Eur J Plant Pathol (2018) 152:977–986
https://doi.org/10.1007/s10658-018-1439-2

SI: PLANT PATHOLOGY FOR INNOVATIVE AGROECOLOGY

Transgenic crops and beyond: how can biotechnology contribute to the sustainable control of plant diseases?

Biotechnology for plant disease control: GMOs and beyond

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Accepted: 13 February 2018 / Published online: 22 February 2018

Abstract Disease resistance is without argument the best technological approach to control diseases in plants since no management input is required by the grower once the resistant variety has been planted. The biggest problems in using disease resistance lie in the facts that effective sources of resistance are not available for many important diseases, especially those caused by necrotrophic pathogens; and that pathogen populations adapt to the utilisation of novel sources of resistance, most notably for air-borne biotrophic pathogens. Several biotechnological approaches have been developed to produce disease resistant plants, the most recent known as NBT – New Breeding Technologies. This review focuses on recent advances in those technologies which adapt the knowledge obtained using molecular genetic approaches for the study of plant-microbe interactions to combat plant diseases.

Keywords New breeding technologies · Gene editing · HIGS · Gene editing · CRISPR-Cas · Disease resistance · GMO · Marker-assisted selection · Cisgenic · New breeding technologies

Introduction

Plant diseases can devastate crops despite the best efforts of skilled farmers supported by the sustained efforts of plant breeders and the global agrochemical industry. Disease resistance is a major contributor to managing specific diseases in many agricultural systems, but the success in utilisation of resistance is under constant pressure from evolving and migrating pathogens. Whereas many sources of disease resistance (viz race-specific resistance genes) are available for many biotrophic and hemibiotrophic pathogens, the same cannot be said for many necrotrophic pathogens. Furthermore, it is very difficult and/or slow to breed some major crops, e.g., bananas, potatoes and perennials (mostly trees and bushes), and, especially for the perennials, market forces often favour specific varieties, such as Cavendish bananas and specific wine grape varieties (Collinge et al. 2016).

Biotechnological approaches are proven for some crop-disease combinations but are largely underutilised. Thus there are still only three widely publicised examples of crops used globally which exhibit useful enhanced disease resistance. The first two of these concern virus resistance: Papaya, *Carica papaya,* exhibiting resistance to Papaya Ringspot Virus PRSV and Summer squash, *Cucerbita pepo*, exhibiting resistance to three viruses, namely cucumber mosaic virus (CMV), zucchini yellow mosaic virus (ZYMV) and watermelon mosaic virus 2 (WMV 2) (see Fuchs and Gonsalves 2007)). The third example concerns maize plants (*Zea mays*) designed to confer insect resistance using the BT toxin Cry1Ab from the bacteria *Bacillus thuringiensis.* These transgenic, insect resistant varieties nevertheless exhibit a

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degree of disease resistance against Fusarium spp., especially *Fusarium verticillioides* (Collinge et al. 2008; Munkvold et al. 1999; Magg et al. 2002). Other transgenic crops have been developed and tested in field trials. In some cases these are also approved for release onto the market; examples include potatoes for virus resistance (Bravo-Almonacid and Segretin 2016; Kaniewski and Thomas 2004; Ricroch and Henard-Damave 2016).

I have previously reviewed the biology and technologies underlying many different approaches which aim to provide disease resistance using transgenic plants and therefore refer to these for detail of approaches and results (Chen et al. 2012; Collinge 2016; Collinge et al. 2008; Collinge et al. 2010; Collinge et al. 2016). This review will focus on a few recent successes in addition to the previous studies and in particular present biotechnological approaches aiming towards disease control which do not result in transgenic plants in the traditional sense.

How can the host inhibit pathogen development?

Plants possess many physiological tools which can inhibit microbial growth. As pathogenic microbes include both bacteria and eukaryotes from different kingdoms (e.g., Chromista, Protozoa and Opisthokonts – which include both animals and fungi), the physiology of the microbe varies enormously as does thus the nature of an appropriate antimicrobial tool. Plants produce specialised metabolites which can inhibit the growth of specific microbial taxa, but the microbes adapted to a particular host species possess mechanisms to disarm these antibiotic substances and/or suppress their production. Plants also produce antimicrobial proteins and enzymes which damage microbial cell walls. These defences can be constitutively produced or can be induced following pathogen attack. Collectively, these induced defences are now termed MAMP (or PAMP)-triggered immunity. The next level of defence includes the ability to undergo localised programmed cell death, the hypersensitive response, which is effective in inhibiting those biotrophic pathogens which require a living host. The mechanisms underlying this process, termed effector-triggered immunity, which are still not fully elucidated, are based on the ability of the host to recognise effector molecules or the result of their action on the host.

The study and elucidation of the processes described above has led to many attempts to strengthen these defences by adding or even removing specific components of host defence using molecular genetic, i.e. transgenic, approaches. Few of them have had any major effect on improving disease control. Many examples have been reviewed previously (most recently in Collinge 2016). Other approaches have looked at the tools used by the pathogen to infect successfully (Ahmed et al. 2016a). Of course it is not feasible to mutate a pathogen itself to reduce its ability to infect; the healthy pathogens would always outcompete the mutants, so no strategy analogous to the use of sterile male insects can be used: they compete for mates but do not contribute to future generations. It is, however, feasible to develop biological control agents using this approach (Jensen et al. 2016). But that is an entirely different story!

What is biotechnology?

