obtaining rootstock that is adapted to different environmental conditions (EMBRAPA, sd.). Materials resistant to the phytonematode *T. semipenetrans* Cobb have been identified, such as *Sevenoaks buxifolia* Ten. and several selections of *Poncirus trifoliata* (L.) Raf. (Hutchison and O'bannon 1972). However, the only source of resistance that has been incorporated into commercially acceptable citrus rootstock was derived from *P. trifoliata* (Verdejo-Lucas and Kaplan 2002).

The banks and germplasm collections of Coffea arabica crops are in the Agribusiness Research Company of Minas Gerais (Empresa de Pesquisa Agropecuária de Minas Gerais), in the Agribusiness Institute of Campinas (Instituto Agronômico de Campinas), in the Agribusiness Institute of Paraná (Instituto Agronômico do Paraná), in the Program for Technical Support to Coffee Growers of the Ministry of Agriculture, Livestock, and Supply (Programa de Apoio Técnico à Cafeicultura no Ministério da Agricultura, Pecuária e Abastecimento – MAPA), in the Federal University of Lavras (Universidade Federal de Lavras) and in the Federal University of Viçosa (Universidade Federal de Viçosa). These banks have a great potential for applications in breeding programs, especially as the sources of variation of different genetic traits of agronomic interest, such as nematode resistance (Sakiyama et al. 2005). For *M. exigua*, Fazuoli et al. (1977) evaluated 1,692 plants and selected 1,106 (6.3 %) with an absence of root-knot galls. According to the authors, the hybrids from *C. arabica x C. canephora* Pierre ex A. Froehner were the most promising, as they provided the greatest number of resistant plants.

The main Brazilian tomato germplasm banks are at Embrapa Vegetables (Embrapa Hortaliças) and at the Federal University of Viçosa (Universidade Federal de Viçosa). The sources of resistance to the nematodes *M. incognita, M. arenaria* and *M. javanica* were cultivars carrying the *Mi* gene from the wild tomato (*Lycopersicon peruvianum* (L.) P. Miller) (Hussey and Janssen 2002). According to Davis et al. (2000), the *Mi* gene of tomato cultivars was effective against *M. javanica* and against races 1, 2, 3, and 4 of *M. incognita.*

5.8 Genetic Control and Relation Among Traits

The number of genes that control soybean resistance to *H. glycines* has been reported by several authors, ranging from one to four, and are dominant or recessive depending on the source of the resistance and the race studied (Dias et al. 2009). Estimates of heritability, in the narrow sense, for RILs (Recombinant Inbred Lines) derived from Hartwig, were 80.67 % for race 3 and 77.97 % for race 9 (Cervigni et al. 2007). The magnitude of the genotypic determination coefficient, obtained by Matsuo et al. (2011), was equal to 93.63 % for the number of females and 91.38 % for the number of eggs, which indicates that the observed differences among the genotypes were predominantly genetic in nature.

For *M. javanica*, soybean resistance is quantitative in nature, and the genes are located at different loci (Luzzi et al. 1995). The number of genes, the transgressive segregation, and the additive effects of dominance and epistasis can vary according to the combination of genotypes used (Silva et al. 2001a). For *M. incognita,* studies have indicated a high heritability and few genes involved in the inheritance of soybean resistance (Li et al. 2001; Tamulonis et al. 1997b). According to Luzzi et al. (1994), the moderate resistance of the Forrest cultivar is determined by a

single gene (*Rmi1/rmi1*) located in the linking group O, with the dominant allele being responsible for the moderate resistance.

Genetic studies of the inheritance of the soybean resistance to *R. reniformis*, which were conducted with segregating populations derived from the Forrest (resistant) x Ranson (susceptible) cross, showed that the resistance is recessive and controlled by alleles at a single locus (Williams et al. 1981) or by two loci with unequal effects (Harville et al. 1985).

In banana crops, the resistance to *R. similis* is controlled by one or more genes, which allows for the incorporation of the resistance allele from Pisang Jary Buaya into diploid and tetraploid plants (Rowe 1991). According to Dochez (2004), the resistance to *R. similis* in populations of diploid plants is controlled by two dominant genes with additive effects of the interaction.

Until 2000, the mode of inheritance of the resistance to *T. semipenetrans* was not known, which is of extreme importance for the development of a resistant rootstock (Ling et al. 2000). Those authors observed that the segregating population, which was developed by the LB6-2 [Clementine mandarin (*C. reticulata* Blanco) x Hamlin' orange (*C. sinensis* (L.) Osbeck)] x Swingle citrumelo (*C. paradisi* Macfad. x *P. trifoliata*) cross, showed a continuous distribution of the resistance that suggested polygenic control.

The coffee plant resistance to *M. exigua* is controlled by a major dominant gene, designated locus *Mex-1* (NOIR et al. 2003). In tomato crops, the *Mi* gene is dominant and is located on chromosome 6 (Hussey and Janssen 2002). Based on sequence analysis, the following three candidate genes were identified in the genomic region of *Mi-1*: *Mi-1.1*, *Mi-1.2* and *Mi-1.3* (Williamson and Roberts 2010). In their review, those authors presented a summary of the genes involved in the resistance to the root-knot nematode *Meloidogyne* spp., which indicated the host plant, the number, and mode of gene inheritance and their specificities in terms of virulence and temperature sensitivity.

To quantify the degree of association among traits related to the resistance to *M. incognita*, race 1, in F_4 progenies of lettuce (*Lactuca sativa* L.), Carvalho Filho et al. (2011) evaluated the incidence of root-knot galls, scoring for the number of root-knot galls, the number of egg masses, and the number of eggs. Considering all the combinations, the genetic correlations were above 0.80, except for the incidence of root-knot galls x number of eggs and for the number of root-knot galls x number of eggs. The authors concluded that there are high positive genetic correlations among the traits analyzed and that a selection based on the number of eggs can provide the greatest gains.

5.9 Stress Induction and Selection Strategy

Stress can be considered as the sum of every factor that negatively affects the plant performance, such as several environmental and/or management factors (Borém and Ramalho 2011) and biotic factors including nematodes. Thus, breeding

programs of different species have worked to select desirable materials from stress inductions, which were aimed at developing resistant cultivars.

For soybean crops, MAPA has published standardized protocols for analyzing the reaction of genotypes to different diseases, such as cyst nematodes (*H. glycines*) and root-knot nematodes (*M. incognita* and *M. javanica*). These protocols are part of the instructions for performing the tests for distinguishability, homogeneity, and stability of soybean cultivars (MAPA 2009). For the other species of nematodes, there were no standardized methodologies for the protection of cultivars in the National Cultivars Protection Service.

For the analysis of the reaction of soybean genotypes to *H. glycines*, seeds of the cultivars to be tested should be germinated in sand together with the race differentiators (Pickett, Peking, PI 88788, PI 90763, and Hartwig) and the standards for susceptibility (Lee 74) and resistance (PI 437654). Two days after emergence, six seedlings/cultivars are transplanted into clay pots (one seedling/pot) containing a sterile mixture of soil and sand (1:3). Simultaneous to the transplanting, each seedling is inoculated with 4,000 eggs of the cyst nematode race to be tested. The pots prepared in this manner are kept in a greenhouse with temperatures between 25 and 30 °C for 28–30 days. After this period, each seedling is removed from the pot, and its root system is washed under a strong jet of water in a 20-mesh sieve coupled to a 60-mesh sieve for the recovery of female nematodes. The female suspensions are transferred to an American-style cup and subsequently are quantified with the aid of an acrylic plate grid and a stereomicroscope (magnifier). A female index (FI) is calculated for each cultivar, where FI (%) = (mean number of females obtained from the cultivar/mean number of females obtained from Lee 74) x 100. Cultivars with FI < 10 % are classified as resistant, with FI from 10 to 30 % as moderately resistant; and FI > 30 % as susceptible (MAPA 2009).

To analyze the reaction of soybean genotypes to *M. javanica*, seeds of the cultivars to be tested should be germinated in sand together with the standards for resistance (PI 595099, CD 201, CD 208, MG/BR 46 Conquista, BRS Celeste, etc.) and for susceptibility (BRSMT Pintado, BRS 133, Embrapa 20, etc.). Subsequently, six seedlings/cultivars should be transplanted into plastic tubes (one seedling/tube) containing a sterile mixture of soil and sand (1:3). Two days after transplanting, each seedling is inoculated with 5,000 eggs and second stage juveniles of a pure nematode population that multiplied on the Santa Cruz tomato plants or on a susceptible soybean cultivar. The tubes prepared in this manner are kept in a greenhouse at temperatures of 25–30 °C for 45–60 days. After this period, each plant is carefully removed from the container and, after washing the excess soil from the roots, given a score (0–5) according to the intensity of root-knot galls (0 means no root-knot galls and 5 is the maximum intensity). Cultivars with a score of up to 2 (average of six replicates) are labeled as resistant, while those with a score of 2–3 are moderately resistant and above 3 are susceptible. In assigning the scores, the behavior of standard cultivars should always be taken into account (MAPA 2009).

To analyze the reaction of soybean genotypes to *M. incognita*, the seeds of the cultivars to be tested plus the standards for resistance (PI 595099, CD 201, CD 202, CD 208, MG/BR 46 Conquista, etc.) and for susceptibility (BRS Celeste, Santa Rosa, BRSMT Pintado, BRS 133, Embrapa 20, etc.) are germinated in sand and transplanted (six seedlings/cultivar) into plastic tubes (one seedling/cultivar per tube) containing a sterile mixture of soil and sand (1:3). Two days after transplantation, each seedling is inoculated with 5,000 eggs and second stage juveniles of a pure nematode population that multiplied on Santa Cruz tomato plants or on a susceptible soybean cultivar. The tubes prepared in this manner are kept in a greenhouse at a temperature of 25–30 °C for 45–60 days. After this period, each plant is carefully removed from the container and, after washing the excess soil from the roots, receives a score (0–5) according to the intensity of root-knot galls (0 means no root-knot galls and 5 is the maximum intensity). Cultivars with a score (average of six replicates) from 0 to 1 are classified as resistant, 1–2 are moderately resistant and above 2 are susceptible. In assigning the scores, the behavior of the standard cultivars should always be taken into account (MAPA 2009).

During the screening process for resistance or tolerance to *Pratylenchus* spp., several obstacles can be identified, such as the different reproductive and pathogenic capacities among populations of the same species of *Pratylenchus*, differences in the host response and in the reproduction of nematodes between experiments, and the lack of information on the effect of root development in response to nematode reproduction (Dewaele and Elsen 2002). Lopes (2011) evaluated soybean and pearl millet genotypes (*Pennisetum glaucum* R. Br.) in *P. brachyurus* in a greenhouse. In that work, the seeds were germinated in sand, and seven days after emergence, the seedlings (six plants/genotype) were transplanted into individual pots (450 dm^3) containing substrate (sand:commercial substrate, 2:1), which was autoclaved (120 °C for 1 h). Subsequently, 100 specimens (juveniles + adults)/plant were inoculated. After 90 days, the reproductive factors and the phenotypic traits of the plants were assessed. The performance of the genotypes was based on the work of Cook and Evans (1987). Further details on the plant screening with *Pratylenchus* spp. were published by DeWaele and Elsen (2002).

The methods indicated to induce plant stress, such as inoculation of soybean with *Rotylenchulus*, and to identify tolerant genotypes are usually adequate; however, most of them are adaptations of methods originally developed for tests of resistance to other nematode species (Robinson, 2002). When developing a new method for the *Rotylenchulus* species, a more or less unique combination of biological traits can be considered, such as a short life cycle, a broad range of hosts, sexual reproduction, adaptation to fine texture soils, and significant plant damage (Robinson 2002). The reproduction of *R. reniformis* in soybean cultivars is affected by the number of nematodes in the inoculum, and the optimum concentration of nematodes (eggs + juveniles) for multiplication of *R. reniformis* is 3.25 and 3.75 per cm^3 of substrate for the susceptible and resistant genotypes, respectively (Cardoso et al. 2010). To study the reaction of soybean genotypes to the reniform nematode, Asmus (2008) germinated the seeds in sand and, 2 days

after transplanting, inoculated 1,000 eggs on each plant. A total of 70 days after inoculation, the number of nematodes on the soybean roots was quantified according to the works of Jenkins (1964) and Coolen and D'Herde (1972), which were used to estimate the reproduction factor.

In banana crops, Santos (2011) used seedlings (five replicates of each genotype) micropropagated and acclimatized for 21 days in a growth chamber and for 15 days in a greenhouse. After the acclimation period, the seedlings were inoculated. After 60 days, the reaction of the banana plantlets to *R. similis* was evaluated based on the reproduction factor according to the scale published by Sasser and Freckman (1987).

In coffee crops, Silva et al. (2007) evaluated the reaction of genotypes, together with the standards for resistance (Apoatã IAC 2258) and susceptibility (Catuai Vermelho IAC 44), towards *M. exigua.* Seeds were germinated in sand that was previously treated with methyl bromide, and the seedlings (six per genotype) were transplanted into individual pots when they were at the "matchstick" stage. The inoculation (5,000 eggs per plant) was performed after the plants developed 3–4 pairs of leaves. At 110 days after inoculation, the number of root-knot galls and the number of eggs per root system were assessed. The eggs were collected according to the method of Boneti and Ferraz (1981) and quantified in a Peter's counting chamber under a light microscope. These data were used to determine the reproduction factor (RF = Fp/Ip, where Fp = final population and Ip = initial population of the nematode).

A general consideration for the screening of plants for resistance to the nematode *T. semipenetrans*, with information on the inoculum, inoculation, and screening protocols, is presented in Verdejo-Lucas and Kaplan (2002). The methods reported for different cultures can be used in breeding programs. However, with the exception of the protocols described in MAPA (2009), it is up to the breeders to identify the best strategy for analyzing the reaction according to the resource availability and methodology accepted by scientists in the field. Different protocols and considerations towards methods for the selection of plants resistant to nematodes are in Sasser and Carter (1985), Veech and Dickson (1987), Starr (1990), Starr et al. (2002a, b) and Starr and Mercer (2010).

5.10 Breeding Methods and Selection Strategies

A breeding program for resistance and/or tolerance to nematodes should be carefully planned based on a determination of the goals and the genetic, financial, and human resources available. In addition, knowledge of the mode of reproduction of the species and the genetic control of resistance are of paramount importance for choosing the best breeding method and selection strategy for the desired trait.

The introduction of germplasm or the selection of individual plants within different genetic pools can be considered as an option for the breeding of species in which the identification of the sources of resistance and studies related to the

genetic control are at the initial stages or when the breeding program has nematode-resistant cultivars and wishes to introduce them to another cultivation region.

In breeding by hybridization, the goal is the fusion of genetically different gametes that is aimed at developing hybrid individuals heterozygous for one or more loci (Borém and Miranda 2009), which can result in the combination of favorable alleles in a new strain after several selection cycles.

For the identification of potential progenitors in the germplasm, the pathogenic species at the site or region for which the new cultivar is recommended should be known because the variabilities within the nematode species may contribute to breaking resistance (Starr and Mercer 2010). Therefore, it is critically important to know the genetic variability of both the host plant and the nematode species of interest and the interaction between the resistance genes and environmental conditions for the development of resistant genotypes. As an example of resistance supplantation, it should be mentioned the study by Dias et al. (1998) confirmed the resistance supplantation of Hartwig by a field population of cyst nematodes obtained in Sorriso, Mato Grosso, which were classified as 4^{+}. An example of the interaction between the resistance gene and environmental conditions is the *Mi-1* gene, which becomes ineffective at high soil temperatures (>28 °C) (Williamson and Roberts 2010).

After identifying the source of nematode resistance, it is important to know its genetic control. When the resistance is controlled by only one or a few genes, the use of simple crossings or backcrossings can be good options. However, if the trait is controlled by many genes, multiple crosses or recurrent selections can be used. With the possibility of commercially exploiting heterosis, either by autogamous or allogamous plants, F_1 seeds resistant to nematodes can be marketed.

The interaction between the breeder and the nematologist is of paramount importance. The plant breeder must know the number of individuals that a nematologist can accurately evaluate in a year or in a specific period of the year, and the nematologist must know the number of plants that the breeder wishes to be evaluated. This is important because a greater number of evaluated plants lead to a faster progression of the program, thereby increasing the likelihood of success in developing a resistant cultivar (Starr and Mercer 2010). There are reliable methods for the rapid identification of resistant genotypes (Fazuolli et al. 1977; Dias et al. 2009; Matsuo 2009) and modern molecular biology tools that can be used at different stages of the breeding program. It is noteworthy that, even when making selections based on rapid identification or genetic markers, it is important to perform phenotypic evaluations of promising genotypes with the pathogen of interest under controlled conditions and in the destination area of the new cultivar to confirm the genetic resistance.

The segregation of populations of soybean cultures for the development of nematode-resistant cultivars has been conducted by means of genealogical methods, backcrossing, SSD (Single Seed Descent), and SPD (Single Pod Descent). In general, the selection of resistant strains is performed by pathogen inoculation and/or molecular marker-assisted selection.

As a result of soybean breeding programs, there are currently approximately 60 cultivars resistant to *H. glycines* and 80 cultivars resistant or moderately resistant to *M. javanica* and/or *M incognita.* Some cultivars (BRSGO Chapadões, M-Soy 8378 RR, M-Soy 8360 RR, M-Soy 8585 RR, M-Soy 8045 RR, BRS Aurora, CD 219 RR, TMG 103 RR, etc.) are more resistant (smaller reproduction factors) to *P. brachyurus* and seem to better tolerate this parasite. Despite considerable genetic variability for *R. reniformis* resistance, there are few materials that exhibit resistance together with other desirable traits, such as precocity and herbicide resistance (Dias et al. 2009; Dias et al. 2010). Genotypes with a resistance to both *H. glycines* and *Meloidogyne* spp. (e.g., Centennial, Hartwig, Forest, etc.) are considered to be potential new sources of resistance to nematodes of the genus *Meloidogyne* (Silva et al. 2001a). In 2011, the MT Foundation (Fundação de Apoio à Pesquisa Agropecuária de Mato Grosso – Fundação MT) (2011) presented, based on preliminary evaluations, cultivars with a reproduction factor $\leq$1.0 for *P. brachyurus* (Anta 82, TMG1176RR, TMG1179RR, TMG1182RR, TMG132RR, TMG115RR, TMG801, and FMT Tabarana). To reduce the time necessary to identify and recommend soybean cultivars resistant to *R. reniformis,* Asmus (2008) suggested focusing the studies on the genotypes that have a proven resistance to *H. glycines*.

Other crops such as cotton (*Gossypium* L.), rice (*Oryza sativa* L.), sugarcane (*Saccharum* L.), millet (*P. glaucum),* maize (*Zea mays* L.), sorghum (*Sorghum bicolor* L. Moench), and vegetable crops have also been bred for nematode resistance.

Pípolo and Ferraz (1999) reported some aspects to be considered in a breeding program for nematode resistance, as follows: amount of inoculum that represents the virulence and variability of that species; nematode multiplication capacity for inoculations at appropriate times; assessment of new sources of germplasm with genetic variability for the study of other types of resistance; artificial and field evaluations and the use of methods that enable consistency and efficiency of infestation; damage assessments using appropriate scales within the categories susceptible and resistant; and the choice of a selection method capable of providing an effective gain in a breeding program.

5.11 Biotechnology: Quantitative Trait Loci (QTL), Marker-Assisted Selection and Transgenesis

The term QTL (Quantitative Trait Loci) refers to loci where quantitative traits are controlled. The ability to detect a QTL depends on the magnitude of its effect on the trait, on the size of the segregating population, on the recombination frequency between the marker and the QTL and on the heritability of the trait under consideration (Lanza et al. 2000). With the advent of molecular markers, it became possible to map and characterize polygenes of quantitative markers. An important

aspect in detecting QTLs using molecular markers is the need for the population under study to be in linkage disequilibrium (Cruz et al. 2009).

For soybean crops, studies report the identification of QTLs for the resistance to *H. glycines* (Yue et al. 2001), *M. javanica* (Tamulonis et al. 1997a), *M. incognita* (Tamulonis et al. 1997b; Li et al. 2001) and *R. reniformis* (Ha et al. 2007). The identification and validation of molecular markers, including RFLP (Restriction Fragment Length Polymorphism), RAPD (Random Amplified Polymorphic DNA), SSR (Simple Sequences Repeats), and AFLP (Amplified Fragment Length Polymorphism), linked to soybean resistance alleles in different races of *H. glycines* were presented in several studies (Dias et al. 2009). Details about the marker-assisted selection for soybean resistance to cyst nematodes can be found in Young and Mudge (2002). For *M. javanica*, SSR (Silva et al. 2001b) and RFLP (Mienie et al. 2002) markers were identified. For *M. incognita*, RFLP (Tamulonis et al. 1997b) and SSR (Li et al. 2001; Ha et al. 2004) markers were observed.

For citrus, Ling et al. (2000) identified a major gene that contributes to the resistance to *T. semipenetrans*. In a QTL analysis, it was observed that this major gene explained 53.6 % of the phenotypic variation. This finding confirmed the existence of other genes that contribute to the observed continuous phenotypic variation. That study identified the RAPD and SCAR (Sequence-Characterized Amplified Regions) markers that can be useful in breeding programs for the selection of rootstocks.

For banana crops, contrasting genotypes were analyzed in terms of susceptibility and resistance to the nematodes *R. similis, M. incognita, M. javanica,* and *M. arenaria*, which were based on the RAPD and SSR markers (Santos 2011). The author identified polymorphic markers that are promising for studies of genetic mapping and marker-assisted selection for nematode resistance.

Biotechnological methods that are applied to coffee crops, such as techniques of tissue culture, molecular markers, genetic mapping, and genetic transformation, have proven to be an important tool to support conventional breeding programs, thereby assisting in the maintenance and conservation of the germplasm used for the development of new cultivars in a short time (Morais and Melo 2011). Noir et al. (2003) identified RFLP markers associated with the resistance to *M. exigua*, which are presumably in the *Mex-1* locus. The association of these markers with *Mex-1* was confirmed by analysis of a group of genotypes involving introgressions of *C. arabica* that is resistant or susceptible to *M. exigua* in the field. These markers represent, according to the authors, an important starting point for enhancing breeding programs based on backcrossing and early seedling selection.

Identification of the root-knot nematode-resistant gene *Mi* is a routine practice in many tomato breeding programs; however, results of field assessments can be misleading due to variations of the nematode population and soil temperature. Therefore, marker-assisted selection has become an important tool in this species (Danso et al. 2011). According to those authors, the use of the SCAR marker that was strongly linked to the *Mi-1.2* gene (specific primer) in the identification and marker-assisted selection of tomato genotypes resistant to root-knot nematodes is reliable and efficient. Yaghoobi et al. (2005) have identified molecular markers

flanking the gene at 0.25 cM, which co-segregates with the *Mi-3* gene. These markers can be used to assist in the introduction of *Mi-3* in tomato cultivars through conventional breeding or cloning strategies. Details on the physical and genetic location of the root-knot nematode-resistant gene *Mi* in the tomato by using molecular markers based on the recombination in progenies of controlled crosses of *L. peruvianum* plants with and without resistance can be found in Kaloshian et al. (1998).

As a consequence of the limited number of genotypes that are resistant to nematodes and the fact that, in some species, resistant genotypes have not yet been identified, Atkinson et al. (2010) reported that there is a need for new approaches for the development of plants resistant to root-knot nematodes. Genetic engineering provides an effective and durable strategy for the development of plants resistant to *Meloidogyne* spp.

Proteinase inhibitors, *Cry* proteins and RNAi are potential strategies for the development of transgenic plants resistant to nematodes, but more studies must be conducted to determine the effectiveness of these transgenic plants (Atkinson et al. 2010). That review presents the commercial prospects on the development of transgenic plants resistant to nematodes of the genus *Meloidogyne*.

A new biotechnology approach to banana crops has been used to confer resistance to Cavendish (AAA) selections using RNAi. In that work, it was observed that the RNAi is ingested when the nematodes feed on plants and that the RNAi silencing mechanism is activated in the reproductive organs, thereby resulting in defective embryos. Plants are currently being field-tested in controlled experiments (Khayat and Ortiz 2011).

