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Abstract: The growing human population will require a significant increase in agricultural production. Changes in the climatic and environmental conditions under which our crops are grown has resulted in the appearance of new diseases, while genetic changes within the pathogen have resulted in the loss of previously effective sources of resistance. We need to intensify the identification and characterisation of new sources of resistance, and use our knowledge of plant-pathogen interactions to develop novel approaches to achieve effective resistance within our crops. Advanced genetic and statistical methods of analysis enable global screens of genetic diversity to be undertaken, identifying new resistance genes, while studies of plant-pathogen interactions are uncovering the mechanisms by which the resistance is achieved. Informed deployment of major, race-specific and partial, race-nonspecific resistance, either by conventional breeding or transgenic approaches, will enable us to produce crop varieties with effective resistance, without impacting on other agronomic importance crop traits.

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Cover Letter

14th August 2012

Dear Rhiannon Macrae

Attached is the invited review, title “Plant-pathogen interactions: disease resistance in modern agriculture”, for the Trends in Genetics special issue “Genetic Pastures”.

Authors: Dr Lesley A Boyd, Dr Chris Ridout, Dr Donal O’Sullivan, Prof Jan Leach and Dr Hei Leung.

Regards

Lesley Boyd

Plant-pathogen interactions: disease resistance in modern agriculture

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Keywords: Crop Disease Resistance, PAMP-Triggered Immunity, Effector-Triggered Immunity, Quantitative Trait Loci, Genome Wide Association Analysis

Abstract

The growing human population will require a significant increase in agricultural production. Changes in the climatic and environmental conditions under which our crops are grown has resulted in the appearance of new diseases, while genetic changes within the pathogen have resulted in the loss of previously effective sources of resistance. We need to intensify the identification and characterisation of new sources of resistance, and use our knowledge of plant-pathogen interactions to develop novel approaches to achieve effective resistance within our crops. Advanced genetic and statistical methods of analysis enable global screens of genetic diversity to be undertaken, identifying new resistance genes, while studies of plant-pathogen interactions are uncovering the mechanisms by which the resistance is achieved. Informed deployment of major, race-specific and partial, race-nonspecific resistance, either by conventional breeding or transgenic approaches, will enable us to produce crop varieties with effective resistance, without impacting on other agronomic importance crop traits.

The impact of plant disease on global agriculture

The human population has more than doubled in the past 50 years and is projected to increase to over 9 billion by 2050 [1]. The issues underlying global food security are among the most pressing issues in international politics. Although many factors contribute to the disproportionate distribution of food around the world, it is clear that food security will require an increase in sustainable agricultural production, and particularly the production of the cereals wheat, rice and maize [http://faostat.fao.org]. Biotic stresses, including biotrophic and necrotrophic fungal, bacterial and viral pathogens, are a major constraint to production. For wheat, significant changes in the pathogen threat have occurred in recent years. For example, in 1999 in eastern Africa, a new race of *Puccinia graminis*, the causal agent of stem rust, overcame the race-specific resistance gene *Sr31* present in 70% of wheat cultivars [2]. In 2000, a new and more aggressive race of *P. striiformis*, the stripe rust pathogen, appeared in the USA causing wide-spread epidemics across the Midwest [3]. In Brazil a new field disease of wheat was discovered in 1986 caused by the rice blast fungus *Magnaporthe oryzae* [4].

These examples highlight the urgent need to better understand crop-pathogen interactions and how this knowledge can be applied in agricultural strategies. As phrased by Prof. Robert McIntosh and Zakkie Pretorius, “Wheat breeding for disease control is not complete unless we encompass all aspects of global pathogen variability and epidemiology, resistance discovery and genetic characterization, germplasm development (pre-breeding), and capture of resistance in competitive high-yielding, high-quality varieties with sufficient adaptability for adoption in agriculture.” [5]. Here we discuss scientific advances in our understanding of the

genetics of plant-pathogen interactions and developments in methods of identification and assessment of genetic variability for disease resistance, with an eye towards developing approaches to achieve sustainable disease resistance in our crops.

Our current understanding of plant-pathogen interactions and resistance

The interactions between plant and pathogen involve two-way communication. Not only must the plant be able to recognise and defend itself against a potential pathogen landing on its surface, but the pathogen must be able to manipulate the plant's biology to create a suitable environment for its growth and reproduction. Both plant and pathogen have evolved a suite of genes which enable this communication. Currently the active defence responses of the plant are considered to operate at two levels [6]. The first line of active plant defence involves the recognition of pathogen (or 'microbe')-associated molecular patterns, PAMPs (or 'MAMPs'), which trigger general plant defence responses referred to as PAMP-Triggered Immunity (PTI). The pathogen is able to suppress the different components of PTI by "effector" proteins delivered into the plant. The second line of plant defence involves the recognition of specific effectors (now referred to as Avirulence (Avr) proteins) by resistance (R-) genes within the plant, triggering what is often perceived as a stronger resistance response and referred to as Effector-Triggered Immunity (ETI). This R/Avr-gene recognition has been termed gene-for-gene resistance [7]. For a current review of R-gene application and future application in crop disease management see [8].

PTI and crop resistance

PAMPs are essential pathogen molecules that cannot be deleted or mutated and are highly conserved amongst a diverse range of microbial species. The best characterised plant recognised PAMPs are flg22, a subunit of the most conserved region of the bacterial mobility protein flagellin [9] and elf18, an epitope within EF-Tu, a bacterial elongation factor and the most abundant bacterial protein [10]. Chitin, a constituent of fungal and insect cell walls is the best-studied fungal PAMP [11]. Other PAMPs include fungal xylanase, oomycete heptoglugans and bacterial lipopolysaccharides [12].

Recognition of PAMPs by plant Pattern Recognition Receptors (PRRs) initiates PTI, a series of defence reactions sufficient to repel most invading microbes. In recent years there have been significant advances in our understanding of PAMP-PRR interactions and the subsequent signalling events [12]. The bacterial PAMPs flg22 and elf18 are recognised by the *Arabidopsis* PRRs Flagellin Sensing 2 (FLS2) [13] and EF-Tu Receptor (EFR) [14], both of which are leucine-rich repeat receptor-like kinases (LRR-RLKs). Chitin is recognized in rice by the LysM domain-containing receptor-like protein (RLP) Chitin Elicitor Binding Protein (CEBiP) [15] and requires the RLK Chitin Elicitor Receptor Kinase 1 (CERK1) to trigger PTI [16]. PRRs have also been found that can detect the peptides or cell wall fragments released during infection or wounding, referred to as Damage-Associated Molecular Patterns (DAMPs) [17].