In the context of plant disease resistance, biotechnology can be considered to encompass transgenic plants (GMO – genetically manipulated organisms) and "New Breeding Technologies" – NBT, and in a broader sense, biological control, which I do not address in this review. The GMO technologies have been under considerable negative pressure from certain NGOs worldwide and, though certain solutions are in wide use outside Europe, very few transgenic crops with an effect on plant diseases are used in Europe as several countries simply do not permit their use (Coca et al. 2016; Collinge 2016). In legislative terms, the permission to utilise genetic engineering in plant breeding has been implemented in essentially two parallel tracks which are exemplified by approaches taken in Europe and North America (Zetterberg and Björnberg 2017). The former is "process based", where the technology of genetic engineering is subject to regulation. Thus, release of GMO crops in the EU is subject to Directive 2001/18/EC concerning release and Regulation 2003/1829 concerning use of the products in food and or fodder before use in production. The latter approach (North American) is based on the physiological or biochemical characteristics of the organism and therefore is product based or phenotype based, which means that the alterations introduced into the crop are taken into account,

and not the means by which the changes were achieved (Camacho et al. 2014). In other words biotechnological methods for plant breeding are assumed to be substantially equivalent to their non-GM crops and therefore are not subjectable to special legislation (Zetterberg and Björnberg 2017). The legislation concerns the trait introduced and whether that can cause harm (e.g., ecological impact, poisonous). As European scientists are essentially currently prevented from using GMO technologies and are keen to see their efforts in understanding disease resistance translated into practise, various paths are being taken to ensure that the knowledge gained from molecular genetics approaches is being applied in other ways, collectively known as "New Breeding Technologies". However, the European legislation has been in place since 1990 and could not foresee the development of these new technologies, the products of which can be indistinguishable from natural mutants. Specifically, introduction of changes through the physical introduction of nucleotides sequences (oligonucleotides as DNA or RNA) whether through a transient process or through stable introduction (GMO) is covered by the GMO legislation. There is a current process to draw up a new legal framework to ensure that the new knowledge and approaches can be used for the benefit of European agriculture (Laaninen 2016).

Disease resistance by GMO

As reviewed previously (Chen et al. 2012; Collinge et al. 2008; Collinge et al. 2010; Collinge et al. 2016), several approaches have been, and are still being used to develop transgenic disease resistance. Briefly, several crops with specific diseases represent priority targets for GMO approaches. As mentioned in the introduction, these crops include bananas and potatoes since conventional plant breeding is not feasible or too slow. However, there are many diseases, especially those caused by necrotrophic fungi, for which no effective source of resistance is known. An important example is the Fusarium head blight complex of cereals which introduce mycotoxins to grain. In potatoes (*Solanum tuberosum*), the main issue is that new sources of resistance to the most devastating pathogen, *Phytophthora infestans*, are found in other species in the genus of *Solanum* and that conventional breeding is very slow (Jo et al. 2016).

The challenge for the bananas (and plantains, Musa spp) lies in the fact conventional plant breeding is not possible in this sterile, triploid species: breeding is done in tetraploid and diploid progenitors which are hybridised and hybrids selected (Ghag and Ganapathi 2017; Ortiz and Swennen 2014). The vast majority of bananas traded and consumed in the industrial world are of one clonal variety, namely Cavendish and its variants. The current focus internationally is on concerns over the threat posed by *Fusarium oxysporum* f. sp. *cubense* (*Foc*), the causal agent of Panama disease, since Cavendish bananas are susceptible to the new *Foc* Tropical Race 4 (TR4) (Ordonez et al. 2015; Ploetz 2015). History is repeating itself: *Foc* was originally described as a devastating disease of the Gros Michel cultivar, the exported banana until its demise in the 1950s (Butler 2013). The industry replaced Gros Michel plants with the Cavendish variety, which is resistant to the original *Foc* strain, believed to be race 1. There are several other important diseases affecting bananas which are arguably most significant in areas where bananas and plantains form an important part of the staple diet: Black Sigatoka caused by the Ascomycete *Pseudocercospora fijiensis* (formally known as *Mycosphaerella fijiensis*), Xanthomonas wilt caused by *Xanthomonas campestris* pv. *musacearum*, Moko disease caused by *Ralstonia solanacearum* and blood bacterial wilt caused by *R. syzygii* subsp. *celebesensis* (Blomme et al. 2017). We reviewed this area extensively in our previous paper (Collinge et al. 2016) and there is a new study reporting field trials utilising both transgenic and cisgenic GMO approaches (Dale et al. 2017). Briefly, several studies have demonstrated HIGS (see below) conferring resistance in transgenic banana to *Foc*. For the sake of record, biological control, in the broad sense, may also offer approaches for combating some of these diseases (Blomme et al. 2017; Kumakech et al. 2017; Xue et al. 2015). Interestingly, mutants have been derived from Cavendish bananas which exhibit promising resistance to *Foc* TR4 (Molina et al. 2016).

Biotechnologies for plant breeding

Biotechnological approaches to crop improvement can, in essence be divided into three categories, although there are variants which fall into grey zones. These categories are: (1) marker-assisted selection (MAS) in plant breeding, (2) genetic engineering, and (3) genome editing, also known as precision breeding (Sauer et al. 2016; Songstad et al. 2017;), which includes gene

editing technologies, e.g., CRISPR-Cas9 (Belhaj et al. 2013), Oligonucleotide Directed Mutagenesis (ODM). New Breeding Technologies (NBT) are defined as methods which use knowledge and techniques of molecular genetics to introduce new phenotypes into crops (Andersen et al. 2015). The term NBT has evolved as new technologies have emerged: "older" literature considered marker assisted selection as the main NBT technology (Brennan and Martin 2007). Subsequently zinc finger and related targeted mutagenesis technologies such as CRISPR-Cas9 have been invented and been added to the list and the consensus now is to no longer consider MAS as a NBT. Nevertheless, as the technologies are often based on molecular genetic methods and knowledge, I am including a few examples here. I refer to some recent views for details of the techniques and other applications in plants (Sauer et al. 2016; Songstad et al. 2017; Belhaj et al. 2013; Andersen et al. 2015).

Marker-assisted breeding

It is generally accepted that the process of plant breeding can be upgraded by increasing the efficiency of breeding technologies. Upgrades introduced include improved data tracking and the use of molecular markers (Langridge and Fleury 2011). The development of the use of DNA markers (marker-assisted selection – MAS) to assist conventional plant breeding programmes is undoubtedly a major advance for the field since it enables both more rapid and accurate process than breeding without MAS. The results of crossing are the same and natural: the difference lies in the ability to select for progeny which have acquired the desired trait whilst rejecting those progeny which have acquired undesirable traits. This is particularly useful in backcrossing programmes where genetic uniformity can be selected for. The use of markers in particular makes it feasible to pyramid many forms of disease resistances, which cannot be achieved easily by resistance phenotyping alone since the same phenotypes have to be used to track the introgression of different genes into the breeding lines.