References

Asmus GL (2008) Reação de genótipos de soja ao nematoide reniforme. Trop Plant Pathol 33:69–71

Atkinson HJ, Urwin PE, Hussey RS (2010) Plant Biotechnol Control. In: Perry RN, Moens M, Starr J (eds) Root-knot nematodes. CAB International, Wallingford, pp 338–362

Boneti JIS, Ferraz S (1981) Modificações do método de Hussey & Barker para extração de ovos de Meloidogyne exigua em raízes de cafeeiro, vol 6. Fitopatologia Brasileira, Brasília, DF, n. 3, p 553

Borém A, Miranda GV (2009) Melhoramento de plantas. 5ª. Edição. Viçosa: Editora UFV. 529 p

Borém A, Ramalho MAP (2011) Estresses abióticos: desafios do melhoramento de plantas nas próximas décadas. In: Fritche-Neto R, Borém A (eds) Melhoramento de Plantas para Condições de Estresse Abióticos. Editora Suprema, pp 9–28

Canto-Sáenz M (1985) The nature of resistance to *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1959. In: Sasser JN, Carter CC (eds) An advanced treatise on *Meloidogyne*, Volume I: Biology and Control. North Carolina State University Graphics, pp 225–231

Cardoso PC, Asmus GL, Gonçalves MC (2010) Efeito da concentração de inóculo sobre a reprodução de *Rotylenchulus reniformis* em cultivares de soja. Revista Agrarian 3:51–55

Carvalho Filho JLS, Gomes LAA, Silva RR, Ferreira S, Carvalho RRC, Maluf WR (2011) Parâmetros populacionais e correlação entre características da resistência a nematoides de galhas em alface. Revista Brasileira de Ciências Agrárias 6:46–51

Cervigni GDL, Schuster I, Sediyama CS, Barros EG, Moreira MA (2007) Inheritance pattern and selection criteria for resistance to soybean cyst nematode races 3 and 9. Pesquisa Agropecuária Brasileira 42:1413–1419
Chitwood DJ (2002) Phytochemical based strategies for nematode control. Annu Rev Phytopathol 40:221–249
Cook R, Evans K (1987) Resistance and tolerance. In: Brown RH, Kerry BR (eds) Principles and practice of nematode control in crops. Academic Press, Orlando, pp 179–231
Coolen WA, D'Herde CJA (1972) Method for the quantitative extraction of nematodes from plant tissue. Ghent: State Nematology and Entomology Research Station, p 77
Cruz CD, Bhering LL, God PIVG (2009) Mapeamento de QTLs em populações derivadas de cruzamentos controlados. In: Borém A, Caixeta ET (eds) Marcadores Moleculares. Editora Folha de Viçosa, pp 483–531
Danso Y, Akromah R, Osei K (2011) Molecular marker screening of tomato (*Solanum lycopersicum* L.) germplasm for root-knot nematodes (*Meloidogyne* species) resistance. Afr J Biotechnol 10:1511–1515
Davis EL, Hussey RS, Baum TJ, Bakker J, Schots A (2000) Nematode parasitism genes. Annu Rev Phytopathol 38:365–396
DeWaele D, Elsen A (2002) Migratory endoparasites: *Pratylenchus* and *Radopholus* species. In: Starr JL, Cook R, Bridge J (eds) Plant resistance to parasitic nematodes. CAB International, Wallingford, pp 175–206
Dias WP, Asmus GL, Silva JFV, Garcia A, Carneiro GES (2010) Nematoides. In: Almeida AMR, Seixas CDS (eds) Soja: Doenças Radiculares e de Hastes e Inter-Relações com o Manejo do Solo e da Cultura. Embrapa Soja, pp 173–206
Dias WP, Ribeiro NR, Pivato A, Molina D (2008) Avaliação da reação de genótipos de soja ao nematoide das lesões radiculares (*Pratylenchus brachyurus*). In: XXX Reunião de Pesquisa de Soja da Região Central do Brasil, Resumos. Rio Verde: Comigo e Embrapa Soja, pp137–139
Dias WP, Silva JFV, Carneiro GES, Garcia A, Arias CAA (2009) Nematoide de cisto da soja: biologia e manejo pelo uso da resistência genética. Nematologia Brasileira 33:1–16
Dias WP, Silva JFV, Kiihl RAS, Hiromoto DM, Abdelnoor RV (1998) Quebra da resistência da cv. Hartwig por população de campo do nematoide de cisto da soja (*Heterodera glycines*). Pesquisa Agropecuária Brasileira 33:971–973
Dochez C (2004) Breeding for resistance to *Radopholus similis* in East African highland bananas (*Musa* spp.). Katholieke Universiteit Leuven. 2004. 222 p (Tese de doutorado)
EMBRAPA—Empresa Brasileira de Pesquisa Agropecuária. sd. Variabilidade genética e melhoramento de bananeira. In: Recursos Genéticos e Melhoramento de Plantas Para o Nordeste Brasileiro. Embrapa. 23p. Disponível em: http://www.cpatsa.embrapa.br/catalogo/livrorg/citros.pdf Acesso em: 29 de outubro de 2011
Fazuoli LC, Monaco LC, Carvalho A (1977) Resistência do cafeeiro a nematoides: I—Testes em progênies e híbridos para *Meloidogyne exigua*. Bragantia 36:297–307
Fazuolli LC, Carvalho A, Lordello RRA (1977) Estudo de métodos de infestação para avaliação precoce da resistência do cafeeiro a *Meloidogyne javanica*. Bragantia 36:231–238
Ferraz S, Freitas LG, Lopes EA, Dias-Arieira CR (2010) Manejo sustentável de fitonematoides. UFV, Viçosa, p 306p
Freitas JA, Azevedo SM, Maluf WR, Silveira MA, Martins LS (2000) Efeito do alelo *Mi* na reação de resistência do tomateiro à *Meloidogyne* spp. Acta Scientiarum Agronomy 22:907–910
Fundação MT (2011) Boletim de pesquisa de soja 2011. Fundação MT, Rondonópolis, p 492
Ha BK, Robbins RT, Han F, Hussey RS, Soper JF, Boerma HR (2007) SSR Mapping and confirmation of soybean QTL from PI 437654 conditioning resistance to reniform nematode. Crop Sci 47:1336–1343
Ha BK, Bennett JB, Hussey RS, Finnerty SL, Boerma HR (2004) Pedigree analysis of a major QTL conditioning soybean resistance to southern root-knot nematode. Crop Sci 44:758–763

Hartman JB, Vuylsteke D, Speijer PR, Ssango F, Coyne DL, Waele DD (2010) Measurement of the field response of Musa genotypes to *Radopholus similis* and *Helicotylenchus multicinctus* and the implications for nematode resistance breeding. Euphytica 172:139–148

Harville BG, Green A, Birchfield W (1985) Genetic resistance to reniform nematodes in soybeans. Plant Dis 69:587–589

Helms DM, Matthews WC, Temple SR, Roberts PA (2004) Registration of 'Cariblanco N' Lima bean. Crop Sci 44:352–353

Huang JS (1985) Mechanisms of resistance to root-knot nematodes. In: Sasser JN, Carter CC (eds) An Advanced Treatise on *Meloidogyne,* Volume I: biology and control. North Carolina State University Graphics, Raleigh, pp 165–174

Hussey RS, Janssen GJW (2002) Root-knot nematodes: *Meloidogyne* species. In: Starr JL, Cook R, Bridge J (eds) Plant resistance to parasitic nematodes. CAB International, Wallingford, pp 43–70

Hutchison DJ, O'bannon JO (1972) Evaluating the reaction of citrus selections to *Tylenchulus semipenetrans*. Plant Dis Rep 56:747–751

Inomoto MM, Silva RA (2011) Importância dos nematoides da soja e influência da sucessão de cultura. In: Siqueri F, Caju J, Moreira M (ed) Boletim de Pesquisa de Soja 2011. Fundação MT, pp 392–399

Jenkins WR (1964) A rapid centrifugal-flotation technique for separating nematodes from soil. Plant Disease Reporter 48:692

Jones JDG, Dangl JL (2006) The plant immune system. Nature 444:323–329

Kaloshian I, Yaghoobi J, Liharska T, Hontelez J, Hanson D, Hogan P, Jesse T, Wijbrandi J, Simons G, Vos P, Zabel P, Williamson VM (1998) Genetic and physical localization of the root-knot nematode resistance locus *Mi* in tomato. Mol Gen Genet 257:376–385

Kaplan DT, Keen NT, Thomason IJ (1980a) Studies on the mode of action of gliceollin in soybean incompatibility to the root-knot nematode, *Meloidogyne incognita*. Physiol Plant Physiol 16:319–325

Kaplan DT, Keen NT, Thomason IJ (1980b) Association of mode of glyceollin with the incompatibility response of roots to *Meloidogyne incognita*. Physiol Plant Physiol 16:309–318

Khayat E, Ortiz R (2011) Genetics of important traits in Musa. In: Pillay M, Tenkouano A (eds) Banana breeding: progress and challenges. CRC Press, New York, pp 71–83

Lanza MA, Guimarães CT, Schuster I (2000) Aplicações de marcadores moleculares no melhoramento genético. Informe Agropecuário 21:97–108

Li Z, Jakkula L, Hussey RS, Tamulonis JP, Boerma HR (2001) SSR mapping and confirmation of the QTL from PI 96354 conditioning soybean resistance to southern root-knot nematode. Theor Appl Genet 103:1167–1173

Ling P, Duncan LW, Deng Z, Dunn D, Hu X, Huang S, Gmitter Jr, FG (2000) Inheritance of citrus nematode resistance and its linkage with molecular markers. Theor Appl Genet 100:1010–1017

Lopes LR (2011) Densidade populacional, hospedabilidade e manejo de lavoura de soja, no controle do *Pratylenchus brachyurus* no Mato Grosso. Viçosa: UFV, 2011. 65 p (Dissertação de mestrado)

Luc M, Bridge J, Sikora RA (2005) Reflections on nematology in subtropical and tropical agriculture. In: Luc M, Sikora RA, Bridge J (eds) Plant parasitic nematodes in subtropical and tropical agriculture, 2nd edn. CAB International, Wallingford, pp 01–10

Luzzi BM, Boerma HR, Hussey RS (1994) A gene for resistance to the southern root-knot nematode in soybean. J Hered 85:484–486

Luzzi BM, Tamulonis J, Hussey RS, Boerma HR (1995) Inheritance of resistance to the javanese root-knot nematode in soybean. Crop Sci 35:1372–1375

MAPA—Ministério da Agricultura, Pecuária e Abastecimento. 2009. Formulários para proteção de cultivares. Disponível em: http://www.agricultura.gov.br/vegetal/registros-autorizacoes/protecao-cultivares/formularios-protecao-cultivares Acesso em: 29 de outubro de 2011

MAPA–Ministério da Agricultura, Pecuária e Abastecimento (2011) Exportações. Disponível em: http://www.agricultura.gov.br/vegetal/exportacao Acesso em: 01 de novembro de 2011

Matsuo É (2009) Estratégias para análise do comportamento de genótipos de soja ao nematoide de cistos (*Heterodera glycines*): Viçosa: UFV, 2009. 85 p (Dissertação de mestrado)
Matsuo É, Sediyama T, Cruz CD, Oliveira RDL, Oliveira RCT, Nogueira APO (2011) Genetic diversity in soybean genotypes with resistance to *Heterodera glycines*. Crop Breed Appl Biotechnol 11:304–312
Mienie CMS, Fourie H, Smit MA, Van Staden J, Botha FC (2002) Identification of AFLP markers in soybean linked to resistance to *Meloidogyne javanica* and conversion to sequence characterized amplified regions (SCARs). Plant Growth Regul 37:157–166
Miranda DM, Favoreto L, Ribeiro NR (2011) Nematoides: um desafio constante. In: Fundação MT (ed) Boletim de Pesquisa de Soja 2011. Fundação MT, pp 400–414
Morais TP, Melo B (2011) Biotecnologia aplicada ao melhoramento genético do cafeeiro. Ciência Rural 41:753–760
Noir S, Anthony F, Bertrand B, Combes MC, Lashermes P (2003) Identification of a major gene (*Mex*-1) from *Coffea canephora* conferring resistance to *Meloidogyne exigua* in *Coffea arabica*. Plant Pathol 52:97–103
Oliveira RP (2006) Biotecnologia em citros. Embrapa Clima Temperado, Pelotas, p 36
Pípolo VC, Ferraz LCCB (1999) Resistência a nematoides. In: Destro D, Montalván R (eds) Melhoramento Genético de Plantas. Editora UEL, pp 517–542
Ribeiro NR, Dias WP, Homechin M, Silva JFV, Francisco A (2007) Avaliação da reação de genótipos de soja ao nematoide das lesões radiculares. In: XXIX Reunião de Pesquisa de Soja da Região Central do Brasil, Resumos. Campo Grande: Uniderp e Embrapa Soja, pp 62–63
Roberts PA (2002) Concepts and consequences of resistance. In: Starr JL, Cook R, Bridge J (eds) Plant resistance to parasitic nematodes. CAB International, Wallingford, pp 23–41
Roberts PA, Mathews WC, Veremis JC (1998) Genetic mechanisms of host plant resistance to nematodes. In: Barker KR, Pederson GA, Windham GL (eds) Plant and nematode interactions. American Society of Agronomy, pp 209–238
Roberts PA, Matthews WC, Ehlers JD, Helms D (2008) Genetic determinations of differential resistance to root-knot nematode reproduction and galling in lima bean. Crop Sci 48:553–561
Roberts PA (1990) Resistance to nematodes: definition, concepts and consequences. In: Starr JL (ed) Methods for evaluating plant species for resistance to plant-parasitic nematodes. Society of Nematology, Maryland, pp 01–15
Robinson AF (2002) Reniform nematode: *Rotylenchulus* species. In: Starr JL, Cook R, Bridge J (eds) Plant resistance to parasitic nematodes. CAB International, Wallingford, pp 153–174
Rowe PR (1991) Avances genéticos em banana e plátano. Augura Bogotá 17:19–83
Sakiyama NS, Pereira AA, Moura WM, Zambolim L (2005) Melhoramento do café arábica. In: Borém A (ed) Melhoramento de Espécies Cultivadas. Editora UFV, pp 203–223
Santos JRP (2011) Caracterização genética e molecular de acessos de bananeira a *Radopholus similis* e *Meloidogyne incognita*. Brasília: UNB. 2011. 244 p (Tese de doutorado)
Sasser JN, Carter CC (1985) An Advanced treatise on *Meloidogyne*. Vol. 1: biology and control. raleigh: North Carolina State University Graphics, 422 p
Sasser JN, Freckman DW (1987) A world perspective of nematology: the role of the Society. In: Veech JA, Dickson DW (eds). Vistas on nematology. Society of Nematologists, Hyatsville, Maryland, pp 7–14
Sediyama T, Teixeira RC, Reis MS (2005) Melhoramento da Soja. In: Borém A (ed) Melhoramento de Espécies Cultivadas. 2. ed. Editora UFV, pp 553–603
Sikora RA, Bridge J, Starr JL (2005) Management practices: an overview of integrated nematode management technologies. In: Luc M, Sikora RA, Bridge J (eds) Plant parasitic nematodes in subtropical and tropical agriculture, 2nd edn. CAB International, Wallingford, pp 793–826
Silva JFV (2001) Resistência genética de soja a nematoide do gênero *Meloidogyne*. In: Silva JFV (ed) Relações Parasito-Hospedeiro nas Meloidoginoses da Soja. Embrapa Soja e Sociedade Brasileira de Nematologia, pp 95–127
Silva JFV, Ferraz LCCB, Abdelnoor RV, Arias CAA (2001a) Identificação de marcadores moleculares de microssatélites associados à resistência de genótipos de soja a *Meloidogyne javanica*. Nematologia Brasileira 25:79–83

Silva JFV, Ferraz LCCB, Arias CA (2001b) Herança da resistência a *Meloidogyne javanica* em soja. Nematropica 31:211–219

Silva RV, Oliveira RDL, Pereira AA, Sêni DJ (2007) Respostas de genótipos de *Coffea* spp. a diferentes populações de *Meloidogyne exigua*. Fitopatologia Brasileira 32:205–212

Starr JL (1990) Methods for evaluating plant species for resistance to plant-parasitic nematodes. The Society of Nematologists, Maryland, p 87

Starr JL, Bridge J, Cook R (2002a) Resistance to plant-parasitic nematodes: history, current use and future potential. In: Starr JL, Cook R, Bridge J (eds) Plant resistance to parasitic nematodes. CAB International, Wallingford, pp 01–22

Starr JL, Cook R, Bridge J (2002b) Plant resistance to parasitic nematodes. CAB International, Wallingford, p 258

Starr JL, Mercer CF (2010) Development of resistance varieties. In: Perry RN, Moens M, Starr JL (eds) Root-knot nematodes. CAB International, Wallingford, pp 326–337

Tamulonis JP, Luzzi BM, Hussey RS, Parrott WA, Boerma HR (1997a) DNA markers associated with resistance to javanese root-knot nematode in soybean. Crop Sci 37:783–788

Tamulonis JP, Luzzi BM, Hussey RS, Parrott WA, Boerma HR (1997b) RFLP mapping of resistance to southern root-knot nematode in soybean. Crop Sci 37:1903–1909

Torres MA (2010) ROS in biotic interactions. Physiologia Plantarum 138:414–429

Trudgill DL (1991) Resistance and tolerance of plant parasitic nematodes in plants. Annu Rev Phytopathol 29:167–192

Vanderplank JE (1963) Plant disease: epidemic and control. Academic Press, New York, p 349

Veech JA, Dickson DW (1987) Vistas on nematology: a commemoration of the twenty-fifth anniversary of the society of nematologists. E.O. Painter Printing Co, Florida, p 509

Verdejo-Lucas S, Kaplan DT (2002) The citrus nematode: *Tylenchulus semipenetrans*. In: Starr JL, Cook R, Bridge J (eds) Plant resistance to parasitic nematodes. CAB International, Wallingford, pp 207–219

Williams C, Gilman DF, Fontenot DS, Birchfield W (1981) Inheritance of reaction to the reniform nematode in soybean. Crop Sci 21:93–94

Williamson VM, Roberts PA (2010) Mechanisms and genetics of resistance. In: Perry RN, Moens M, Starr JL (eds) Root-knot nematodes. CAB International, Wallingford, pp 301–325

Wingard SA (1953) The nature of resistance to disease, the Yearbookof agriculture. US Department of Agriculture, Washington, pp 165–173

Yaghoobi J, Yates JL, Williamson VM (2005) Fine mapping of the nematode resistance gene *Mi-3* in *Solanum peruvianum* and construction of a *S. lycopersicum* DNA contig spanning the locus. Mol Genet Genomics 274:60–69

Young ND, Mudge J (2002) Marker-assisted selection for soybean cyst nematode resistance. In: Starr JL, Cook R, Bridge J (eds) Plant resistance to parasitic nematodes. CAB International, Wallingford, pp 241–258

Yue P, Arelli PR, Sleper DA (2001) Molecular characterization of resistance to *Heterodera glycines* in soybean PI 438489B. Theor Appl Genet 102:921–928

Chapter 6
Breeding for Resistance to Insect Pests

Alexandre Augusto de Morais and José Baldin Pinheiro

Abstracts The relationship between man and insect pests exists since the beginning of agriculture. Historically, the pest control most widely used is the chemical, however, despite its importance in controlling pests in various crops, its intensive use has been environmentally harmful, causing problems such as selection of populations of insect pests resistant to the products used outbreaks of secondary pests, reduction of natural enemies, undesirable residues in food poisoning in humans, animals, and environmental contamination. The alternative would be the development of resistant cultivars, it has significant advantages in the use of insecticides, and is considered an alternative strategy, or at least complement, the use of insecticides. Furthermore, does not represent additional burden brings farmers and does not require transfer of a new technology. The methods used in breeding programs for resistance to insects are similar to those used for other traits of agronomic interest. In the literature, the methods used to incorporate resistance genes in plants are arthropods: mass selection, pedigree method, bulk, single seed descent, backcross, and recurrent selection. The procedures used in plant breeding have made significant progress with the development of techniques of molecular biology and genetic engineering. The transfer of exogenous genes for agronomic species can be considered the most significant advances in the biological sciences in recent years. Unlike what happens in the classic improvement where a large part of the genome is transferred by hybridization, genetic engineering techniques allow isolated genes are engineered and introduced in cultivars. Biotechnology is not intended to replace the conventional breeding, but

A. A. de Morais · J. B. Pinheiro (✉)
Agronomic Engineer, "Luiz de Queiroz" College of Agriculture/University of São Paulo, São Paulo, Brazil
e-mail: jbaldin@usp.br

A. A. de Morais
e-mail: moraisaa@gmail.com

R. Fritsche-Neto and A. Borém (eds.), *Plant Breeding for Biotic Stress Resistance*,
DOI: 10.1007/978-3-642-33087-2_6, © Springer-Verlag Berlin Heidelberg 2012

the progress is undeniable and the contributions they have given to basic research and advanced genetics, as well as the development of cultivars with recognized contribution to agriculture.

Keywords Insect resistance · Pest infestations · Plant defenses · Genetic resistance · Control tactics

6.1 Introduction

As man ceased to be nomadic, the soil lost its vegetal coverage traits alongside with the beginnings of agriculture. The loss of the plant balance compelled other living beings to readapt, including the phytophagous insects; the prevalence of adapted insects increased significantly, and they began to compete with man for nourishment. Over time, this effect became increasingly more evident, such that phytophagous insects represent an important factor in today's agricultural production costs. According to Robinson (1996), such organisms are responsible for 20 % of the agricultural production losses worldwide when post-harvest damage is not taken into account. In Brazil, approximately 7 % of the production lost in 1997 was due to insect attacks (Bento 1999).

From a historical perspective, the most widely used method of pest control is chemical; however, despite its importance in the control of pests affecting several crops, the intensive use of chemicals is ecologically noxious and has resulted in problems such as the selection of pest-insect populations that are resistant to the products employed, outbreaks of secondary pests, reductions in the population natural enemies, undesirable residues in foodstuffs, the poisoning of human beings and animals, and environmental contamination (Smith 1970).

Integrated pest management might represent an option for minimizing insect attacks and maintaining the pest populations below the level of economic damage. One of the methods used for this purpose is the development of pest-resistant plant cultivars (Gallo et al. 2002). This method stabilizes productivity because it exhibits significant advantages over the use of insecticides. Thus, it is considered an alternative, or at least as a complementary strategy, to pesticides. In addition, it does not impose an additional onus on farmers, nor does it require the transfer of new technology. Pest-resistant cultivars are also compatible with the other strategies used in integrated pest management.

The selection of plants resistant to insects first emerged as an art in the earliest times of agriculture. Even before the domestication of plants for agricultural purposes, the plants susceptible to arthropods died before producing seeds. When the susceptible plants did produce seeds, they were damaged and achieved low rates of germination. Consequently, the resistant plants survived due to adaptation and natural selection. Together with the start of domestication approximately 10,000 years ago, when wheat began to be cultivated in the area that now

corresponds to Iran, Iraq, Israel, Jordan, Syria, and Turkey, the process of empirical selection of the best plants by man became more intense (Smith 2005).

The earliest reports on insect-resistant plants date from the eighteenth and early nineteenth centuries. Havens made the first report on a genotype resistant to insects in 1792 when he described the *Underhill* wheat cultivar resistant to the Hessian fly (*Mayetiola destructor*). However, the actual use of genotypes to control pests was first reported by Lindley (1831), who specifically recommended the *Winter Majetin* and *Siberian Bittersweet* apple cultivars of the genus *Malus* spp. because they exhibited resistance to the wooly apple aphid (*Eriosoma lanigerum*). By approximately 1860, plant resistance played an important role in French–American relationships. Grape phylloxera (*Phylloxera vitifoliae*), which is native to North America, was accidentally introduced to French vineyards. Although this pest attacks both the roots and leaves, the greatest damage is caused by the attack to the roots, in which phylloxera forms tuberosities that break and allow for the entrance of soil microorganisms, which cause the putrefaction of the roots and the death of the plant. In 25 years, the grape phylloxera had destroyed one-third (approximately 10 million hectares) of the French vineyards and ruined the wine production of France, with a cost equivalent to more than 2 billion dollars in current terms. The collaboration between American and French entomologists led to the identification of resistant American varieties of grape (which produced inferior wine) to be used as rootstock for the susceptible European varieties (which produced superior wine). Thus, the control of that pest was achieved, and this technique is still used today (Smith 2005; Vendramim and Nishikawa 2001; Ventura and Pinheiro 1999; Panda and Khush 1995). The breeding of plants to resist pests intensified at the end of the nineteenth century, when Mendel's laws were rediscovered. In general, the study of resistance as a science in a strict sense might be divided into three periods: the period before World War II, when research evolved slowly and parallel to the studies on genetics; the period immediately following World War II, when plant resistance was relegated to the background due to the discovery of the insecticides DDT (dichlorodiphenyltrichloroethane) and BHC (β-hexachlorocyclohexane); and the period starting in 1960, when resistant insects were identified following the indiscriminate use of insecticides. The production of insect-resistant transgenic plants through the application of molecular biology might be considered a new period (Smith 2005; Vendramim and Nishikawa 2001; Ventura and Pinheiro 1999).