PTI responses can be readily measured in plants and as such could provide high-throughput methods of screening for the variation in the genes underlying PTI in

crops (Figure 1). One of the earliest cellular responses following PAMP elicitation is the oxidative burst produced by NADPH oxidases [18]. Activation of respiratory burst oxidase homolog D (RbohD) produces superoxide (O_2-), which is converted to hydrogen peroxide (H_2O_2) by superoxide dismutase [19]. H_2O_2 accumulation can be measured with luminol-based detection methods to provide a quantitative evaluation of the PTI response. Early induced genes such as MAP kinases and WRKY transcription factors also provide a quantitative measure of PTI responses. Increased transcription of these genes can be detected within 15 minutes after PAMP application. Later PTI responses include callose deposition and the production of salicylic acid and ethylene [20].

The rice R-gene *Xa21* confers resistance to all tested isolates of *Xanthamonas oryzae* pv. *oryzae*, the rice blight pathogen. The corresponding Avr gene *Ax21* was found to be conserved not only in *X. oryzae* pv. *oryzae* but in *Xanthamonas* spps of soybean, tomato, pepper, citrus and *Brassica* plant species, and outside the genera in the plant bacteria *Xylella fastidiosa* [21]. *Ax21* therefore represents a conserved effector, not only within a species, but across bacterial pathogens. Although *Xa21* is not found in all rice genotypes it is now considered by some to be a PRR and *Ax21* a PAMP [22]. *Xa21* could potentially be used to confer resistance in other crop species where *Xanthamonas* spps are a pathogen of agronomic importance.

Other examples of cloned resistance genes that confer resistance to multiple pathogens include the tomato *Mi1* gene [23], which confers resistance towards root-knot nematodes, potato aphids, whitefly, viruses, bacteria and fungi, the lettuce gene *Dm3* [24], which confers resistance to downy mildew and lettuce root aphid, and the

tomato *Pto* gene, which when over-expressed confers resistance to the bacteria *P. syringae* pv. *tomato* and *X. campestris* pv. *vesicatoria* and the fungus *Cladosporium fulvum* [21, 25]. The multi-pathogen resistance conferred by these genes may also have wider applications in resistance if incorporated into other crop species.

ETI and crop resistance

To achieve successful infection a pathogen must be able to manipulate the cellular environment of the host plant, not only suppressing the plant's natural defence responses, but also altering the cellular environment to allow it to grow and reproduce. This is achieved by the production of an arsenal of proteins, collectively known as effectors, which target plant defence pathways and metabolism [26]. The rice blast fungus *M. oryzae* secretes Slp1, an effector protein that sequesters chitin oligosaccharides to prevent their binding to CEBiP, thereby suppressing PTI in rice [27]. The Pep1 effector from *Ustilago maydis,* the maize smut fungus, inhibits peroxidise activity in maize, suppressing the oxidative burst [28]. Increasing numbers of pathogen effectors are being identified. Understanding the role of these effectors in suppressing the general PTI plant defence responses will enable us to develop new approaches to disease resistance.

X. oryzae pv. oryzae produce Transcription Activator-Like Effectors (TALEs) that bind to TALE-specific DNA sequences within the promoter regions of plant genes, activating gene transcription to facilitate bacterial colonisation [29]. Rice resistance genes effective against *X. oryzae* include R-genes which are constitutively expressed, triggering ETI upon recognition of a pathogen *Avir* effector, but also R-genes which are induced directly by the *Avir* protein. The recessive R-gene *xa13* is

believed to be an allelic variant of the rice gene *Os8N3. Os8N3* is transcriptionally activated by the TALE PthXo1, which is required for bacterial multiplication and disease development, but no transcript of *Os8N3* was detected in *xa13* genotypes of rice [30]. The recessive R-gene *xa5* is thought to interfere with the function of multiple TALEs. *Xa5* encodes for TFIIAgamma, a component of the RNA II polymerase complex. Many TALEs may use this complex via interaction with TFIIAgamma to activate transcription, and the *xa5* allelic variant prevents this interaction [29]. Genetic variants of the plant target genes of effectors could thus provide novel sources of resistance.

In a novel approach, TALEs have been used to create mutations within the promoter regions of target plant genes by fusing the DNA recognition repeats of native or customised TALEs with an endonuclease, creating TALE Nucleases (TALENs) [31,32]. TALENs were successfully used to generate site-specific DNA modifications in the promoters of plant TALE target genes, resulting in a modified promoter to which the TALE could no longer bind [33, 34]. The rice gene *OsSWEET14* encodes a member of the SWEET sucrose-efflux transporter family and is co-opted by the *X. oryzae* pv. *oryzae* effectors *AvrXa7* and *PthXo3* to divert sugars for the benefit of the bacteria [35]. Using tailor-made TALENs DNA sequence modifications, mainly small deletions, were generated in the *AvrXa7* and *PthXo3* binding sites of *OsSWEET1.* These modifications prevented the TALEs from activating transcription of the gene, while showing no apparent disruption of the normal function of OsSWEET14 on rice development [36].

Whole-genome sequencing of plant pathogens has enabled bioinformatic approaches to identify candidate effectors [37-39]. Small proteins with signal peptides that would allow secretion of the protein into the plant are considered potential effector candidates. In the oomycete pathogens *Phytophthora spps.,* the conserved sequences RXLR and CRN define families of effectors [40]. All *P. infestans Avr* genes identified to date belong to the RXLR class of effectors. These genes encode secreted proteins with a RXLR motif required for translocation into the plant cell, followed by diverse and rapidly evolving C-terminal effector domains [41]. In *P. infestans* these families of effector protein are expanded 2-fold or more by the insertion of transposable elements and other repetitive sequences compared to *P. sojae* and *P. ramorum* [37]. The dynamic nature of these regions of the pathogen's genome may explain the great diversity of effectors and the rapidity with which *P. infectans* overcomes resistance in potato.

Functional, *in planta* screening of candidate effectors would allow the identification of matching Avir-R-gene interactions. In potato a rapid screening method for *P. infestans* effectors was used to identify new R-genes in related *Solanum* spps [42]. R-genes that recognise highly-conserved, non-redundant effectors essential for pathogenicity represent potentially durable sources of resistance [43].

