ARTÍCULO DE REVISIÓN / REVIEW ARTICLE

UNRAVELING THE MOLECULES HIDDEN IN THE GRAY SHADOWS OF QUANTITATIVE DISEASE RESISTANCE TO PATHOGENS

Descifrando las moléculas ocultas en las sombras grises de la resistencia cuantitativa a patógenos

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ABSTRACT

One of the most challenging questions in plant breeding and molecular plant pathology research is what are the genetic and molecular bases of quantitative disease resistance (QDR)?. The scarce knowledge of how this type of resistance works has hindered plant breeders to fully take advantage of it. To overcome these obstacles new methodologies for the study of quantitative traits have been developed. Approaches such as genetic mapping, identification of quantitative trait loci (QTL) and association mapping, including candidate gene approach and genome wide association studies, have been historically undertaken to dissect quantitative traits and therefore to study QDR. Additionally, great advances in quantitative phenotypic data collection have been provided to improve these analyses. Recently, genes associated to QDR have been cloned, leading to new hypothesis concerning the molecular bases of this type of resistance. In this review we present the more recent advances about QDR and corresponding application, which have allowed postulating new ideas that can help to construct new QDR models. Some of the hypotheses presented here as possible explanations for QDR are related to the expression level and alternative splicing of some defense-related genes expression, the action of "weak alleles" of R genes, the presence of allelic variants in genes involved in the defense response and a central role of kinases or pseudokinases. With the information recapitulated in this review it is possible to conclude that the conceptual distinction between qualitative and quantitative resistance may be questioned since both share important components.

Keywords: breeding, complex traits, genome, gene expression, plant immunity, quantitative disease resistance (QDR), quantitative trait loci (QTL).

RESUMEN

Una de las preguntas más desafiantes del fitomejoramiento y de la fitopatología molecular es ¿cuáles son las bases genéticas y moleculares de la resistencia cuantitativa a enfermedades?. El escaso conocimiento de cómo este tipo de resistencia funciona ha obstaculizado que los fitomejoradores la aprovecharlo plenamente. Para superar estos obstáculos se han desarrollado nuevas metodologías para el estudio de rasgos cuantitativos. Los enfoques como el mapeo genético, la identificación de loci de rasgos cuantitativos (QTL) y el mapeo por asociaciones, incluyendo el enfoque de genes candidatos y los estudios de asociación amplia del genoma, se han llevado a cabo históricamente para describir rasgos cuantitativos y por lo tanto para estudiar QDR. Además, se han proporcionado grandes avances en la obtención de datos fenotípicos cuantitativos para mejorar estos análisis. Recientemente, algunos genes asociados a QDR han sido clonados, lo que conduce a nuevas hipótesis sobre las bases moleculares de este tipo de resistencia. En esta revisión presentamos los avances más recientes sobre QDR y la correspondiente aplicación, que han permitido postular nuevas ideas que pueden ayudar a construir nuevos modelos. Algunas de las hipótesis presentadas aquí como posibles explicaciones para QDR están relacionadas con el nivel de expresión y el splicing alternativo de algunos genes relacionados con la defensa, la acción de "alelos débiles" de genes R, la presencia de variantes alélicas en los genes implicados en la respuesta de defensa

y un papel central de quinasas o pseudoqinasas. Con la información recapitulada en esta revisión es posible concluir que la distinción conceptual entre resistencia cualitativa y cuantitativa puede ser cuestionada ya que ambos comparten importantes componentes.

Palabras clave: expresión génica, fitomejoramiento, genoma, inmunidad vegetal, loci de caracteres cuantitativos, rasgos complejos, resistencia cuantitativa a enfermedades.

INTRODUCTION

The study of traits that show simple inheritance has been the focus of most genetic research in plants. Genes for thousands of monogenic traits have been characterized in plants that belong to a wide variety of taxonomic groups. The study of these traits is straightforward because the phenotype reveals the underlying genotype without ambiguity (St. Clair, 2010). However, the phenotypic variation observed in natural populations is governed mainly by multiple genes and, to a lesser extent, by single genes, indicating that the complex inheritance of traits is the rule rather than the exception. In model plants, as well as in agronomically important crops, although single genes that control morphology, productivity, yield, food quality and disease resistance have been described extensively, the real genetic bases of these traits, in most cases, depends on the concerted and simultaneous action of multiple genes.

The study of the genetic bases of plant resistance has not escaped the above-mentioned oversimplification. The response phenotypes of individuals with qualitative resistance have a discrete (categorical) distribution and the genes involved segregate following the expected Mendelian ratios (St. Clair, 2010). The association of plant resistance with single genes was first proposed by Flor with the well-known gene-by-gene model (Flor, 1955). Since then, a lot has been accomplished in understanding how these genes control responses to pathogens. The number of single genes associated with plant immunity that have been cloned and characterized provided a broad view of molecular mechanisms that control plant immunity, but quantitative resistance has not been considered to the same extent. In this review, we describe the most recent efforts that have been made to elucidate molecular bases of this major type of resistance. We refer to studies that are not only worthy of being included in new immunity models, but also are helpful in understanding how quantitative genetics of the resistance have been studied. It is important to note the lack of knowledge concerning on genomic regions that explain quantitative disease resistance (QDR). We also aim at explaining how quantitative resistance works at the molecular level by describing QDR genes that have been cloned and functionally validated and by highly their common characteristics.