6.2 The Notion of Plant Resistance to Insects

By definition, plant resistance to insects is the relative addition of the hereditary qualities of plants that influence the intensity of insect damage (Painter 1951). However, Rossetto (1973) defines plant resistance to insects as one individual that is less damaged than another individual under the same conditions as a function of its genotypic constitution. Resistance is relative. Therefore, measuring resistance

requires comparison of the investigated genotype to other genotypes. Resistance is hereditary. Therefore, the progenies of a resistant genotype should maintain their behavior when tested in the same conditions under which resistance was initially detected. Resistance is specific. For example, one genotype might be resistant to a given pest-insect, but susceptible to others. In other words, a resistant genotype suffers less damage but not less attack by the pest-insect.

As mentioned above, resistance is relative because several genotypes might exhibit different degrees of resistance. When a genotype is not harmed at all by insects, this is not an instance of resistance, but of immunity; when a genotype exhibits less damage than the average exhibited by the investigated population, this is an instance of high resistance; when the damage exhibited by a genotype is slightly less than the average of the investigated population, the resistance is moderate; when a genotype exhibits damage similar to the average of the investigated population, the genotype is considered susceptible; and finally, when a genotype exhibits more damage than the average of the investigated population, that genotype is considered highly susceptibility.

6.3 Categories of Resistance

The literature commonly describes three types of plant resistance to arthropods, which Painter (1951) originally defined as *non-preference*, *antibiosis*, and *tolerance*. Kogan and Ortman (1978) suggested replacing the term *non-preference* with *antixenosis* because *non-preference* only concerns insect behavior and *antixenosis* occurs when a plant or variety is less used by insects for nutrition, oviposition, or as shelter compared to others under equal conditions. Antixenosis is characterized by the morphological or chemical factors of the investigated plant that alter the behavior of insects and lead to the selection of an alternative host. Physical barriers such as thick epidermal layers, wax deposits on the leaves, stalks, or fruits, and the number of trichomes on plants might compel insects to search for alternative hosts. Resistant plants might contain no or low levels of phytochemicals that stimulate the nutrition and oviposition of insects. Resistant plants might also contain exclusive phytochemicals that repel or inhibit the nutrition and oviposition of pests, or substances that are toxic for the pests that use the plant for nourishment.

Antibiosis occurs when plants negatively affect the biology of insects. Such effects vary from mild to lethal and might result from the morphological and chemical defenses of plants. Antibiosis might cause the lengthening of the insect developmental period, a reduction in their size and weight, mortality during the larval or nymph stages, a reduction in fecundity, fertility, the oviposition period, etc.

Tolerance might be defined as the ability of a plant to stand or recover from insect damage compared to other plants under the same conditions. Consequently, the productive potential of its genotype is less reduced. According to Smith (2005),

five primary factors are involved in such an ability: (i) an increased photosynthetic rate (ii) a high relative growth rate (iii) increased branching or tillering after the break of apical dominance (iv) high levels of carbon storage in the roots, and (v) the ability to redirect the above-mentioned carbon to sinks (growth points). From the perspective of breeding, this means selecting genotypes with great vigor and growth to tolerate pest attacks.

In contrast to antibiosis and antixenosis, tolerance involves traits that are exclusively related to the plant, whereas the former two processes derive from the combined traits of the plants and insects. Nevertheless, tolerance often occurs in combination with antibiosis and antixenosis. With regard to the total effect of plant resistance on the arthropod population, cultivars with tolerance require less antibiosis or antixenosis than the cultivars without it.

Resistance by means of tolerance exhibits some advantages: the population of arthropods does not decrease upon exposure to tolerant plants, in contrast to the plants that are resistant through antibiosis or antixenosis. Thus, the odds the emergence of a biotype that is able to interrupt this mechanism of resistance are lower because the selective pressure exerted by tolerant plants is lower than the selective pressure of plants that exhibit resistance by means of antibiosis or antixenosis.

6.4 Factors that Affect the Expression of the Insect Resistance

When testing the resistance of a given group of genotypes to a pest-insect, a series of variations must be taken into account, such as the age of the insect, the part of the plant tissue that will be used, and the type of assessment that will be performed. These variations must be accounted for because each variable might influence the resistance of plants in laboratories, greenhouses, and even in the field. Induced environmental changes exert significant effects on the expression of insect resistance. Such stress conditions might considerably affect the growth and metabolism of the plants and thus the conditions of resistance. Therefore, a better understanding of the plant, insect, and the environmental variables involved in resistance is needed.

Rosetto (1973) classified these factors in three groups corresponding to the plants, the insects, and the environment. Based on these categories, the variables might be summarized as follows: (i) plant-related factors: age, infestation location, previous infestation by other insects; (ii) insect-related factors: phase and age, species, race and biotype, pre-imaginal conditioning, population, and size; and (iii) environmental factors: climatic and edaphic (humidity, temperature, and nutrients) factors and infestation by other insects (predation and parasitism, planting season, size of parcels, adjacent plants, previous crop).

Table 6.1 Soybean resistance to *H. zea* (adapted from Beland and Hatchett 1976)

Varieties	LM (%)[a]	DP[b]	PW (mg)[c]	DE[d]
Six week old plants				
PI 229358	62.5	21.3 a	230.2 b	11.3
Ed 73-375	55.0	19.8 a	218.1 b	11.2
BRAGG	32.5	18.2 b	271.7 a	11.0
DAVIS	20.0	16.4 c	273.7 a	10.8
Nine week old plants				
PI 229358	97.5	36.0 a	107.5 b	–
Ed 73-375	100.0	–	–	–
BRAGG	25.0	21.3 b	231.6 a	10.8
DAVIS	20.0	20.1 b	245.8 a	10.5

[a] Larval mortality
[b] Days to pupation
[c] Pupal weight
[d] Days to emergence

6.5 Plant-Related Factors

1. Plant density
   The foliar density affects the expression of arthropod resistance. Webster et al. (1978) observed that the *Oulema melanopus* (L) attack on oat is influenced by the population density of the plant. Those authors found that this insect attacks denser plantations more frequently.
2. Plant height
   The plant height might also affect the expression of resistance. Smith and Robinson (1983) found a correlation between the height of rice cultivars and infestation by *Ancyloxypha numitor* (F.), with the shortest plants being damaged the most.
3. Age of the foliar tissue
   The resistance of the foliar tissue might vary throughout the plant lifecycle. In some plants, the interaction between the pest-insect and the resistance of the infested foliar structure might occur later as a function of the phenological development of the plant. Beland and Hatchett (1976) investigated the attack of *Helicoverpa zea* on soybean, assessing some parameters related to the insect (Table 6.1) at two crop developmental stages, namely, at 6 and 9 weeks. Table 6.1 shows that the effect of the soybean varieties on the caterpillars was more noticeable when the caterpillars fed on the leaves of 9-week-old plants, although satisfactory varietal differences were also found at 6 weeks. Those authors suggest that the varieties "PI 229358" and "ED 73-375", which proved to be highly resistant at 9 weeks, might occasionally behave as susceptible plants during the initial stage of their development.
4. Plant phenology
   Alterations in the phenology of plants affect the relationship between pest-insects and their hosts. The case of sorghum resistance to *Stenodiplosis*

sorghicola (Coquillet), which was documented by Diarisso et al. (1998), provides a good example. The authors observed that the panicles of resistant sorghum opened during the night and closed during the day, thus hindering insect oviposition. Conversely, the susceptible genotypes blossomed during the day and thus permitted oviposition.

5. Type of foliar tissue
   In general, the younger foliar tissues (that are less lignified) are preferred by pest-insects over the older foliar tissues. The younger foliar tissues of the soybean are attacked by the caterpillar *Helicoverpa zea* and the mite *Tetranychus urticae* more frequently than the older tissues (Rodriguez et al. 1983; McWilliams and Beland 1977).
6. Infestation of the foliar tissue by diseases
   Resistance to insects might also result from the immune response of plants to a disease attack. Endophytic fungi have been studied in this regard. Funk et al. (1983) reported on the influence of the *Acremonium lolii* fungus on the resistance of the grass *Lolium perenne* L. to the *Listronotus bonariensis* (Kuschel) beetle and several species of Lepidoptera of the genus Crambus. Clay and Cheplick (1989) found that fungi from the Balansiae tribe, which cause rust in gramineous plants, produce alkaloids that bestow resistance to the fall armyworm (*Spodoptera frugiperda*) on the infected plants.
7. Plant mortality
   The number or percentage of dead plants is a parameter that might serve to assess resistant plant varieties. This method is primarily used in plants at their initial stages of development, when the attack by pest-insects might cause their death.

6.6 Insect-Related Factors

When conducting assays on plant resistance to pest-insects, one must take into account not only the plant genotype but also the pests. Thus, attention must be paid to the following factors that influence the response of the genotype of interest to the investigated pest-insect:

(a) Insect age: the age of the insects directly influences their ingested biomass, rate of oviposition, and survival. These factors, in turn, are reflected in the resistance or susceptibility of the investigated genotype. Therefore, the age of the insects used in studies on resistance must be standardized to ensure assay accuracy.
(b) Gender: differences in the behavior of males and females of the target species might influence the expression of resistance. Female phytophagous insects often consume more foliage than the males due to their need for additional protein for oviposition.

(c) Density and length of infestation: establishing the intensity and length of infestation is of paramount importance to avoid mistakes in the identification of resistance. The initial point includes establishing the level of damage (the minimal number of insects needed to cause economic damage to the crop).
(d) Pre-imaginal conditioning: this trait is exhibited by some adult insects that prefer to feed or lay eggs on the same plant they previously used as nourishment. Such behavior varies among different species and must be assessed on an individual basis. When this phenomenon is present in the investigated insect, its effect must be isolated in the tests comparing varieties because the results might otherwise be invalidated. Therefore, to test the response of a genotype to attack (feeding) by a given pest, a genotype different from the one used to raise the insect must be selected.
(e) Insect activity period: the time (day and night) of insect activity might affect the assessment of plant resistance. Thus, before resistance assays are started, the period of highest insect activity must be established to avoid drawing incorrect conclusions.
(f) Insect biotypes: by definition, biotypes are races or populations of an insect species that are morphologically similar, but biologically and ecologically different. Thus, the identification of different pest-insect biotypes prior to assay commencement is of paramount importance. Table 6.2 describes the number of insect biotypes in some crops.

6.7 Environmental Factors

Humidity: the relative humidity of the air, the host humidity, and the soil humidity must be taken into account. The soil humidity interacts with other factors such as insect species, plant age, etc., and might primarily influence sucking insects. Thus, a plant genotype might behave as resistant or susceptible as a function of the humidity conditions in which the test is performed. This parameter is important, for instance, in pests of stored grain (Chap. 7). A lack of water in the soil might induce the hydrolysis of leaf proteins and thus cause increased concentrations of soluble nitrogen in the sap or alter the sap pressure in the phloem.

Temperature: the influence of temperature on the expression of resistance has been widely studied by several authors, with most studies investigating aphids. Special attention must be paid to the significant effects of temperature on insects. For instance, the reproductive ability of the aphid *Macrosiphum avenae* (F.) is remarkably affected by temperatures below 10 °C or above 25 °C (Fig. 6.1). Another example was described by Ohm et al. (1997), who studied the effect of temperature on the expression of the *H19* and *H27* genes, which bestow wheat with resistance to *M. destructor*. According to those authors, the *H19* gene exhibits full expression at 19 °C, impaired expression starting at 23 °C and no expression at 26 °C. However, the expression of the *H27* gene is not affected by temperature variations.

Table 6.2 Number of insect biotypes present in crops (Vendramin and Nishikawa 2001)

Insect	Host plant	NO of biotypes
Acyrthosiphon pissum	Alfalfa	4
Amphorophora rubi	Raspberry	4
Aphis craccivora	Peanut	2
Brevicoryne brassicae	Cole	7
Callosobruchus chinensis	Pea	2
Eriosoma lanigerum	Apple	3
Mayetiola destructor	Wheat	9
Nilaparvata lugens	Rice	5
Ostrinia nubilalis	Maize	3
Phylloxera vitifoliae	Vine	2
Rhopalosiphum maidis	Maize	4
Schizaphis graminum	Wheat	7
Therioaphis maculata	Alfalfa	6

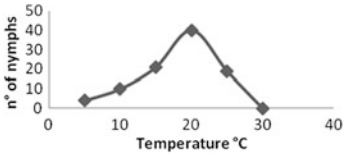

Fig. 6.1 Effect of temperature on the reproductive ability of *M. avenae Source* Adapted from Fagundes and Arnt (1975)

Nutrients: the expression of resistance might be affected by both macro- and micronutrients in either the insects (as a function of the nutritional demands of each species) or the plants. Table 6.3 describes the effects of fertilization on some crops and pest-insects (Lara 1978). Nitrogen (N)-poor soils tend to reduce the concentration of amino acids and starches, whereas N-rich soils reduce proteolysis, thereby decreasing the level of N in the sap. Phosphorus (P) deficiency tends to increase the levels of soluble N by inhibiting the metabolism of proteins. Potassium (K) deficiency is also correlated with the accumulation of soluble N and carbohydrates due to an increase in the proteolysis rate and the inhibition of protein synthesis. The large differences in the nutrient concentrations of soils also lead to different concentrations of nutrients in different parts of the plants, thus leading to the differential expression of resistance. Together with nutritional variations, such nutrient variations might influence insect biology and, consequently, plant resistance. Therefore, it is worth emphasizing that edaphoclimatic factors might act on both plants and insects, making the most predominant factor difficult to identify.

Infestation by other insects: plants are subjected to infestation by countless pests under normal field conditions. Infestation often alters the physiology of plants and, consequently, their behavior in regard to a given insect. However, such physiological alterations are not necessarily required for variations in the infestation by other species to occur. Interspecific relationships might also influence the observations when a single niche is shared by the investigated species and another species. In some cases, the interspecific relationships favor one of the species without reaching the level of commensalism because both species are independent of each other. Such factors might eventually influence resistance.

Table 6.3 The influence of fertilization on the insect population and plant resistance

Crop	Insect	Nutritional situation	Plant resistance
Alfalfa	*Therioaphis maculata*	N deficiency	No influence
		P deficiency	Increased resistance
		K deficiency	Reduced resistance
Rice	*Nilaparva lugens*	N fertilization	Increase of population
Oat	*Rhopalosiphum fitchii*	N fertilization	Increase of population
Fava bean	*Aphis rumicis*	Full fertilization	Increase of population
Apple tree	*Tetranichus telarius* *Panonychus ulmi*	> Mg and K levels > N level	Increase of population
Nasturtium	*Mysus persicae*	N, P, K, Ca, or Mg deficiency	No influence
Crop	Insect	Nutritional situation	Plant resistance
Sorghum	*Blissus leucopterus*	Full fertilization	Reduced resistance
	B. leucopterus	P fertilization	Increase in population
	Chilo zonellus	P fertilization	Increase in population
Wheat	*Schizaphis graminum*	> N level	Increase in population
	Sitophilus oryzae	N fertilization	Reduced resistance

Adapted from Lara (1978)

Predation and parasitism: as the population density of a pest increases, the efficiency of its natural enemies tends to increase. As a rule, the natural enemies initially respond to the stimuli originating in the plants, and then to those of their host. Such features might influence the pest infestation and the damage it causes, thus confounding the assessment of resistance.

Time of planting/sowing: all of the factors discussed above might vary as a function of the time when plants are cultivated. In addition, the length of the day varies with the seasons of the year and affects plant physiology. In this regard, the time of planting/sowing might also influence resistance. Of the factors already described, the population density of the pest is one of the most important because it exhibits the widest variations as a function of the planting time.

Density of planting/sowing: as mentioned above, the density of planting/sowing affects pest infestation and the resistance of plants. This effect might be attributed to the greater visual attraction induced by the contrast between clean and planted soil. When plantings become denser, the number of plants (available food) increases, and the insect population might also grow.

Plot size: based on the same principle as that underlying the effects associated with the density of planting/sowing, the plot size might affect the level of infestation by pest-insects.

Adjacent plants: many pests are able to reside on other plant species when their preferential host is absent. This process might apply to weeds, other cultivated species, or cultivars. Therefore, such plants function as alternative hosts for pests beyond the standard cultivation season.

Previous crop: crop rotation is a widely employed strategy to control the damage caused by diseases and pests by interrupting their reproductive cycles. A previously cultivated crop might also indirectly influence the expression of resistance by inducing environmental modifications.

6.8 Breeding Strategies

The inheritance of pest resistance has been studied since the beginning of the twentieth century, when Harlan (1916) showed that resistance to the mite *Eriophyes gossypii* Banks in cotton crops was an inheritable trait. From that moment onwards, the genetics of pest resistance have been documented and reviewed (Smith, 2005). The development of resistant varieties might also include considerations of the genetic variability of the pest populations and the cultivated species (Vendramim and Nishikawa 2001; Ventura and Pinheiro 1999). This feature is particularly important when the target insect includes biotypes that are able to attack resistant varieties exhibiting specific genes, and thus restrict the effectiveness of the resistant plant to a definite period.

Strategies based on the genes exhibiting the largest and smallest effects must be applied to the development of arthropod-resistant cultivars. Horizontal, or polygenic, resistance (also known as field resistance) is quantitative and is controlled by the expression of several genes, each contributing a small additive effect. The major advantage of horizontal resistance is that it allows the control of a wide range of insect biotypes. However, this type of resistance is more difficult to introduce in other cultivars. The individual parental alleles might not be identified, and the frequency of desirable individuals in the offspring of a given crossing is difficult to predict. Vertical, or monogenic, resistance is the product of a single gene with a large effect, and results in high levels of resistance against some pest-insect populations. This resistance strategy exhibits a shorter duration than horizontal resistance because the continuous use of genotypes exhibiting this type of resistance might contribute to the selection of resistant biotypes. Unlike horizontal resistance, vertical resistance is controlled by a few genes with large effects. These genes might be identified and transferred from one genotype to another, and segregation is predicted based on the parental generation.

6.9 Germplasm and Genetic Variability

A breeding program aiming at developing insect resistance includes several stages. According to Jenkis (1981) and Wiseman (1987), a series of data on the plant, insect, and their mutual interaction are required. Thus, the key pests affecting the crop of interest must be identified.

Key pests include any organism that might compete with man for the food he produces. Thus, insects are classified as pests when their population density causes economic losses to man. From a conceptual point-of-view, the term key pest is applied to a given insect species that attacks a crop and causes more damage to it than any other pest. In this regard, a single crop might be associated with more than one key pest.

Generally, researchers who seek insect-resistant materials return to the center of species diversity because resistant sources occur more frequently in the areas where the pest is endemic or coevolved with the host plant (Panda and Khush 1995). Commercial materials might also prove to be good sources of resistance. However, these traits are not assessed in specific assays. Consequently, breeding programs should increase the use of this type of assessment to shorten the time needed to develop new resistant cultivars because such materials would already be selected for other desirable agronomic traits. The use of exotic germplasm that have not been selectively bred is complex; however, the introgression of genetic variability from such exotic materials into elite materials has been more widely used for insect resistance (Rasmusson and Phillips 1997). When the genetic control of the resistance trait is quantitative, the sources of exotic resistance must undergo a *pre-breeding* process to form a base-population specifically synthesized with a wide genetic basis. In general, breeding for insect resistance does not differ from breeding for other agronomic traits and might be implemented in breeding programs as an additional assessment.

This approach was also advocated by Ventura and Pinheiro (1999), who stated that once the key pest against which resistance is desired has been chosen, the sources containing the resistance genes must be identified; that is, the sources that are less attacked and/or damaged. Such sources might be commercial, exotic or non-adapted cultivars, wild species, and even transgenic varieties. In general, elite germplasm (a commercial cultivar or line) tends to be preferred for the development of a new cultivar or to incorporate resistance because it is easy to obtain and has the further advantage of already having been bred for other traits. When this option is not feasible, the candidate germplasm should exhibit agronomic traits relevant to producers, such as the desired lifecycle, plant height, resistance to disease, and good productivity, whenever possible. Thus, the initial goal of a breeding program must be based on the definition of the key pests. It is important to work with pests that are considered key, this is, those pests that cause the most direct harm to the crop, resulting in production losses and indirect damage such as an increase in production costs. However, whenever the incorporation of resistance is easily achieved, it must also be performed. Ventura and Pinheiro (1999) claim that any breeding program aimed at developing and using resistant cultivars must include three stages: (i) the identification of the sources of resistance; (ii) the incorporation of the alleles in commercial cultivars by means of breeding methods; and (iii) the development of new cultivars.

The control of insects can be approached on three levels: (i) the environment, which is approached through cultivation practices; (ii) the insect, the use of insecticides, and biological control; and (iii) the host, which is approached through genetic control. Thus, the focus of this chapter is genetic resistance as an additional strategy for the control of insects.

Table 6.4 Methods of pest-insect resistance gene incorporation

Method	Autogamous	Allogamous
Massal selection	X	X
Genealogical method	X	
Population method	X	
Single seed descent	X	
Backcrossing	X	
Recurrent selection	X	X

6.10 Main Breeding Methods

The breeding methods used in insect resistance programs are similar to those used for other traits of agronomic interest. Basically, the gene choice must take into account the inheritance of the involved trait (oligogenic or polygenic) as well as the reproductive system of the targeted species. Some important peculiarities of the genotype selection process are discussed next.

As mentioned above, oligogenic, and more specifically monogenic, resistance is characterized by a discontinuous phenotypic distribution. Therefore, the resistant individuals are easily distinguished from the susceptible ones. In general, a gene of interest is identified from a source within the available genetic set; that is, its source might be an elite line or even exotic or wild material. However, the approach to polygenic inheritance and thus a continuous contribution is different.

The differences in the genetic structures of autogamous and allogamous populations also influence the choice of the breeding method. Populations of autogamic species essentially comprise homozygous lines. Thus, the individual plants of such species are homozygous, and the final goal of the breeding program is to obtain pure lines. With regard to allogamous species, the fundamental condition of commercial cultivars is heterozygosity, and thus the final goal of the breeding program is to obtain and select lines to produce hybrids or open pollination varieties (Ventura and Pinheiro 1999).

Several breeding methods are used for developing insect resistance (Table 6.4). According to the literature, the most widely employed methods to incorporate insect resistance genes into plants are massal selection, the genealogical method, single seed descent, backcrossing, and recurrent selection. The use of such genes is particularly dependent on the reproductive system of the species to be bred. More details on such methods might be found in Borém and Miranda (2009).

Massal selection involves the individual selection of resistant plants in each generation. The seeds of resistant plants are sown in bulk, thus forming a resistant population. The aim of this method is to select several sources of resistance in each of several selection cycles. The breeding of resistance is usually performed at the

Table 6.5 Pest-insect-resistant plants obtained by means of recurrent selection

Crop	Pest-insect(s)	Reference(s)
Brassica campestrus	*Hyadaphis erysimi*	Barnes and Cuthbert (1975)
Gossypium hirsutum	*Anthonomous grandis*	Bird (1982)
	Heliothis virescens	
	Pseudatomoscelis seriatus	
	Lygus lineolaris	
Ipomoea batatas	*Cylas formicarius elegantulus*	Jones and Cuthbert (1973)
		Jones et al. (1976)
Medicago sativa	*Hypera postica*	Hanson et al. (1972)
	Therioaphis maculata	Graham et al. (1965)
Solanum tuberosum	*Empoasca fabae*	Sanford & Ladd (1987)
Zea mays	*Helicoverpa zea*	Widstrom et al. (1982)
		Butron et al. (2002)
	Ostrinia nubilalis	Klenke et al. (1986)
	Sitophilus zeamais	Widstrom (1989)
	Spodoptera frugiperda	Widstrom et al. (1992)

initial selection, which is then followed by two to five additional cycles of selection. Massal selection was effectively used to breed resistance to the potato leafhopper *Empoasca fabae* (Harris) (Sanford and Ladd 1983). Nevertheless, due to large-scale, non-controlled environmental effects, this method is seldom used in breeding programs and is restricted to programs that are focused on purifying poorly bred cultivars and species (Panda and Khush 1995).