A major barrier to achieving this in a "rational" way is a general lack of detailed knowledge of the genetic and physiological basis of relevant traits. In other words, the ability to map the genetic variation in plant genomes in any detail and link genetic differences to specific traits requires the ability to assign a trait to a specific gene (Poland and Rife 2012). The major advance in recent times thus lies in the ability to harness data generated from comparative next-generation sequencing programmes within important crop species (Deschamps et al. 2012; Salvi and Tuberosa 2015). Once agronomically important genes have been identified, gene-specific markers can be developed to assist introduction by crossing, or they can be introduced into breeding lines by cisgenic or marker-assisted breeding, or, perhaps in some cases by genome editing. Next-generation sequencing has also motivated the implementation of genome wide selection (GWS) in major crops, which, in contrast to MAS, can build up beneficial alleles around loci that only confer minor effects to disease resistance. Because of their inferior and/or indirect effects on disease resistance, such loci are not identified by classical QTL approaches. However, the aggregation of perhaps thousands of minor-effect loci may confer what is normally characterized as broad-host or horizontal disease resistance.

Genetic engineering

Genetic engineering is usually defined as a molecular process that introduces a gene or gene construct to a recipient organism usually at a random position by a process which could not occur spontaneously. The resulting strain is then termed a GMO (genetically modified organism). Although the origin of the gene is of no importance, in terms of the legal regulation of the process, technology is classified in two forms depending on whether the introduced gene originates from a different species – in which case they are transgenic – or from the same species – when the term cisgenic is applied (Holme et al. 2013). Many argue that cisgenesis should be considered a NBT and exempt from GMO law since, hypothetically, the product can be developed by natural crossing. This is particular pertinent for crops like potato where introgression of a resistance gene can take decades (Jo et al. 2016), or valuable cultivars such as wine grape varieties or Cavendish bananas. In the case of wine grape varieties, a certain level of genetic variation exists where the plants within a cultivar may be selected for adaptation for different climates and terroir around the world. In these cases it is argued that the introduction of one or few genes to provide pest or pathogen resistance can be considered not to represent so substantial a genetic change as to necessitate changing the name of the variety. Another promising approach which

has potential for perennial woody crops such as grapevines and fruit trees (where many of the same constraints apply) is the use of transgenic rootstocks for certain diseases. This can work where the transgene produces a product – whether protein or signal RNA molecule (see below) which can be transported in the vascular tissue. The perceived advantages are twofold, and are political and practical: the consumed product itself is not transgenic and different vine or fruit tree cultivars can be grafted onto the same transgenic rootstock (Cantu et al. 2016).

HIGS (host-induced gene silencing) and SIGS (spray-induced gene silencing)

RNA interference (RNAi) technologies have proven their worth in the development of transgenic virus resistant plants, where RNA molecules inhibit gene expression, often by destroying specific mRNA molecules. (Fuchs and Gonsalves 2007; Groen et al. 2017). HIGS (and SIGS) are methods which exploit gene silencing in an ingenious manner to control diseases caused by fungi (or insects and nematodes). This is made possible following the remarkable demonstration that at least some pathogenic fungi (and invertebrates) can take up double stranded RNA molecules from their local environment including the host.

The procedure to achieve HIGS (or SIGS) is first to identify genes which are essential for the growth of the pathogen in vitro and/or *in planta.* Thus the genes do not need to encode pathogenicity factors per se, but merely to be essential for the development of the pathogen. This can be achieved by "intelligent design", for instance by looking for fungicide targets, or screening for lethal or near-lethal mutants in the pathogen. As mentioned above, clearly, any mutant strain of a pathogen would be outcompeted in nature or the field by unaffected strains. Gene constructs transcribed to make double stranded or hairpin RNA molecule which targets the essential pathogen function consumers are prepared. The essential test for the strategy is whether an RNAi construct or small RNAs can be designed that can be introduced into the pathogen which knock out the function in vivo, thereby reducing viability. These constructs are then introduced into the host making transgenic strains for HIGS. For example, by using a double-stranded RNA hairpin construct, the expression of the *Foc* SGE1 gene, a phloem effector protein of the SIX group, was reduced and pathogenicity in banana reduced by approximately 95%, compared to that of the wild-type strain (Fernandes et al. 2016). This result implies that this is an appropriate target for HIGS.

HIGS has also been demonstrated for insects (Taning et al. 2016) and nematode pests (Niu et al. 2010). This approach was initially used to make transgenic banana and barley (*Hordeum vulgare*) plants exhibiting resistance against Fusarium and powdery mildew pathogens (Nowara et al. 2010; Pliego et al. 2013; Ahmed et al. 2016b; Koch et al. 2013; Ghag et al. 2014). More recent and promising examples include maize (*Zea mays*) with reduced levels of aflatoxins (Thakare et al. 2017) and wheat with resistance to stripe rust, caused by *Puccinia striiformis* (Qi et al. 2017).

SIGS is attractive for several reasons. As a research tool it is easier to prepare and spray double-stranded RNA (dsRNA) molecules onto a plant than it is to make a transgenic plant, and in the politically negative climate surrounding GMOs in much of Europe, spraying with a chemical is seen to be more acceptable (Mitter et al. 2017). Furthermore, the time-consuming process of making the transgene can be bypassed in order to determine whether the approach is likely to work, saving resources. Koch and colleagues had previously demonstrated that HIGS designed using fungal genes encoding essential components for ergosterol production could protect the transgenic barley lines against the head blight pathogen *Fusarium graminearum* (Koch et al. 2013). In a subsequent study they demonstrated that the same pathogen could be targeted using SIGS, although the efficiency of control was lower (Koch et al. 2016). Likewise, several recent studies have demonstrated that this approach can also be used for combating viruses (Mitter et al. 2017).

