



PLANT PATHOGEN RESISTANCE BIOTECHNOLOGY

Edited by
David B. Collinge

WILEY Blackwell

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*To Andrea,
Mikkel and Jakob
Tak for jeres støtte*

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Foreword

It is almost a cliché to point out that the agricultural production systems of the planet are facing a series of unprecedented challenges.

The world population is predicted to grow to more than 8 billion within 20 years, approaching 10 billion in 2050 (<http://esa.un.org/wpp/>).

Urbanization of the population is reducing the available area of agricultural land by encroachment and affecting adjacent areas with pollution and increased water demand.

The advanced economic growth and social development of regions, especially in Asia, is driving demand for meat-based diets with the knock-on effect of increasing the cultivation of commodity crops (e.g., maize, soybean) for animal feed purposes whilst simultaneously elevating greenhouse gas emissions (Smith et al., 2007).

Climate change is challenging the sustainability of traditional cropping systems via stochastic temperature fluctuations, rising CO₂ levels, increased frequency of extreme weather events and by moving climate zones.

Faced with these multiple challenges, global agriculture must adopt more dynamic, efficient and sustainable production methods to increase food and fodder production to feed a growing population with fewer resources (FAO). Finally, climate changes

alone present several independent factors affecting the palette of disease and disease control. In particular, emerging pathogens (and pests) find favourable conditions in new regions and, secondly, the increased unpredictability of the weather is leading to an increase in and unpredictability of abiotic stresses, such as drought, heat and cold, thereby altering risk patterns for specific diseases (Chakraborty and Newton, 2011). In turn, the latter leads to the need to understand the subtle interactions between these abiotic stress factors, the hormones regulating the ability of the plant to adapt to abiotic stress and microorganisms exhibiting different lifestyles. These range from beneficial endophytes and symbionts to harmful pathogens, and indeed there are examples where the same microbe can act as a benign if not beneficial endophyte under some conditions and as a harmful pathogen under others. While plant diseases can devastate crops, they can often be controlled by cultural practice, disease resistance, biological control and the use of pesticides. A level of complexity for the biologist attempting to unravel the nature of plant defence and the influence of abiotic factors, however, lies in the fact that evolution is based on adaptation of the tools available. This means that many of the same tools and their regulators are

used in radically different processes in the plant where signal transduction processes regulate, e.g., growth and development as well as responses to biotic and abiotic stress. Examples of genes include those encoding different classes of receptors and components of signal transduction such as protein kinases as well as transcription factors. The regulators include phytohormones such as abscisic acid and cytokinins and ions such as Ca^{2+} . Plants are well capable of defending themselves against most pathogens through innate immunity, as the mechanisms of disease resistance are termed at the cellular level, and disease resistance is the most cost-effective and environmentally friendly way of protecting crops from diseases: the plants themselves do the job. However, successful pathogens overcome the plants' defences and, indeed, effective natural disease-resistance is often not available for the breeder. This is especially true for some hemibiotrophs and necrotrophs. In these cases, transgenic strategies may afford a viable alternative for crop production. Thus, the main aim of this book is to provide an in-depth overview of the current strategies available to develop transgenic-based disease-resistant plants, whilst also presenting the knowledge gained to date in this area and thus evaluating the potential of such strategies for disease control.

No magic bullet has been developed to combat fungal and bacterial diseases effectively, but an increased understanding of the underlying biology suggests several approaches, which may be combined – pyramided – to provide sustainable resistance. The strategies differ depending both on the organisms to be controlled as well as on the lifestyle strategy used by the pathogen and these are exemplified in the different chapters. Disease resistance (or, at this level, immunity) is triggered by the recognition in the host of molecules produced by the pathogen, or by the perturbations that pathogen

molecules have on plant immunity. The response event leads to inhibition of pathogen development through several independent physiological mechanisms which are activated concomitantly. Strategies for developing transgenic disease resistance attempt to exploit the recognition events, the signalling pathways regulating the immune response or the tools actually responsible for pathogen arrest. The different chapters of the first part of the book explore examples of these mechanisms in order to highlight the depth of knowledge gained from research in this field to date and demonstrate the potential for how this information can be exploited for biotechnological purposes for targeted plant breeding.

The second part of the book provides contrasting case studies of globally important crops, namely coffee, grapevine, potato and rice and their diseases, where effective and durable disease resistance to the major pathogens has not been achieved by conventional breeding, and describes the strategies which are being tested to assist pathogen defence of for these diverse crops.

