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How can we exploit functional genomics approaches for understanding the nature of plant defences? Barley as a case study

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Abstract The concept 'functional genomics' refers to the methods used for the functional characterisation of genomes. The methods utilised provide new opportunities for studying the nature and role of defence mechanisms in plants. Unlike *Arabidopsis*, poplar and rice, the full genomic sequence of barley is not available. In this case, the analysis of barley gene expression data plays a pivotal role for obtaining insight into the functional characterisation of individual gene products. Many genes are activated transcriptionally following attack by pathogens and these often contribute to the defence mechanisms which underlie disease resistance. The use of large-scale complementary DNA library constructions and genome-wide transcript profiles of plants exposed to biotic stress provide the data required to drive hypotheses concerning the function of newly identified genes. In this paper, we illustrate how publicly available gene expression data has proved valid for studies of plant defence responses; enabling a cost-effective workflow starting from isolated gene transcripts to elucidation of biological function upon biotic stress.

Keywords Barley · Functional genomics · Plants · NAC transcription factors · *Hordeum vulgare* · *Blumeria graminis* f.sp. *hordei* · Pathogen

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Introduction

Plants are constantly under attack by microorganisms. However, only a few of these are potential pathogens capable of causing disease on a particular plant species. Even for host pathogen species, only few pathogen attacks actually develop to cause a successful infection. More often than not, the plant succeeds in repelling attack through deployment of its defences, with disease resistance as the result. Before we describe the means by which functional genomic approaches can be deployed for the elucidation of molecular components engaged in plant defences, a brief summary of plant defensive strategies is presented.

Plant defences comprise the production of antimicrobial compounds (Field et al. 2006; Hammerschmidt 1999) and proteins (van Loon et al. 2006), chemical and physical changes to secondary cell walls (Mörschbacher and Mendgen 2000), and the induction of programmed cell death, known as the hypersensitive response (HR; Jabs and Slusarenko 2000). Some defence mechanisms are essentially constitutive, that is, they are always produced at a particular stage in the host's development.

Others are first induced or activated when a pathogen attacks the host. Many of the same defences are activated in a particular host by different pathogen species (Collinge et al. 2002). Even in compatible interactions, plant defences comprise an effective barrier to pathogens, often limiting the rate at which the pathogen invades the host tissues (Trujillo et al. 2004). However, the particular mechanisms which are effective against a specific pathogen will depend on physiology of the pathogen, which in part reflects its taxonomic group, e.g., fungus, bacteria or virus.

The employment of different life style strategies by different pathogens also plays a role in the efficacy of specific defence mechanisms used by the attacked host plant. The extremes are represented by necrotrophy, in which the pathogen destroys and consumes the host tissues, and biotrophy, in which the pathogen parasitizes living tissue. Hemibiotrophs in essence utilise both strategies at different phases of their life cycles. Defence mechanisms, for example, the HR, differ in their effectiveness against pathogens using these different strategies, and the regulation of the activation of these mechanisms differs too; thus salicylic acid signalling and jasmonic acid signalling are associated, at least in *Arabidopsis*, with defence against biotrophs and necrotrophs, respectively (Glazebrook 2005; Zimmerli et al. 2004). This generalisation highlights important correlations, though some degree of stimuli-dependent discrepancies have been observed (Zimmerli et al. 2004). Effective resistance thus depends on successful recognition by the host that it is being attacked by the pathogen and deployment of the appropriate defence mechanisms at the right place and at the right time.

In this article, we will look at the means by which plant defences are studied and the tools which can be used for determining whether a particular defence mechanism has a role in disease resistance towards a specific pathogen. As a case study, we will focus on defence against a biotrophic fungal pathogen and use the interaction between barley, *Hordeum vulgare*, and the barley powdery mildew fungus, *Blumeria graminis* f.sp. *hordei* (*Bgh*). Functional genomics encompass a wealth of scientific disciplines not covered in this case study. Thus, high-throughput bioimaging, proteomics and metabolomics approaches are also excellent tools which are being utilised in various biological systems for an improved understanding of genome function, even for those not yet sequenced.

Why barley and *Blumeria graminis*?