The ABC of plant immunity

Since plants are continuously threatened by different kind of pathogens, it is imperative to develop new strategies in order to control plant diseases. The most environmental friendly strategy is to exploit natural mechanisms that plants have evolved to control invading pathogens. The activation of an effective immune response depends on the ability of plants to recognize pathogens. Based on the knowledge on molecules produced by pathogens and their recognition by plants hosts, the so called zig-zag model has outlined how to describe immunity systems in plants (Jones and Dangl, 2006). This model states that plants have evolved immune receptors that are able to recognize pathogen-associated molecular patterns (PAMPs) or specialized effector proteins that are present in particular races/strains of pathogens. The recognition of PAMPs depends on the pattern recognition receptors (PRRs) that constitute the first line of molecular defense, known as PAMP triggered immunity (PTI) (Zipfel, 2014). Adapted races or strains of pathogens translocate effector proteins into plant cells to manipulate host components or suppress PTI (Cui *et al.*, 2015). Plants can recognize pathogen effectors through R proteins and the immunity they activate is known as effector triggered immunity or ETI (Chisholm *et al.*, 2006). Although this resistance is most of the time total, it is race specific and can be easily overcome by point mutation in effectors that escape plant recognition (Houterman *et al.*, 2009). ETI is the molecular explanation of the gene-by-gene model proposed by Flor. According to this model, a plant is resistant when the interaction with the pathogen is incompatible. On the other hand, when the plant is susceptible, the interaction is compatible. In this case, there are only two possible phenotypes, resistant or susceptible, and the intermediates are not considered.

The zig-zag model does not include intermediate phenotypes and, consequently, is somewhat partial. A novel "invasion model" was recently proposed in which the classification of the plant immunity is based on the pathogen invasion patterns (IPs) (Cook *et al.*, 2015). These IPs are a large spectrum of molecules that are produced and/or released during invasion and are perceived by invasion pattern receptors (IPRs). The function of IPs can vary from microbiological physiology to host defense suppression and, consequently, they trigger a wide range of continuous defense responses. Plant responses can be either symbiotic or not, depending on the ability of the plant to recognize the IPs with IPRs and activate an IPTR (IP-triggered response). This new model also takes into account the fact that there is a complex interaction of multiple receptors and ligands at the same time and that the outcome of the interaction result from the combined result of all of them. The invasion model was developed as an alternative to the adopted

classifications that separate PTI from ETI and in which PAMPs are defined from the host perspective while the effectors are considered from the pathogen perspective (Cook *et al.*, 2015). In its application, the invasion model emphasizes the identification and understanding of molecules produced by the pathogen. Therefore, it is necessary to develop a model that uses the idea of defense as a continuum of responses, with a synergy and interaction between components from the invasion model, but that also shows the plant perspective of the model and its application.

Quantitative resistance enters into the game

In plant populations, when the response to a pathogen is a continuous phenotypic value, varying from highly susceptible to highly resistant individuals, it is considered as quantitative resistance (Huard-Chauveau *et al.*, 2013). QDR is controlled by several genes, each one contributing, to a different degree, to the reduction of the disease (St. Clair, 2010). The term polygenic, or oligogenic, resistance is frequently associated with QDR because of its inherent genetic architecture (Mackay *et al.*, 2009; Niks *et al.*, 2015). Although the concept of QDR is well-defined, it is widely used and sometimes misunderstood or misused. Partial resistance is the most widely used concept in literature to describe an intermediate phenotype when resistance is not total (Niks *et al.*, 2015). It is important to emphasize that this definition must be used to just describe a phenotype and not to define the genetic basis underlying the response (Niks *et al.*, 2015). QDR is often called field resistance because it has been evaluated frequently in polycyclic field conditions (Niks *et al.*, 2015); however, QDR should not been used as a synonym of field resistance since it has also been observed and assessed under greenhouses and controlled conditions.

Traditionally, QDR has been associated with two important concepts: broad spectrum and durable resistance; however, it is important to stress that these two concepts are not strictly exclusive to QDR. Durable resistance is a concept that was defined by Johnson in 1981 to refer to the resistance that retains its effectiveness in crops that are widely cultivated in an environment that is favorable to the pathogen, but this does not mean that it has to be permanent (Johnson, 1983). QDR has been considered as more durable than qualitative resistance and, consequently, more reliable. However, experimental evidence for this assumption is scarce (St. Clair, 2010). On the other hand, broad spectrum resistance occurs when the defense is effective against two or more types of pathogen species or to several strains or isolates of the same species of pathogen (Kou and Wang, 2010). The term is often used as a synonym for QDR, but it is important to note that there are several examples of strain-specific quantitative resistance loci (QRLs) (Poland *et al.*, 2009). On the other hand, PRRs can confer a broad spectrum defense (Zipfel, 2014) and single *R* genes can also mediate broad-spectrum resistance (Xiao *et al.*, 2001; Zhao *et al.*, 2004; Narusaka *et al.*, 2009) or can be engineered to achieve this type of resistance (Segretin *et al.*, 2014; Giannakopoulou *et al.*, 2015).