A third section combines national and regional surveys of the actual use of transgenic crops including those conferring disease resistance in the field coupled with those currently in development and regulatory pipelines. This section of the book presents several case studies in which the authors in question were asked to answer the following questions: Which transgenic crops are grown? What is the economic and agronomic impact of these studies? Are there transgenic disease resistant crops among these? In addition, BT maize is grown in many countries to control European Corn Borer (*Ostrinia nubilalis*) and the corn earworm (*Helicoverpa zea*), but are there studies from their country showing enhanced resistance to Fusarium and reduced levels of mycotoxins compared to the non-transgenic crop (see (Clements et al., 2003; Duvick

2001)))? Is there promising work aiming to introduce disease-resistant crops in the foreseeable future? The reader is also referred to the pro-GM (genetically modified) lobby ISAAA's (International Service for the Acquisition of Agri-biotech Applications) annual reports <http://www.isaaa.org/> where the latest reports that "18 million farmers in 27 countries planted biotech crops in 2013, reflecting a five million, or three percent, increase in global biotech crop hectareage" (James, 2013). The penetration in the domestic market for some of these transgenic varieties exceeds 90% in some countries, according to the IAAA.

Several chapters impinge on the issues perceived by society as being important in relation to the extent that GM technology can be implemented, seen in relation to the approaches taken by those countries who are focused on the need both to thrive agronomically and economically whilst respecting public opinion on an issue of intense debate. It is no secret that there is considerable opposition against GM food amongst consumers worldwide, but the nature of this opposition differs geographically. This means that only about 30 countries use GM crops in commercial agriculture, although many others import GM plant products either for fodder, industrial purposes (including cotton) or other consumer products (e.g., cut flowers). Many more use GM microorganisms in industry for the production of enzymes or medicines, and there is little or no opposition against these applications. Within those countries which have adopted the GM technology, the main crops have often reached a very high level of penetration in the potential market: again, according to ISAAA (*ibid*), 96% rape (canola) is GM in Canada, in the USA over 90% maize, cotton and soybean are GM. In India and China, over 90% of the cotton is GM and in India 18 million farmers use GM. In other words, 90% of farmers using GM crops

are in developing countries (James, 2013). Economy is the driving force. Farmers cannot be expected to plant a crop for more than one season unless it pays – or they are persuaded.

The need to feed populations across the world is not equally distributed. The pressure is greatest in Asia which includes some of the world's most densely-populated countries. Among these are India and China, which are currently experiencing a rapid economic development that is leading to a shift from being largely vegetarian to omnivore, meaning that the requirement of fodder is increasing accordingly. It is estimated that the demand for rice will at least double by 2050 (see Chapter 12 by San Segundo et al.). Europeans (and North Americans) can (still) afford to import the food and fodder that cannot be produced locally, so the incentive to accept GM food is perhaps therefore lower (Brookes and Barfoot, 2013; Klümper and Qaim, 2014).

The wide and carefully regulated use of GMs in Argentina (see Chapter 13 by Bravo-Almonacid and Segretin) has led to the development of an innovative culture to develop new solutions aimed at local problems. Although all GM crops grown commercially at present originate from well-known international companies, e.g., Monsanto and Syngenta, many new crops (often termed "events") have been developed and are passing through the regulatory pipeline leading to commercial release (e.g., transgenic lines for PVY resistance in potato). There is a much lower incentive in Europe to develop GM crops; however, although the European moratorium reduces the incentive to look for GM solutions to solve serious problems, it stimulates alternative, more refined technologies, e.g., cisgenics (Holme et al., 2013), and gene targeting approaches such as CRISPR (clustered regularly interspaced short palindromic repeats) (Belhaj et al., 2013) in the host and to

target the pathogen using siRNA by HIGS (host-induced gene silencing) (Fairbairn et al., 2007; Ghag et al., 2014; Pliego et al., 2013). The development and potential for these “soft GM” technologies has led to a renewed debate in the EU. These issues are discussed in more detail in Chapters 1 and 4. See also European Academies Science Advisory Council, 2013 (Hartung and Schiemann, 2014).

Much disease resistance has been introduced by crossing in from related plant species. For example, in tomato the *Cf* genes conferring resistance to *Cladosporium fulvum* originate from, e.g., *Solanum pimpinellifolium* (Kruijt et al., 2004), various grasses in the tribe tritici to wheat (Kleinhofs et al., 2009) and *Solanum* spp (see Chapter 10). Plant breeding by introgression is intrinsically less precise than genetic engineering since many fragments of chromosome from the donor species are introgressed. Of course, errors also occur with genetic engineering, but these can be eliminated for further use by selecting only the verified clean insertion events. What might the consequences be if disease resistance is transferred? Is there any evidence that disease controls the populations of wild relatives? These are among the questions addressed in Chapter 20.

Organisation of the book

- An introduction to the problems of diseases, life style strategies and taxonomic groups of pathogens, the nature of plant immunity, and its exploitation for disease resistance.
- Biological strategies leading towards disease resistance. Which genes have been used to confer disease resistance and which genes and strategies offer the greatest hope for the future?
- Case studies – should certain crops be prioritized or avoided and which special problems are presented by these? Why is it especially advantageous to use transgenic strategies for these pathogens or crops?
- Status of transgenic crops around the world. Summaries of the current situation and prospects for the future for four countries on different continents where transgenic strategies are widely used.
- Transgenic disease resistance is not the only way of exploiting the knowledge gained from transgenic technology: discussed here is how the status and prospects of how the knowledge gained through experimental molecular genetics and related forms of biotechnology benefit plant protection. The examples chosen represent molecular breeding, induced resistance and biological control.