Bgh is the causal agent of powdery mildew; one of the most important diseases of barley worldwide. The barley–*Bgh* interaction has evolved as a model system for several reasons, both biological and practical (Collinge et al. 2002). Firstly, a very large number of race-specific resistance genes have been described (Jørgensen 1994)–and many of these have been incorporated into near-isogenic lines of barley (Kølster et al. 1986). The resistant phenotype for the majority of these disease resistance genes is associated with the HR. Recently, allelic diversity has become accessible with ecoTILLING lines (Mejlhede et al. 2006) and single-nucleotide polymorphisms (SNP) populations (Rostoks et al. 2005), which offer great potential for exploitation of the natural variation of disease resistance. Thus, ecoTILLING is a polymerase chain reaction (PCR)-based technique that allows identification of allelic variants in known genes of interest, for example R-genes (Mejlhede et al. 2006). A new microarray-based technique has been developed for identifying SNP in specific genes (Rostoks et al. 2005). These methods represent new tools for identification of the allelic variation in known genes, which can be tested phenotypically for their influence on disease resistance. Secondly, the development of the fungus on the host is synchronised, facilitating meaningful experiments where physiological and molecular responses of the barley host can be correlated perfectly with the development of the fungus, using bioimaging analyses (Shen et al. 2007) and transcript profiles among other methods (Caldo et al. 2006; Gjetting et al. 2007; Gregersen et al. 1997; Zierold et al. 2005). Collectively, this has made the barley–*Bgh* interaction among the best-studied pathosystems for investigating plant responses towards pathogen attack.

Historical perspective of transcriptomics in the barley–*Bgh* interaction

The first molecular studies to assay responses in the barley transcriptome to *Bgh* used in vitro translation products by two-dimensional polyacrylamide gel electrophoresis (Collinge et al. 2002; Gregersen et al. 1990; Manners and Scott 1985). The next phase, in the 1990s, was the utilisation of various differential and subtractive hybridisation techniques to

isolate complementary DNA (cDNA) clones (Collinge et al. 2002; Gregersen et al. 1997; Hein et al. 2004), and the differential expression suggested by the screening method was confirmed by northern blotting. In some cases, sequence-based identification was supported by biochemical evidence (see Collinge et al. 2002). For the barley–*Bgh* interaction, these approaches for gene discovery have been superseded largely by the use of expressed sequence tag (EST) libraries which provide a vast open resource of partial–and even full-length–cDNA sequences, representing roughly 500,000 individual cDNA clones, which reflect gene expression in specific tissues and physiological states. We illustrate this in Table 1 with the NAC transcription factor family of barley (see below). EST databases are now providing a corroborative effort to assemble contigs (as sequences assembled from smaller overlapping fragments) encoding full-length or near full-length gene products. For instance, the UniGene database (http://www.ncbi.nlm.nih.gov/sites/entrez?db=unigene) is an in silico experimental system for automatically partitioning GenBank sequences into a non-redundant set of gene-oriented clusters. It uses part of a coding sequence to extract all sequences including transcripts exhibiting homologies to a given query sequence (Boguski and Schuler 1995). From such homology searches, sequences including ESTs with high sequence similarity are grouped into clusters. Subsequently, these UniGene clusters can be used to (1) obtain an indication of the level of transcript accumulation for a given UniGene member for a specific tissue and (2) perform intercluster comparisons for the possible discovery of expressed genes responding to a particular environmental stress factor, physiological state or developmental stage (Zhang et al. 2004). However, it should be stressed that UniGene clusters are dynamic entities, and represent the current best model for interpreting the coding sequences of genes in the databases expressing these 'tags' at the time of sampling.

EST data, (e.g. Zhang et al. 2004) also provided the basis for the design of an Affymetrix GeneChip®, which in barley carries 22,792 gene sequences (Close et al. 2004; Shen et al. 2005). Parallel to the development of this Affymetrix Barley1 GeneChip®, dotted filter array technology has also been utilised for barley–*Bgh* interactions (Zierold et al. 2005). Each of these technologies offers its advantages and disadvantages which we will discuss below. However, all hybridization-based transcriptome techniques suffer the limitation that a specific gene will not be present unless the cDNA is prepared from a tissue in the physiological state where it is expressed. This problem is essentially solved once the entire genomic sequence is available for the species in question. Although the barley genome is large (5,000 Mb), it is predicted that a draft sequence for barley will be available within a few years.[1]

Transcriptomics in barley today

The Affymetrix Barley1 GeneChip® microarray has been used for several studies of gene expression in barley after *Bgh* inoculation (Caldo et al. 2004, 2006) as well as for other interactions in barley involving biotic, namely *Fusarium graminearum* (Boddu et al. 2006), and abiotic (Svensson et al. 2006) stress, namely cold. Likewise the dotted filter array has been utilised for barley–*Bgh* interactions (Eichmann et al. 2006; Gjetting et al. 2007; Zierold et al. 2005). The advantage of the dotted filter array compared to the microarray is that it is straightforward to add new sequences to the study as they are discovered. A disadvantage lies in reduced sensitivity and specificity. This makes it difficult to distinguish closely related and weakly expressed gene sequences from each other. The advantage of both array technologies is that it is possible to study the expression of a large number of genes simultaneously. Thus detailed time course studies with appropriate biological replicates using array technologies have been conducted (Caldo et al. 2004, 2006) and much of the data from these and other studies can be accessed through the public Affymetrix-specific data repository PLEXdb (www.plexdb.org).