As for CRISPR-Cas described below, a potential pitfall with HIGS and SIGS technology is the risk that a specific construct will only target a single pathogen genotype and efforts need to be taken to ensure that allelic variation within a particular pathogen species is covered, and at the same time, that the sequences targeted are not so conserved as to be present in beneficial microbes. The R&D efforts are therefore not inconsiderable to ensure the product is targeted correctly.

Natural mutants conferring disease resistance

There are relatively few examples of recessive resistance genes which confer disease resistance in crop

plants (van Schie and Takken 2014). Loss of function can, of course, be readily achieved by conventional mutation, though these are often associated with deleterious effects, typically manifested as yield loss (Glazebrook 2001; Schulze-Lefert and Vogel 2000). Gain of function mutation can also be achieved by natural processes and selected for by MAS but the rate by which this can occur is inherently lower and therefore not considered feasible in practice. The best known of the former is the *mlo* gene which confers resistance in barely (*Hordeum vulgare*) against the powdery mildew fungus *Blumeria graminis*. The original barley *mlo* mutant, which has been used for decades in spring barley varieties, was obtained originally as a radiation-induced mutation, though it has since been found in Ethiopian land races of barley (Acevedo-García et al. 2014; Ge et al. 2016; Jørgensen 1992). It should be noted that the *mlo* mutation provides enhanced susceptibility of barely to certain hemibiotrophic or necrotrophic fungi and may be predicted to do so in wheat too (Jarosch et al. 2003; McGrann et al. 2014). After many decades of use, it was discovered that the pea resistance gene *er*1, which confers resistance to *Erysiphe pisi* is in fact an *mlo* homologue (Pavan et al. 2011) and *ol*-2 in tomato confers resistance to *Oidium neolycopersici* (Bai et al. 2008; Nekrasov et al. 2017). Many other genes conferring resistance to pathogens are dominant resistance genes and are associated with biotrophic (or to a lesser extent hemibiotrophic) pathogens such as those causing rusts, powdery and downy mildews. New specificities in these cases often arise following gene duplication (McHale et al. 2006).

Gene editing and resistant plants

Genome editing or precision breeding concerns a process which changes the sequence of the target gene. This can result in sequence substitutions, small deletions or insertions which can change the nature of proteins, or result in complete loss of function of the specific gene product. These changes can be achieved using several technologies of which the currently best known are transcription activator–like effector nuclease (TALEN) and clustered, regularly interspaced, short palindromic repeats (CRISPR-Cas9) (Belhaj et al. 2013). Gain of function has also been achieved using CRISPR-Cas9 and has great potential (Chen et al. 2017).

There are several examples of improved disease resistance achieved using different genome editing approaches. Thus both CRISPR-Cas9 and a related technology TALEN were used in hexaploid wheat (*Triticum aestivum)* to create *mlo* varieties by knocking out the three homeologous genes (Wang et al. 2014). Likewise this strategy is being used in grapevine (*Vitis vinifera*) to confer resistance to *E. necator* (Pessina et al. 2016) and tomato against powdery mildew (Nekrasov et al. 2017), where resistance has been achieved by suppressing the expression of four genes by RNA interference. The valuable knowledge gained from this targeted genetic approach can be applied to conventional MAS breeding programmes using TILLING, and indeed mutants in individual homeologous wheat *mlo* genes have been identified in the three component genomes from mutant populations in hexaploid wheat (Acevedo-Garcia et al. 2017).

One of the drawbacks of classical CRISPR-Cas9 lies in the need to make transgenic lines to introduce the CRISPR-Cas9 ribonuclease into the genome of the target plant. For this reason alone, breeding lines developed using this technology have considered to be transgenic, even though it is possible to lose the CRISPR-Cas9 construct through segregation in progeny whilst retaining the engineered mutation. A recent development bypasses the need to make a GMO: instead the enzyme complex has been introduced transiently into protoplasts of several diverse and important crop and model plant species (Arabidopsis, lettuce (*Lactuca sativa*), petunia (*P. x hybrida*), rice (*Oryza sativa*), tobacco (*Nicotiana tabacum*) and wheat), with the mutants recovered from the regenerated plants (Subburaj et al. 2016; Woo et al. 2015; Zhang et al. 2016). This approach has been demonstrated recently for apple (*Malus domestica*) and grapevine (*Vitis vinifera*) to target host genes necessary for infection with the fireblight bacteria, *Erwinia amylovora* and the powdery mildew fungus *Erysiphe necator*, respectively (Malnoy et al. 2016).

Citrus crops suffer from several important bacterial diseases including canker and Huanglongbing (HLB) caused by *Xanthomonas citri* and *Candidatus* Liberibacter, respectively. Breeding citrus for resistance can be challenging and time-consuming for several reasons. The CsLOB1 gene encodes a transcription factor which is manipulated by *Xanthomonas* spp. to facilitate infection. In a recent study, the CsLOB1 genes (there are

two) of Duncan grapefruit (*Citrus x paradisi*) were targeted by CRISPR-Cas9 and resistant lines identified. The regenerated plants were apparently phenotypically normal and no potential off-target genes had been affected, i.e. gene sequences close to the target (Jia et al. 2017). The perceived risk of off-target mutation is a drawback of gene-editing technologies. Indeed this is a serious concern for the use of CRISPR-Cas9 in therapeutic medicine (Schaefer et al. 2017). This is ultimately less of a concern in plant breeding for the simple reason that such off-target mutants can be discarded.

It is feasible to use mutagenesis and CRISPR-Cas9 to target susceptibility factors in the host, in other words, genes where the functional allele enables the pathogen to infect (van Schie and Takken 2014). The examples detailed above all represent genes where a knock-out (or knock-down) results in resistance, in other words recessive resistance genes. This approach is less appropriate for dominant resistance genes, though as our understanding of the nature of disease resistance increases, examples will undoubtedly arise. The approach is particularly valuable when a family of genes contributes to the phenotype and it is necessary to knock-out two or more genes at once, since, in these cases, the stone can kill two or more birds, exemplified by *mlo* and *CsLOB1* above, which is more arduous using mutation coupled with MAS.