How to study complex traits and QDRs

Continuous traits do not exhibit single phenotype inheritance, such as those occuring in progenies that segregate according to the Mendelian rules (e.g. 3:1 or 15:1). However, this is not a consequence of distinct genetic mechanisms *per se*. Indeed, the variation in the phenotype observed for a quantitative trait is the result of a multiple genotypic expression of segregating alleles. In addition, it is highly influenced by the environment. As a consequence, it is not possible to clearly address a given phenotype to a particular genotype. For this reason, the Mendelian mechanisms in the study of quantitative traits is masked despite the fact that each gene could be segregated in a Mendelian mode (Griffiths, 2005).

For the above reasons, how to study quantitative traits has represented a new challenge to classical geneticists. The first studies on complex quantitative traits in plants included the association between the size of the seed (a quantitative trait) and the seed coat color (a qualitative trait) (Sax, 1923). The initial idea of quantitative loci mapping was first proposed by Thoday (1961), based on the observation that segregating single gene markers could be linked with loci associated with complex traits. Later, the term quantitative trait loci (QTL) was used for the first time to name the different loci that determine several quantitative traits in tomato (Tanksley *et al.*, 1982).

The logic behind QTL detection is to determinate the relationship between DNA variation, as captured by DNA-based markers, and the observed phenotypic variation (Mackay *et al.*, 2009). The identification of QTLs starts with the construction of a genetic map, where a large group of molecular markers are positioned in linkage groups (chromosomes) based on recombination frequencies. Once obtained, these markers are associated with the phenotypic trait of interest, using the fact that genes responsible for a particular trait and linked molecular markers cosegregate *via* chromosomal recombination during meiosis (Collard *et al.*, 2005).

In the past, the challenge was to increase the number of molecular markers present in genetic maps for QTL mapping purposes. During the 90s, the development of DNA-based markers revolutionized the ability to detect DNA variations (Phillips and Vasil, 2013). Molecular markers, such as RFLP (Restriction Fragment Length Polymorphism), RAPD (Random Amplified Polymorphic DNA), AFLP (Amplified fragment length polymorphism) and SSR (Simple Sequence Repeat), contributed significantly to the development of high-dense genetic maps, allowing the dissection of qualitative and quantitative traits. Despite significant efforts, the genetic maps obtained through the use of these markers were generally low- density maps because of the lack

of markers representing the complete set of recombination events. Thus, the first versions of maize and tomato maps contained only 50 RFLP markers each (Helentjaris *et al.*, 1988) and the first potato map had 135 RFLP markers (Bonierbale et al., 1988). Moreover, at that time, the QTL intervals were large, usually ranging from 10 to 30 cM (Glazier *et al.*, 2002). These limitations were overcome by massive sequencing technologies (Ansorge, 2009). These new genotyping technologies allowed for the high throughput identification of SNPs (Single Nucleotide Polymorphism), which have become the most widely used molecular marker. Nowadays, hundreds or thousands of widely distributed SNPs and their positions in the genome (provide the reference genome is accessible) can be identified in a relatively short period of time and at a low cost. Thereby, in recent years, the number of molecular markers and, therefore, map resolutions have increased, which ultimately leads to the reduction of QTL interval lengths to a few cM (Gautami *et al.*, 2012; Stephens *et al.*, 2014; Soto *et al.*, 2015).

The association mapping (AM) approach emerged at the turn of the 21st century as an alternative to the linkage mapping approach for QTL identification. In this case, the analysis is based on the phenomenon of linkage disequilibrium (LD) and the availability of historical recombination events at the population level (Zhu *et al.*, 2008). AM take advantage of the explosion of new genome-scale data, allowing a higher resolution in loci localization assignment, compared with linkage mapping (Zhu *et al.*, 2008). In this case, two strategies for the dissection of complex traits can be followed. The first one is the candidate gene approach and the second one is genome wide association studies (GWAS) (Brachi *et al.*, 2011). While in the QTL linkage mapping approach, quantitative candidate genes are located within an interval; in AM, a direct association between complex traits sand the polymorphic markers, usually SNPs, is achieved (Rafalski, 2002). However, both approaches seem to be complementary in the sense that their ultimate goal is the detection of the genes underlying the quantitative complex trait for further cloning. Several examples of the use of the association mapping approach in QDR studies for the more limiting diseases can be found in recent literature (Benson *et al.*, 2015; Gutiérrez *et al.*, 2015; Arruda *et al.*, 2016; Iquira *et al.*, 2015; Olukolu *et al.*, 2016; Turuspekov *et al.*, 2016). However, despite the broad use of this approach, no genes detected by AM for plant QDR have been cloned so far.