Case study: EST libraries and their exploitation for studying the NAC transcription factors of barley

Members of the large plant-specific gene family encoding NAC transcription factors share a common N-terminal domain, comprised of five highly conserved motifs. The domain is termed NAC from its

[1] http://pgrc.ipk-gatersleben.de/etgi/publications/whitepaper_barley_physmap_and_sequence.pdf; http://www.ars.usda.gov/research/projects/projects.htm?ACCN_NO=411452

Table 1 Gene-oriented UniGene clusters of NAC transcript sequences

Barley NAC UniGenes	Transcripts[a]	Rice homologue (% iden.)	*Arabidopsis* homologue (% iden.)	cDNA source
Hv.6550 (*HvNAC4*)	12[a]	Os01g0816100 (79.4%)	ATAF2 (At5g63790) (62%)	*B. graminis* inoc. leaf, seed, stem, root
Hv.1425	88[a]	Os03g0815100 (85.4%)	ATAF2 (At5g63790) (65.6%)	*B. graminis* inoc. leaf, seed, callus, root
Hv.984	3	Os03g0624600 (55.9%)	–	Root
Hv.6308	38	Os02g0822400 (71.4%)	ANAC051/ANAC052 (At3g10490) (58%)	*B. graminis* inoc. leaf, seed, callus, flower
Hv.5295	28[a]	Os08g0562200 (81.4%)	ANAC053 (At3g10500) (49.3%)	*B. graminis* inoc. leaf, callus, flower
Hv.5097	8[a]	Os03g0327800 (76.2%)	ATAF2 (At5g63790) (56%)	Seed, leaf, root
Hv.5147	7	Os07g0683200 (85.5%)	ANAC032 (At1g77450) (71.6%)	Leaf, flower, root
Hv.877	10	Os04g0460600 (80.5%)	ANAC092 (At5g39610) (69.8%)	Root, leaf
Hv.17199	13	Os09g0493700 (68.2%)	ANAC051/ANAC052 (At3g10490) (48.4%)	*B. graminis* inoc. leaf, seed
Hv.2154	5	–	–	Seed
Hv.4825	26[a]	Os08g0157900 (69.8%)	–	*B. graminis* inoc. leaf, stem, callus
Hv.2154	14	Os08g0200600 (69.9%)	(At3g12977) (64.7%)	Seed
Hv.15755 (*HvNAC6*)	47[a]	Os01g0884300 (86.5%)	ATAF1 (At1g01720) (76.5%)	*B. sorokiniana* inoc. leaf, root, callus
Hv.19392	10	Os07g0684800 (61.1%)	ANAC092 (At5g39610) (67.6%)	Seed
Hv.19815	6	Os04g0536500 (56.4%)	ANAC092 (At5g39610) (72.4%)	Seed, flower, *F. graminearum* inoc. leaf
Hv.6910	3	Os06g0131700 (46.1%)	ANAC092 (At5g39610) (64.4%)	Seed
Hv.18811	3	Os04g0536500 (58%)	ANAC092 (At5g39610) (61.2%)	Seed
Hv.18323	7	Os07g0684800 (75.7%)	ANAC092 (At5g39610) (67.6%)	*B. graminis* inoc. leaf, root, callus
Hv.17687	3	Os11g0294400 (57.1%)	–	Flower, meristem
Hv.21351	7[a]	Os11g0126900 (73%)	RD26 (At4g27410) (64.7%)	*B. graminis* inoc. leaf, callus, seed
Hv.21779 (*HvNAC1*)	8[a]	Os12g0610600 (81.8%)	NAC1 (At1g56010) (48.8%)	*B. graminis* inoc. leaf, stem, root
Hv.19852	24	Os11g0184900 (80.9%)	ANAC032 (At1g77450) (75.3%)	*B. graminis* inoc. leaf, stem, root
Hv.25370	6	Os06g0675600 (84.2%)	–	Leaf, seed, root
Hv.24230	3	Os03g0624600 (71.2%)	ANAC058 (At3g18400) (75.7%)	Root, stem
Hv.16999	24	Os03g0327100 (84.3%)	ANAC092 (At5g39610) (75.8%)	Root, leaf, seed, callus
Hv.16783	3	–	ANAC074 (At4g28540) (63.7%)	Root, seed

[a] Full-length mRNA available

first identified members *NAM*, *ATAF1*,*2* and *CUC2* (Aida et al. 1997; Souer et al. 1996). Genes encoding NAC transcription factors have been reported to be induced by both abiotic and biotic stresses. Furthermore, modulation of the expression of individual members has resulted in improved salt and drought tolerance, and enhanced resistance towards *Fusarium oxysporum*, in rice and *Arabidopsis* (Hu et al. 2006; Lu et al. 2006). We have isolated several NAC gene members from barley, using differential display and cDNA library screening techniques for transcripts expressed in barley upon *Bgh* inoculation (Gregersen and Collinge 2001; Jensen et al. 2007). We have shown subsequently that *HvNAC6* (*H. vulgare NAC6*) has a positive role in penetration resistance against *Bgh* (Jensen et al. 2007). In the following, we will present how public transcript data repositories can be used in a data-driven approach for developing

hypotheses on the functionality of specific genes of interest. We will use expression profiles from NAC gene members as a case study, but any gene of interest can be exploited, as long as a transcript sequence originating from the gene of interest is present on the array platform to be analyzed.