The population method is used to incorporate resistance genes into autogamous species. The main difference between this method and the genealogical method is the mixture of seeds from the synthesized populations without any concern for their origin. Natural selection is the only force operating until the F_5 generation. As a disadvantage, the population method might only be used when the segregating population is present in locations where the pest infestation exhibits high levels.

Recurrent selection is used to increase the frequency of arthropod resistance alleles that are scattered among several sources of resistance. After each cycle, the resistant plants are selected from the offspring of an earlier crossing of resistant individuals, resulting in a gradual increase in the average level of resistance. Recurrent selection allows for the production of resistant cultivars with a minimum degree of endogamy and the introduction of additional sources of resistance to be used in the following selection cycles. Table 6.5 describes some examples of the use of recurrent selection to increase the resistance of different crops to arthropod pests.

The genealogical method considers the F_2 generation of the synthesized population, with the insect-resistant individuals being selected. By natural self-fertilization, these individuals give rise to F_3 families that might be assessed for their resistance to the pest of interest. Thus, selection is performed among and within families, ensuring that the best individuals from the best families are selected. The F_4 generation is obtained from these individuals, with selection continuing up to the most advanced endogamic generations (F_6 to F_8), when a high

level of homozygosity is achieved. At this stage, the genotype is fixed and pure lines are obtained. Taking allogamous species into account, the selection of individual plants from each synthesized population is performed on the grounds of their resistance to insects. A hybrid is first obtained, and the full F_1 offspring is selected and replanted. The best F_2 plants are selected and their seeds are sown as F_3 families. In the F_3 generation, approximately 25–50 seeds from the resistant families are selected. In the F_4 generation, one sample from each F_3 family (seeds of 50–100 plants) is sown and selection is performed within the families. In the F_5 generation, the samples from the selected F_4 families (seeds of 100–500 plants) are sown and assessed for resistance and yield. Such an assessment is performed to eliminate the low-yield and poorly resistant families. In the following generations, the families with better resistance, yield, and other agronomic traits are selected. The advantage of this method is that a large amount of susceptible plant material is eliminated at the beginning of the program, thus allowing for the thorough assessment of the resistant plants over several years. The main disadvantage of this method is that only a limited number of lines can be used due to the time required for planting, harvesting, and acquiring data. This method was used to increase the resistance levels of rice *Oryza sativa* (L.), *Nephotettix virescens* (Distant), *Nilaparvata lugens* (Stal), and *Orseolia oryzae* (Wood-Mason) (Khush 1980).

The backcrossing method involves crossing individuals with one "recurrent" progenitor obtained from a hybrid and selecting the offspring based on resistance. Thus, the recurrent progenitor is an elite cultivar susceptible to the pest-insect of interest, and the non-recurrent or donor progenitor is a source of resistance. Backcrossing can be used as a quick method of incorporating vertical resistance to arthropods into agronomically superior cultivars. Highly productive rice and soybean cultivars exhibiting arthropod resistance were obtained using this method (Khush 1978; Smith and Brim 1979).

6.11 Inheritance and Relationships Between Traits

The genetics and inheritance of resistance to pest-insects in species used for the production of foodstuffs, fibers and forage have been widely discussed by several reviews (Smith 2005; Panda and Khush 1995; Gatehouse et al. 1994). This section includes a short summary focused on the different reproductive systems.

6.11.1 Cotton

Tobacco budworm (Heliothis virescens): diallel analysis indicates that the additive effect explains 90 % of the total genetic variance of cotton with regard to tobacco budworm resistance and the number of gossypol glands (Wilson and Lee 1971; Wilson and Smith 1977).

Pink bollworm (Pectinophora gossypiella): Wilson and George (1979) assessed the combined resistance of a group of cotton cultivars and lines against seed damage. When one of these lines was crossed with cultivars exhibiting a lack of floral nectaries, resistance was inherited due to dominant or epistatic effects (Wilson and George 1983). The genetic action that contributes to resistance in the offspring is additive, and few genes determine resistance.

6.11.2 Soybean

The resistance of soybean to defoliating insects involves several genes. Estimates of the inheritability of resistance to *Trichoplusia ni* (Hubner) (Luedders and Dickerson 1977) and *E. varivestis* (Sisson et al. 1976) point to quantitative inheritance. F_2 plants arising from the crossing of one *Pseudoplusia includens* (Walker) resistant parent and one *Pseudoplusia includens* (Walker) susceptible parent also indicated the presence of partial dominance or quantitative inheritance (Kilen et al. 1977). In the case of soybean resistance to a complex of stink bugs, a genetic analysis of the components of means suggests that additive effects predominate in the following stink bug resistance traits: the percentage index of pod damage (PIPD) and percentage of spotted seeds (PSS). The variance components analysis revealed that the dominance effects are greater than the additive effects with regard to the grain filling period (GFP) and leaf retention (LR) traits. Thus, the selection of stink bug resistant genotypes might be performed in earlier generations in regard to PIPD and PSS and in generations with higher levels of endogamy in the case of GFP and LR (Godoi 2009).

6.11.3 Bean

The resistance of the lima bean (*Phaseolus lunatus* L.) to the leafhopper *Empoasca kraemeri* Ross and Moore is due to the quantitative effects of several genes and is a recessive trait (Lyman and Cardona 1982). Additive and dominant genetic effects explain the resistance of the common bean (*Phaseolus vulgaris* L.) to *E. kraemeri* (Kornegay and Temple 1986). Some evidence also points to transgressive segregation in some offspring as a result of the crossing of resistant and susceptible bean plants.

6.11.4 Potato

The offspring resulting from the crossing of cultivated potatoes and several *Solanum* species exhibiting different glandular trichome densities exhibit

inheritable resistance to the aphid *Myzus persicae* that ranges from 50 to 60 %. Resistance is expressed as a partially dominant trait (Sams et al. 1976). However, Gibson (1979) established that only one dominant gene controls the resistance of *S. tarijense* and *S. berthaultii* to the aphid *S. persicae*, whereas the offspring of a *S. phureja* and *S. berthaultii* cross-exhibit the action of two genes in the expression of resistance. Mehlenbacher et al. (1984) studied the heritability of lobed type A and type B trichome densities in *S. berthaultii* leaves, finding that resistance to *M. persicae* is associated with the density of trichomes and the size of the droplets they exude; for this reason resistance is rated a quantitative trait. Resistance to the potato tuber moth *Phtorimaea operculella* (Zeller) derives from *S. sparsipilume* and is controlled by a small number of larger genes (Ortiz et al. 1990).

6.11.5 Maize

Recurrent selection was used to increase the levels of maize resistance to *Helicoverpa zea* and *Spodoptera frugiperda* (Butron et al. 2002). General combining ability (GCA) is increasingly used because it is more descriptive of the *H. zea* and *S. zeamais* resistance studies than the dominance and epistatic effects obtained from specific combining ability (SCA) (Widstrom et al. 1972; Widstrom and McMillian 1973). GCA and SCA are used to explain the variations in maize resistance to *S. frugiperda* (Williams et al. 1998). The resistance of maize to *H. zea* -induced defoliation is also quantitatively inherited (Widstrom and Hamm 1969). Wiseman and Bondari (1995) used several populations of maize segregating for resistance to *H. zea,* expressed as the effects of antibiosis of maize stigmata on caterpillars, to establish the resistance inheritance pattern. These authors found that in some populations, the additive-dominant genetic variance model could not explain the resistance when the hypothesis that more than one pair of genes controls maize resistance to *H. zea* and that some of the genes interact in a non-allelic manner was taken into account. Maize spike and thatch resistance to the corn stalk borer *Sesamia nonagrioides* (Lefebvre) is also quantitatively inherited, and the interaction of dominant, additive, and epistatic effects controls the action of the genes (Cartea et al. 1999, Cartea et al. 2001). Additive and dominant effects explain a large part of the variation in maize resistance to the aphid *Rhopalosiphum maidis* (Fitch) (Bing and Guthrie 1991) and the spotted stalk borer *Chilo partellus* (Swinhoe) (Pathak 1991).

In general, phenological, genetic and environmental factors, and limited manpower might influence the inheritance of resistance to pest-insects. The genes and the number of loci associated with a given resistance factor might also affect the advancement of inheritance research. Well-adjusted genetic and experimental designs that allow the accurate study of inheritance mechanisms are needed to bypass this problem because the samples are otherwise destroyed, preventing the tests from being repeated.

6.12 Biotechnology: QTL, Marker Assisted Selection, and Transgenics

Plant breeding procedures have undergone significant advancement due to the development of molecular biology and genetic engineering techniques. In this section, these techniques are summarily discussed in their application to insect resistance.

Molecular biology began when James Watson and Francis Crick elucidated the structure of DNA in 1953. At the beginning of the 1970s, techniques employing recombinant DNA were developed after restriction enzymes were discovered. These novel advances allowed scientist to cut the DNA molecule at specific sites, reconnect such fragments, and sequence their bases. Such innovations together with the optimization of plant tissue culture and transformation techniques allowed for the emergence of genetic engineering.

Although biotechnology does not aim to and is not capable of replacing conventional breeding, its advancement and contributions to basic and advanced genetic studies and the development of important cultivars are undeniable.

The transfer of exogenous genes to agronomic species might be considered one of the most significant advances in the biological sciences in recent years (Panda and Khush 1995). Unlike classic breeding, in which a large part of the genome is transferred by means of hybridization, genetic engineering techniques allow for isolated genes to be *engineered* and introduced to cultivars. After the initial report describing the development of transgenic tobacco plants, several other transgenic versions of dozens of plant species were produced. The best-known and most widely studied pest-insect resistance genes are those that express protease inhibitors, alpha-amylase inhibitors, and lectins from the bacterium *Bacillus thuringienses* (*Bt*). Protease inhibitors, which represent an additional insect resistance factor manipulated by genetic engineering, are polypeptides that participate in natural plant defenses against the action of insects. Protease inhibitors occur naturally in the reserve tissues of a large number of species, such as tobacco, potato, tomato, and soybean, and the genes responsible for this trait might bestow resistance to insects. More recent studies have shown that soybean trypsin inhibitors abolish the activity of *Spodoptera frugiperda* intestinal proteases. Alpha-amylase inhibitors also act on the digestive system of some insects, where they inhibit the digestion of carbohydrates. The most widely studied alpha-amylase inhibitors are those of the common bean and wheat. Lectins are a diverse group of proteins present in the seeds of several plant species. Lectins bind to sugars and provide important protection against insect attacks. The greatest insecticide activity of this type of protein is exhibited by the lectins of common bean and wheat germ, which bind to chitin.

Wild and/or non-domestic species contain many genes important for insect resistance. In this regard, molecular markers might be used to identify genes of interest. The most widely used molecular markers are RFLPs (*restriction fragment length polymorphisms*), RAPD (*random amplified polymorphic DNA*), AFLPs

(*amplified fragment length polymorphisms*), SSRs (*single sequence repeats*), and SNPs (*single-nucleotide polymorphisms*). These markers may be used to simultaneously select for genes of interest and against undesirable genomic segments, thus reducing the linkage drag and the time needed to obtain new cultivars. Several applications were immediately suggested for this new technique and classified by Borém and Caixeta (2009) as:

(i) Short-term applications
This type of application basically comprises the identification and discrimination of genotypes and might be performed under several circumstances because each individual exhibits a sequence of nucleotides that comprises its DNA. Therefore, differences among such sequences might be used to identify individuals. This process makes the identification of paternity, the identification and protection of cultivars, the certification of genetic purity, and the monitoring of the crossings performed in diversity and genetic distance studies possible, therefore potentially supplying useful data for the choice of progenitors in breeding programs. This process also makes the characterization of resistance sources possible.

(ii) Middle- and long-term applications
These types of applications include the use of markers to quantify the genetic variability at the DNA level and correlate it with a phenotype. These processes are characterized by genetic mapping and might also be applied to the traits associated with insect resistance. These processes might also be used to perform the indirect selection of complex traits. An assessment of the genotype reaction is not always simple because the insects must be present at a level that allows the satisfactory discrimination of the genotypes. Therefore, indirect selection by means of markers might contribute to the development of faster breeding programs.

One additional application is known as marker assisted selection (MAS), in which the selection process is based on the marker associated with the locus of the investigated trait.

Currently, one of the more widely used applications of molecular markers is the introgression of genes by means of backcrossing. This method is commonly used to incorporate insect and disease resistance into superior genotypes. These markers might be useful in such programs by only allowing for the backcrossing of those individuals that contain the resistance gene and exhibit maximal similarity with the recurrent progenitor. It is possible to apply this procedure by comparing the band patterns of the progenitors and the offspring, which leads to a considerable savings in time and resources.

One additional molecular tool is the use of ESTs (*expressed sequence tags*), which allows for the rapid identification of expressed resistance genes. Approximately, 30 insect resistance genes were mapped in six species that were cultivated to develop resistance to insects in the orders Homoptera, Hemiptera, Diptera, Lepidoptera, and Coleoptera. One example of the use of cloning is tomato *Mi* gene, which was studied by Rossi et al. (1998) and initially identified as a gene for

resistance to the root-knot nematode *Meloidogyne incognita*. The *Mi* gene codes for a protein that belongs to a family of plant proteins that are associated with disease resistance and characterized by leucine-rich nucleotide sites. The same authors used the positional cloning technique to show that this gene was the *Meu-1* gene described by Kaloshian et al. (1995), which bestows resistance to the aphid *Macrosiphum euphorgiae*. This example is one of the earliest instances of transferring resistance genes to phylogenetically different organisms. Once a gene for insect resistance is cloned, a large number of accessions might be identified by searching for additional alleles at the same locus, some of which might bestow higher levels of resistance than the initial cloned version.

The construction of genetic maps has had a great impact on genetic analysis because it allows the localization of genomic regions that control important traits, such as insect resistance.

Some of the insect resistance genes are linked to a morphological trait that cosegregates together with pest resistance in the population, thus allowing for the selection of plants based on a particular morphological trait and insect resistance. However, such morphological traits are rare and usually harmful for the plants. Conversely, molecular markers might contribute to the timely and efficient mapping of resistance genes as a function of the availability of a large number of polymorphic genetic markers, which allow scientists to construct linkage maps easily.

Acknowledgment The authors would like to acknowledge the graduate program in genetics and plant breeding of ESALQ/USP for their support in the publication of the current chapter.

References

Barnes WC, Cuthbert FP (1975) Breeding turnips for resistance to the turnip aphid. Hort Sci 10:59–60

Beland GL, Hatchett JH (1976) Expression of antibiosis to the bollworm in two soybean genotypes. J Econ Entomol 69:557–560

Bento JMS (1999) Perda por insetos na agricultura. Ação Ambiental 2:19–21

Bing JW, Guthrie WD (1991) Generation mean analysis for resistance in maize to the corn leaf aphid Homoptera: Aphididae. J Econ Entomol 84:1080–1082

Bird LS (1982) Multi-adversity diseases, insects and stresses resistance MAR in cotton. Plant Dis 66:172–176

Borém A, Caixeta ET (2009) Marcadores Moleculares. UFV, Viçosa

Borém A, Miranda GV (2009) Melhoramento de Plantas. UFV, Viçosa

Butron A, Widstrom NW, Snook ME, Wiseman BR (2002) Recurrent selection for corn earworm Lepidoptera: Noctuidae resistance in three closely related corn southern synthetics. J Econ Entomol 95:458–462

Cartea ME, Malvar RA, Butrón A, Vales MI, Ordás A (1999) Inheritance of antibiosis to Sesamianonagrioides Lepidoptera: Noctuidae in maize. J Econ Entomol 92:994–998

Cartea ME, Malvar RA, Vales MI, Butrón A, Ordás A (2001) Inheritance of resistance to ear damage caused by *Sesamianonagrioides* Lepidoptera: Noctuidae in maize. J Econ Entomol 94:277–283

Clay K, Cheplick GP (1989) Effect of ergot alkaloids from fungal endophyte-infected grasses on fall armyworm *Spodoptera frugiperda*. J Chem Ecol 15:169–182
Diarisso NY, Pendleton BB, Teetes GL, Peterson GC, Anderson RM (1998) Spikelet flowering time: Cause of sorghum resistance to sorghum midge Diptera: Cecidomyiidae. J Econ Entomol 87:514–522
Fagundes AC, Arnt TA (1975) Efeito de diferentes temperaturas sobre o pulgão da espiga *Macrosiphumavenae* (F.). Anais da sociedade entomológica do Brasil 4:28–32
Funk CR, Halisky PM, Johnson MC, Siegel MR, Stewart AV, Amhad S, Hurley RH, Harvey IC (1983) An endophytic fungus and resistance to sod webworms: association in Loliumperenne L. Bio/Technology 1:189–191
Gallo D, Nakano O, Neto SS, Carvalho RPL, Batista GC, Filho EB, Parra JRP, Lopes JRS, Omoto C (2002) Entomologia Agrícola. FEALQ, Piracicaba
Gatehouse AM, Boulter D, Hilder VA (1994) Potential of plant-derived genes in the genetic manipulation of crops for insect resistance. Plant Genet Manip Crop Prot 7:155–181
Gibson RW (1979) The geographical distribution, inheritance and pest-resisting properties of stickytipped foliar hairs on potato species. Potato Res 22:223–237
Godoi CRC, Pinheiro JB (2009) Genetic parameters and selection strategies of soybean genotypes resistant to stink bug-complex. Genet Mol Biol 32:328–336
Graham JH, Hill RR, Barnes, D K, Hanson CH (1965) Effect of three cycles of selection for resistance to common leaf spot in alfalfa. Crop Sci 5:171–173
Hanson CH, Busbice JH, Hill RR, Hunt OJ, Oakes AJ (1972) Directed mass selection for developing multiple pest resistance and conserving germplasm in alfalfa. J Environ Qual 1:106–111
Harlan SC (1916) The inheritance of immunity to leaf blister mite *Eriophyesgossypii* Banks in cotton. West Ind Bull 17:162–166
Havens JN (1792) Observations on the Hessian fly. Trans New York Soc Agron Pt 1:89–107
Jenkins JN (1981) Breeding for insect resistance. In: Frey KJ (ed) Plant breeding II. Iowa University Press, Ames
Jones A, Duke PD Cuthbert FP Jr (1976) Mass selection in sweet potato: breeding for resistance to insects and diseases and for horticultural characteristics. J Am Soc Hort Sci 101:701–704
Jones A, Cuthbert PF (1973) Associated effects of mass selection for soil-insect resistance in sweet potato. J Am Soc Hort Sci 98:480–482
Kaloshian I, Lange WH, Williamson VM (1995) An aphid-resistance locus is tightly linked to the nematode-resistance gene *Mi*, in tomato. PNAS 92:622–625
Khush GS (1980) Breeding rice for multiple disease and insect resistance. In: Rice Improvement in China and Other Asian Countries. International Rice Research Institute, Los Banos, pp 219–238
Khush GS (1978) Biology techniques and procedures employed at IRRI for developing rice germplasm with multiple resistance to diseases and insects. Jap Trop Agric Res Ctr Res Ser 11:8
Kilen TC, Hatchett JH, Hartwig EE (1977) Evaluation of early generation soybeans for resistance to soybean looper. Crop Sci 17:397–398
Klenke JR, Russell WA, Guthrie WD (1986) Distributions for European corn borer Lepidoptera: Pyralidae ratings of S1 lines from 'BS9' corn. J Econ Entomol 79:1076–1081
Kogan M, Ortman EF (1978) Antixenosis - A new term proposed to define Painter's "nonpreference" modality of resistance. Bull Entomol Soci Am 24:175–176
Kornegay JL, Temple SR (1986) Inheritance and combining ability of leafroller defense mechanisms in common bean. Crop Sci 26:1153–1158
Lara FM (1978) Princípios de resistência de plantas à Insetos. Ceres, Piracicaba, p 318
Lindley G (1831) A guide to the orchard and kitchen garden
Luedders VD, Dickerson WA (1977) Resistance of selected soybean genotypes and segregating populations to cabbage looper feeding. Crop Sci 17:395–396
Lyman JM, Cardona C (1982) Resistance in lima beans to a leafhopper, *Empoascakrameri*. J Econ Entomol 75:281–286

McWilliams JM, Beland GL (1977) Bollworm: effect of soybean leaf age and pod maturity on development in the laboratory. Ann Entomol Soc Am 70:214–216
Mehlenbacher SA, Plaisted RL, Tingey WM (1984) Heritability of trichome density and droplet size in interspecific potato hybrids and relationship to aphid resistance. Crop Sci 24:320–322
Ohm HW, Ratcliffe RH, Patterson FL, Cambron SE (1997) Resistance to Hessian fly conditioned by genes *H19* and proposed gene *H27* of durum wheat line PI422297. Crop Sci 37:113–115
Ortiz R, Iwanaga M, Raman KV, Palacios M (1990) Breeding for resistance to potato tuber moth, *Phthorimaeaoperculella* Zeller, in diploid potatoes. Euphytica 50:119–125
Painter RH (1951) Insect resistance in crop plants. McMillian, New York
Panda N, Khush GS (1995) Host plant resistance to insects. Guildford, Biddles
Pathak RS (1991) Genetics of sorhgum, maize, rice and sugarcane resistance to the cereal stem borer *Chilo* spp. Insect Sci Applic 11:689–699
Rasmusson DC, Phillip RL (1997) Review and interpretation: plant breeding progress and genetic diversity from the new variation and elevated epistasis. Crop Sci 37:303–310
Robinson RA (1996) Return to resistance: breeding crops to reduce pesticide dependence. AgAccess, Davis
Rodriguez JG, Reicosky DA, Patterson CG (1983) Soybean and mite interactions: Effects of cultivar and plant growth stage. J Kans Entomol Soc 56:320–326
Rossetto CJ (1973) Resistência de Plantas a Insetos. ESALQ, Piracicaba
Rossi M, Goggin FL, Milligan SB, Kaloshian I, Ullman DE, Williamson VM (1998) The nematode resistance gene Mi of tomato confers resistance against the potato aphid. PNAS 95:9750–9754
Sams DW, Lauer FI, Radcliffe EB (1976) Breeding behavior of resistance to green peach aphid in tuber-bearing *Solanum* germplasm. Am Potato J 53:23–29
Sanford LL, Ladd LT (1987) Genetic transmission of potato leafhopper resistance from recurrent selection populations in potato *Solanum tuberosum* L. Gp. tuberosum. Am Pot J 64:655–662
Sisson VA, Miller PA, Campbell WV, Van Duyn JW (1976) Evidence of inheritance of resistance to the Mexican bean beetle in soybeans. Crop Sci 16:835–837
Smith CM (2005) Plant resistance to arthropods–molecular and conventional approaches. Springer, New York
Smith CM, Brim CA (1979) Field and laboratory evaluations of soybean lines for resistance to corn earworm leaf feeding. J Econ Entomol 72:78–80
Smith CM, Robinson JF (1983) Effect of rice cultivar height on infestation by the least skipper, *Ancyloxypha numitor* F. Lepidoptera: Hesperiidae. Environ Entomol 12:967–969
Smith RF (1970) Pesticides: their use and limitations in pest management. In: Rabb RL, Guthrie FE (ed) Concepts of pest management. North Caroline State University, Raleigh
Vendramim JD, Nishikawa MA (2001) Melhoramento para resistência a insetos In: Nass LL, Valois AC, Melo IS, Valadares-Inglis MC (ed) Recursos genéticos e melhoramento de plantas. Fundação MT, Rondonópolis
Ventura MU, Pinheiro JB (1999) Resistência de plantas a insetos. In: Destro D, Montalvan R (eds) Melhoramento Genético de Plantas. Universidade Estadual de Londrina, Londrina
Webster JA, Smith Jr DH, Gage SH (1978) Cereal leaf beetle Coleoptera: Chrysomelidae: influence of seeding rate of oats on populations. Great Lakes Entomol, 11:117–120
Widstrom NW (1989) Breeding methodology to increase resistance in maize to corn earworm, fall armyworm and maize weevil. In: Mihm, JA (ed) Toward insect resistant corn for the third world: proceedings of the international symposium on methodologies for developing host plant resistance to corn insects. CIMMYT, Mexico
Widstrom NW, Bondari K, McMillian WW (1992) Hybrid performance among maize populations selected for resistance to insects. Crop Sci 32:85–89
Widstrom NW, Wiseman BR, McMillian WW (1972) Resistance among some maize inbreds and single crosses to fall armyworm injury. Crop Sci 12:290–292.
Widstrom NW, Wiseman BR, McMillian WW (1982) Responses to index selection in maize for resistance to ear damage by the corn earworm. Crop Sci 22:843–846

Widstrom NW, Hamm JJ (1969) Combining abilities and relative dominance among maize inbreds for resistance to earworm injury. Crop Sci 9:216–219
Widstrom NW, McMillian WW (1973) Genetic factors conditioning resistance to earworm in maize. Crop Sci 13:459–461
Williams WP, Davis FM, Buckley PF (1998) Resistance to southwestern corn borer in corn after anthesis. Crop Sci 38:1514–1517
Wilson FD, George BW (1979) Combining ability in cotton for resistance to pink bollworm. Crop Sci 19:834–836
Wilson FD, George BW (1983) A genetic and breeding study of pink bollworm resistance and agronomic properties in cotton. Crop Sci 23:1–4
Wilson FD, Lee JA (1971) Genetic relationships between tobacco budworm feeding response and gland number in cotton seedlings. Crop Sci 11:419–421
Wilson FD, Smith JN (1977) Variable expressivity and gene action of gland-determining alleles in *Gossypiumhirsutum* L. Crop Sci 17:539–543
Wiseman BF, Bondari K (1995) Inheritance of resistance in maize silks to the corn earworm. Entomol Exp Appl 77:315–321
Wiseman BR (1987) Technological Advances for determining resistance in maize to *Heliothis-zea*. In: Mihm JA (ed) International symposium on methodologies for developing host plant resistance to corn insects: proceedings of the international symposium on methodologies for developing host plant resistance to maize insects 7. CIMMYT, Mexico

Chapter 7
Breeding for Resistance to Insect Pests of Stored Grain

Alexandre Augusto de Morais and José Baldin Pinheiro

Abstract The stored grain pests cause serious damage annually. According to FAO the losses from pest attack stored grain is estimated at 10 %. These losses arise from several factors; beginning in the field where the grain already harvested may contain insect eggs, and go to bad storage conditions, which may favor the development of these pests and other insect infestations. The mechanisms of plant resistance to the pest of stored grains are similar to those used for insect pests resistance of crops and these mechanisms can be classified as pre-harvest and post-harvest. The mechanisms of pre-harvest are those capable of preventing the grain in already infested field and are often related to the architecture of the reproductive structure of the plant. The post-harvest mechanisms are those that will prevent the stored grain to be infested by pests and are related to the storage of metabolites produced by the plant and will influence the hardness and moisture content of grain. The study of reaction resistance to stored grain pests is a major challenge for plant breeding, since the organism under study is the seed and that this finding is in latency stage. In the presence of the insect pest, the grain will not signal biochemicals that will trigger defense mechanisms. So their defense mechanism is summarized only in the stored metabolites, which give greater or lesser resistance by pest attack.