Regulation of NBT

There is currently a political process aiming to update biotech legislation in Europe to allow the use of new technologies. The industrial lobby group NBT Platform has prepared an informative analysis and guide to the different technologies (Anon 2013). Several potentially subtle and therefore controversial criteria are proposed to determine whether a technology is GMO. For example, in their analysis, any artificial change of less than 20 bp could have occurred naturally by random mutation and therefore should not be considered to be GMO irrespective how the sequence was altered. There is a current international discussion as to which of these biotechnologies can be defined as producing GMOs (Andersen et al. 2015; Laaninen 2016). Some authors consider the use of DNA markers and association genetics as NBTs, whereas others draw the line at gene targeting approaches such as CRISPR-Cas and TALENS. It is important that the communication about the technologies, and their limitations and risks, are taken seriously to avoid the situation encountered with GMO in Europe.

Closing remarks

The planet is facing increasing challenges, most of which are caused by mankind. The population is projected to increase a further 15–20% by the middle of the present century. Globally, the consumption of meat has doubled over the last half century. Populations are more urban and often occupy best agricultural land, and use water. Climate changes provide a wealth of challenges to agriculture. Global trade increases the rate of migration of pests and pathogens according to OECD and FAO statistics. Disease resistance is the best means of controlling pathogens when it is available, but globally our efforts into understanding the biology of plant-pathogen interactions and the search for natural disease resistance in our threatened genetic heritage in locally adapted land races is hampered by inadequate resources. In this paper I have reviewed some recent and very promising developments which have arisen largely from recent advances in understanding the nature of plant-microbe interactions. Over the last couple of decades, the biotechnological approaches to improving the control of pathogens has moved from adding single genes which encode antimicrobial factors to exploitation of our understanding of the regulation of cellular processes and the tools used by pathogens to thwart the natural defences of the host. The two most promising novel technologies concern the exploitation of signalling RNA molecules and of a form of natural site directed mutagenesis. The former is gene silencing and was first discovered as the tool used by plants to defend themselves against viruses. This tool has been exploited for two decades to provide transgenic virus resistant plants, and the concept is now being developed as a spray to control viruses and fungi. The second technique gene editing or site directed mutagenesis was discovered as a pathogenicity mechanism used by certain phytopathogenic bacteria and is now being developed as a non-transgenic technology to make targeted mutations in plants to control pathogens. HIGS, SIGS and gene editing are smart approaches with great potential, but these techniques suffer from potential off-target issues (Schaefer et al. 2017) – in other words they may target other genes in the host plant or beneficial organisms (e.g., endophytes or mycorrhiza), or, in the case of SIGS, in consumers. Of course, although these issues are also valid for any pesticide in use, they may hinder broad application of this technology. Ultimately, scientists failed to convince the European public that

transgenic technologies are not harmful. The major challenge is to prove and then explain to the public and legislators that other tools developed are safe and will benefit mankind in a sustainable manner. Efforts to prevent public refusal of these new biotechnologies have to be made now at the early stage of technology development to convince society of their safety and benefit for facilitating actual application in (especially in relation to the GMO story) European agriculture.

Acknowledgements I am grateful to my colleagues Solveig K Christiansen, Rosanna C. Hennessy, Christian S. Jensen, Athina Koutouleas, Edward C. Rojas Tayo and Jeppe Thulin Østerberg for valuable comments and input.

Compliance with ethical standards

Conflict of interest No external funding is involved in this study therefore I cannot envisage any potential conflicts of interest (financial or non-financial).

Human and animals studies This review article does not contain any studies with human participants or animals performed by any of the authors.

References

Acevedo-García, J., Kusch, S., & Panstruga, R. (2014). Magical mystery tour: MLO proteins in plant immunity and beyond. *New Phytologist, 204*, 273–281. https://doi.org/10.1111/nph.12889.

Acevedo-Garcia, J., Spencer, D., Thieron, H., Reinstädler, A., Hammond-Kosack, K., Phillips, A. L., et al. (2017). Mlo-based powdery mildew resistance in hexaploid bread wheat generated by a non-transgenic TILLING approach. *Plant Biotechnology Journal, 15*(3), 367–378. https://doi.org/10.1111/pbi.12631.

Ahmed, A. A., McLellan, H., Aguilar, G. B., Hein, I., Thordal-Christensen, H., & Birch, P. R. J. (2016a). Engineering barriers to infection by undermining pathogen effector function or by gaining effector recognition. In D. B. Collinge (Ed.), *Plant pathogen resistance biotechnology* (pp. 23–50). New York: WILEY Blackwell.

Ahmed, A. A., Pedersen, C., & Thordal-Christensen, H. (2016b). The barley powdery mildew effector candidates CSEP0081 and CSEP0254 promote fungal infection success. *PLoS One, 11*(6). https://doi.org/10.1371/journal.pone.0157586.

Andersen, M. M., Landes, X., Xiang, W., Anyshchenko, A., Falhof, J., Østerberg, J. T., et al. (2015). Feasibility of new breeding techniques for organic farming. *Trends in Plant Science, 20*, 426–434.

Anon (2013). The regulatory status of plants resulting from New Breeding Technologies. http://www.nbtplatform.org/. Accessed 25/10–17 2017.

Bai, Y. L., Pavan, S., Zheng, Z., Zappel, N. F., Reinstadler, A., Lotti, C., et al. (2008). Naturally occurring broad-spectrum powdery mildew resistance in a central American tomato accession is caused by loss of Mlo function. *Molecular Plant-Microbe Interactions, 21*(1), 30–39.

Belhaj, K., Chaparro-Garcia, A., Kamoun, S., & Nekrasov, V. (2013). Plant genome editing made easy: Targeted mutagenesis in model and crop plants using the CRISPR/Cas system. *Plant Methods, 9*, 39. https://doi.org/10.1186/1746-4811-9-39.

Blomme, G., Dita, M., Jacobsen, K. S., Pérez Vicente, L., Molina, A., Ocimati, W., et al. (2017). Bacterial diseases of bananas and Enset: Current state of knowledge and integrated approaches toward sustainable management. [review]. *Frontiers in Plant Science, 8*(1290). https://doi.org/10.3389/fpls.2017.01290.