Quantitative disease resistance and PTI in crops

R-genes commonly express dominant and clear resistant phenotypes and as such were traditionally favoured by breeders for the ease by which they can be selected within a breeding program. However race-specific R-genes are often effective for only a short period of time, as virulence rapidly arises within the pathogen

population. In recent years crop breeders and researcher alike have shown a greater interest in sources of Durable Resistance, a term defined as “resistance that remains effective when deployed over extensive acreage and time in an environment favourable for the disease” [44, 45]. Although many of these sources of resistance have only partial effects, representing Quantitative Trait Loci (QTL), by combining these genes, crop varieties with acceptable levels of disease resistance can be obtained [46, 47].

Durable QTL include the wheat leaf rust resistance genes *Lr34* and *Lr46*. These loci, *Lr34/Yr18/Pm38* and *Lr46/Yr29/Pm39,* have also been shown to confer resistance to stripe rust and powdery mildew, and thus are examples of both race-nonspecifc and multi-pathogen sources of resistance [48]. The *Lr34* gene encodes a protein resembling ABC transporters [49]. The rice *pi21*allele, which confers partial race-nonspecific resistance to the blast pathogen *M. oryzae,* is also considered durable [50]. Cloning of *pi21* identified a loss-of-function mutation in a gene encoding a proline-rich protein containing a heavy metal transport-detoxification domain and putative protein-protein interaction motifs. The wild-type gene *Pi21* appears to be required for *M. oryzae* infection; the mutant resistance allele *pi21* still allows but slows *M. oryzae* colonisation.

Work in several crop species suggests a relationship between disease resistance QTL and the defence responses triggered by PTI. Although further work is required to substantiate this, the association could provide new approaches to identifying and screening QTL in crop improvement programmes. In wheat, five quantitative trait loci (QTL) conferring multi-pathogen resistance to combinations of Septoria

tritici blotch, caused by *S. tritici*, Stagonospora glume blotch, caused by *S. nodorum* and Fusarium head blight, caused by *F. graminearum* and *F. culmorum* have been found in European wheat cultivars. These QTL may well represent genes conferring general defence responses which are effective towards multiple pathogens. Because these pathogens are genetically unrelated and infect different plant tissues, the resistance mechanism may be triggered by widely-conserved pathogen molecules similar to PAMPs [51].

In soybean correlations were observed between pathogen resistance against *Pseudomonas syringae* and *Sclerotinia sclerotiorum* and the levels of PAMP-triggered oxidative burst and defence gene induction. Four QTL were identified, one related to total ROS production and three related to PAMP-induced gene expression [52]. In maize, 29 QTL for resistance to Northern Leaf Blight (*Setosphaeria turcica* (anamorph *Exserohilum turcicum*) have been reported [53]. Genome-wide nested association mapping identified multiple plant defence-related genes associated with these QTL, including five RLK, consistent with their involvement in PTI. Similar associations between RLK and QTL against Southern leaf blight (*Cochliobolus heterostrophus*) have also been reported in maize [54].

Applications of quantitative disease resistance and PTI in crop improvement

Pyramiding QTL such *Lr34/Yr18/Pm38* and *Lr46/Yr29/Pm39* has proven effective within the CIMMYT spring wheat breeding program [55]. However little is known about how these different sources of resistance act together or what the best combinations to achieve durable resistance are. Cloning of QTL genes will enable the mechanisms behind each source of resistance to be determined, and allow

genes conferring different mechanisms of resistance to be combined to optimize durability. In rice QTL loci that co-localised with defence-related genes were pyramided by marker-assisted selection to develop rice cultivars resistant to *M. oryzae* (Box1). The similarities in sequence, gene organization and roles in disease resistance of germin-like protein (GLP) family members in rice and other cereals, including barley [56] and wheat [57], suggest that resistance contributed by the rice chromosome 8 OsGLP is a broad-spectrum, general mechanism conserved among the Gramineae [58].

With new knowledge about the PRRs, co-receptors and downstream events involved in PTI, the candidate gene approach could be used to identify new QTL genes for breeding. Genes identified in one crop could also be informative in related species. The barley homologue of the rice PRR gene CEBiP has been shown to have a role in basal resistance against *M. oryzae* [59], while EFR, found only in the Brassicaceae, when transformed into tomato conferred resistance to bacterial pathogens, including species of *Agrobacterium*, *Xanthomonas* and *Ralstonia* [60].

Application of cross species R-gene introduction in crop improvement

Many classical R-genes that mediate plant-pathogen ETI have now been cloned from several crop species [8]. These R-genes either recognise the pathogen through direct binding of the R-protein to the *Avr* pathogen effector, or indirectly through the interaction with another plant protein, the ‘guardee’, following its modification by the pathogen *Avr* effector (guard hypothesis). A well-studied example of a guardee protein is *RIN4* in *Arabidopsis*, which is required for resistance to *Pseudomonas syringe* pv. *tomato* mediated by the R-genes *RPM1* and *RPS2* [61,62].

The transfer of R-genes between sexually incompatible plant species using transgenic technologies has been proposed as a means of achieving durable pathogen resistance [63]. Such approaches allow different R-gene alleles to be combined as R-gene stacks, presenting the pathogen with an extensive arsenal of R-genes to evade if it is to circumvent recognition. Novel R-gene alleles, conferring new recognition specificities can be generated by directed mutagenesis [64] or domain swapping between R-genes [65]. Mutations generated within the potato R-gene *Rx*, which confers resistance to the potato virus X (PVX), generated new alleles effective against additional strains of the PVX virus, as well as a virus of the tree species poplar, poplar mosaic virus [64].

The transfer of R-genes between species is however not without problems (Box 2). Many R-genes effectively expressed in the donor species are lost in the recipient owing to R-gene suppression [46,66]. Studies using the transgenic approach to transfer R-genes have found that the R-gene triggered defence reactions in the absence of a pathogen or specifically increased susceptibility to other pathogens [63]. R-genes may require additional genes to function, such as R-gene guardees that are not found in the recipient species [62].