Keywords Insect resistance · Stored grain insects · Direct feeding damage · Stored metabolites · Defense mechanisms

A. A. de Morais · J. B. Pinheiro (✉)
"Luiz de Queiroz" College of Agriculture, University of São Paulo, São Paulo, Brazil
e-mail: jbaldin@usp.br

A. A. de Morais
e-mail: moraisaa@gmail.com

R. Fritsche-Neto and A. Borém (eds.), *Plant Breeding for Biotic Stress Resistance*,
DOI: 10.1007/978-3-642-33087-2_7, © Springer-Verlag Berlin Heidelberg 2012

7.1 Introduction

The pests of stored grain cause serious damage every year. According to data from the food and agriculture organization (FAO), the losses associated with pest attacks on stored grain are estimated at 10 %. In Brazil, the corresponding estimate is 20 % (Gallo et al. 2002). Such losses derive from several factors beginning in the field, where the harvested grain might contain insect eggs, and extending to poor storage conditions, which might favor the development of such pests and infestation by others. Therefore, knowledge of the types and severity of the pests that attack stored grain is needed to establish breeding programs aimed at developing resistance.

According to Gallo et al. (2002), pests of stored grain are classified as internal and external primary, secondary, and associated pests. Internal primary pests are insects that perforate the grain endosperm, feed on its internal content, and complete their cycle inside the grain. Such insects pave the way for an attack from other insects and opportunistic fungi. External primary pests are insects that feed on the external portion of the grain and, after destroying this section, feed on the internal portion as well. Such insects are unable to break the grain's protective envelope. Secondary pests are unable to attack the whole grain, but feed on damaged or defective grain, grain that suffered mechanical damage during harvest, or even the subproducts of the stored grain (flours, brans, grain meals, and rations). Finally, associated pests are insects that do not attack grain, but feed exclusively on debris and fungi. However, their presence impairs the appearance and quality of the stored products. Table 7.1 summarizes the main pest insects that attack stored products in Brazil.

7.2 Mechanisms of Resistance to Pests of Stored Grain

Like the pests of major crops, the pests of stored grain are also subjected to the same resistance mechanisms involved in cycle of pests. These mechanisms are antixenosis, antibiosis, and tolerance (Fig. 7.1).

Antixenosis, also known as nonpreference, occurs when insects use a plant for nourishment, shelter, or oviposition less frequently than another plant under equal conditions. In the case of the pests of stored grain, insects might exhibit a preference for specific types of grain.

Antibiosis occurs when insects usually feed on a given plant that causes adverse effects on their biology and directly or indirectly affects their reproductive potential. According to Painter (1968), some of the causes of antibiosis are toxins, growth, and/or reproduction inhibitors, and a lack of or deficiency in a nutritional element, especially in regard to the carbohydrate-amino acid ratio.

Tolerance is the ability of plants to withstand or recover from the insect damage that normally causes serious harm to more susceptible hosts. Such resistance essentially depends on the plant itself rather than on the insect–plant relationship.

Table 7.1 Insect pests of stored grain in Brazil (Gallo et al. 2002)

Pest	Crop										
	Peanut	Rice	Cacao	Coffee	Flours	Bean	Tobacco	Maize	Soybean	Sorghum	Wheat
Acanthoscelides obtectus						x					
Anagastha kuehniella		x			x						
Araecerus fasciculatus				x		x					
Cadra cautella			x								
Callosobruchus maculatus						x					
Cathartus quadicollis							x				
Corcyra cephalonica	x	x	x	x				x		x	
Ephestia eutella			x								
Laemophloeus minutus								x		x	x
Lasioderma serricorne							x		x		
Oryzaephilus surinamensis								x			
Plodia interpunctella		x				x		x	x		x
Pyralis farinalis					x					x	
Rhyzopertha dominica		x									x
Sitophilus oryzae		x						x		x	x
Sitophilus zeamais		x						x		x	x
Sitotroga cerealella		x						x	x	x	x
Stegobium paniceum					x						
Tenebrio molifor					x						
Tenebrioides mauritanicus								x			
Tribolium castaneum								x			x
Tribolium confusum								x			
Zabrotes subfasciatus						x					

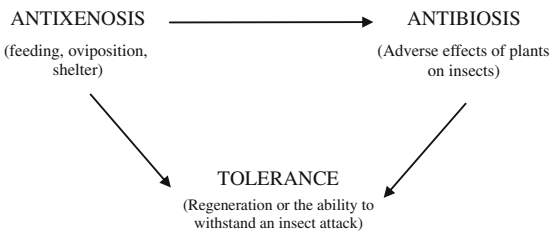

Fig. 7.1 Types of insect resistance (Painter 1951, 1968)

The environment might also influence this type of resistance because more vigorous plants or plants that are not subjected to other types of stressors might better tolerate attack. However, the effect of tolerance cannot be directly observed in the case of seeds because they are considered to be in a state of latency. In this case, tolerance might be observed at the time of sowing, when the grain serves as a seed.

7.3 Resistance Mechanisms

As mentioned above, the plant resistance mechanisms used against pests of stored grain are the same as those used in the resistance to crop pests. The resistance mechanisms are classified as pre- or post-harvest.

Pre-harvest mechanisms are mechanisms that prevent infested grain from arriving from the field. Some of these mechanisms are related to the reproductive structure of plants. For instance, Hinds (1914) provided the earliest report on breeding for the protection of the vegetative structure to prevent attacks by *Sitophilus zeamais* in the field, mentioning husks that were well adhered to maize ears, as Santos (2006) observed (Fig. 7.2). In rice, the disposition of the lemma and palea represent a factor of resistance to insect attack during storage (Link et al. 1971).

The post-harvest defense mechanisms are those that prevent the infestation of stored grain by pests. These mechanisms are related to the storage of metabolites produced by the plants and are influenced by the hardness and water content of the grain.

7.4 Chemical Defense Mechanisms

The plant defense mechanisms used against insect attacks often include factors that act on the metabolism of insects, such as protein inhibitors. Three types of enzymes are particularly important for digestion in insects: proteases, amylases, and lipases. Protease inhibitors are frequently concentrated in the seeds and tubercles of plants and are mainly found in gramineous, leguminous, and solanaceous plants (Sadasivam and Thayumanavan 2003). The plant-based protease inhibitors combine with the digestive enzymes of insects and inactivate them. This type of association is quite stable. Such protease inhibitors appear in the reserve

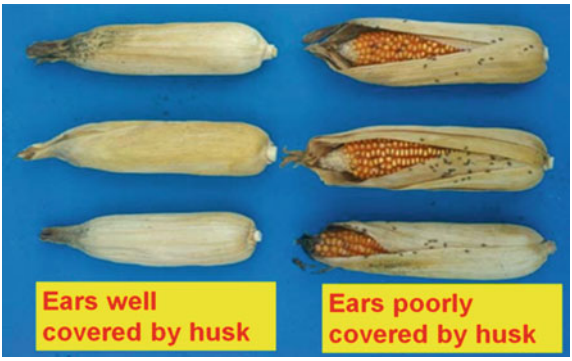

Fig. 7.2 Protection of grain by the husk (Santos 2006)

tissues of a wide variety of plants, such as tobacco, potato, tomato, and soybean (Johnson et al. 1989). One example of a protease inhibitor is the cowpea trypsin inhibitor, which protects cowpeas against insects in the orders Lepidoptera, Orthoptera, and Coleoptera by altering the development and survival of the insects. Other studied inhibitors include those found in potatoes, which are classified as type II inhibitors and hinder the activity of trypsin- and chymotrypsin-related proteases. Cysteine-type protease inhibitors were isolated from rice after the grin efficiently inhibited such enzymes in the intestine of the beetles *S. oryzae* and *Tribolium castaneum* (Panda and Khush 1995).

Alpha-amylase inhibitors also act on the metabolism of some insects by inhibiting the digestion of carbohydrates. Most of the studies that investigated such inhibitors were conducted with bean plants and wheat. Lecithins are a heterogeneous group of proteins occurring in the seeds of several plant species. These proteins bind to sugars and protect the plants from insect attacks. The lecithin with the greatest insecticide activity is found in the common bean and wheat germ and binds to chitin (Vendramim and Nishikawa 2001; Hilder and Boulter 1999). Arcelin, also a lecithin, is responsible for the resistance of *P. vulgaris* to *Zabrotes subfasciatus* (Boheman), as arcelin is a toxic protein commonly found in the seeds. The presence of arcelin is a dominant trait (Romero et al. 1986; Kornegay et al. 1993). Resistance to woodworm *Acanthoscelides obtectus* (Say) also derives from a wild accession of *Phaseolus*; however, resistance is inherited and involves the complementary effects of two separate recessive genes (Kornegay and Cardona 1991).

Non-protein amino acids (NPA) constitute another important group. More than 900 NAP have already been identified in plants. NPAs participate in plant defense by means of two mechanisms: by blocking the synthesis or absorption of protein amino acids, or through incorporation into proteins, which renders the protein nonfunctional because their tertiary structure or active site is altered. One example is afforded by cavanine, which is structurally similar to arginine and thus interferes with the incorporation of the latter into structural components and biochemical reactions; cavanine thus becomes a powerful antimetabolite of arginine. This deleterious property has a deterrent effect on the nourishment of insects (Sadasivam and Thayumanavan 2003).

7.5 Factors that Influence the Manifestation of Resistance

The factors responsible for the manifestation of resistance are similar to those that influence the manifestation of resistance to the pest insects of major crops.

7.6 Insect-Related Factors

7.6.1 Phase and Age of Insects

Accurate control of the insect development stage is needed to ensure better precision in study results. For instance, upon designing a study to assess the damage caused by *Sitophilus zeamais,* sexually mature insects are needed for oviposition to occur in seeds. The lack of this control might result in the use of sexually immature individuals or those undergoing the final phase of oviposition, which might affect infestation and consequently lead to an error in the interpretation of the results. As a whole, same-age or same-instar insects must be used in resistance tests to avoid errors in genotype discrimination. The use of a large number of individuals is recommended to minimize this problem, as is completing a large number of replicates.

7.6.2 Pre-Imaginal Conditioning

This trait points to the fact that adults prefer to feed or lay eggs on the plant they used previously for nourishment. Such behavior varies among species; therefore, each instance must be studied on an individual basis and the effects must be isolated for the results to be valid. In the case of weevil *Sitophilus zeamais*, the use of adults in studies has paramount importance; therefore, mass breeding of the insects is needed. This species does not exhibit pre-imaginal conditioning, but might be raised in another grain and then tested in maize.

7.6.3 Size of the Infesting Population

One individual can manage resistance to a given pest insect up to a specific level of infestation. After this point, the genotype might start exhibiting damage similar to the susceptible genotypes. As a whole, small populations cause little damage to varying genotypes and impair the differentiation between resistant and susceptible plants. The opposite is also true; that is, large populations might cause the same level of damage in resistant and susceptible plants as a function of the high selective pressure represented by the high level of infestation. Thus, establishing the appropriate level of infestation for resistance assays is of paramount

importance in validating the selection process or identifying sources of resistance. Rosetto (1972) tested several *Sitophilus zeamais* adult population sizes, concluding that the ideal ratio was 20 individuals for parcels containing 10 g of maize. Sexing is another important feature that avoids the exclusive use of either males or unfertilized females. This task is easily accomplished when sexual dimorphism is present (the males differ morphologically from the females). However, when sexual dimorphism is not present, the process of sexing is slow and frequently harms or even causes the death of the insects. One strategy to bypass this problem is to establish the ideal number of randomly chosen insects that will result in the birth of a reasonable number of insects. In the case of *Sitophilus zeamais*, Rossetto (1972) reported that the use of 20 adult insects affords a reasonable rate of births; that is, this number reduces the odds of harvesting only male or only female insects to infest the parcels.

7.7 Environmental Factors

The main factors deserving mention are the relative humidity of the air (RH%), the humidity of the seeds, and the environmental temperature. Pests of stored grain obtain the humidity required by their vital processes from their food. For this reason, grain humidity is a critical factor in pest survival.

The optimal grain humidity for the development of most insects varies between 12 and 15 %. With humidity levels below 10 %, almost no insect is able to infest grain. Therefore, insect infestation most likely varies in accordance with variations in the humidity of grain. To avoid such factors and ensure uniform infestation in resistance assays, the humidity of the seeds must be standardized (Puzzi 2000). Oil-rich seeds require lower levels of humidity compared to amylaceous seeds under similar environmental storage conditions because they are hydrophobic and thus absorb less water (Brooker et al. 1992).

With regard to temperature, most insects that attack stored grain have a subtropical origin and do not hibernate. Such insects develop more intensively in areas of high temperature. However, the ideal temperature for the development of the investigated insects ought to be known to achieve a satisfactory degree of precision in the results.

7.8 Breeding Strategies and Methods

In the breeding of resistance to pests of stored grains, traits that exhibit high heritability and additive genetic effects might be selected based on the individual performance of lines or populations. When a trait has low heritability or its inheritance is associated with non-additive genetic effects, the selection of genotypes must be based on the performance of hybrids. To avoid repetition, further details on breeding methods may be found in Chap. 6.

7.9 Mapping Loci for Pest Resistance

The identification of quantitative trait loci (QTL) associated with resistance is still sparsely attempted. Nevertheless, initial studies indicate that the field might advance with the development of resistant genotypes. As an example of this line of research, García-Lara et al. (2009) sought to identify QTLs for resistance to *Sitophilus zeamais* in a tropical maize population. These authors found five QTLs that explained 28 % of the phenotypic variation and 50 % of the genetic variation of the investigated population. The authors also observed that the resistance trait is dominant. The biochemical basis of *Sitophilus zeamais* resistance was reported in a later study by García-Lara et al. (2010), who found 17 QTLs linked to biochemical compounds that bestow resistance to *Sitophilus zeamais* scattered across the maize genome.

7.10 Transgenics

Specific studies on the use of transgenics in the development of pest resistance in stored grain are scarce. Some chemical compounds in the seeds have been studied, including lecithin, whose antibiotic activity might be expressed by the whole plant.

7.11 Final Considerations

The study of resistance to pests of stored grain poses a major challenge to plant breeding. The challenge lies in the fact that the subject of study is the seed, which is in a state of latency. In the presence of pest insects, grain does not signal biochemical compounds to trigger defense mechanisms. Therefore, the defense mechanisms of a seed are restricted to stored metabolites, which bestow greater or lesser pest resistance. The environmental storage conditions might also interfere with the selection process because these conditions influence the pest insects' developmental rate and, consequently, might mask resistance. When resistance assays are conducted, the temperature and humidity must be appropriate and standardized to achieve satisfactory experimental precision. The mapping of QTLs is a complementary strategy to advance the study of plant resistance to pest insects of stored grain.

Acknowledgments The authors would like to acknowledge the graduate program in genetics and plant breeding of ESALQ/USP for their support in the publication of the current chapter.

References

Brooker DB, Bakker-Arkema FW, Hall CW (1992) Grain equilibrium moisture content. In: Brooker DB, Bakker-Arkema FW, Hall CW (eds) Drying and storage of grains and oilseeds. AVI, Van Nostrand Reinhold, New York

Gallo D, Nakano O, Neto SS, Carvalho RPL, Batista GC, Filho EB, Parra JRP, Lopes JRS, Omoto C (2002) Entomologia Agrícola. FEALQ, Piracicaba

García-Lara S, Burt AJ, e Arnason JT, Bergvinson DJ (2010) QTL mapping of tropical maize grain components associated with maize weevil resistance. Crop Sci 50:815–825

García-Lara S, Khairallah MM, Vargas M, Bergvinson DJ (2009) Mapping of QTL associated with maize weevil resistance in tropical maize. Crop Sci 49:139–149

Hinds WE (1914) Reducing insect injury in stored corn. Journal of Economic Entomology 7:203–211

Hilder VA, Boulter D (1999) Genetic engineering of crop plants for insect resistance—a critical review. Crop Prot 18:177–191

Johnson R, Narvaez J, An G, Ryan C (1989) Expression of proteinase inhibitors I and II in transgenic tobacco plants: effects on natural defense against *Manduca sexta* larvae. Proc Natl Acad Sci 86:9871–9875

Kornegay JL, Cardona C (1991) Inheritance of resistance to *Acanthoscelides obtectus* in a wild common bean accession crossed to commercial bean cultivars. Euphytica 52:103–111

Kornegay J, Cardona C, Posso CE (1993) Inheritance of resistance to Mexican bean weevil in common bean, determined by bioassay and biochemical tests. Crop Sci 33:589–594

Link D, Rossetto CJ, Igue T (1971) Resistência relativa de variedades de arroz em casca, ao ataque de *Sitophilus oryzae* Linné, 1763, *Sitophilus zeamais* Motschusky, 1855 e *Sitotroga cerealella* Olivier, 1819 em condições de laboratório. Universidade Federal de Santa Maria, Santa Maria

Painter RH (1951) Insect resistance in crop plants. Macmillan, New York

Painter RH (1968) Crops that resist insects provide a way to increase world food supply. Kansas Agric Exp Stn Bull 520:22

Panda N, Khush GS (1995) Host plant resistance to insects. Biddles, Guildford

Puzzi D (2000) Abastecimento e armazenagem de grãos. Instituto Campineiro de Ensino Agrícola, Campinas

Rossetto, CJ (1972) Resistência de milho a pragas da espiga *Helicoverpa zea* Boddie, *Sitophilus zeamais* Motschulsky e *Sitotroga cerealella* Oliver. Thesis, Escola Superior de Agricultura Luiz de Queiroz

Romero AJ, Yandell BS, Bliss FA (1986) Bean arcelin 1 Inheritance of a novel seed protein of *Phaseolus vulgaris* L and its effect on seed composition. Theor Appl Genet 72:123–128

Sadasivam S, Thayumanavan B (2003) Molecular host plant resistance to pests. CRC Press, New York

Santos JP (2006) Controle de Pragas Durante o Armazenamento de Milho. EMBRAPA/CNPMS, Sete Lagoas

Vendramim JD, Nishikawa MA (2001) Melhoramento para resistência a insetos In: Nass LL, Valois AC, Melo IS, Valadares-Inglis MC (ed) Recursos genéticos e melhoramento de plantas. Fundação MT, Rondonópolis

Chapter 8
Breeding for Weed Management

Roberto Fritsche-Neto, Júlio César DoVale, Lino Roberto Ferreira, Francisco Affonso Ferreira and Antônio Alberto da Silva

Abstract The highly adaptive and multiplying capacities of weeds are defining characteristics that are useful in perpetuating these species in various environmental conditions. However, the physiological bases that explain these advantages are complex and have not been fully elucidated. With the introduction of transgenic cultivars, other concerns have been attributed to weeds, due to the possibility of gene flow between crops and invasive species. This gene flow can change the fitness of the latter, both for biotic and abiotic factors, thereby generating significant changes in the invasive species' rusticity, invasiveness, and competitiveness, making them stronger colonizers. Given these findings, many recent studies have examined cultivars' ability to compete with weeds. This chapter addresses competition theories between crops and weeds, several physiological bases for stress caused by competition for resources needed for plant growth and improvement strategies adopted to obtain cultivars with greater competitive abilities.

Keywords Plant improvement · Efficient use of resources · Competitive ability

R. Fritsche-Neto (✉) · L. R. Ferreira · F. A. Ferreira · A. A. da. Silva
Federal University of Viçosa (Universidade Federal de Viçosa – UFV), Viçosa, Brazil
e-mail: roberto.neto@ufv.br

J. C. DoVale
Foundation University of Tocantins (Fundação Universidade do Tocantins – Unitins), Tocantins, Brazil
e-mail: juliocvale@gmail.com

L. R. Ferreira
e-mail: lroberto@ufv.br

F. A. Ferreira
e-mail: faffonso@ufv.br

A. A. da Silva
e-mail: aasilva@ufv.br

R. Fritsche-Neto and A. Borém (eds.), *Plant Breeding for Biotic Stress Resistance*,
DOI: 10.1007/978-3-642-33087-2_8, © Springer-Verlag Berlin Heidelberg 2012

8.1 Introduction

A total of 7 billion people currently inhabit the world, and this number should continue to increase until approximately 2,050, a year in which, according to UN forecasts, it will level off, reaching a staggering 9.1 billion people. In emerging countries, designated BRIC (Brazil, Russia, India, China, and South Africa), living conditions are improving, thereby increasing demand for food, especially of animal origin. With population and per capita income growth, agriculture will face greater pressure to increase food production in both quantity and quality at an affordable cost.

As population increases, there is a parallel reduction in cultivated areas. Food production in cultivated areas must, therefore, increase to meet demand, which can only be achieved by increasing cultivated species' productivity. To ensure this increase, it is necessary to improve cultivation practices, including increased fertility, soil conservation, and especially weed management, which can lead to a 20–30 % reduction in crop yields, even reaching 90 % in the absence of weed control methods (Blanco 1972).

Environmental factors control plants' growth and development. According to Radosevich et al. (1996), these factors are the "resources" and "conditions" for expressing cultivars' genetic potential. Resources are consumables, such as water and nutrients, and can be exhausted with high demand or might not be available for the species, due to adverse conditions, such as light for plants in the shade and CO_2 for species that reach quick saturation. Conditions are not directly consumable factors, i.e., soil pH, atmospheric and soil temperature, and soil density (compaction level), which affect the availability of natural resources. Certain factors even become limiting factors as the plant develops, being aggravated by the presence of other plants that also require the same resources in the same space, often generating a competitive relationship between them (Silva et al. 2007a).