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Chapter 1

The Status and Prospects for Biotechnological Approaches for Attaining Sustainable Disease Resistance

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1.1 Introduction

Plant pathogens constitute major constraints on crop yield. In fact, a recent conservative estimate suggests that crop diseases are responsible for average annual yield losses of 10% (Chakraborty and Newton, 2011). For example, late blight of potatoes, caused by *Phytophthora infestans*, is estimated to cause annual losses of over €5 billion worldwide (Chapter 9). Another disease complex, Fusarium head blight, represents a more complex problem because the disease not only affects yield, but also contaminates food and fodder with mycotoxins which impact negatively on the health of both humans and livestock (Buerstmayr and Lemmens, 2015).

Several factors suggest that the negative impact of advancing plant diseases is increasing. For example, increasing areas of monoculture with reduced rotation to meet food productivity and profitability increases

crop vulnerability to pathogenic microorganisms. This is matched by the erosion of crop management systems as witnessed by, for instance, the alarming increase in fungicide resistance within cereal pathogens (Cools and Fraaije, 2012). Furthermore, the passive spread of opportunistic pathogens has increased as a consequence of globalisation, which has promoted open markets across continents. A recent example in Europe is the East Asian fungus *Hymenoscyphus fraxineus*, a saprophyte of *Fraxinus mandshurica*. This was not known as a pathogen before colonisation and subsequent decimation of European ash (*Fraxinus excelsior*) populations was observed in Eastern Europe about 20 years ago (reviewed by McKinney et al., 2014). Climate changes are also assisting the spread of crop pathogens, as evidenced by the devastating migration of coffee rust (caused by *Hemileia*

vastatrix) strains across the central and northern parts of South America into coffee plantations at higher altitudes, which were previously not attacked (Ghini et al., 2011).

But how can the alarming progression of crop diseases be halted? There are several methods which can contribute to the control of plant diseases. Good farm management is always a prerequisite, but other measures, especially disease resistance obtained by classical breeding and the use of pesticides, are highly important to secure food production worldwide. Furthermore, biological control and induced resistance are promising alternatives, especially in sustainable and integrated pest management strategies (Strange and Scott, 2005; Chapters 17 and 18). Yet, when taken individually, each of these methods has its limitations, and none can stand alone to solve all the problems in the effort to feed the increasing world population.

We believe that the employment of biotechnology-based approaches can contribute towards developing more effective and higher levels of disease control. The development of transgenic disease resistant plants is only one – albeit the most obvious – way of exploiting these biotechnological approaches (Campbell et al., 2002; Chen et al., 2012; Collinge et al., 2008; Collinge et al., 2010; Fuchs and Gonsalves, 2007; Gurr and Rushton, 2005a; Gurr and Rushton 2005b). Indirect biotechnological approaches, such as marker-assisted breeding, as well as the exploitation of association genetics and genomic selection, are closely-linked methods where the identification of genes responsible for specific traits can be used to develop gene-specific molecular markers to accelerate the process of conventional breeding and/or make it more efficient (Mammadov et al., 2007; Moose and Mumm 2008; Chapter 19, this volume). In addition, the development and understanding of alternative control measures, including induced disease resistance (Chapter 17) and biological control (Chapter 18), has benefited from the

application of multiple biotechnological approaches coupled with molecular and cellular approaches.

Among the thousands of species of plant pathogenic microorganisms, only a small minority have the capacity to infect a broad range of plant species. Most pathogens instead exhibit a high degree of host specificity and only cause disease in one or a few hosts. On the other hand, most hosts are susceptible to a number of pathogenic species. Therefore, different host-pathogen interactions represent different challenges, agronomically, biologically and ecologically. This chapter provides an overview of the mechanisms of disease resistance, which show the greatest potential for being targeted by GM approaches, and discusses how our increased understanding of the processes of plant defence can lead to improved disease control. In addition, the technical and biological constraints which are likely to hamper the successful development of GM crops are exemplified and discussed.

1.2 Factors to consider when generating disease-resistant crops

Disease resistance or, at the cellular level, plant immunity, is complex and depends on a plethora of independent but interacting physiological mechanisms. This section introduces important pathogen and host factors involved in the interaction between pathogens and their hosts. This is the platform for successful manipulation of the plant to achieve resistance.

1.2.1 The diversity and life styles of microbial pathogens

Many types of organisms can cause diseases in plants. Prokaryotes and eukaryotes themselves are highly diverse, and the latter encompasses three important kingdoms: Fungi, Chromista (oomycetes) and Protozoa