Identification and exploitation of natural variation in disease resistance

The vast majority of resistance genes deployed in today's crop varieties were bred in the traditional manner using resistance sources from the primary, secondary and to a more limited extent the tertiary gene pool (Box 2). The almost bewildering array of diversity present in these natural gene pools means that researchers targeting novel

resistances must deploy their finite resources carefully to maximise efficiency. The Focused Identification of Germplasm Strategy (FIGS) is a method to rationally select germplasm collection sites that are most likely to have produced selection pressure for the trait in question [67]. In the search for disease resistance, factors taken into account include the climatic conditions considered optimal for the development of the disease in question, including minimum/maximum temperatures and relative humidity, the geolocation of accessions previously demonstrated to contain resistance, and ecological factors such as the natural range of pathogen secondary host species. Although still in its infancy, FIGS has already been successfully used to increase the efficiency of searches for novel resistances in wheat to stem rust [68] and mildew [69].

Bi-parental mapping populations have been extensively used to genetically characterise sources of disease resistance in many of our crops, and although effective, they are limited by the genetic diversity that can be examined in each population. Genome-Wide Association Screens (GWAS) offer a more global approach, allowing the assessment of the genetic variability underlying a trait of interest in a diverse genetic collection. Examples identified through GWAS include lettuce spot blotch [70] and mildew [71] resistance, maize southern [72] and northern leaf blight [73] resistance, as well as resistance to rust [74] and tan spot [75] in wheat.

Although natural variation for disease resistance is extensive, our increasing understanding of the genes involved in plant-pathogen interactions provides an opportunity to utilise variation in target plant genes generated through mutation and

identified using TILLing procedures [76]. The classic example of mutation identifying an agronomically valuable source of disease resistance is that of the powdery mildew resistance gene in barley, *mlo,* which confers broad-spectrum resistance to all isolates of the fungus *Blumeria graminis* f. sp. *hordei* (Box 3). More recently several natural alleles of the barley transcription elongation factor *eIF4E,* which confer bymovirus resistance, were identified in a collection of 1090 barley landraces [77].

Concluding remarks

Research into plant-pathogen interactions for crop improvement currently falls into two broad approaches. The first focuses on the identification of the pathogen factors, PAMPs and effectors that are conserved across isolates and essential for pathogen survival, and unable to withstand modification. The plant resistance genes which recognise these conserved pathogen factors would theoretically have a greater possibility of remaining effective over time because of the constraint on the pathogen factor to evolve.

The second approaches the problem from the plant's perspective. Through the identification and study of QTL for partial and often durable resistance, research is showing that many of these QTL encode genes directly involved in resistance. Expressing resistant alleles of these genes prevents the pathogen from exploiting its host or activates general defence mechanisms used by the plant to restrict pathogen growth. Often these genes have the added bonus of conferring resistance to multiple pathogen species. Through the cloning of these QTL it is possible to determine the mechanisms of resistance and thereby accumulate resistance genes with contrasting

and complementary mechanisms that can be used in conjunction to provide long-term pathogen resistance.

These approaches are equally valid and complementary, with plant target genes of pathogen effectors being identified as previously cloned resistance genes, and they both work towards the same goal of breeding long-term, stable disease resistance within nutritious and high-yielding crops that will feed the ever increasing human population.

References

1 FAO (2010) The state of food security in the world – addressing food insecurity in protracted crises. Rome: Food and Agriculture Organization of The United Nations. http://www.fao.org/docrep/013/i1683e/i1683e.pdf

2 Pretorius, Z.A. *et al.* (2000) Detection of virulence to wheat stem rust resistance gene *Sr31* in *Puccina graminis* f. sp. *tritici* in Uganda. *Plant Disease* 84, 203.

3 Hovmøller, M.S. *et al.* (2008) Rapid global spread of two aggressive strains of a wheat rust fungus. *Molecular Ecology* 17, 3818-3826.

4 Urashima, A.S. *et al.* (1993) Host range, mating type and fertility of *Pyricularia grisea* from wheat in Brazil. Plant Disease 77, 1211-1216.

5 McIntosh, R.A. and Pretorius, Z.A. (2011) Borlaug Global Rust Initiative provides momentum for wheat rust research. *Euphytica* 179, 1-2.

6 Jones, J.D.G. and Dangl, J.L. (2006) The plant immune system. *Nature* 444, 323-329.

7 Flor, H.H. (1942) Inheritance of pathogenicity in *Melampsora lini*. *Phytopathology* 28, 365-391.

8 Gururani, M.A. *et al.* (2012) Plant disease resistance genes: Current status and future directions. *Physiol. Mol. Plant Pathol.* 78, 51-65.

9 Felix, G. *et al.* (1999) Plants have a sensitive perception system for the most conserved domain of bacterial flagellin. *Plant J.* 18, 265-276.

10 Kunze, G. *et al.* (2004) The N terminus of bacterial elongation factor Tu elicits innate immunity in *Arabidopsis* plants. *Plant Cell* 16, 3496-3507.

11 Felix, G. *et al.* (1993) Specific perception of subnanomolar concentrations of chitin fragments by tomato cells – induction of extracellular alkalinisation, changes in protein-phosphorylation, and establishment of a refractory state. *Plant J.* 4, 307-316.

12 Monahan, J. and Zipfel, C. (2012) Plant pattern recognition receptor complexes at the plasma membrane. *Current Opinion in Plant Biology* 15, 349-357.

13 Gomez-Gomez, L. and Boller, T. (2000) FLS2: An LRR receptor-like kinase involved in the perception of the bacterial elicitor flagellin in Arabidopsis. *Molecular Cell* 5, 1003-1011.

14 Zipfel, C. *et al.* (2006) Perception of the bacterial PAMP EF-Tu by the receptor EFR restricts *Agrobacterium*-mediated transformation. *Cell* 125, 749-760.

15 Kaku, H. *et al.* (2006) Plant cells recognize chitin fragments for defense signaling through a plasma membrane receptor. *PNAS* 103, 11086-11091.

16 Miya, A. *et al.* (2007) CERK1, a LysM receptor kinase, is essential for chitin elicitor signaling in *Arabidopsis*. *PNAS* 104, 19613-19618.

17 De Lorenzo, G. *et al.* (2011) Engineering plant resistance by constructing chimeric receptors that recognize damage-associated molecular patterns (DAMPs). *FEBS Letters* 585, 1521-1529.

18 Torres, M.A. *et al.* (2006) Reactive oxygen species signalling in response to pathogens. *Plant Physiol.* 141, 373-378.

19 Lamb, C. and Dixon, R.A. (1997) The oxidative burst in plant disease resistance. *Ann. Rev. Plant Physiol. and Plant Mol. Biol.* 48, 251-275.