Bravo-Almonacid, F. F., & Segretin, M. F. (2016). Status of transgenic crops in Argentina. In D. B. Collinge (Ed.), *Biotechnology for plant disease control* (pp. 275–283). New York: Wiley Blackwell.

Brennan, J. P., & Martin, P. J. (2007). Returns to investment in new breeding technologies. *Euphytica, 157*, 337–349.

Butler, D. (2013). Fungus threatens top banana. *Nature, 504*, 195–196.

Camacho, A., Van Deynze, A., Chi-Ham, C., & Bennett, A. B. (2014). Genetically engineered crops that fly under the US regulatory radar. [opinion and comment]. *Nature Biotechnology, 32*(11), 1087–1091. https://doi.org/10.1038/nbt.3057.

Cantu, D., Roper, M. C., Powell, A. L. T., & Labavitch, J. M. (2016). To long life and good health: untangling the complexity of grape diseases to develop pathogen resistant varieties. In D. B. Collinge (Ed.), *Plant Pathogen Resistance Biotechnology (pp. 193-215)*. New York: Wiley Blackwell.

Chen, Y. J., Lyngkjaer, M. F., & Collinge, D. B. (2012). Future prospects for genetically engineering disease resistance plants. In G. Sessa (Ed.), *Molecular plant immunity* (pp. 251–275). New York: Wiley.

Chen, Y. Y., Wang, Z., Ni, H., Xu, Y., Chen, Q. J., & Jiang, L. (2017). CRISPR/Cas9-mediated base-editing system efficiently generates gain-of-function mutations in Arabidopsis. [journal article]. *Science China Life Sciences, 60*(5), 520–523. https://doi.org/10.1007/s11427-017-9021-5.

Coca, M., López-García, B., & Segundo, B. S. (2016). Transgenic crops in Spain. In D. B. Collinge (Ed.), *Plant pathogen resistance biotechnology* (pp. 295–304). New York: Wiley Blackwell.

Collinge, D. B. (2016). *Biotechnology for plant disease control* (first ed.). New York: Wiley Blackwell.

Collinge, D. B., Lund, O. S., & Thordal-Christensen, H. (2008). What are the prospects for genetically engineered, disease resistant plants? *European Journal of Plant Pathology, 121*(3), 217–231.

Collinge, D. B., Jørgensen, H. J. L., Lund, O. S., & Lyngkjær, M. F. (2010). Engineering pathogen resistance in crop plants - current trends and future prospects. *Annual Review of Phytopathology, 48*, 269–291.

Collinge, D. B., Mullins, E., Jensen, B., & Jørgensen, H. J. L. (2016). The status and prospects for biotechnological approaches to attaining sustainable disease resistance. In D. B.

Collinge (Ed.), *Biotechnology for plant disease control* (pp. 1–20). New York: Wiley Blackwell.

Dale, J. L., James, A., Paul, J.-Y., Khanna, H., Smith, M., Peraza-Echeverria, S., et al. (2017). Transgenic Cavendish bananas with resistance to fusarium wilt tropical race 4. *Nature Communications, 8*(1), 1496. https://doi.org/10.1038/s41467-017-01670-6.

Deschamps, S., Llaca, V., & May, G. D. (2012). Genotyping-by-sequencing in plants. *Biology, 1*(3), 460.

Fernandes, J. S., Angelo, P. C. S., Cruz, J. C., Santos, J. M. M., Sousa, N. R., & Silva, G. F. (2016). Post-transcriptional silencing of the SGE1 gene induced by a dsRNA hairpin in *Fusarium oxysporum* f. sp *cubense*, the causal agent of Panama disease. *Genetics and Molecular Research, 15*(2), gmr.15027941. https://doi.org/10.4238/gmr.15027941.

Fuchs, M., & Gonsalves, D. (2007). Safety of virus-resistant transgenic plants two decades after their introduction: Lessons from realistic field risk assessment studies. *Annual Review of Phytopathology, 45*(1), 173–202.

Ge, X. T., Deng, W. W., Lee, Z. Z., Lopez-Ruiz, F. J., Schweizer, P., & Ellwood, S. R. (2016). Tempered mlo broad-spectrum resistance to barley powdery mildew in an Ethiopian landrace. *Scientific Reports, 6*. https://doi.org/10.1038/srep29558.

Ghag, S. B., & Ganapathi, T. R. (2017). Genetically modified bananas: To mitigate food security concerns. *Scientia Horticulturae, 214*(Supplement C), 91–98. https://doi.org/10.1016/j.scienta.2016.11.023.

Ghag, S. B., Shekhawat, U. K. S., & Ganapathi, T. R. (2014). Host-induced post-transcriptional hairpin RNA-mediated gene silencing of vital fungal genes confers efficient resistance against fusarium wilt in banana. *Plant Biotechnology Journal, 12*(5), 541–553. https://doi.org/10.1111/pbi.12158.

Glazebrook, J. (2001). Genes controlling expression of defense responses in Arabidopsis-2001 status. *Current Opinion in Plant Biology, 4*(4), 301–308.

Groen, S. C., Wamonje, F. O., Murphy, A. M., & Carr, J. P. (2017). Engineering resistance to virus transmission. *Current Opinion in Virology, 26*(Supplement C), 20–27. https://doi.org/10.1016/j.coviro.2017.07.005.

Holme, I. B., Wendt, T., & Holm, P. B. (2013). Intragenesis and cisgenesis as alternatives to transgenic crop development. *Plant Biotechnology Journal, 11*(4), 395–407.

Jarosch, B., Jansen, M., & Schaffrath, U. (2003). Acquired resistance functions in mlo barley, which is hypersusceptible to Magnaporthe grisea. *Molecular Plant-Microbe Interactions, 16*(2), 107–114.

Jensen, D. F., Karlsson, M., Sarrocco, S., & Vannacci, G. (2016). Biological control using microorganisms as an alternative to disease resistance. In D. B. Collinge (Ed.), *Plant pathogen resistance biotechnology* (pp. 341–363). New York: Wiley Blackwell.