Recently, two approaches had been proposed for studies on quantitative traits. First, there is extreme-phenotype GWAS (XP-GWAS), a new approach combining bulk segregant analysis (BSA) and GWAS (Yang *et al.*, 2015). The second approach takes advantage of the use of clustered regularly interspaced short palindromic repeats (CRISPR-Cas9) and allow to obtain targeted mitotic recombination events without needing to develop directed crosses (Sadhu *et al.*, 2016).

Through this approach, high frequency double strand breaks (DSB) are induced in regions of interest in mitotic cells. Then, the intrinsic cell reparation by homologous recombination (HR), generates recombination events that lead to the formation of a recombinant. Thus, the high efficiency of CRISPR-Cas9 mediate recombination events within 20 kb of the targeted site has been demonstrated. Comparing this rate of recombination with that obtained by random meiotic segregation, the later would require more than seven thousand individuals (Sadhu *et al.*, 2016). The application of XP-GWAS and CRISPR-Cas9 approaches and their potential use in QDR characterization is highly promising.

In recent years, the concept of the set of all the information supported experimentally irrespective of the followed methodology, about QTLs and their allelic variation for a trait in one species, has received the name QTLome (Salvi and Tuberosa, 2015). Beyond constructing a QTLome, it is necessary to find a way to integrate and give a global meaning to the whole QTL information. This is the challenge of statistical QTL meta-analyses. The detection of common QTLs and the identification of co-locating resistance candidate genes from different experiments and populations have been recently achieved using QTL meta-analyses in maize to find resistance genes for virus diseases (Wang *et al.*, 2016), leaf rust in wheat (Soriano and Royo, 2015) and verticillium wilt in cotton (Zhang *et al.*, 2015).

A new era for QDR studies: phenotyping has the last word
The greatest aim for QDR studies in the past century was to increase molecular markers in mapping populations to capture all the allelic variants of genes that govern complex traits. Advances in high-throughput sequencing technologies have overcome this limitation, at least partially. However, the current challenge is to produce quality phenotype data, increasing molecular information and representing the bedrock of a new era of plant quantitative trait studies that will contribute to a better understanding of QDR (Basu *et al.*, 2015).

Advances in automated precision phenotyping or high-throughput phenotyping apply technologies principally based on image, thermal, spectra and digital sensors, from which quantitative phenotypic information can be generated (Araus and Cairns, 2014). There are several advantages of these approaches. First, the reduction of subjectivity in the determination of disease incidence and symptoms during a particular plant-pathogen interaction. Second, the increase in the number of plants that can be evaluated. Finally, the increase in the reproducibility and the possibility to collect data at numerous time points (Mutka and Bart, 2015).

Some of the more sophisticated technologies for high-throughput phenotyping applied in QDR studies are hyper-spectral imaging, chlorophyll fluorescence imaging and thermal imaging (Mutka and Bart, 2015). Plant diseases produce different spectral reflectance patterns and plants

suffering biotic stresses display changes in chlorophyll fluorescence emission (Baker, 2008). It has been shown that pathogens can change plant tissue temperature during the infection process. With these recent technologies, all of these parameters can be measured, even in the early plant phenological stages (Mutka and Bart, 2015). Wheat and sugarcane are some crops where these technologies have been used for detection and study of QDR (Mahlein *et al.*, 2012; Bauriegel and Herppich, 2014; Mutka *et al.*, 2016). Despite the fact that these techniques require a large number of previous evaluations in order to set the parameters for each disease, their potential in phenotyping plant disease is undeniable.

Phenotype has also been studied from an "omics" view (Salvi and Tuberosa, 2015). This new phenotyping method includes transcripts, proteins and metabolites, such as elements directly related to the phenotype, which have led to approaches such as expression-QTLs (eQTLs), protein-QTLs (pQTLs) and metabolite-QTLs (mQTLs), respectively. eQTLs and mQTLsare the more used given their progress in collection, automation and analysis of data (Salvi and Tuberosa, 2015).

From theory to practice: QDR in breeding

The complexity of QDR represents a challenge and an opportunity for plant breeding. A breeding scheme focused on obtaining qualitative disease resistance is relatively simple. It would be enough to introduce a single *R* gene into a susceptible plant background to confer resistance. On the other hand, in the case of QDR, the introduction of a gene from a QTL can confer a reduction, but not absence of disease. Thus, it would not be possible to get complete resistance until all of the resistance responsible loci are identified. In addition, in contrast to the current relative large repertoire of isolated *R* genes, the isolation of genes governing QDR for future use in breeding programs has not been an easy task.

For decades, marker-assisted selection (MAS) (Xu and Crouch, 2008) and gene pyramiding (Brun *et al.*, 2010) efforts have been directed toward the use of QTLs with major effects, explaining more than 20 % of phenotypic variance, for introduction into plant resistance breeding programs (Collard *et al.*, 2005). Some examples with great success in achieving high levels of resistance are found in rice (Bustamam *et al.*, 2002), common bean (Miklas *et al.*, 2006) and pearl millet (Sehgal, 2016), but unfortunately this has not been the case for most crops, including staple crops, such as cassava.