20 Zipfel, C. & Robatzek, S. (2010) Pathogen-Associated Molecular Pattern-Triggered Immunity: Veni, Vidi…? *Plant Physiol.* 154, 551-554.

21 Chen, X. and Roland, P.C. (2011) Innate immunity in rice. *Trends in Plant Science* 16, 451-459.

22 Han, S-W. *et al.* (2011) Secretion, modification, and regulation of *Ax21*. *Current Opinion in Microbiology* 14, 62–67.

23 Milligan, S.B. *et al.* (1998) The root-knot nematode resistance gene Mi from tomato is a member of leucine zipper, nucleotide binding, leucine-rich repeat family of plant genes. *Plant Cell* 10, 1307-1319.

24 Meyers, B.C. *et al.* (1998) The major resistance gene cluster in lettuce is highly duplicated and spans several megabases. *Plant Cell* 10, 1817-1832.

25 Tang, X. *et al.* (1999) Overexpression of *Pto* activates defence responses and confers broad resistance. *Plant Cell* 11, 15-29.

26 Koeck, M. *et al.* (2011) The role of effectors of biotrophic and hemibiotrophic fungi in infection. *Cellular Microbiology* 13, 1849-1857.

27 Mentlak, T.A. *et al.* (2012) Effector-Mediated Suppression of Chitin-Triggered Immunity by Magnaporthe oryzae Is Necessary for Rice Blast Disease. *Plant Cell* 24. 322-335.

28 Hemetsberger, C. *et al.* (2012) The *Ustilago maydis* Effector Pep1 suppresses plant immunity by inhibition of host peroxidise activity. *PLoS Pathog.* 8(5): e1002684. doi:10.1371/journal.ppat.1002684.

29 Bogdanove, A.J. *et al.* (2010) TAL effectors: finding plant genes for disease and defence. *Current Opinions in Plant Biology* 13: 394-401.

30 Yang, B. *et al.* (2006). *Os8N3* is a host disease-susceptibility gene for bacterial blight of rice. *PNAS* 103, 10503-10508.

31 Li, T. *et al.* (2011) TAL nucleases (TALNs): hybrid proteins composed of TAL effectors and FokI DNA-cleavage domain. Nucleic Acids Res. 39, 359-372.

32 Li, L. *et al.* (2012) Rapid and highly efficient construction of TALE-based transcriptional regulators and nucleases for genome modification. *Plant Mol. Biol.* 78, 407-416.

33 Mahfouz, M.M. *et al.* (2011) De novo-engineered transcription activator-like effector (TALE) hybrid nuclease with novel DNA binding specificity creates double-stranded breaks. *PNAS* 108, 2623-2628.

34 Cermak, T. *et al.* (2011) Efficient design and assemble of custom TALEN and other TAL effector-based constructs for DNA targeting. Nucleic Acids Research 39, e82.

35 Chen, L-Q. *et al.* (2012) Sucrose Efflux Mediated by SWEET Proteins as a Key Step for Phloem Transport. *Science* 335, 207-211.

36 Li, T. *et al.* (2012) High-efficiency TALEN-based gene editing produces disease-resistant rice. *Nature Biotechnology* 30, 390-392.

37 Haas, B.J. *et al*. (2009) Genome sequence and analysis of the Irish potato famine pathogen *Phytophthora infestans*. *Nature* 461, 393-398.

38 Dean, R.A. *et al.* (2005) The genome sequence of the rice blast fungus *Magnaporthe grisea*. *Nature* 434, 980-986.

39 Oberhaensti, S. *et al.* (2011) Comparative sequence analysis of wheat and barley powdery mildew fungi reveals gene colinearity, dates divergence and indicates host-pathogen co-evolution. *Fungal Genetics and Biology* 48, 327-334.

40 Schornack, S. *et al*. (2009) Ten things to know about oomycete effectors. *Mol. Plant Pathol.* 10, 795-803.

41 Vleeshouwers, V.G.A.A. *et al.* (2011) Understanding and Exploiting Late Blight Resistance in the Age of Effectors. *Annu. Rev. Phytopathol.* 49, 507–531.

42 Oh, S-K. *et al.* (2009) In planta expression screens of *Phytophthora infectans* RXLR effectors reveal diverse phenotypes, including activation of the *Solanum bulbocastanum* disease resistance protein Rpi-blb2. *Plant Cell* 21, 2928-2947.

43 Whisson, S.C. *et al.* (2011) Exploiting Knowledge of Pathogen Effectors to Enhance Late Blight Resistance in Potato. *Potato Research* 54, 325-340.

44 Johnson, R. (1981) Durable resistance, definition of genetic control, and attainment in plant breeding. *Phytopathology* 71, 567-568.

45 Johnson, R. (1984) A critical analysis of durable resistance. *Annu. Rev. Phytopathol.* 22, 309–330.

46 Boyd, L.A. (2005) Can Robigus defeat an old enemy? - Yellow rust of wheat. Centenary Review, *Journal of Agricultural Science* 143, 233-243.

47 Boyd, L.A. (2006) Perspectives: Can the durability of resistance be predicted? *Journal of the Science of Food and Agriculture* 86, 2523-2526.

48 Lillemo, M. *et al.* (2008) The adult plant rust resistance loci *Lr34/Yr18* and *Lr46/Yr29* are important determinants of partial resistance to powdery mildew in bread wheat line Saar. *Theor. Appl. Genet.* 116, 1155–1166.

49 Krattinger, S.G. *et al.* (2009) A putative ABC transporter confers durable resistance to multiple fungal pathogens in wheat. *Science* 323, 1360–1363.

50 Fukuoka, S. et al. (2009) Loss of function of a proline containing protein confers durable disease resistance in rice. *Science* 325, 998-1001.

51 Miedaner, T. *et al.* (2012) Broad-spectrum resistance loci for three quantitatively inherited diseases in two winter wheat populations. *Molecular Breeding* 29, 731-742.

52 Valdés-López, O. (2011) Identification of Quantitative Trait Loci Controlling Gene Expression during the Innate Immunity Response of Soybean. *Plant Physiol.* 157, 1975-1986.

53 Poland, J.A. *et al.* (2011) Genome-wide nested association mapping of quantitative resistance to northern leaf blight in maize. *PNAS* 108, 6893–6898.

54 Kump, K.L. *et al.* (2011) Genome-wide association study of quantitative resistance to southern leaf blight in the maize nested association mapping population *Nature Genet.* 43,163–168.