Weeds' strong adaptive and multiplying capacities are defining characteristics for perpetuating these species in various environmental conditions. However, the physiological bases that explain these advantages are complex and have not been fully elucidated. According to Silva et al. (2007a), the competition between weeds and cultivated plants affects them both; however, weeds almost always outperform cultivated species. This outperformance usually occurs because true weeds have specific characteristics that enable them to use limiting resources efficiently and rapidly. Moreover, weeds can continue developing even when some resources are scarce (Radosevich et al. 1996). Other characteristics of these plants, including fast emergence and growth, high seedling vigor, rapid leaf expansion, and dense canopy formation, in addition to lower susceptibility to abiotic stress, fast-growing root systems, and greater capacities for producing and releasing allelopathic chemicals, are also determining factors of their competitive ability (Sanderson and Elwinger 2002). Faster emergence and early growth makes these plants have access to environmental resources, especially water and light, before cultivated plants. Plants with these characteristics, thus, generally have an advantage under

competitive conditions, as they limit the access of crops to growth resources, especially light (Gustafson et al. 2004).

With the introduction of transgenic cultivars, other concerns have been attributed to weeds. The main concern is the possibility of gene flow between cultivated and invasive species, which can change the fitness of these plants for biotic and abiotic factors, thus generating significant changes in their rusticity, invasiveness, and competitiveness, turning them into even stronger colonizers. Given these findings, many recent studies have examined cultivars' ability to compete with weeds, especially because this competition can reduce costs and environmental impacts (Balbinot et al. 2003).

This chapter addresses competition theories among cultivated and weed plants, several physiological bases for the stress caused by competing for resources needed for plant growth and improvement strategies adopted to obtain cultivars with greater competitive abilities. Greater focus is given to abiotic stress and allelopathy because previous chapters have addressed biotic factors.

8.2 Theoretical Basis for Competition Between Weeds and Cultivated Plants

Most research aiming to quantify the impact of weed interference on the growth and development of cultivated species is based on the theories of Grime and Tilman (Radosevich et al. 1996). Grime (1979) classified plants based on vital characteristics and adaptation to stress and disturbances. Stress refers to phenomena that limit photosynthetic performance and plant growth, including limitations in light, water, and essential nutrients and the availability of space for root growth. The disturbance refers to partial or total vegetation destruction and may be due to biotic pressures, e.g., predation and parasitism, or non-periodic abiotic factors, e.g., windstorms, fire and soil erosion. According to their classifications, plants adapted to low levels of stress and disturbance are considered competitors (C), those adapted to high stress but low disturbance are regarded as tolerant (S) and those adapted to low stress levels and high disturbance are considered ruderal (R) (Fig. 8.1). Based on this classification, both weeds and cultivated plants can be considered ruderal in an agricultural area.

This classification says little about the dynamic competition of weeds within a particular cultivation system. Grime (1979) defines competition as the tendency of neighboring plants to use the same resources; the plant's capacity to capture resources greatly influences competitive success. Based on this theory, the competitive ability is positively correlated with the relative growth rate and determined by the capacity to exploit the environmental resources rapidly, rather than to tolerate resource depletion.

Tilman (1982) proposed his theory based on resource competition. At a given time, plants can extract resources to a certain level (R*), below which the

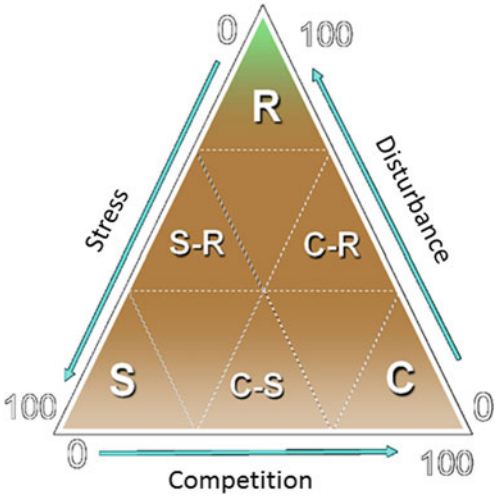

Fig. 8.1 Adaptive types of plants, according to competitive ability

population cannot self-sustain, i.e., the growth rate equals the mortality rate. According to his analysis, the species with the lowest R* can completely displace all other species until reaching equilibrium. This equilibrium occurs spontaneously after resource depletion (Fig. 8.2). This equilibrium is important in cultivation systems because the resources are rarely extracted at the R* level, and the disturbance is managed to ensure the success of a specific class of plant (ruderal).

Based on these theories, certain plants are good competitors for rapidly using a resource or by being able to continue to grow at low levels of a given resource.

Despite several concepts for competition and thousands of studies to quantify weed interference on the growth and development of cultivated species, little is known about the causes that lead to reduced productivity. Although stress causes a reduction in expressing the potential yield of cultivated species, the cause of this stress is often unknown. According to Linquist (2000), weeds generally have no direct effect on the physiological status of cultivated species. However, both cultivated plants and weeds exert a direct effect on resource availability. Moreover, both have a unique response to the amount of resources available in a particular environment. Weeds, thus, indirectly cause productivity losses for cultivated plants, through they influence the resources required for cultivated plant growth. These indirect effects can, in turn, cause abiotic and/or biotic stresses, as detailed below.

8.3 Stresses Caused by Competing with Weeds

Competition for any resource leads a plant to compete for one or more resources. The limitation of a resource, thus, commonly generates, in certain situations, more than one type of stress for the plant. Ramalho (2009) illustrated this situation well:

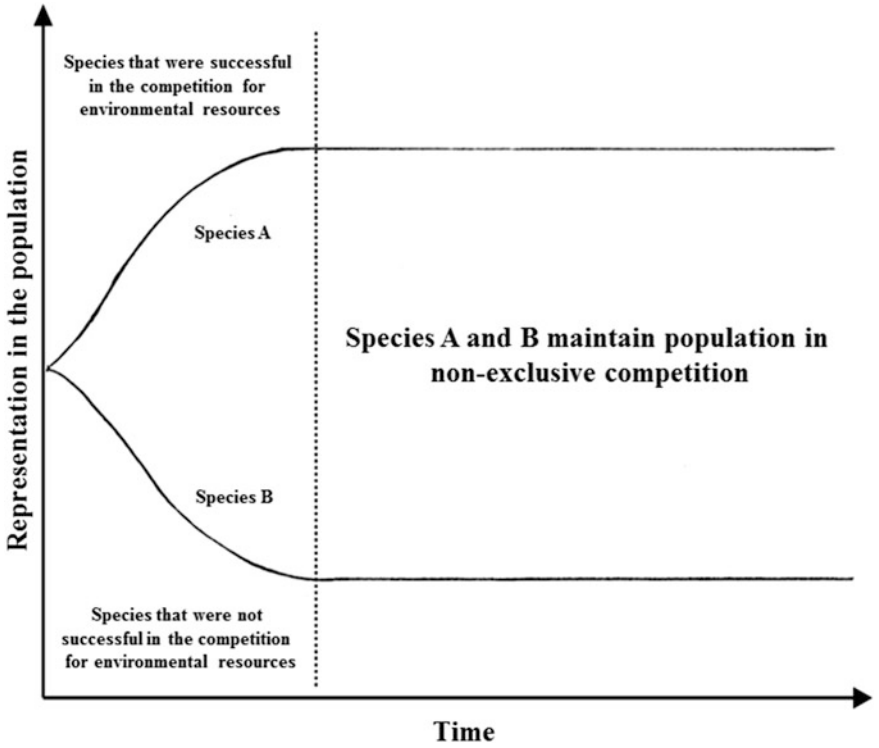

Fig. 8.2 Representation of two hypothetical species in the population after a period of competition for an environmental resource. *Source* Adapted from Tilman (1982)

when a plant is subjected to water stress and high temperatures, pest incidents increase. There is the possibility of a triple stress, two abiotic and one biotic. Despite these factors, this chapter individually discusses the most common stresses on cultivated plants due to competition with weeds, neglecting their possible interactions.

8.4 Abiotic Stresses

8.4.1 Water Competition Stress

Throughout evolution, non-cultivated species achieved high survival rates under the most adverse conditions, allowing greater competitive advantages while obtaining available resources, including water. In some agroecosystems, especially the tropics, agronomic plant species commonly become completely withered on hot days, while weeds remain turgid, without any sign of water stress (Silva et al. 2007a). The greater capacity for various weed species to extract water from the

soil and use it more efficiently can be attributed to the higher soil volume exploitation rate in the weeds' root systems, as well as their morphological and physiological characteristics, such as the capacity of roots for osmotic adaptation (Radosevich et al. 1996). These traits and unique photosynthetic routes make these plants highly competitive for environmental resources, as they are interrelated.

The Spanish needle (*Bidens pilosa*), a common weed in Brazil, can extract soil water at tensions three times lower than those observed for soybean and bean plants (Fig. 8.3). According to Procópio (2004b), this species may have high survivability in soil with little water because, in the initial phase of its development, there is a greater allocation of photoassimilates to root production, which leads to a low shoot/root ratio. At later development stages, this phenomenon allows greater soil exploration in the search for water (Procópio et al. 2002). During dry periods, *B. pilosa* and other weeds that possess this characteristic may become more competitive, especially in soybean and bean cultures, due to higher soil water absorption capacity.

According to Silva et al. (2007a), certain species can use less water per unit of produced dry mass, i.e., they exhibit high efficiency in using this resource. In this sense, such plants with low water requirement should be more productive during periods of limited water availability and, thus, more competitive (Radosevich et al. 1996). However, Procópio et al. (2002) observed that certain weed species may have different water use efficiency (WUE) values during the cycle (Table 8.1), which leads to a differentiated competition for this resource at different growth stages.

Differences in WUE values among species are important factors in their competitive abilities; however, this characteristic is not the only mechanism used for water competition (Silva et al. 2007a). Pearcy et al. (1981) observed that metabolism influenced the difference in WUE values between *Chenopodium album* (C_3) and *Amaranthus retroflexus* (C_4). The C_3 species probably circumvented water deficiency by stomatal control because the WUE for species with this type of metabolism tends to be lower.

Aside from factors related to plant metabolism, water stress adaptation involves a higher water absorption capacity per unit root mass. This increase can be achieved by selecting genotypes that show more robust root systems, i.e., deeper and more branched, which can better exploit deeper soil layers. A hallmark of plants subjected to water deficit is the increase in carbon allocation to the root system (Rajabi et al. 2009).

8.4.2 Light Competition Stress

During normal photosynthetic system operations, the chemical reactions in which water is oxidized to oxygen, the reducing agent nicotinamide adenine dinucleotide phosphate (NADP) is reduced and adenosine triphosphate (ATP) is synthesized are known as the thylakoid reactions because most occur within these organelles. These reactions require the presence of light (Fig. 8.4).

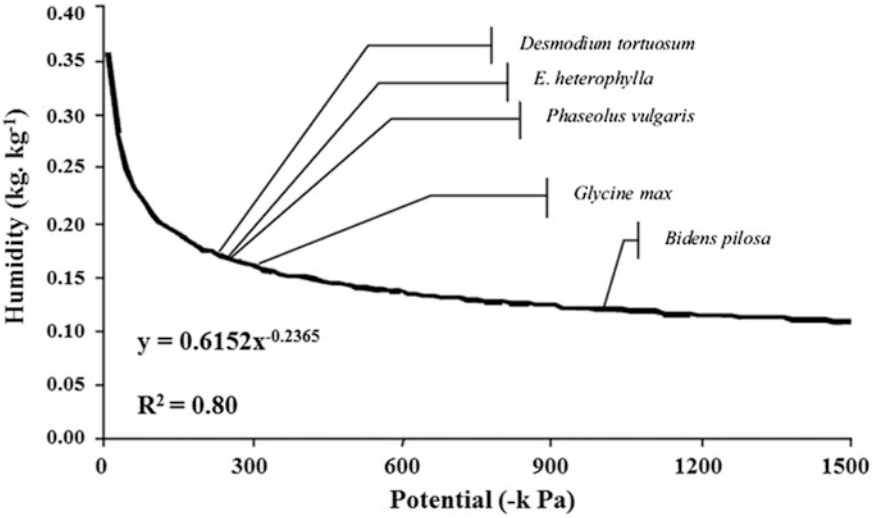

Fig. 8.3 Soil water potential at the permanent wilting point for soybean (*Glycine max*), bean (*Phaseolus vulgaris), E. heterophylla, Bidens pilosa* and *Desmodium tortuosum,* upon induction of water stress. *Source* Adapted from Procópio et al. (2004b)

Table 8.1 Water use efficiency (WUE) before and after flowering for the species soybean (*Glycine max*), bean (*Phaseolus vulgaris), E. heterophylla, Bidens pilosa* and *Desmodium tortuosum*

Plant species	WUE = g of dry matter produced kg^{-1} of H_2O provided	
	Before flowering	After flowering
Phaseolus vulgaris	0.073	0.316
Glycine max	0.168	2.088
Euphorbia heterophylla	0.015	0.250
Bidens pilosa	0.017	1.367
Desmodium tortuosum	0.112	0.963

Source Adapted from Procópio et al. (2002)

In the stroma, both ATP and NADPH are consumed according to the Calvin cycle in a series of reactions controlled by enzymes that reduce CO_2 to carbohydrates (triose phosphate). This consumption requires light not only to regulate the synthesis of energy sources required for photosynthesis but also to activate certain key enzymes of this process, including rubisco.

Björkman (1981) observed that when light-hungry species, as is the case for most cultivated species, receive gradual increases of photosynthetically active radiation, this generates an increasing photosynthetic response up to a certain point, begins to stabilize and finally reaches the saturation point (Fig. 8.5). After reaching the saturation point, further photon flux increases no longer affect the photosynthetic rates. This saturation indicates that factors other than the incident

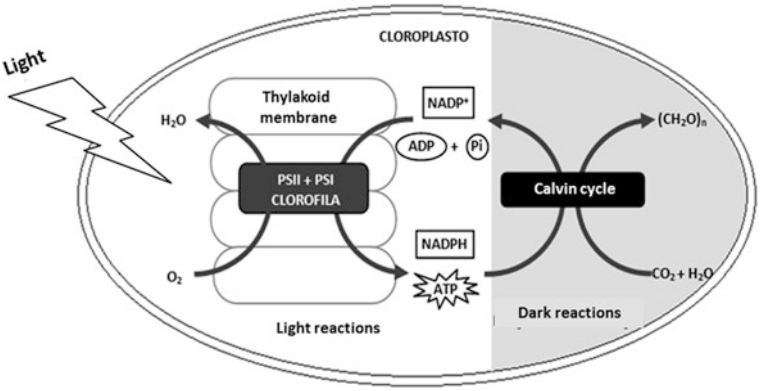

Fig. 8.4 Light and carboxylation photosynthesis reactions in vascular plant chloroplasts. In the thylakoid membranes, chlorophyll excitation in the electron transport system (PSII and PSI) by light induces ATP and NADPH formation. *Source* Adapted from Taiz and Zeiger (2009)

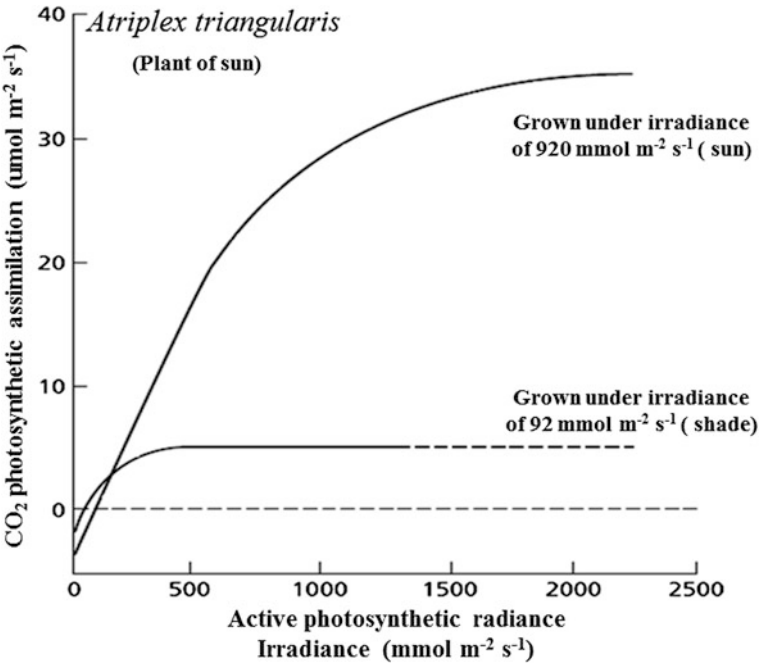

Fig. 8.5 Photosynthetic light response of a sun plant grown under sun (920) and shade (92 µmol m^2 s^{-1}) conditions. *Source* Adapted from Björkman (1981)

light, including the electron transport rate, rubisco activity, or triose phosphates metabolism, become limiting factors for photosynthesis.

After the saturation point, photosynthesis is commonly referred to as CO_2-limited, which reflects the inability of the Calvin cycle enzymes to follow the absorbed light energy that produces ATP and NADPH (Taiz and Zeiger 2009). In contrast, the saturation point of a light-hungry species is well below optimum when shading influences growth. When this shading is less intense, the plants tend to etiolate because there is a greater allocation of assimilates to photosynthetically active structures, rather than reproductive structures. Given these circumstances, certain crops show lodging and other sharp productivity reductions. However, when a leaf or branch cannot maintain itself autotrophically, abscission tends to occur. The plant does not invest assimilates in a condition where the developing leaves do not have enough light to support their photosynthetic rates. The demand for sinks (i.e., new leaves) by sucrose thus decreases, its export diminishes and triose phosphates are converted to starch, which in turn, cover the chloroplasts. The function of these organelles may be impaired, leading to a photosynthesis retroinhibition in photosynthetically active parts. This finding explains the low yields obtained in this culture condition.

Santos et al. (2003) evaluated the radiation use efficiency (RUE) of crops, including soybean and beans, and the weed species *E. heterophylla, Bidens pilosa*, and *Desmodium tortuosum.* These authors observed that the cultivated species have higher RUE values. The greater competitive capacity of weeds can be attributed to higher population and better use of resources, such as water. Genetic improvement programs in plants have developed modern cultivars, based on ideotypes that further maximize RUE. Selection is based on a few traits, such as the presence of erectophile leaves, i.e., with leaf angles >60° in the upper portion and plagiophile leaves, with leaf angles between 30 and 60° in the lower portion. These angles increase the RUE along the plant vegetative canopy, making it more competitive, as it promotes shading late emerging species, while not affecting production drastically; it interferes with harvesting operations and increases the seed bank.

8.4.3 Nutrient Competition Stress

Of the competition factors between cultivated plants and weeds, nutrients, especially nitrogen (N), phosphorus (P), and potassium (K), have great importance in understanding the production loss of agricultural crops (Procópio et al. 2004a). Weeds have a great ability to extract these elements from the environment, which are essential to their growth and development; consequently, they compete with crops. These nutrients are often in quantities below those demanded by crops (Silva et al. 2007a).

Table 8.2 Wheat production (kg ha^{-1}) in competition with wild oat at three N-fertilization levels

Wild oat (plants m^{-2})	N application at pre-planting (kg ha^{-1})			
	0	67	134	Mean
	Wheat production (kg ha^{-1})			
0	6,990	7,520	7,650	7,390
4	6,430	6,660	6,640	6,580
8	6,460	6,100	6,140	6,230
16	5,940	5,200	5,470	5,540
32	5,400	4,120	3,450	4,320
Mean	6,240	5,920	5,870	

Source Adapted from Carlson and Hill (1986)

Competition for nutrients largely depends on the amount present in the soil and the species in the area. Fertilizers can be used to alter competitive relationships, which may favor weeds in certain circumstances and crops in others, as long as the competing species exhibit different responses to nutrient applications (Armstrong et al. 1993). Soybeans under optimum fertilization accumulate 10, 20, and 5 times more N, P and K, respectively, compared to the Mexican clover (*Richardia brasiliensis*) (Pedrinho et al. 2004). However, Carson and Hill (1986) observed that greater N application increased the competitiveness of *Avenafatua*, negatively affecting wheat production (Table 8.2).

The high competitiveness of weeds for nutrients can cause stress to cultivated plants, due to the differential allocation pattern of fixed carbon. Under conditions of low N or P availability, there is reduced leaf growth, due to decreased synthesis of amino acids and proteins that are essential for the formation of plant tissue (Fig. 8.6). Leaf area reduction leads to lower light interception and reduced carbohydrate (assimilates) synthesis. The roots require less investment of assimilates per unit length. Under stress, the partition of assimilates to the root system is greater, as this compartment must ensure plant survival and shoot growth (Zhu and Lynch 2004). The shoot/root ratio is, thus, reduced. According to Mollier and Pellerin (1999), the increase in the partition of assimilates to the roots under stress conditions can be up to two times greater than under optimal culture conditions. Depending on the plant development stage, 25–50 % of the photoassimilates that are produced per day are allocated to the root system for its growth, cellular maintenance, and ion absorption (Marschner 1995).

However, the ability to compete for a certain nutrient is not related only to the ability to extract it from the ground but in using it. Procópio et al. (2004a) observed that although bean plants have high N absorption efficiency, the species *Bidens pilosa* (Spanish needle) and *Euphorbia heterophylla* (Mexican fireplant) make better use of the smaller amounts of N absorbed, resulting in higher dry matter production. To obtain more competitive cultivars, it is thus important to consider selection based on both the root system and intracellular activity, which brings a new complicating factor to the process.

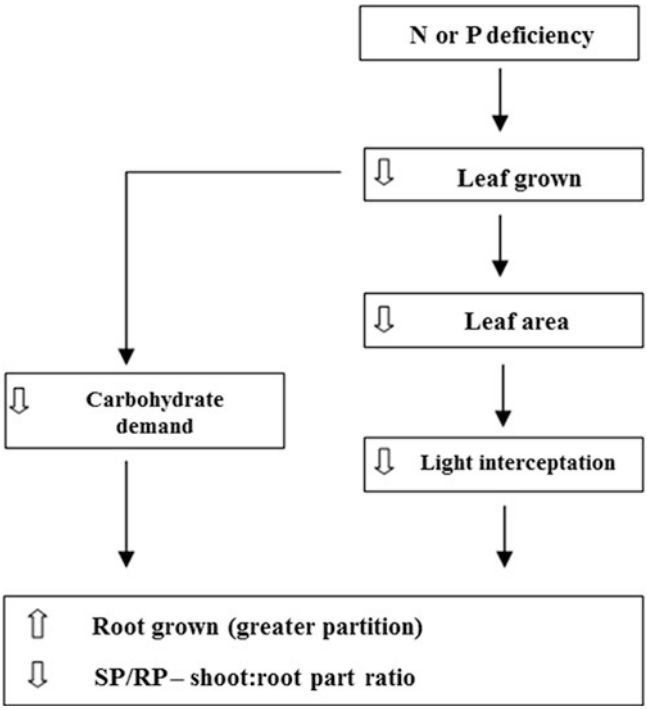

Fig. 8.6 Diagram of the effects caused by N and P deficiency in the shoot and root system

8.5 Biotic Stresses

8.5.1 Stress Caused by Pests and Diseases

Weeds in coexistence with cultivated plants, besides affecting cultural practices and harvesting, can serve as alternative hosts to pests, diseases, nematodes, and parasitic plants. For nematodes, weeds make it impracticable to establish control programs, due to the rotation with non-susceptible crops. Brazil alone has 57 weed species that act as alternative hosts for *Meloidogyne javanica,* an important nematode of soybean crops. Among these species are species with broad and widespread occurrence in the agricultural environments of Brazil, including *Brachiaria plantaginea*, *Digitaria adscendens*, *Eleusine indica*, *B. pilosa, and Ageratum conyzoides* (Pitelli 1987).

As described in previous chapters, pest attacks and disease emergences in cultivated species may involve physiological processes aiming to repair damaged cells and tissues, thereby ensuring structural and physiological integrity. However, plants respond to biotic damage in different ways, ranging from a simple compensatory response, with no fitness or performance reduction for the species, to an

induced response that involves increasing the concentration of certain compounds to prevent further damage (Haile 2000). However, severe pest attacks may damage tissues responsible for photosynthesis, thus reducing the carbon assimilation and fixation rates and consequently biomass production. Furthermore, pests may damage conductive vessels, causing disorders in transporting water and nutrients to different plant compartments.