In plant breeding programs focusing on QDR, one of the limitations to be considered is the genetic linkage between the genes conferring resistance and closely linked undesirable genes, a phenomenon called linkage drag (Summers and Brown, 2013). Undesirable genes may affect the commercially

accepted gene pool and, therefore, modify the quality and crop yield. If linkage drag is not eliminated or decreased, the use of the QTL in the program will be impractical. The MAS strategy has counteracted this phenomenon. Through high throughput genotyping and the use of haplotype analysis of the introgressed region (QTL), linkage drag in seedlings can be detected and tracked in order to subsequently backcross these individuals to resistant varieties lacking drag. This strategy was applied to detect and remove the linkage drag around the *Rpv12* gene and confer resistance to powdery mildew in wine grapes (*Vitis vinifera* L.) (Venuti *et al.*, 2013); alternatively, the marker-assisted recurrent selection (MARS), combined with genomic selection (GS) (Heffner *et al.*, 2009), can also contribute to solving the linkage drag problem for QDR (Summers and Brown, 2013). GS selects plant material carrying whole genome molecular marker that are associated with resistance to a specific pathogen through the prediction of the phenotype using breeding values (BV) (Falconer and Mackay, 1996). These BVs are obtained by the compilation of molecular marker scores, phenotypic data evaluation of several germplasm and populations under a range of environmental conditions and (if it is available) pedigree information. Thus, MARS would increase the frequency of insertion of the desirable gene, decreasing the incorporation of undesirable ones and speeding up the detection of resistance loci with GS.

Another limiting factor in exploiting QTL with the aim of generating new varieties is the effect of the environment on the QTL. Multi-environment analyses in QDR studies offer the opportunity to detect the QTL x environment interaction (Q x E), conditional QTLs (El-Soda *et al.*, 2014) and QTL stability during seasons and crop cycles. Special attention should be given to an eventual QxE interaction in plant quantitative resistance that is widely influenced by the environment and in which heritability values are usually low (Ntare and Williams, 1998).On the other hand, the functional validation of candidate generation an important part of QDR studies, which can be carried out by overexpressing or down regulating the candidate gene by applying genetic engineering (Mittler and Blumwald, 2010) or exploiting the mutant collections (Cavanagh *et al.*, 2008).

In a large number of QDR studies the phenotypic evaluation (host response to the pathogen) is done after an artificial inoculation, employing a particular strain or a group of strains, allowing for the detection of QTL associated with these strains and leaving aside other genomic regions involved in resistance to other strains. When these QTLs are introgressed in particular varieties and evaluated under naturally diseased fields, where different pathogen strains or races can be found, it is possible to obtain unsatisfactory results. For this reason, it is mandatory that a breeding program starts with the knowledge on the dynamics and diversity of the pathogen populations.

Molecular explanation of quantitative resistance

Although important progress in understanding and analyzing complex traits has been accomplished in recent years, knowledge on the molecular basis of the QDR is still scarce. Several hypotheses have been generated to explain the function of the genes that control the QDR (Poland *et al.*, 2009). Five genes have been cloned from QTLs, which has enriched the proposed models. The rice *Pi21* gene, which encodes for a protein that has a heavy metal–transport/detoxification domain, confers resistance to several races of *Magnaporthe oryzae* (Fukuoka *et al.*, 2009). The QDR genes from wheat *Yr36* (resistance to *Puccinia striiformis* f. sp. *tritici*) and *Lr34* (resistance to *Puccinia striiformis*, *P. triticina* and to *Blumeria graminis*) encode for a Kinase-START protein and a pleiotropic drug resistance subfamily of ABC transporters, respectively (Fu *et al.*, 2009; Krattinger *et al.*, 2009). In addition, the *RKS1* gene, which encodes for an atypical kinase identified in the model plant *Arabidopsis thaliana*, confers resistance to most *Xanthomonas campestris* races, and to the pathovars *raphani*, *incanae* or *armoraciae* (Huard-Chauveau *et al.*, 2013). Finally, the receptor-like protein coded by the *ZmWAK* gene of maize that confers resistance to *Sphacelotheca reiliana* was cloned recently (Zuo *et al.*, 2015). Considering these discoveries and the gaps in QDR knowledge, different explanations of how it works are plausible.