55 Singh, R.P. *et al.* (2011) Race non-specific resistance to rust diseases in CIMMYT spring wheats. *Euphytica* 179, 175-186.

56 Schweizer, P. and Stein, N. (2011) Large-scale data integration reveals colocalization of gene functional groups with meta-QTL for multiple disease resistance in barley. *Mol. Plant-Microbe Interact*. 24, 1492-1501.

57 Christensen, A. *et al.* (2004) The germinlike protein GLP4 exhibits superoxide dismutase activity and is an important component of quantitative resistance in wheat and barley. *Mol. Plant-Microbe Interact*. 17, 109–117.

58 Breen, J. and Bellgard, M. (2010) Germin-like proteins (GLPs) in cereal genomes: gene clustering and dynamic roles in plant defence. *Funct. Integr. Genomics* 10, 463–476.

59 Tanaka, S. *et al*. (2010) *HvCEBiP*, a gene homologous to rice chitin receptor *CEBiP*, contributes to basal resistance of barley to *Magnaporthe oryzae*. *BMC Plant Biology* 10, 288-298.

60 Lacombe, S. *et al.* (2010) Interfamily transfer of a plant pattern-recognition receptor confers broad-spectrum bacterial resistance. *Nature Biotechnol.* 28, 365-369.

61 Mackey, D. *et al.* (2002) RIN4 interacts with *Pseudomonas syringae* type II effector molecules and is required for *RPM1*-mediated resistance in *Arabidopsis*. Cell 108, 743-754.

62 Eitas, T.K and Dangl, J.L. (2010) NB-LRR proteins: pairs, pieces, perception, partners and pathways. Curr. Opin. Plant Biol. 13, 472-477.

63 Wulff, B.B.H. et al. (2011) Improving immunity in crops: new tactics in an old game. Current Opinion in Plant Biology 14, 468-476.

64 Farnham, G. and Baulcombe, D.C. (2006) Artificial evolution extends the spectrum of viruses that are targeted by a disease resistance gene from potato. *PNAS* 103, 18828-18833.

65 Brunner, S. *et al.* (2010) Intragenic allele pyramiding combines different specificities of wheat Pm3 resistance alleles. *Plant J.* 64, 433-445.

66 Nelson, J. C. *et al.* (1997) Mapping genes conferring and suppressing leaf rust resistance in wheat. *Crop Science*, 37, 1928-1935.

67 Mackay, M., and Street, K. (2004) Focused identification of germplasm strategy-FIGS. In *54th Australian cereal chemistry conference and the 11th wheat breeders' assembly*, pp 138-141, Royal Australian Chemical Institute, Melbourne, Australia.

68 Endresen, D.T.F.*, et al.* (2012) Sources Of Resistance To Stem Rust (Ug99) In Bread Wheat And Durum Wheat Identified Using Focused Identification Of Germplasm Strategy. *Crop Science* 52, 764-773.

69 Bhullar, N.K.*, et al.* (2009) Unlocking wheat genetic resources for the molecular identification of previously undescribed functional alleles at the Pm3 resistance locus. *Proceedings of the National Academy of Sciences* 106, 9519-9524.

70 Roy, J.K.*, et al.* (2010) Association mapping of spot blotch resistance in wild barley. *Mol Breed* 26, 243-256.

71 Spies, A.*, et al.* (2011) Allele mining in barley genetic resources reveals genes of race-non-specific powdery mildew resistance. *Front Plant Sci* 2, 113.

72 Kump, K.L.*, et al.* (2011) Genome-wide association study of quantitative resistance to southern leaf blight in the maize nested association mapping population. *Nat Genet* 43, 163-168.

73 Poland, J.A.*, et al.* (2011) Genome-wide nested association mapping of quantitative resistance to northern leaf blight in maize. *Proceedings of the National Academy of Sciences* 108, 6893-6898.

74 Yu, L.X., *et al.* (2011) Association mapping and gene-gene interaction for stem rust resistance in CIMMYT spring wheat germplasm. *Theor Appl Genet* 123, 1257-1268.

75 Gurung, S., *et al.* (2011) Identification of novel genomic regions associated with resistance to *Pyrenophora tritici-repentis* races 1 and 5 in spring wheat landraces using association analysis. *Theor Appl Genet* 123, 1029-1041.

76 Marroni, F., *et al.* (2011) Large-scale detection of rare variants via pooled multiplexed next-generation sequencing: towards next-generation Ecotilling. *The Plant Journal* 67, 736-745.

77 Hofinger, B.J., *et al.* (2011) An exceptionally high nucleotide and haplotype diversity and a signature of positive selection for the eIF4E resistance gene in barley are revealed by allele mining and phylogenetic analyses of natural populations. *Molecular Ecology* 20, 3653-3668.

78 Liu, B. et al. (2009) What it Takes to Achieve Durable Resistance to Rice Blast? In *Advances in Genetics, Genomics and Control of Rice Blast Disease* (Wang, X. and Valent, B., eds), pp. 385-402, Springer Publishers Inc.

79 Liu, B. *et al.* (2004) Candidate defense genes as predictors of quantitative blast resistance in rice. *Mol Plant Microbe Interact* 17, 1146-1152.

80 Wu, J.L. *et al.* (2004) Association between molecular markers and blast resistance in an advanced backcross population of rice. *Theor Appl Genet* 108, 1024-1032.

81 Davidson, R.M. *et al. Rice* (2010) Allelic Diversity and Relationships to Early Stress Responses. *Rice* 3, 43-55.

82 Manosalva, P.M. *et al.* (2009) A germin-like protein gene family functions as a complex quantitative trait locus conferring broad-spectrum disease resistance in rice. *Plant Physiol.* 149, 286-296.

83 Stukenbrock, E.H., and McDonald, B.A. (2008) The Origins of Plant Pathogens in Agro-Ecosystems. *Annual Review of Phytopathology* 46, 75-100.

84 Miklis, M.*, et al.* (2007) Barley MLO modulates actin-dependent and actin-independent antifungal defense pathways at the cell periphery. *Plant Physiol* 144, 1132-1143.

85 Humphry, M.*, et al.* (2011) Durable broad-spectrum powdery mildew resistance in pea er1 plants is conferred by natural loss-of-function mutations in PsMLO1. *Molecular Plant Pathology* 12, 866-878.

86 Bai, Y.*, et al.* (2007) 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, 30-39.

87 Consonni, C*., et al.* (2006) Conserved requirement for a plant host cell protein in powdery mildew pathogenesis. *Nat Genet* 38, 716-720.