8.5.2 Stress Caused by Allelopathic Substances

Allelopathy is defined as any direct or indirect effect, harmful or beneficial, that one plant exerts on another, by producing chemical compounds that are released into the environment (Ferreira and Aquila 2000). These compounds, known as allelochemical substances, can be produced in various plant organs; their amounts and compositions depend upon the species and environmental conditions (Silva et al. 2007). The compounds are released into the environment through different methods, including root exudation, leaching, volatilization, and plant residue decomposition in the soil (Fig. 8.7).

The cuticles of neighboring plants can directly absorb the allelochemicals through vapor condensation in the dew, or they can reach the soil, where they are absorbed by the roots (Almeida 1988). The selective permeability of the cytoplasmic membrane may be lost and die shortly thereafter. Another way to release allelochemical substances in the soil is using crop residue decomposition. Miró et al. (1998) observed that residues of *Ilex paraguariensis* showed allelopathic effects on maize growth, even after 60 days of incorporating residues into the soil (Table 8.3).

As mentioned above, competition among weeds and cultivated species is established when they compete for a common developmental factor that is necessary for both plants, i.e., water, light, or nutrients, while allelopathy occurs by adding a factor to the environment. Most studies found in the national and international literature do not separate the effects of competing for production factors from the allelopathic effects. These studies show reports on the interference (competition and allelopathy) of weeds on the growth and/or development of cultivated species. Studies showing the allelopathic effects of cultivated plants on weeds are less common, as cultivated plants have undergone artificial selection throughout their evolution for other traits unrelated to competitive ability (Silva et al. 2007c). To improve palatability and reduce the toxicity of certain forage species, alleles that control producing allelopathic substances, e.g., tannins and alkaloids, have most likely been deleted. Recent research with rice, corn, sorghum, barley, and wheat has indicated the possibility of exploiting the allelopathic effect of these cultures as an auxiliary tool in the management of weeds, thereby reducing the dose and number of herbicide applications (Kim and Shin 2003). Improvement strategies and methods that are important for developing cultivars with allelopathic and/or competitive characteristics are discussed below.

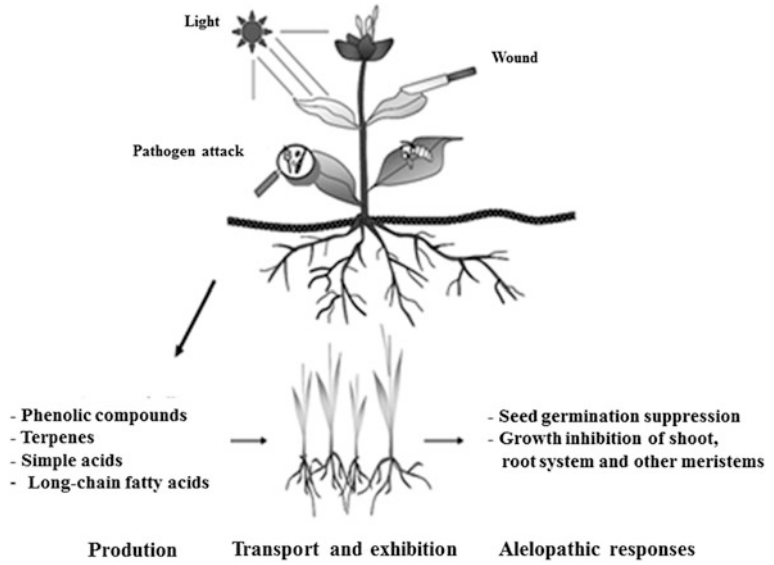

Fig. 8.7 Induction of allelochemical substances from environmental stresses. *Source* Adapted from Kim and Shin (2003)

Table 8.3 Effect of incorporating *Ilex paraguariensis* mature fruits into the soil on the growth of corn sown immediately and after 60 days. Data observed at 30 days of corn emergence. Treatments: Control = no fruits; 22 (22 g of fruit per pot); 50 (50 g of fruit per pot)

Parameters	Treatments					
	0 days			60 days		
	Control	22	50	Control	22	50
Plant height	27.5 a	23.7 b	16.5 c	31.2 a	22.1 b	19.3 c
Shoot dry matter (mg)	260 a	180 b	120 c	97 a	63 b	56 b
Root dry matter (mg)	210 a	180 b	90 c	60 a	60 a	40 a

Source Adapted from Miró et al. (1998)

8.6 Germplasm and Genetic Variability

Weed control through genetic improvement employs two strategies: (i) improving cultivated species to compete with weed species and (ii) improving cultivated species to tolerate herbicides that are non-selective for weeds.

Table 8.4 Shoot dry mass (SDM, kg ha^{-1}) yield in maize cultivars with different genetic structures, intercropped with Brachiaria

Cultivar	Genetic structure	SDM	Tukey's (1 %)
DKB 390	Simple hybrid	10,535	A
DKB 455	Triple hybrid	6,280	B
DKB 789	Double hybrid	7,278	B
UFVM 100	Variety	6,280	B

8.6.1 Improving for Competitiveness

The germplasm of some species contains large differences in the performance of genotypes and consequently cultivars when competing with weeds. To this effect, contrary to what has often been discussed, exploiting heterosis may be more important than genetic variability to stress tolerance. This importance can be observed in Table 8.4 (unpublished data generously provided by Paulo Igor Barbosa e Silva) in which the simple hybrid, despite having no genetic variability, presented superior performance than other types of maize cultivars that possess genetic variability. These results may partly explain why hybrids have superior performance than open-pollinated varieties, even in cultures with low technology. Moreover, these results can help redirect improvement programs to biotic and abiotic stress conditions.

There are several characteristics that can be used in plant improvement to increase competitiveness, including selecting plants with erectophile leaves for higher RUE or plants with fast initial establishment. For maize, using premature cultivars with more erect leaves allow its productivity to almost triple (Sawasaki and Paterniani 2004).

Other characteristics that can be related to greater competitiveness are root system morphology, for which there are large variations in size, growth rate, and axial and lateral root proportions (Fig. 8.8). These variations are not just between species, as there is great genetic variability for these traits within cultivated species that are susceptible to selection (Fig. 8.9).

In this context, the specific root density, which is root length by volume, can improve nutrient and soil water acquisition for the increased root surface area without increasing carbon allocation to the root (Marschner 1995). Another characteristic that may be important is the root system depth, which allows greater exploration of the soil profile and greater water and nutrient absorption (Garnett et al. 2009). Although the root characteristics have considerable impact on efficient resource use and crop productivity and there is great genetic variability, these factors are rarely considered in selecting cultivars in a genetic improvement program. The main limitation in using them is the measurement difficulty and their quantitative inheritance mode (Tuberosa et al. 2002).

From a physiological perspective, other characteristics that could be considered in improving competitiveness are plants' efficiencies in absorbing soil water and

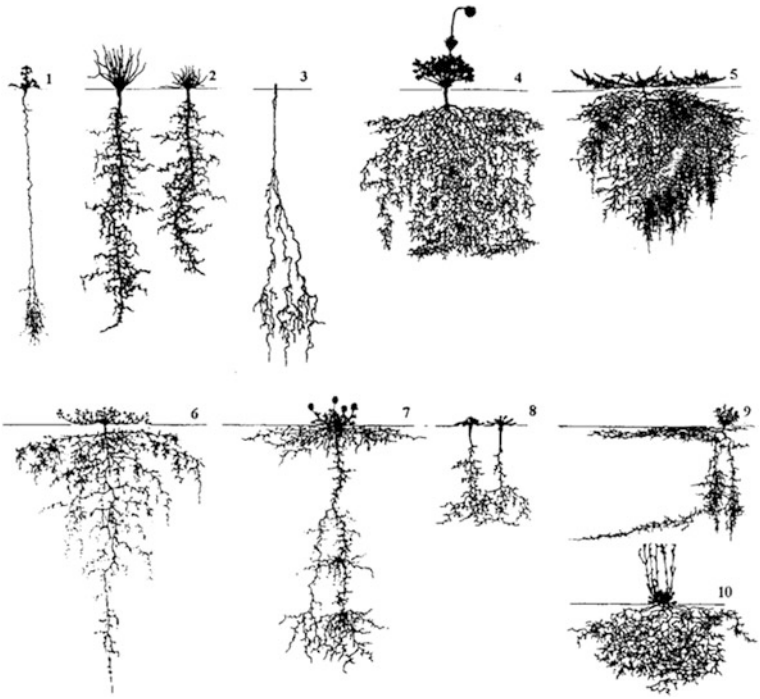

Fig. 8.8 Examples of variability in root system morphology. 1. *Fryngium country*; 2. *Scorzonera villosa*; 3. *Chondrilla juncea*; 4. *Pulsatilla pratensis*; 5. *Genista germanica*; 6. *Trigonella balansae*; 7. *Trifolium trichocephalum*; 8. *Carum caucasicum*; 9. *Onosma arenarium*; 10. *Silene otites. Source* Adapted from Lynch (1995)

nutrients, as well as the efficiency in using these resources to form photosynthetic and reproductive structures. Such traits as efficiency of absorption (EAb) and use (EUs) of nitrogen, phosphorus and water can be help plants overcome the capacity competition with weeds. For these characteristics, high genetic variability has been observed in several cultivated species. Maize has contrasting performance between different lines in the EUs of N and P under the low availability of these elements in the soil (Fig. 8.10).

In such species as rice and wheat, a number of genetic improvement programs have recently sought to develop cultivars that exhibit allelopathy against major crop weeds. Allelopathy can be used in biological weed control, wherein applying this technique involves two culture stages: the vegetative and post-harvest stages. In the vegetative growth phase, "allelopathic" cultivars directly suppress the growth and development of some weed species. In the post-harvest stage, the residues of these cultivars suppress weed establishment at the beginning of the next culture. Wu et al. (1998) observed that wheat accession residue with allelopathic potential differed in annual ryegrass suppression. Subsequent studies showed that root exudates of seedlings of 453 wheat accessions differentially inhibited root growth of annual

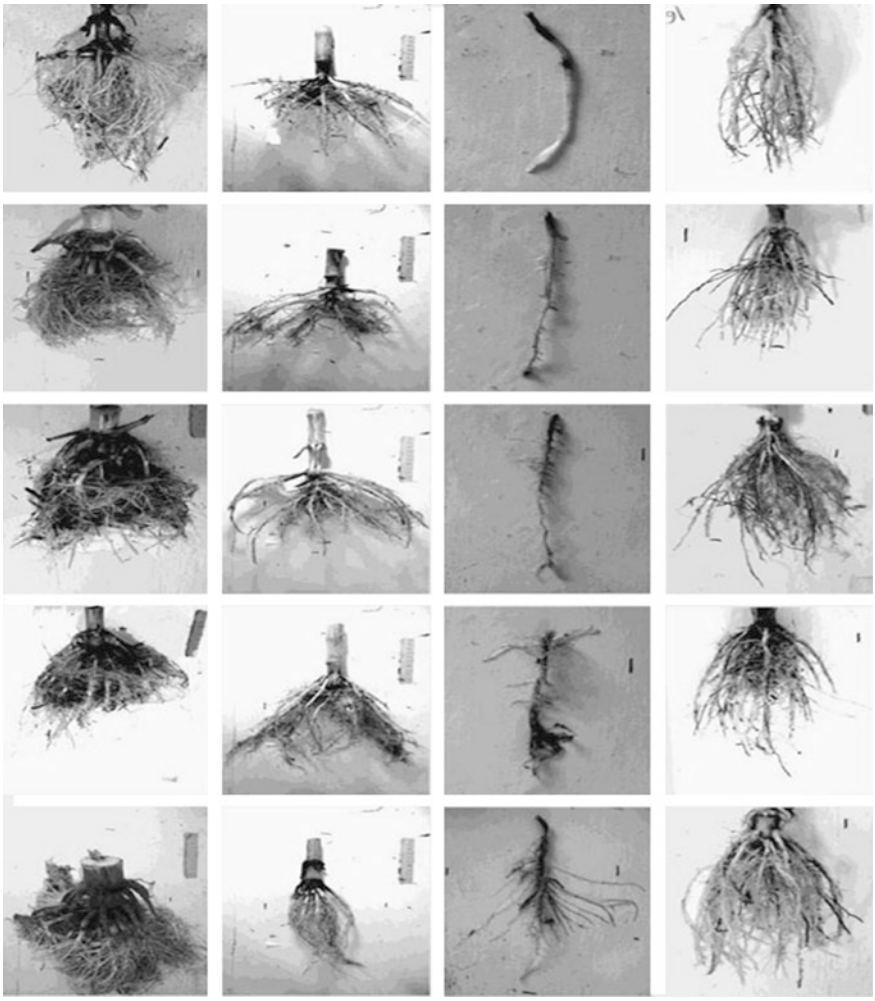

Fig. 8.9 Variability in maize genotypes according to morphology and root growth. *Source* Adapted from Trachsel et al. (2011)

ryegrass at a range of 10–91 %, indicating the existence of great genetic variability for the trait.

Similar results regarding the genetic variability and possible use of allelopathy are reported in rice to suppress weeds such as blue mudplantain (*Heteranthera limosa*) and barnyard grass (*Echinochloa cruz-galli*) (Fig. 8.11). Dilday et al. (1989) evaluated approximately 5,000 lines and found that approximately 4 % of these showed some allelopathic activity against blue mudplantain.

Using cultivars with high allelopathic activity is possible and could reduce the need for herbicides. Developing allelopathic cultivars has thus been

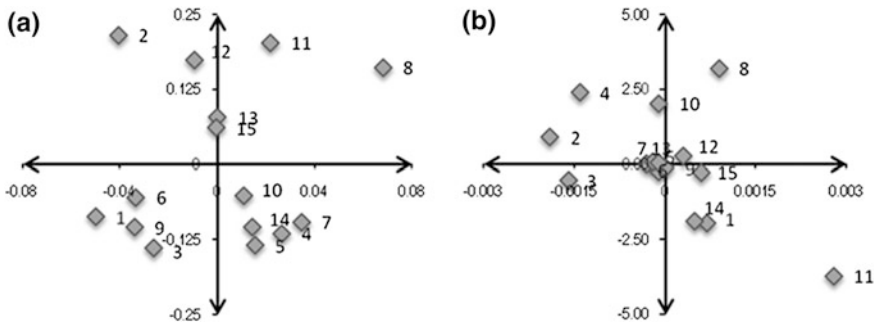

Fig. 8.10 Genotypic values between the EUs of nitrogen (N) and phosphorus (P) (EUtN and EUtP, respectively) and EAb of N and P (EAbN and EAbP, respectively), under low N availability (panel A) and P (panel B) in the soil, in 15 tropical maize lines. *Source* Adapted from Silva (2011)

increasingly recognized as a possible component of integrated weed management (WU et al. 1998).

8.6.2 Non-Selective Herbicide Resistance

Another weed control possibility is identifying and selecting cultivars tolerant to herbicides. This selection can be achieved through biotechnology, using mutation or exploiting the natural genetic variability of the germplasm of cultivated species. Conventional genetic improvement combined with biochemical and molecular biology techniques can effectively develop cultivars tolerant to herbicides and help lower the costs of crop production and the losses inherent to phytotoxicity caused by the herbicides. Studies are still required that characterize the natural tolerance of the germplasm to these products.

Ferreira et al. (2010) sought to identify sugarcane cultivars tolerant to non-selective herbicides. The authors observed that among the cultivars studied, the SP80-3280 and CTC2 were the most tolerant to the ten most commonly used herbicides in sugarcane culture.

8.7 Inheritance and Relationships Among Traits

Identifying and quantifying gene effects that control certain characteristics allow assessing the efficiency of different improvement strategies. Characteristics like early maturation, erectophile angle, and greater RUE are often mono- or oligogenic of high heritability with easier selection based on individual performance, enabling rapid selection gains.

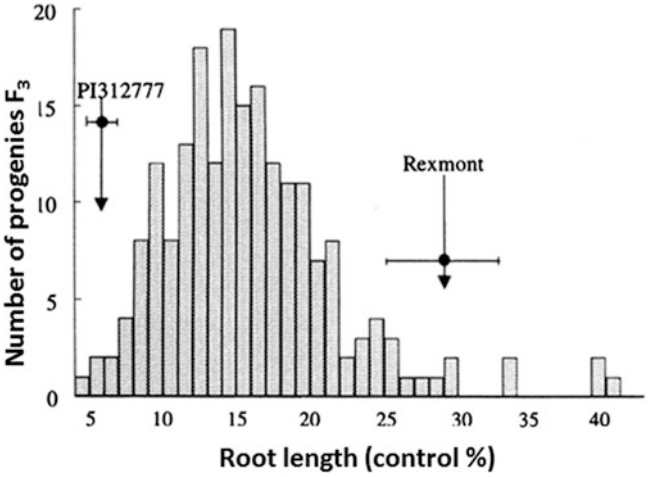

Fig. 8.11 Frequency distribution of root length of lettuce treated with the allelopathic extracts of 192 F_3 progenies of rice derived from biparental cross (PI312777 x Rexmont), with contrasting allelopathy. *Source* Adapted from Okuno and Ebana (2003)

Table 8.5 Estimates of heritability in the broad ($\hat{h}^2_g$), strict ($\hat{h}^2_A$) and average degree of dominance ($\hat{gmd}$) of dry root weight (DRW, *g*), lateral root length (RLat, *m*), and axial (RAxi, *m*), evaluated in 188 genotypes of maize at low nitrogen availability

Trait	Parameters		
	$\hat{h}^2_g$	$\hat{h}^2_A$	$\hat{gmd}$
DRW	0.79	0.69	0.52
RLat	0.17	0.11	1.05
RAxi	0.56	0.36	1.05

Source Adapted from Lima (2010)

In contrast, several studies related to the genetic bases of root characteristics indicate that these characteristics are quantitative in nature. Lima (2010), in a study on maize with low N availability, demonstrated that over 50 % of the variability expressed is genetic in nature for most root traits (Table 8.5). Furthermore, the estimates of the average degree of dominance ($\hat{gmd}$), obtained using the ratio between dominance variance and additive deviations, revealed the existence of overdominance in the control of the lengths of lateral (RLat) and axial (RAxi) roots, as observed in other studies (Fig. 8.12).

For the EUs of N, the additive and non-additive genetic effects are influenced by N levels in the soil (Chun et al. 2005) and the germplasm studied. Souza et al. (2008) observed the greater importance of the additive effects for EUs of N in maize for low N availability. Consequently, selection could be performed in the lines, as the hybrids obtained from these crosses would also be EVs of UN.

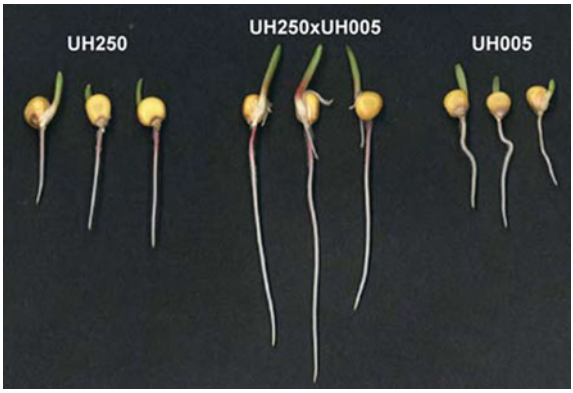

Fig. 8.12 Heterosis for root length at the seedling stage (simple hybrid and the respective parental lines UH250 and UH005). *Source* Adapted from Paschold et al. (2010)

However, Lima (2010) reported the opposite effect, i.e., the non-additive effects were more important. Selecting superior genotypes should, thus, be performed only considering the performance of obtained hybrids.

For the EUs of P, Parentoni et al. (2000) evaluated a diallel of maize lines under conditions of high and low P availability and found that the non-additive genetic effects were greater than the additives. In rice, Chaubey et al. (1994) studied the genetics of the EUs of P (measured as the relative ability to affiliate under contrasting P conditions) and also concluded that there was a predominance of non-additive genetic effects in controlling the trait studied.

The genetic mechanisms controlling allelopathy are poorly understood due to their low heritability and high environmental influence (AHN et al. 2005). However, results obtained using QTL (Quantitative Trait Loci) analyses in rice indicate that non-additive effects are more important in expressing the trait (Okuno and Ebana 2003).

The inheritance and expression of a trait depend not only on nuclear gene dosage effects but also on the maternal effect, i.e., the contribution of the female parent to the genotype of its offspring. Consequently, if there is significant maternal effect, the choice of the female parent in a particular crossing will differ. Several studies, both of quantitative genetics and at the molecular level, have shown that cytoplasmic factors greatly contribute to the variation and inheritance of quantitative and qualitative traits in plants. Quantitative analysis of maternal effects using reciprocal crosses is a strategy that may increase the efficiency of genetic improvement, as physiological parameters, including photosynthetic efficiency, CO_2 gas exchange, and energy production (mitochondria), can be related to characteristics, e.g., the EUs of N, P, water, and radiation.

Besides knowing the inheritance, estimating correlations among traits allow the breeder to know the changes that occur in certain characteristics as functions of the selection made in others. Furthermore, using indirect evaluation and selection methods has great interest because they accelerate the selection process, immediately discarding genotypes with higher susceptibility and concentrating resources on the potentially superior genotypes (Pereira 2011). Considering the

Table 8.6 Gains with the indirect selection of the efficiency of use (EUsN), utilization (EUtN) and absorption (EAbN) of nitrogen (N) through the activity of glutamine synthetase (GS) in low N availability, in tropical maize

Indirect selection gain (%)	EUsN	EUtN	EAbN
Glutamine synthetase	24.93	21.16	24.01

Source Adapted from Oliveira (2009)

possibility of indirect selection for EUs of N and its components, Oliveira (2009) estimated their gains using the activity of glutamine synthetase (GS) in tropical maize lines, under low N availability. The gains observed were positive and had high value for all traits (Table 8.6), demonstrating that GS can be used to select and develop maize cultivars with high EUs of N.

Pereira (2011) attempted to identify morphological traits in early plant stages related to the EUs of N and P in tropical maize. According to the author, the shoot dry matter at the stage of six fully expanded leaves has a high direct effect (0.99) on these and can be used to indirectly select these traits.

Considering the relationship of allelopathy with the other traits, this is not correlated with plant height and root length for wheat (WU et al. 2000), and its evaluation and selection should be performed directly. However, for rice, the results indicate that cultivars with colored lemma or palea have lower allelopathic effect than strains that do not have it (Chung et al. 2003). Moreover, there is a great impediment to using allelopathy in rice, as the trait has a strong negative relationship with grain yield (Ahn et al. 2005). Effective allelopathy use in this case, thus, depends on the germplasm genetic variability and the lack of negative correlation between allelopathy and agronomically important traits.

8.8 Stress Induction: Phenological Stage, Intensity, and Duration

Stress management is a crucial point to obtain success in genetic improvement programs for biotic stress conditions. If the induced stress is too severe in competition with weeds, as mentioned above, it could "overshadow" the genetic variability and make the selection impractical.

Few studies have aimed to select genotypes with higher competitiveness with weeds. Most studies are conducted under field conditions, although several researchers conducted these studies in greenhouses; in both cases, the aim is the same: to clearly simulate stress conditions under real cultivation scenarios. Duration, intensity, and uniformity are factors that should be considered to establish the appropriate stress management. The duration should be long enough for the stress to coincide with the critical plant growth stages, and there should be nutritional efficiency traits susceptible to improvement. The intensity must be severe enough to affect important productivity characteristics. Management must

be uniform in time and space to easily observe the genetic variance and possible gains.

8.9 Improvement Strategies and Methods

As for other traits, in improving for competitiveness against weeds, individual performance may select for traits that show high heritability and additive genetic control, i.e., the individual performance of lines or populations. When the trait has low heritability or presents inheritance due to non-additive gene effects, genotype selection should be based on hybrid performance for allogamous species. Moreover, if there is significant maternal effect, as mentioned above, the choice of the female parent in a particular cross will differ.

As discussed earlier in this chapter, the genetic parameters for characteristics that provide greater competitive capacity may vary depending on the stress levels, species, and germplasm studied. It is, thus, essential to study the germplasm inheritance in each genetic improvement program. Other important studies to assist selection are identifying and quantifying genetic correlations among competitiveness traits and among these and agronomic traits. The aim is to verify whether it is possible to perform indirect selection for traits with low heritability and/or difficult analysis, either due to experimental difficulties or high measurement costs.