QDR as a continuous response that depends on gene expression intensity

The expression level of genes involved in plant resistance can play important roles on the intensity of the expressed resistance. Transcriptomic analyses have allowed the identification of global changes in the expression profiles of genes involved plant immunity. Through expression analysis, it was possible to demonstrate that, during incompatible, compatible and non-host interactions, gene expression profiles were almost the same and that differences consisted in the intensity and kinetics of their induction (Tao *et al.*, 2003). Other studies support the overlap between PAMP and ETI at the gene expression level (Navarro *et al.*, 2004; Bozsó *et al.*, 2009; Bozso *et al.*, 2016). For QDR, several studies have reported a direct relationship between the expression level of some genes and the degree of resistance response. The maize *ZmWAK* gene is induced after pathogen inoculation. This gene is highly expressed in the mesocotyl and, at a lesser extent in the coleoptile of resistant lines, and the expression level of *ZmWAK* can be associated with the degree of pathogen growth restriction in mesocotyl and coleoptiles (Zuo *et al.*, 2015). Likewise, the *RKS1* gene expression is correlated with the resistance level in different *Arabidopsis* accessions to *Xanthomonas campestris* pv. *campestris* (Xcc) strain 568 (Huard-Chauveau *et al.*, 2013). Finally, the induction of the expression of susceptibility increases the susceptibility of plants, as demonstrated for the susceptibility

Pi21 gene from rice. In this case, transgenic plants showing higher expression of this gene were more susceptible to a virulent race of *Magnaporthe oryzae* (Fukuoka *et al.*, 2009).

QDR can result not only from gene expression level, but also from pattern expression of genes. The wheat *Lr34* gene, which was recently cloned from a QTL involved in wheat partial resistance to rust fungi and powdery mildew, is expressed in similar levels in resistant and susceptible plants. However, its expression level significantly varies between wheat seedlings and adult plants, where it is more effective (Krattinger *et al.*, 2009). In this case, the quantitative response depends not on the genetic background of the plant, but on the alteration of defense gene expression by different factors, such as the developmental stage of the plant, tissue or organ location, etc.

The above examples suggest a correlation between the transcription level of QDR genes and the degree of resistance (Fig. 1). For instance, Huard-Chauveau *et al.* (2013) established, that the expression of RKS1-L in natural accessions was negatively correlated with the disease
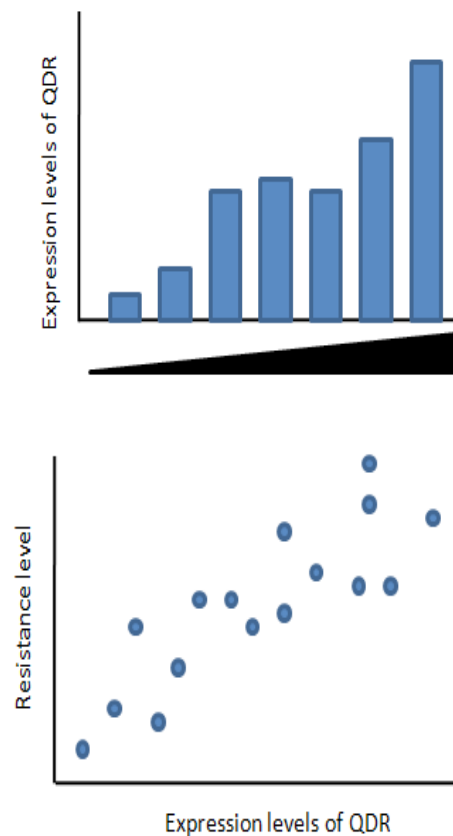

Figure 1. Model explaining the quantitative nature of resistance. As discussed in the text, the expression level of a group of particular genes involved in immunity contributes to the final output of the phenotype. The output intensity is determinate by the number of genes induced and their expression level.

intensity. A question could be what is the factor that determines the induction level of gene expression? During the expression of plant resistance, it is considered that the induction of defense gene expression result from the activation of a signal pathway, which in turn is dependent on pathogen recognition. According to this, gene expression level could be conditioned depending on the ability, specificity and strength of the interaction between pathogen-derived molecules and plant receptors.

Another aspect related to the transcriptional control of resistance level is the requirement for the presence of alternatively spliced transcripts. The classical example is the N gene from tobacco, initially known as conferring total resistance in tobacco to Tobacco Mosaic Virus (TMV). Through alternative splicing, both short N_S and long N_L transcripts are produced. During the initial phase of infection, the N_L version, encoding the full-length N protein, is more abundant, but, four hours after inoculation, a short truncated version is more abundant. If only one of the two variants is present, complete resistance is lost (Dinesh-Kumar and Baker, 2000). Concerning the specifically QDR related gene, above mentioned *RKS1* gene, two transcripts were identified, with differences in length between resistant and susceptible *Arabidopsis* accessions (Huard-Chauveau *et al*., 2013). Another example is the *Yr36* gene from wheat, which can result in up to six alternative transcript variants; however, only one of them encodes for a protein containing a complete START domain. This transcript is differentially regulated by temperature and is the only one that is up-regulated after inoculation with the fungus *Puccinia striiformis* f. sp. *tritici* (Fu *et al*., 2009).

To summarize, the transcription level differences shown by QDR genes suggest that it is necessary to incorporate the information on gene expression into the DNA variation data in order to achieve a systematic genetic approach and, thus, gain a better understanding of the molecular bases of the quantitative response (Mackay *et al*., 2009).