88 Thomas, W.T.B*., et al.* (1998) Identification of a QTL decreasing yield in barley linked to Mlo powdery mildew resistance. *Molecular Breeding* 4, 381-393.

89 Piffanelli, P*., et al.* (2004) A barley cultivation-associated polymorphism conveys resistance to powdery mildew. *Nature* 430, 887-891.

Figure Legends

Figure 1

Methods of studying PAMP-Triggered Immunity (PTI) in crops

A Production of reactive oxygen species (ROS) from leaf discs of Brassica cultivars after addition of PAMPs

ROS was measured with a luminometric assay expressed as Relative Light Units (RLU). The assay was performed in a 96-well microtitre plate with 8 replicate discs per cultivar. ROS measurement is a potentially high-throughput method, although the method needs to be optimised, and may not be suitable for all crop species.

B Induced relative gene expression in barley cultivars after addition of PAMP

Gene expression was measured 60 minutes after vacuum infiltration of the PAMP solution. Relative Expression (RE) of the PAMP responsive gene *HvCEBiP* (relative to barley Elongation Factor 1^{α} as control) after treatment with the PAMPs chitin and flg22. Not as high-throughput as method **A**, although can produce more consistent results. Selected genes need to be PAMP-responsive and up-regulated within 60 mins.

C Induced resistance against *Pseudomonas syringae* pv. *syringae* in wheat

PAMP solutions were syringe-infiltrated into leaves at the central zone (red) and the extent of the infiltration recorded (black ringed zone). After 24 h bacteria were

infiltrated into the central zone and Colony Forming units recorded after 3 days. Induced resistance can be used to compare small numbers of cultivars (10-40) and provides a direct readout of how PTI affects pathogen growth.

D Callose formation observed 24h after syringe-infiltration of chitin into barley leaves

Bright callose flecks were visible in the highlighted zone after staining with aniline blue. Callose formation, lignin accumulation and metabolite changes are not appropriate methods for high-throughput screening. They may be useful for characterising PTI in selected varieties or mutants.

Figure 2

Breeding for durable blast resistance in rice

A cross was generated between the rice line TXZ-13, susceptible to the rice blast pathogen *Magnaporthe oryzae* and the resistant rice line SHZ-2. The backcross line was selected using three DNA markers, one for a major resistance gene and two for QTL for rice blast resistance. The resulting line exhibited effective resistance for over 20 seasons in the field.

Glossary

Avir: Avirulence gene, the product of which, as defined by Flor's gene-for-gene hypothesis, is recognised by a plant R-gene, activating ETI.

CEBiP: Chitin Elicitor Binding Protein. Plant PRR which binds the PAMP chitin.

CERK1: Receptor-Like Kinase required for CEBiP triggered PTI.

DAMP: Damage-Associated Molecular Pattern. Plant peptides or cell wall fragments released during pathogen infection or wounding.

EFR: EF-Tu Receptor. Plant PRR which binds the PAMP EF-Tu.

ETI: Effector Triggered Immunity. Plant defence responses activated following the recognition by the plant of pathogen effectors.

FLS2: Flagellin Sensing 2. Plant PRR which binds the PAMP flg22.

GWAS: Genome Wide Association Screen: The GWAS approach systematically screens a genome-wide array of markers against the phenotypes of interest to identify statistical associations between markers and phenotypes.

PAMP: Pathogen-Associated Molecular Pattern: Conserved pathogen molecules recognised by the plant. Also known as Microbe-Associated Molecular Patterns (MAMPs).

PRR: Pattern Recognition Receptor.

PTI: PAMP Triggered Immunity. Plant defence responses activated following the recognition by the plant of PAMPs.

QTL: Quantitative Trait Locus. A genetic region which contributes to a phenotype displaying a continuous distribution.

R-gene: Resistance gene.

RLK: Receptor-Like Kinase. Protein containing a receptor-recognition and a functional kinase domain.

TALE: Transcription Activator-Like Effectors. TALEs bind to TALE-specific DNA sequences within the promoter regions of plant genes activating gene transcription.

TALEN: Transcription Activator-Like Effector Nucleases. A fusion protein between the plant gene DNA recognition repeats targeted by TAL effectors and the DNA cleavage domains of FoKI, a bacterial type IIS restriction endonuclease.

Box 1

Informed resistance gene selection in breeding for disease resistance in rice

Analysis of crop varieties in germplasm breeding programs suggests that effective and durable resistance would be best achieved by combining both quantitative trait loci (QTL) and single resistance (*R*) genes into elite crop varieties [78]. The incorporation of QTL for crop improvement however has not been widely adopted. This is due in part to the lack of reliable markers for accumulation of the QTL, with the genes that contribute to the quantitative trait being largely unknown. Determining which genes confer the QTL phenotypes is complicated by the imprecision of QTL mapping, and because the effects of each QTL are small and often vary with environment. Recent approaches that build on knowledge of plant defence responses show promise for targeted incorporation of resistance QTL for blast and sheath blight into rice [79,80]. Convergent evidence, based on co-localization with a disease resistance QTL, comparative sequence analysis, targeted gene expression profiling, gene silencing studies, and functional analyses of gene family members, has implicated several members of a germin-like protein (GLP) gene family in contributing to a disease resistance QTL on rice chromosome 8 [81,82]. The QTL is correlated with a polymorphism created by an insertion in the promoter of *OsGLP8-6,* one of 12 GLP family members clustered on chromosome 8 [81]. The *OsGLP8-6* promoter insertion adds *cis*-elements associated with early gene induction during plant defence and after wounding, and the presence of the insertion is correlated with higher induced expression of *OsGLP8-6* and increased resistance to both rice blast and sheath blight diseases. In addition to the *OsGLP8* gene family, other defence response genes have been shown to function in QTL-directed resistance

effective against a broad spectrum of diseases including bacterial blight, sheath blight and rice blast [79].

Knowledge of variation in genes responsible for QTL function allows design of molecular markers to facilitate accumulation of the QTL into elite germplasm [79,80]. However, because QTL alone might not suffice when disease pressure is high, in practical breeding QTL should be used in combination with major R-genes. This approach has been practiced with considerable success [78]. Rice lines that exhibited high levels of resistance in multi-location trials were constructed by using marker-assisted selection to combine QTL candidate genes and major blast R-genes [79] (Figure 2). These lines have shown high levels of blast resistance after continuous testing in disease hot spots in southern China for over 20 cropping seasons (10 years)([82]; Liu Bin et al., unpublished data).