Jia, H., Zhang, Y., Orbović, V., Xu, J., White, F. F., Jones, J. B., et al. (2017). Genome editing of the disease susceptibility gene CsLOB1 in citrus confers resistance to citrus canker. *Plant Biotechnology Journal, 15*(7), 817–823. https://doi.org/10.1111/pbi.12677.

Jo, K. R., Zhu, S. Z., Bai, Y., Hutten, R. C. B., Kessel, G. J. T., Vleeshouwers, V. G. A. A., et al. (2016). Problematic crops: 1. Potatoes: Towards sustainable potato late blight resistance by Cisgenic R gene pyramiding. In D. B. Collinge (Ed.), *Biotechnology for plant disease control* (pp. 171–191). New York: Wiley Blackwell.

Jørgensen, J. H. (1992). Discovery, characterization and exploitation of Mlo powdery mildew resistance in barley. *Euphytica, 63*, 141–152.

Kaniewski, W. K., & Thomas, P. E. (2004). The potato story. *AgBioforum, 7*(1&2), 41–46.

Koch, A., Kumar, N., Weber, L., Keller, H., Imani, J., & Kogel, K.-H. (2013). Host-induced gene silencing of cytochrome P450 lanosterol C14α-demethylase–encoding genes confers strong resistance to fusarium species. *Proceedings of the National Academy of Sciences, 110*(48), 19324–19329. https://doi.org/10.1073/pnas.1306373110.

Koch, A., Biedenkopf, D., Furch, A. C. U., Weber, L., Rossbach, O., Abdellatef, E., et al. (2016). An RNAi-based control of fusarium graminearum infections through spraying of long dsRNAs involves a plant passage and is controlled by the fungal silencing machinery. *PLoS Pathogens, 12*(10), e1005901. https://doi.org/10.1371/journal.ppat.1005901.

Kumakech, A., Jørgensen, H. J. L., Collinge, D. B., Edema, R., & Okori, P. (2017). *Azadirachta indica* reduces black Sigatoka in east African highland banana by direct antimicrobial effects against *Mycosphaerella fijiensis* without inducing resistance. *Journal of Agricultural Science, 9*(4). https://doi.org/10.5539/jas.v9n4p61.

Laaninen, T. (2016). New plant-breeding techniques: Applicability of GM rules. *EPRS, PE, 582*, 018.

Langridge, P., & Fleury, D. (2011). Making the most of 'omics' for crop breeding. *Trends in Biotechnology, 29*(1), 33–40. https://doi.org/10.1016/j.tibtech.2010.09.006.

Magg, T., Melchinger, A. E., Klein, D., & Bohn, M. (2002). Relationship between European corn borer resistance and concentration of mycotoxins produced by fusarium spp. in grains of transgenic Bt maize hybrids, their isogenic counterparts, and commercial varieties. *Plant Breeding, 121*(2), 146–154. https://doi.org/10.1046/j.1439-0523.2002.00659.x.

Malnoy, M., Viola, R., Jung, M.-H., Koo, O.-J., Kim, S., Kim, J.-S., et al. (2016). DNA-free genetically edited grapevine and apple protoplast using CRISPR/Cas9 ribonucleoproteins. [original research]. *Frontiers in Plant Science, 7*(1904). https://doi.org/10.3389/fpls.2016.01904.

McGrann, G. R. D., Stavrinides, A., Russell, J., Corbitt, M. M., Booth, A., Chartrain, L., et al. (2014). A trade off between mlo resistance to powdery mildew and increased susceptibility of barley to a newly important disease, Ramularia leaf spot. *Journal of Experimental Botany, 65*, 1025–1037. https://doi.org/10.1093/jxb/ert452.

McHale, L., Tan, X. P., Koehl, P., & Michelmore, R. W. (2006). Plant NBS-LRR proteins: Adaptable guards. *Genome Biology, 7*(4). https://doi.org/10.1186/gb-2006-7-4-212.

Mitter, N., Worrall, E. A., Robinson, K. E., Xu, Z. P., & Carroll, B. J. (2017). Induction of virus resistance by exogenous application of double-stranded RNA. *Current Opinion in Virology, 26*(Supplement C), 49–55. https://doi.org/10.1016/j.coviro.2017.07.009.

Molina, A. B., Sinohin, V. O., Fabregar, E. G., Ramillete, E. B., Loayan, M. M., & Chao, C. P. (2016). Field resistance of Cavendish somaclonal variants and local banana cultivars to tropical race 4 of fusarium wilt in the Philippines. In *International Society for Horticultural Science (ISHS),*

Leuven, Belgium (1114 ed., pp. 227-230). https://doi.org/10.17660/ActaHortic.2016.1114.31.

Munkvold, G. P., Hellmich, R. L., & Rice, L. G. (1999). Comparison of fumonisin concentrations in kernels of transgenic Bt maize hybrids and nontransgenic hybrids. *Plant Disease, 83*, 130–138.

Nekrasov, V., Wang, C., Win, J., Lanz, C., Weigel, D., & Kamoun, S. (2017). Rapid generation of a transgene-free powdery mildew resistant tomato by genome deletion. *Scientific Reports, 7*(1), 482. https://doi.org/10.1038/s41598-017-00578-x.

Niu, J. H., Jiang, H., Xu, J.-M., Guo, Y. D., & Liu, Q. (2010). RNAi technology extends its reach: Engineering plant resistance against harmful eukaryotes. *African Journal of Biotechnology, 9*(45), 7573–7582.

Nowara, D., Gay, A., Lacomme, C., Shaw, J., Ridout, C., Douchkov, D., et al. (2010). HIGS: Host-induced gene silencing in the obligate biotrophic fungal pathogen *Blumeria graminis*. *Plant Cell, 22*(9), 3130–3141.

Ordonez, N., Seidl, M. F., Waalwijk, C., Drenth, A., Kilian, A., Thomma, B. P. H. J., et al. (2015). Worse comes to worst: Bananas and Panama disease—When plant and pathogen clones meet. *PLoS Pathogens, 11*(11), e1005197. https://doi.org/10.1371/journal.ppat.1005197.