R weak alleles

As mentioned before, the first step in the activation of plant immunity is pathogen-derived molecules recognition. In ETI, a specific, strong and direct or indirect interaction between R proteins and the corresponding effector (named Avr) results in the activation of a signaling pathway, leading to immunity, which, in most cases, is associated with an HR response. Such as Avr-R recognition results in only one possible phenotype, *i.e.* R. Several studies on QTLs have demonstrated the presence within them of typical qualitative *R* genes, encoding for NB-LRR in QTLs, suggesting that the molecular bases of the pathogen recognition during QDR can exhibited common features with those of ETI. Supporting this hypothesis is the fact that both responses, ETI and QDR, share common molecular components (Roux *et al*., 2014b). In this model, the cause of the partial nature of QDR is the presence of "weak alleles" of *R* genes (Roux *et al*., 2014b). How to explain that an R protein confers only partial resistance? R protein is responsible for the recognition of a specific effector or the activity of it on a pathogenicity target protein. A weak allele of the R protein can correspond to a protein that is able to interact with an effector (or pathogenicity target), but its affinity is not high enough to induce a normal full response.

Allelic variation

The R weak alleles hypothesis can be considered as an extreme case of *R* gene allelic variation resulting in QDR. However, other QDR genes have shown allelic variation. Indeed there is evidence that QDR is associated with allelic variation of genes that differ in structure from canonical R proteins and that are important for plant defense. For example, the recently cloned QDR gene, *ZmWAK,* exhibits alleles differing in seven substitutions and a deletion between the resistant and susceptible maize lines. Although, in this case, polymorphisms affecting protein function were not found (Zuo *et al*., 2015), these polymorphisms could affect the interaction with other molecules or could prevent protein complexes formation. A similar situation was observed for the *RKS1* gene, which, even if it is present in both resistant and susceptible accessions, has several SNPs that have been found to be associated with both phenotypes. These SNPs are located within both the coding and the 5' and 3' regulatory regions. In fact, one of the identified in *RKS1* susceptible alleles corresponds to a stop codon. The authors suggested that mutations could be associated with susceptibility as a consequence of altered RKS1 long transcript expression (Huard-Chauveau *et al*., 2013). Polymorphisms have been also found between resistant and susceptible wheat plants, which are located within the *Lr34* gene; two of them were located in exons and one in an intron. The polymorphisms located in exons are present in the resistance cultivar and correspond to a 3 bp deletion and an SNP that changes the aminoacid tyrosine for histidine and affect the first transmembrane domain of the ABC transporter (Krattinger *et al*., 2009). Resistant and susceptible rice cultivars have seven polymorphisms between them, located in the genomic region that harbors the *Pi21* gene. Two of these polymorphisms correspond to deletions and were associated with the corresponding phenotype. Polymorphisms in this region, between different cultivated rice accessions, allowed for the identification of 12 haplotypes and revealed the natural variation of QDR genes. Only one haplotype was associated with resistance (Fukuoka *et al*., 2009). Further studies that include the intermediate phenotypes that are in between the lines or accessions that have already been evaluated, will resolve the role of polymorphisms in QDR. It is important to stress that these studies were conducted on contrasting lines, representing extreme resistant and susceptible phenotypes, and it would

be interesting to evaluate the expression levels of QDR genes in individuals showing a gradient of phenotypic response.

Polymorphisms can be present even in promoter sequences, *e.g.* in the *ZmWAK* gene; however, no association with function or phenotype has been studied for these variations (Zuo *et al.*, 2015). Additional studies are required to reveal if there is an association between such polymorphisms and QDR. In addition, polymorphisms located in introns or in promoters can modify transcription factor binding and splicing events, resulting in various quantities of transcripts or differential timing and tissue specificity of gene expression (Mackay *et al.*, 2009).

The studies presented here to exemplify allelic variation are important, not just because they show that polymorphisms result in different alleles, but also because they were found in QDR genes that were cloned and validated, and could result in quantitative resistance. The number and nature of polymorphisms found in a QDR gene or in its genomic region could define the level of the phenotype. Huard-Chauveau *et al.*, 2013 suggested that the QDR phenotype could be due to the additive effect or interaction of SNPs present in the identified haplotypes (Huard-Chauveau *et al.*, 2013). Likewise, we propose that there is a highly resistant phenotype exhibited by a haplotype for a QDR gene or its genomic region and that variation of this haplotype would lead to the quantitative nature of the resistance. Moreover, additional SNPs could be located in other genes or other genomic regions that account for the resistance. Furthermore, susceptible alleles could compete with resistant ones for the interaction with scaffold proteins of molecular signaling complexes (Huard-Chauveau *et al.*, 2013). Complementary studies with accessions that represent whole the range of different resistance levels and the corresponding sequence polymorphisms will help to tell if the polymorphisms present in QDR sequences are associated with the phenotype in order to validate this hypothesis.