Box 2

Resistance gene deployment from natural populations (primary, secondary and tertiary gene pools)

Many sources of resistance have been introduced into our crops from external gene pools. The primary gene pool consists of domesticated and wild specimens that are completely interfertile. Although the relative absence of crossing barriers makes deployment of genes from the primary genepool a matter of routine, it should be recognised that moving a resistance gene from a wild accession into a commercial breeding program requires much greater effort than simple reassortment of genes already deployed in elite varieties. The introduction of resistance sources from landrace or cultivated, but maladapted origin requires significant levels of effort on the part of the breeder. The greater the burden of unadapted and undesirable traits in the source of resistance, the more crossing and selection required to obtain a high-yielding, resistant derivative adapted to the growing conditions of choice.

The use of related species (secondary genepool) as sources of resistance offers several advantages, but equally several problems. The additional species can encompass a wider range of genetic diversity which is accompanied by a correspondingly deeper history of co-evolution with pathogens specific to the plant taxa in question [83].

The tertiary genepool consists of species which have incompatible genomes, unable of producing fertile F1 hybrids due to mismatched chromosome numbers/ploidy levels and/or significant levels of non-homology etc. and which therefore require

radical interventions such as ‘bridging crosses’, chemically-induced chromosome doubling and/or embryo rescue in order to introgress so-called ‘alien’ chromosome segments into the background genotype of choice. Several such ‘alien’ introgressions e.g., the replacement of the long arm of wheat chromosome 1B with the short arm of rye (*Secale cereale*) chromosome 1R (known as the 1B/1R translocation) in the 1920’s, which brought resistance to leaf, stripe and stem rust and to powdery mildew, as well as background-dependent alterations in biomass and yield potential, is still maintained in a number of elite wheat varieties and segregates rather like a single dominant locus in breeding programs. Against these advantages most alien introgressions (1B/1R included) carry negative, as well as positive traits, and due to the lack of homology with the recipient genomes, homology-dependent recombination is largely unavailable to separate these negative and positive traits over successive breeding cycles.

Box 3

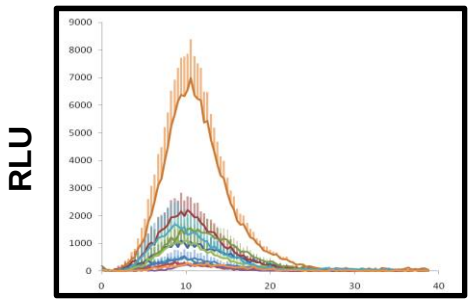
Barley *mlo* resistance: broad-spectrum resistance for powdery mildew from natural and induced sources.

Mlo genes encode seven transmembrane domain proteins unique to and conserved amongst land plants. The most studied is the *Mlo* gene of barley, *HvMlo.* The HvMLO protein senses Ca^{2+} via an interaction with calmodulin and negatively regulates cell death by inhibiting both actin-dependent, vesicle-associated as well as actin-independent defence responses mounted as part of the basal defence reaction [84]. Recessive loss-of-function *mlo* alleles have been shown to confer broad spectrum resistance to a variety of appropriate and inappropriate powdery mildew species in several crops. The barley *mlo* gene has conferred race-nonspecific resistance to *Blumeria gaminis* f. sp. *hordei* for more than 30 years. The recessive *er1* resistance gene of pea, effective against all races of pea powdery mildew for >50 years, is a naturally occurring allele of the *Mlo* homologue, as is the *or-1 gene* in tomato [85,86].

Evidence suggests that MLO function has been exploited as a susceptibility factor by *Eryshiphales* (mildew) spp. for at least 200 million years [87]. As a route to broad-spectrum powdery mildew resistance, MLO is a good target for mutagenesis in crops where naturally occurring alleles do not exist. The tale of the barley *mlo* gene, however, offers a cautionary note. Although a number of induced mutations in the *Mlo* gene have been identified and characterised, none have been deployed in barley breeding with the same success as the spontaneously occurring allele from an Ethiopian landrace (*mlo-11*). *mlo-11*, which unlike induced mutants, came through natural selection and minimized the documented negative yield penalty associated

with loss of *Mlo* function [88]. *mlo-11* was shown to possess partial *Mlo* function through a complex rearrangement of the upstream regulatory sequences which, though greatly reducing *Mlo* expression, still allow partial transcriptional read through to an otherwise unaltered MLO protein [89].

Figure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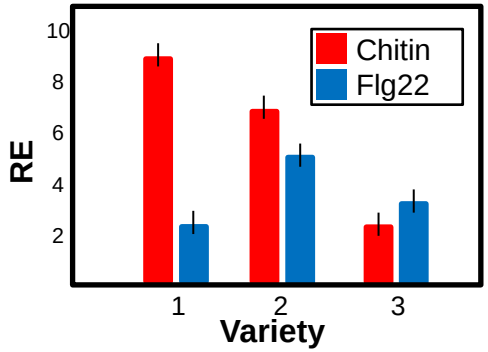
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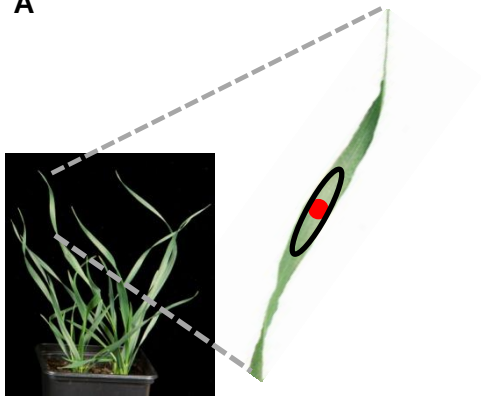

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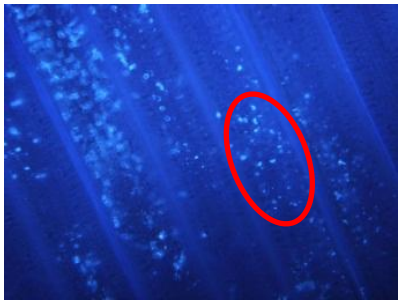

D

Figure

X

Recurrent rice parent TXZ-13 susceptible to blast

Donor rice parent SHZ-2 with history of stable resistance to blast

Resulting backcross line with effective blast resistance