Ortiz, R., & Swennen, R. (2014). From crossbreeding to biotechnology-facilitated improvement of banana and plantain. *Biotechnology Advances, 32*(1), 158–169.

Pavan, S., Schiavulli, A., Appiano, M., Marcotrigiano, A. R., Cillo, F., Visser, R. G. F., et al. (2011). Pea powdery mildew er1 resistance is associated to loss-of-function mutations at a MLO homologous locus. [journal article]. *Theoretical and Applied Genetics, 123*(8), 1425–1431. https://doi.org/10.1007/s00122-011-1677-6.

Pessina, S., Lenzi, L., Perazzolli, M., Campa, M., Costa, S. U., Valè, G., et al. (2016). Knockdown of MLO genes reduces susceptibility to powdery mildew in grapevine. *Horticulture Research, 3*. https://doi.org/10.1038/hortres.2016.16.

Pliego, C., Nowara, D., Bonciani, G., Gheorghe, D. M., Xu, R., Surana, P., et al. (2013). Host-induced gene silencing in barley powdery mildew reveals a class of ribonuclease-like effectors. *Molecular Plant-Microbe Interactions, 26*(6), 633–642.

Ploetz, R. C. (2015). Management of Fusarium wilt of banana: A review with special reference to tropical race 4. *Crop Protection, 73*(Supplement C), 7–15. https://doi.org/10.1016/j.cropro.2015.01.007.

Poland, J. A., & Rife, T. W. (2012). Genotyping-by-sequencing for plant breeding and genetics. *The Plant Genome, 5*(3), 92–102. https://doi.org/10.3835/plantgenome2012.05.0005.

Qi, T., Zhu, X., Tan, C., Liu, P., Guo, J., Kang, Z., et al. (2017). Host-induced gene silencing of an important pathogenicity factor PsCPK1 in *Puccinia striiformis* f. sp. *tritici* enhances resistance of wheat to stripe rust. *Plant Biotechnology Journal*. https://doi.org/10.1111/pbi.12829.

Ricroch, A. E., & Henard-Damave, M. (2016). Next biotech plants: New traits, crops, developers and technologies for addressing global challenges. *Critical Reviews in Biotechnology, 36*(4), 675–690.

Salvi, S., & Tuberosa, R. (2015). The crop QTLome comes of age. *Current Opinion in Biotechnology, 32*(Supplement C), 179–185. https://doi.org/10.1016/j.copbio.2015.01.001.

Sauer, N. J., Mozoruk, J., Miller, R. B., Warburg, Z. J., Walker, K. A., Beetham, P. R., et al. (2016). Oligonucleotide-directed mutagenesis for precision gene editing. *Plant Biotechnology Journal, 14*(2), 496–502. https://doi.org/10.1111/pbi.12496.

Schaefer, K. A., Wu, W. H., Colgan, D. F., Tsang, S. H., Bassuk, A. G., & Mahajan, V. B. (2017). Unexpected mutations after CRISPR-Cas9 editing in vivo. [correspondence]. *Nature Methods, 14*(6), 547–548. https://doi.org/10.1038/nmeth.4293.

Schulze-Lefert, P., & Vogel, J. (2000). Closing the ranks to attack by powdery mildew. *Trends in Plant Science, 5*(8), 343–348.

Songstad, D. D., Petolino, J. F., Voytas, D. F., & Reichert, N. A. (2017). Genome editing of plants. *Critical Reviews in Plant Sciences, 36*(1), 1–23. https://doi.org/10.1080/07352689.2017.1281663.

Subburaj, S., Chung, S. J., Lee, C., Ryu, S.-M., Kim, D. H., Kim, J.-S., et al. (2016). Site-directed mutagenesis in petunia × hybrida protoplast system using direct delivery of purified recombinant Cas9 ribonucleoproteins. [journal article]. *Plant Cell Reports, 35*(7), 1535–1544. https://doi.org/10.1007/s00299-016-1937-7.

Taning, C. N. T., Andrade, E. C., Hunter, W. B., Christiaens, O., & Smagghe, G. (2016). Asian citrus psyllid RNAi pathway–RNAi evidence. [article]. *Scientific Reports, 6*, 38082. https://doi.org/10.1038/srep38082.

Thakare, D., Zhang, J., Wing, R. A., Cotty, P. J., & Schmidt, M. A. (2017). Aflatoxin-free transgenic maize using host-induced gene silencing. *Science Advances, 3*(3). https://doi.org/10.1126/sciadv.1602382.

van Schie, C. C. N., & Takken, F. L. W. (2014). Susceptibility genes 101: How to be a good host. *Annual Review of Phytopathology, 52*(1), 551–581. https://doi.org/10.1146/annurev-phyto-102313-045854.

Wang, Y. P., Cheng, X., Shan, Q. W., Zhang, Y., Liu, J. X., Gao, C. X., et al. (2014). Simultaneous editing of three homoeoalleles in hexaploid bread wheat confers heritable resistance to powdery mildew. *Nature Methods, 32*(9), 947–951.

Woo, J. W., Kim, J., Kwon, S. I., Corvalan, C., Cho, S. W., Kim, H., et al. (2015). DNA-free genome editing in plants with preassembled CRISPR-Cas9 ribonucleoproteins. [research]. *Nature Methods, 33*(11), 1162–1164. https://doi.org/10.1038/nbt.3389.

Xue, C., Ryan Penton, C., Shen, Z. Z., Zhang, R. F., Huang, Q. W., Li, R., et al. (2015). Manipulating the banana rhizosphere microbiome for biological control of Panama disease. *Scientific Reports, 5*, 11124.

Zetterberg, C., & Björnberg, K. E. (2017). Time for a new EU regulatory framework for GM crops? [journal article]. *Journal of Agricultural and Environmental Ethics, 30*(3), 325–347. https://doi.org/10.1007/s10806-017-9664-9.

Zhang, Y., Liang, Z., Zong, Y., Wang, Y., Liu, J., Chen, K., et al. (2016). Efficient and transgene-free genome editing in wheat through transient expression of CRISPR/Cas9 DNA or RNA. [article]. *7*, 12617. doi:https://doi.org/10.1038/ncomms12617.