Kinases and signaling
Kinases are essential components in plant cell biology and regulate different processes, such as biotic stress (Afzal *et al.*, 2008; Parniske, 2008), hormone signaling (Santner and Estelle, 2009), growth (Hematy and Hofte, 2008), cell differentiation and other physiological processes (De Smet *et al.*, 2009). Serine-threonine kinases are important signaling transduction components of both PTI and ETI (Zipfel, 2014) and MAP kinase cascades regulate downstream defense responses (Schwessinger and Zipfel, 2008). Additionally, pseudokinases are described as being important in signaling network control (Huard-Chauveau *et al.*, 2013). In this context, it is reasonable to consider genes involved in the signaling pathway, including MAP kinases, as putative key elements of QDR.

Several proteins that have been characterized as involved in QDR have proved to be kinases or pseudokinases. RKS1

from *Arabidopsis* (Huard-Chauveau *et al.*, 2013) is a typical kinase, ZmWAK from maize contains a kinase domain (Zuo *et al.*, 2015) and Yr36 from wheat has a kinase domain similar to *Arabidopsis* WAK-like kinases (Fu *et al.*, 2009). In addition, through eQTLs in barley, a gene that encodes for a "putative histidin-kinase" was identified as an important component of the resistance to *Puccinia graminisf.* sp. *tritici* (Druka *et al.*, 2008). This biochemical characteristic opens the door to possibilities for the role that these proteins may play in QDR, as for example like transmitting molecular signals.

Miscellaneous
One interpretation of how QDR works at the cellular level considers QDR as corresponding weak PTI or ETI (López, 2011; Kushalappa *et al.*, 2016). In this way, resistance known as qualitative could also be polygenic and be achieved if all the components are present and function correctly (Kushalappa *et al.*, 2016). Therefore, the quantitative counterpart may have missing components. The missing concept here not necessarily corresponds to complete absence of a particular component, but to differential quantities of resistance related metabolites, proteins coded by *R* genes, or PRRs, which in turn are regulated by other genes (Kushalappa *et al.*, 2016). Therefore, the more defense-related components are missing (or diminished), the more resistance is reduced. An alternative, but not excluding, hypothesis states that differences in defense responses are the consequence of the sensitivity of the components to input signals. It was hypothesized that resistant plants display robust responses because they are insensitive to small changes in input signals (Tao *et al.*, 2003); therefore, the remaining range of responses of QDR could be more sensitive to this change. QDR have been recently redefined due to the cloning of some of the corresponding genes and it has been stated that the involved proteins do not belong to a specific group, such as in the case of *R* genes, but may have several functions (Navabi *et al.*, 2005; Poland *et al.*, 2009; Bryant *et al.*, 2014; Roux *et al.*, 2014a). Thus new molecules, which previously have not been described as important during plant-microbe interactions, could be responsible for resistance (Roux *et al.*, 2014b). Nevertheless, it seems that *R* genes actually have roles in QDR, but with a weak contribution, as compared to other genes with different structures and functions (Corwin *et al.*, 2016).

CONCLUSIONS
In the present review, we highlighted the potential but still to be achieved impact of QDR on plant breeding, here is quantitative gap between the amount of published QTLs studies and the application of the generated information at the field scale.

In addition, results coming from QTL studies could lead to false conclusions, since a genomic region could be

identified as responsible for disease resistance because of a statistic artifact. Furthermore, unless QTL detection is done in well-controlled greenhouse or growth chambers conditions, these experiments should have repetitions over different growing cycles and different seasons or weather conditions to be sure that an identified QTL is both real and stable. Moreover, the identification of these QTL is frequently done with only one strain of the pathogen. In some cases strain is being evaluated is not even know when studies are done using natural inoculation. Therefore, QTL validation should include multiple strain inoculations. Plant-microbe interaction is a two-way interaction, so the genetic characteristics of the pathogen are necessary components that should be taken into account. Frequently, QTL are involved in resistance to populations of pathogens; however, no information about the evolution or diversity level of the pathogen is included or old information is often used. Finally, the problem of subjectivity that adds error to QTL studies will be removed with the arrival of precision phenotyping methods that, in the end, will result in much better reproducibility and reliability of these studies.

We gathered results and experiences from different pathosystems for QDR because we wanted to highlight some components of plant defense that are known, but that have not been integrated or incorporated to plant immunity models. Thereby, plant immunity must be seen from a different point of view. We propose here that plant defense is not a simple and single-layered issue, but is instead a synergistic process and represents the sum of several protein interactions that are occurring at the same time. Even hypotheses or mechanisms proposed by other authors, such as Poland *et al*. (2009), may occur simultaneously. Here, we presented different molecular explanations of how QDR work. It is important to note that these explanations are not exclusive. We also suggest that it is necessary to consider alternatives to classically adopted models. A particular and specific model could be valid for a given pathosystem, but not for others. Even though the zig-zag model has helped to understand plant immunity, it just explains a part of it, which represents monogenic interactions in which QDR are not included. To achieve a better understanding of plant immunity, a holistic approach should be considered that integrates the intricate array of interactions between molecules and cells, determining the complexity of phenotypic traits in the frame of the IPs model for example.

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CONFLICT OF INTEREST
The authors declare that there is no conflict of interest.

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