Finally, it is important to quantify the interaction between genotype and stress levels. If the interaction is significant for the characteristics used in selection, then the interaction should not be based on genotype performance in only one environment, as the genes will be expressed differently according to the environment provided. Also not differing from improving other characteristics, the competitiveness improvement methods are chosen according to the species reproductive system, the desired cultivar type, and the heritability and genetic control of the most important characteristics.

The population methods are based on recurrent selection because they aim to gradually increase the frequency of alleles favorable for quantitative traits through repeated selection cycles, without significantly reducing the population's genetic variability. Recurrent selection can be divided into obtaining progenies, progeny evaluation in experiments with repetition and recombining superior progenies to originate the next generation. The improved populations can be used repeatedly to start a new cycle of recurrent selection after recombining selected and superior progenies (Bernardo 2002). When the characteristics show control, particularly of the additive effects, the intrapopulational recurrent selection methods can be used. However, when the heterosis effect occurs (non-additive effects), interpopulational methods are the most suitable.

8.10 Biotechnology

Although the "classic" improvement has made significant contributions in several species and characteristics, the biotechnology has provided and is still producing the best results in weed control. These are mainly due to transgenesis, which allows introducing one or more genes for herbicide tolerance into the cultivars. Management, thus, becomes more efficient and faster at any developmental stage of the culture.

8.10.1 Transgenesis

Research institutions have been developing, through transgenesis, cultivars of different agronomic species resistant to herbicides, including soy, maize, cotton, canola, and sugarcane resistant to glyphosate; maize to imazaquin; and rice and soybeans to glufosinate-ammonium. However, there is no doubt that the next stage of this technology is producing cultivars of maize, soybean, and other species resistant to glyphosate and other herbicide groups with different action mechanisms. The possibility of growing corn, cotton, sugar cane, and soybeans without competition with weeds is attractive to the Brazilian farmer, especially because of the economic benefits, given that the damage resulting from competition can result in a complete production loss. Conversely, misusing this technology may, in a few years of cultivation, select weed biotypes with tolerance and others with resistance to herbicides or even biodiversity reduction, i.e., eliminating numerous plant species and their dependent microorganisms (Silva et al. 2007a).

Producers quickly accepted and adopted the glyphosate-resistant soybean technology, expanding the use of this herbicide. Today there are, on average, three glyphosate applications per soybean cycle for desiccation and two applications after crop emergence. Furthermore, glyphosate is the main herbicide for various crops like fruits, coffee, eucalyptus and desiccation for direct planting (Ferreira et al. 2009).

The glyphosate-resistant soybean technology allows reducing or eliminating the need to apply other herbicides to manage different weed species, which also contributes to increased selective pressure and emergence of resistant biotypes. Moreover, some aspects of the population dynamics of weeds and the possibility of selecting glyphosate-tolerant species must be considered. The type of management and herbicides used in an area change the type and ratio of species that comprise the local population because herbicides do not control the species in the area equally; some species thus end up benefiting and prevailing over others. In these situations, plants of low occurrence in the area can become serious problems for the producer. The continuous and repeated use of the same herbicide or herbicides with the same action mechanism makes species selection inevitable (Silva et al. 2007b). Developing cultivars with two or more genes that confer tolerance to herbicides with different action mechanisms could help manage resistant weeds.

Although this technology has great value to the farmer, it is necessary to use it as part of an integrated weed management program for it to be sustainable over several years.

8.10.2 Mutation

Genes that confer herbicide resistance can be found in the natural biodiversity of cultivated species or obtained by inducing mutation (Brasileiro and Dusi 1999). Mutations are primary sources of genetic variability, resulting from DNA changes that alter the individual's genotype, whether spontaneous or induced (Pinto 1995). Spontaneous mutations are rare and non-targeted with limited use in plant improvement, as the chance that a favorable mutation occurs spontaneously is small. When referring to mutant lines, induced mutation should thus come to mind. The mutant lines obtained by radiation, chemical agents, or somaclonal variation are not subject to any legal restrictions in Brazil, and their production and consumption are allowed without restrictions.

Mutation application can be useful in weed control. Developing mutant lines of rice tolerant to herbicides inhibitors of the enzyme acetolactate synthase (ALS) is well studied (Silva et al. 2007b) because it can be an alternative for controlling red rice, creating opportunities to apply new strategies and increase the flexibility of weed management and control (Duke 1996).

8.10.3 QTL: Quantitative Trait Loci

Quantitative trail loci mapping enables a better understanding of the genetic control and inheritance of traits, helping to choose the best selection strategy. Some studies report the QTL identification for EUs of N in maize (Gallais and Hirel 2004) and rice (Cho et al. 2007) under low soil N availability. Among these QTLs, several are coincident for the glutamine synthetase and nitrate reductase enzymes, both of which are from N metabolism (Hirel et al. 2007).

Regarding root characteristics, Liu et al. (2009) identified several QTLs for root architecture in a population of RILs (Recombinant Inbreed Lines) from corn. The authors also observed that the QTLs for root architecture are colocalized with those for grain yield and N absorption, as reported in other studies. These results confirm that one way to increase nutrient use efficiency is producing a root system that has more efficient absorption.

For allelopathy in both wheat and rice, several QTLs have been identified. In rice, five QTLs, located on chromosomes 3, 5, 6, 7, and 12, explained 36.6 % of the total phenotypic variation for allelopathy to barnyard grass (Okuno and Ebana 2003).

When the QTLs of interest are identified, their molecular linkage disequilibrium markers can be used to transfer them (pyramiding) for a given genotype, provided

they do not occupy the same locus. The advantage of this method is that favorable alleles can be accumulated without agronomic evaluation, saving time and labor (Fritsche-Neto 2011). However, few studies have examined epistatic interactions among pyramid QTLs, i.e., there is no guarantee that accumulating QTLs of interest in a single genotype makes it superior.

Recent advances in statistical genomics and molecular biology will provide, in the near future, more precise QTL identifications. To have consistent QTL results, it is necessary to improve phenotyping. Large mapping populations should thus be used, evaluated at different locations and years, observing the maximum number of traits and using more precise statistical tools, including experiments performed in lattice and mixed model methodology in the statistical analyses. Furthermore, uniformity in the stress environment is essential.

8.10.4 MAS: Marker-Assisted Selection

Destructive methods are usually employed for evaluation and selection based on the phenotype for stress conditions, as when evaluating root characteristics. There are situations in which the characteristics of interest, such as the allelopathic potential, contain complex reactions between the plant and environmental conditions, including water stress, temperature, light, soil, and plant age. Depending on the genetic structure of the plant, it is not possible to use or identify the characteristics identified as superior in hybridizations, as the environmental variation "overshadows" the genetic variation. Consequently, the potential gains to obtain with the selection are reduced.

Marker-assisted selection (MAS) methods, especially the genome-wide selection (GWS), have been proposed to increase the genetic improvement efficiency (Meuwissen et al. 2001). With the GWS, prediction and selection can be performed at early plant stages, thus accelerating the genetic improvement process. GWS provides a direct form of early selection, as it is performed in advance but in genes that will be expressed in adulthood. In contrast, traditional early selection is indirect, as it is performed (using phenotypic observation) in prematurely activated genes, hoping that they partially report genes expressed in adulthood. Fritsche-Neto (2011) compared the GWS method with the recurrent selection for improving the efficiency of absorption (EAb) of N and P in tropical maize, under stress conditions (Table 8.7).

With a GWS cycle per year, it would be possible to obtain additional annual gains of 306.98 and 1,028.01 % for the EAb of N and P, respectively, compared to recurrent selection methods. When considering the possibility of two GWS cycles per year, these values become 990.53 and 3,317.05 %, respectively. With GWS, there is a significant increase in gains using selection per unit time and in the improvement process. This method can revolutionize how selections are made in genetic improvement programs for stress conditions.

Table 8.7 Estimated gains with selection (GS) in additive genetic standard deviations for each method considered and the relative efficiency (RE) of genome-wide selection (GWS), with one (GWS 1) or two (GWS 2) cycles per year, compared to recurrent selection methods (RS), for the efficiency of absorbing nitrogen and phosphorus (EAbN and EAbP, respectively) in maize, under low soil nutrient availability

Parameter	Method	Characteristics	
		EAbN	EAbP
GS	RS	0.65	0.29
	GWS	1.32	1.97
RE (%)	GWS 1	306.98	1028.01
	GWS 2	990.53	3317.05

Source Adapted from Fritsche-Neto (2011)

Although MAS is still considered an expensive method, these markers can reduce the time required, manpower, experimental area, and risks in developing highly productive new cultivars with high tolerance to biotic and abiotic stresses.

8.11 Climate Changes and Weeds

Many researchers argue that the productive scenario of cultivated species will worsen due to climate changes. Most of these species will encounter culture conditions far from the ideal zone. Moreover, these changes are likely to enhance weed growth and development, due to greater genetic diversity for weeds than for agronomic species. Consequently, under limiting conditions of a resource, it is more likely that weeds show greater phenotypic plasticity and productive response. Other researchers have more optimistic predictions on the subject based on the hypothesis of the lower growth response of weeds facing increased temperatures and CO_2 concentrations, as many species have C_4 metabolism. However, this assumption does not consider the large number of weeds with C_3 metabolism present in the fields.

References

Ahn JK, Hahn SJ, Kim JT, Khanh TD, Chung IM (2005) Evaluation of allelopathic potential among rice (*Oryza sativa* L.) germplasm for control of *Echinochloa crus-galli* P Beauv in the field. Crop Prot 24:413–419

Almeida FS (1988) A alelopatia e as plantas. IAPAR, Londrina, p 60

Armstrong RD, Brown RF, Helyar KR (1993) The use of nitrogen, phosphorus and lime to limit the competitive ability of *Aristida armata* in the establishment phase, Australian. J Agric Res 44:167–178

Balbinot AA Jr, Fleck NG, Barbosa Neto JF, Rizzardi MA (2003) Características de plantas de arroz e a habilidade competitiva com plantas daninhas. Planta Daninha 21:165–174

Bernardo R (2002) Breeding for quantitative traits in plants. Stemma Press, Woodbury, p 369
Björkman O (1981) Responses to different quantum flux densities. In: Lange OL, Nobel PS, Osmand CB, Ziegler H (ed.) Encyclopedia of plant physiology: physiological plant ecology I. Springer, New York, p 57–107
Blanco HG (1972) A importância dos estudos ecológicos nos programas de controle de plantas daninhas. O Biológico 38:343–350
Brasileiro ACM, Dusi DMA (1999) Transformação genética de plantas. In: Torres AC, Caldas LS, Buso JA (ed.) Cultura de tecidos e transformação genética de plantas. Embrapa, Brasília, p 679–735
Carlson HL, Hill JE (1986) Wild oat (*Avena fatua*) competition with spring wheat: effect of nitrogen fertilization. Weed Sci 34:29–33
Chaubey CN, Senadhira D, Gregorio GB (1994) Genetic analysis of tolerance for phosphorous deficiency in rice (*Oryza sativa* L.). Theor Appl Genet 89:313–317
Cho YI, Jiang WZ, Chin JH, Piao ZZ, Cho YR, McCouch SR, Koh HJ (2007) Identification of QTLs associated with physiological nitrogen use efficiency in rice. Mol Cells 23:72–79
Chun L, MI G, Li J, Chen F, Zhang F (2005) Genetic analysis of maize root characteristics in response to low nitrogen stress. Plant and Soil 276:369–382
Chung IM, Kim KH, Ahn JK, Lee SB, Kim SH, Hahn SJ (2003) Comparison of allelopathic potential of rice leaves, straw, and hull extractson barnyardgrass. Agron J 95:1063–1070
Dilday RH, Nastasi P, Smith Jr RJ (1989) Potential allelopathic activity of rice (Oryza sativa L.) germplasm. In: 42nd annual meeting of the Southern Weed science society, Anais…. Weed Science Society, Champaign, p 261
Duke SO (1996) Herbicide resistant crops. Lewis Publishers, Boca Raton, p 420p
Ferreira AG, Aquila EA (2000) Alelopatia: uma área emergente da ecofisiologia. Revista Brasileira de Fisiologia Vegetal 12:175–204
Ferreira EA, Germani C, Vargas L, Silva AA, Galon L (2009) Resistência de *Lolium multiflorum* ao Glyphosate. In: Agostinetto D, Vargs L (ed.) Resistência de plantas daninhas no Brasil. Berthie, BraZil, p 271–289
Ferreira RR, Oliveira FTR, DeLite FS, Azevedo RA, Nicolai M, Carvalho SJP, Christoffoleti PJ, Figueira AVF (2010) Tolerância diferencial de variedades de cana-de-açúcar a estresse por herbicidas. Bragantia 69:395–404
Fritsche-Neto R (2011) Seleção genômica ampla e novos métodos de melhoramento do milho. UFV, Viçosa, 28p. (Tese de Doutorado)
Gallais A, Hirel B (2004) An approach of the genetics of nitrogen use efficiency in maize. J Exp Bot 55:295–306
Garnett T, Conn V, Kaiser BN (2009) Root based approaches to improving nitrogen use efficiency in plants. Plant Cell Environ 32:1272–1283
Grime JP (1979) Plant strategies and vegetation processes. Wiley, London, p 254
Gustafson DJ, Gibson DJ, Nickrent DL (2004) Competitive relationships of *Andropogon gerardii* (Big Bluestem) from remnant and restored native populations and select cultivated varieties. Funct Ecol 18:451–457
Haile FJ (2000) Drought stress, insects, and yield loss. In: Peterson RKD, Higley LG (ed.). Biotic stress and yield loss. CRC Press LLC, Boca Raton, p 117–134
Hirel B, Le Gouis J, Ney B, Gallais A (2007) The challenge of improving nitrogen use efficiency in crop plants: towards a more central role for genetic variability and quantitative genetics within integrated approaches. J Exp Bot 58:2369–2387
Kim KU, Shin DH (2003) The importance of allelopathy in breeding new cultivars. In: Labrada R (ed.) Weed management for developing countries, Addendum 1. FAO plant production and protection paper 120. 65p
Lima RO (2010) Herança e correlação de caracteres de raiz e eficiência de uso do nitrogênio em milho. UFV, Viçosa, 38p. (Dissertação de Mestrado)
Linquist JL (2000) Mechanisms of crop loss due to weed competition. In: Peterson RKD, Higley LG (ed.) Biotic stress and yield loss. CRC Press LLC, Boca Raton, p 233–253

Liu J, Chen F, Olokhnuud C, Glass ADM, Tong Y, Zhang F, Mi G (2009) Root size and nitrogen-uptake activity in two maize (Zea mays) inbred lines differing in nitrogen-use efficiency. J Plant Nutr Soil Sc 172:230–236

Lynch J (1995) Root architecture and plant productivity. Plant Physiol 109:7–13

Marschner HM (1995) Mineral nutrition of higher plants. Academic Press, London, p 889p

Meuwissen T, Hayes B, Goddard M (2001) Prediction of total genetic value using genome-wide dense marker maps. Genetics 157:1819–1829

Miró CP, Ferreira AG, Aqüila MEA (1998) Alelopatia de frutos de erva-mate (*Ilex paraguariensis*) no desenvolvimento do milho. Pesquisa Agropecuaria Brasileira 33:1261–1270

Mollier A, Pellerin S (1999) Maize root system growth and development as influenced by phosphorus deficiency. J Exp Bot 50:477–487

Okuno K, Ebana K (2003) Identification of QTL controlling allelopathic effects in rice: genetic approaches to biological control of weeds. Jpn Agric Res Quart 37:77–81

Oliveira LR (2009) Eficiência de uso de nitrogênio e atividade da nitrato redutase e glutamina sintetase em milho. Universidade Federal de Viçosa, 94p. (Tese Doutorado)

Parentoni SN, Vasconcelos CA, Alves VMC, Pacheco CAP, Santos MX, Gama EEG, Meirelles WF, Correa LA, Pitta GVE, Bahia Filho AFC (2000) Eficiência na utilização de fósforo em genótipos de milho. In: 23° Congresso Nacional de Milho e Sorgo, Anais… ABMS, Uberlândia, p 92

Paschold A, Marcon C, Hoecker N, Hochholdinger F (2010) Molecular dissection of heterosis manifestation during early maize root development. Theor Appl Genet 120:383–388

Pearcy RW, Tumosa N, Williams K (1981) Relationships between growth, photosynthesis and competitive interactions for a C_3 and C_4 plant. Oecologia 48:371–376

Pedrinho Júnior AFF, Bianco S, Pitelli RA (2004) Acúmulo de massa seca e macronutrientes por plantas de *Glycine max* e *Richardia brasiliensis*. Planta Daninha 22:53–61

Pereira FB (2011) Relação entre caracteres determinantes da eficiência no uso de nitrogênio e fósforo em milho. UFV, Viçosa, 18p. (Dissertação de mestrado)

Pinto RJB (1995) Introdução ao melhoramento genético de plantas. EDUEM, Maringá, p 275p

Pitelli RA (1987) Competição e controle das plantas daninhas em áreas agrícolas. Série Técnica IPEF 4:1–24

Procópio SO, Santos JB, Pires FR, Silva AA, Mendonça ES (2004a) Absorção e utilização do nitrogênio pelas culturas da soja e do feijão e por plantas daninhas. Planta Daninha 22:365–374

Procópio SO, Santos JB, Silva AA, Costa LC (2002) Análise do crescimento e eficiência no uso da água pelas culturas de soja e do feijão e por plantas daninhas. Acta Scientiarum 24:1345–1351

Procópio SO, Santos JB, Silva AA, Donagemma GK, Mendonça ES (2004b) Ponto de murcha permanente de soja, feijão e plantas daninhas. Planta Daninha 22:35–41

Radosevich S, Holt J, Ghersa C (1996) Physiological aspects of competition. In: Radosevich S, Holt J, Ghersa C (ed.) Weed ecology implications for managements. Willey, New York, p 217–301

Rajabi A, Ober ES, Griffiths H (2009) Genotypic variation for water use efficiency, carbon isotope discrimination, and potential surrogate measures in sugar beet. Field Crop Res 113:48–63

Ramalho MP, Silva GS, Dias LAS (2009) Genetic plant improvement and climate changes. Crop Breed Appl Biotechnol 9:189–195

Sanderson MA, Elwinger GF (2002) Plant density and environment effects orchardgrass-white clover mixtures. Crop Sci 42:2055–2063

Santos JB, Procópio SO, Silva AA, Costa LC (2003) Captação e aproveitamento da radiação solar pelas culturas da soja e do feijão e por plantas daninhas. Bragantia 62:147–153

Silva AA, Ferreira FA, Ferreira LR, Santos JB (2007a) Biologia de plantas daninhas. In: Silva AA, Silva JF, Tópicos em manejo de plantas daninhas. Editora UFV, Viçosa, p 17–62

Silva AA, Vargas L, Ferreira EA (2007b) Herbicidas: resistência de plantas. In: Silva AA, Silva JF Tópicos em manejo de plantas daninhas. Editora UFV, Viçosa, p 280–324

Silva JCV (2011) Identificação de genitores e importância relativa dos efeitos gênicos para os caracteres associados às eficiências no uso de nitrogênio e fósforo em milho. UFV, Viçosa, 90p. (Tese de Doutorado)

Silva MP, Villela FA, Dode LB, Magalhães AM Jr, Tillmann MAA (2007c) Detecção de sementes de arroz mutante tolerante ao herbicida imazethapyr. Revista Brasileira de Sementes 29:130–137

Souza LV, Miranda GV, Galvão JCC, Eckert FR, Mantovani EE, DeLima RO, Guimarães LJM (2008) Genetic control of grain yield and nitrogen use efficiency in tropical maize. Pesquisa Agropecuária Brasileira 43:1517–1523

Taiz L, Zeiger E (2009) Fisiologia vegetal, 4th edn. Artmed, Porto Alegre, p 848p

Tilman D (1982) Resource competition and community structure. Princeton University Press, Princeton 296p

Trachsel S, Kaeppler SM, Brown KM, Lynch JP (2011) Shovelomics: high throughput phenotyping of maize (*Zea mays* L.) root architecture in the field. Plant Soil 341:75–87

Tuberosa RC, Sanguineti MC, Landi P, Giuliani MM, Salvi S, Conti S (2002) Identification of QTLs for root characteristics in maize grown in hydroponics and analysis of their overlap with QTLs for grain yield in the field at two water regimes. Plant Mol Biol 48:697–712

Wu H, Pratley J, Lemerle D, Haig T (2000) Evaluation of seedling allelopathy in 453 wheat (*Triticum aestivum*) accessions by the equal-compartment-agar-method. Australian J Agric Res 51:937–944

Wu H, Pratley J, Lemerle D, Haig T, Verbeek B (1998) Differential allelopathic potential among wheat accessions to annual ryegrass. In: Michalk DL, Pratley JE (ed.) 9th Australian agronomy conference, Anais….Charles Sturt University, Wagga Wagga, p 567–571

Zhu J, Lynch JP (2004) The contribution of lateral rooting to phosphorus acquisition efficiency in maize (*Zea mays* L.) seedlings. Funct Plant Biol 31:949–958

Index

A
African cassava mosaic virus, 59

B
Bacteria, 6
Bacterial diseases, 37
Bacterium, 8
Barley mild mosaic virus (BaMMV), 75
Barley yellow dwarf virus, 60
Barley yellow mosaic virus (BaYMV), 75
Bean common mosaic virus (BCMV), 75
Bean golden mosaic begomovirus, 76
Bean yellow mosaic virus (BYMV), 75
Biotechnology, 88, 96, 98
Breeding for disease resistance, 37
Brome mosaic virus, 59

C
Cauliflower mosaic virus, 59
Chrysanthemum stem necrosis virus (CSNV), 60
Citrus tristeza virus, 60
Climate change, 4, 7
Competitive ability, 138, 140, 148
Cucumber mosaic virus (CMV), 59, 61, 74, 75
Cucurbit yellowing stunting disorder virus, 65

D
Defense mechanisms, 130, 134
Disease, 4, 5, 7–10, 13–15, 17–19

F
Fungus, 8

G
Genetic resistance, 114
Germplasm, 3, 10–12, 16, 17
Global warming, 2, 4, 7, 9
Groundnut ringspot virus (GRSV), 60, 69

H
Horizontal resistance, 3, 7, 8, 13, 14, 19
Host, 3, 6, 7, 10, 14, 18

I
Insect resistance, 107, 113, 115, 121, 122, 130
Insects, 7–9
Interaction, 6, 7, 17, 18

L
Lettuce mosaic virus (LMV), 64, 75

R. Fritsche-Neto and A. Borém (eds.), *Plant Breeding for Biotic Stress Resistance*,
DOI: 10.1007/978-3-642-33087-2, © Springer-Verlag Berlin Heidelberg 2012

M
Melon necrotic spot virus (MNSV), 75
Moroccan watermelon mosaic virus, 65

P
Papaya ringspot virus, 65
Pathogen, 3, 4, 6–11, 13, 17–19
Pea seed borne mosaic virus (PSbMV), 75
Pepper mottle virus (PepMoV), 62, 75
Pepper veinal mottle virus (PVMV), 75
Pepper yellow mosaic virus (PepYMV), 62
Plant breeding, 83
Plant defenses, 120
Plant improvement, 150
Plum pox virus (PPV), 59, 75
Potato leafroll virus (PLRV), 60, 61
Potato virus X, 59, 62, 74
Potato virus Y (PVY), 59, 60, 74, 75

R
Rice dwarf virus (RDV), 75
Rice tungro spherical virus (RTSV), 75
Rice yellow mottle virus (RYMV), 75

S
Soybean mosaic virus, 74
Stored grain insects, 128
Stored metabolites, 127, 134

T
Tobacco etch virus (TEV), 74, 75
Tobacco mosaic virus (TMV), 59, 61, 74, 75
Tomato bushy stunt virus, 60
Tomato chlorotic spot virus (TCSV), 60
Tomato spotted wilt virus (TSWV), 59, 60, 69, 74
Tomato yellow leaf curl virus, 59
Turnip crinkle virus, 74, 75
Turnip mosaic virus (TuMV), 75

V
Variability, 3, 8, 10
Vertical resistance, 7, 8, 11, 19
Virus, 6, 8

W
Watermelon mosaic virus (WMV), 65
Weeds, 5, 7–9

Z
Zucchini yellow mosaic virus (ZYMV), 65, 75