A Basic Local Alignment Search Tool search of European Molecular Biology Laboratory and GenBank databases using a nucleotide sequence encoding the conserved NAC domain yields approximately 400 putative barley NAC derived transcript sequences. However, most of these are partial sequences which can be grouped nevertheless into 26 UniGene clusters based on sequence similarities (Table 1). Each UniGene cluster comprises several partial transcript sequences, ideally making up a contig (i.e. contributing to a composite and potentially complete gene sequence), deciphering the full-length mRNA sequence of the individual gene. Table 1 show that approximately 50% of the current (UniGene Build #48) NAC domain containing UniGene clusters include ESTs originating from *Bgh*-inoculated barley cDNA libraries. As transcripts from biotically stressed barley cDNA libraries are included in approximately 35% of the total number of barley UniGene clusters, NAC members could be over represented in biotically stressed barley cDNA libraries, making them interesting candidates for the understanding of the regulatory mechanisms involved in the barley–*Bgh* interaction.

Apart from the Barley1 Gene Chip®, one such platform is the ~3 k cDNA microarray from the *Bgh*-infected barley epidermis cDNA library developed by Institute of Plant Genetics and Crop Plant Research (Zierold et al. 2005). In barley, the recessive loss-of-function alleles of the *Mlo* gene mediate durable and race-nonspecific resistance towards *Bgh* associated with the rapid formation of large epidermal cell wall-associated structures termed papillae (Jørgensen 1992). By comparing the large-scale transcript responses of *mlo5* mutant plants with wild-type *Mlo* plants upon *Bgh* inoculation, Zierold et al. (2005) aimed at identifying candidate genes mediating durable resistance towards *Bgh* in barley. Investigating the origin of the approximately 400 transcripts representing 26 NAC UniGene clusters, for transcripts originating from the *Bgh*-inoculated DNA library used for spotting the nylon filter used by Zierold and co-workers, we identified 11 gene-oriented NAC clones, of which eight had been successfully spotted on the cDNA array (Fig. 1). Among the spotted clones, two belonged to UniGene clusters *Hv*.21779 and *Hv*.15755, of which we have isolated full-length cDNA clones (*HvNAC*1 and *HvNAC*6, respectively, see Jensen et al. 2007). Interestingly, from our data-mining, we observed *mlo5*-specific up-regulation of *HvNAC6* upon *Bgh* inoculation. In the susceptible *Mlo* wild-type background, no *HvNAC6* induction was observed (Fig. 1), possibly due to a *Mlo*-dependent negative control of *HvNAC6* transcription (Zierold et al. 2005). Another interesting transcript profile is depicted by the HO13D12 cDNA clone (UniGene cluster Hv.1425). HO13D12 abundance shows delayed accumulation in the wild-type plants compared to *mlo5* plants upon *Bgh* attack. As the outcome of race non-specific lines of defence are believed to depend on the timing of most responses towards attacking pathogens (Caldo et al. 2006) the observed delayed induction of HO13D12-specific transcripts in wild-type plants could affect the delicate timing of effective race non-specific resistance. However, the *HvNAC6* expression profile shown in Fig. 1 was not identical to another member of the Hv.15755 UniGene cluster, which did not show any genotype- or treatment-specific regulations (HO05F04, data not shown). Hence caution and thorough investigation of the repositories available should be performed when mining transcripts originating from genomes not yet sequenced. Finally, the generation of working hypotheses should include expression profiling by an alternate approach to the one analysed in silico (e.g., quantitative real-time (qRT)-PCR).

From an independent transcript analysis, we supplemented the *HvNAC6* expression pattern presented in Fig. 1 by using qRT-PCR on a pattern of a *Bgh*-challenged Pallas near-isogenic line (Kølster et al. 1986) and continued with functional studies to examine the possible importance *HvNAC6* for resistance towards *Bgh* (Jensen et al. 2007). For this purpose, we made use of the particle bombardment transformation assay of barley epidermal cells (Shirasu et al. 1999). Individual NAC gene constructs for in vivo gene silencing or over expression were co-transformed with *uidA* the β-glucuronidase reporter gene, thereby providing a reverse genetics tool to study the cell-autonomous interaction outcomes between barley and *Bgh* of transformed cells (Fig. 2). Our studies showed that *HvNAC6* transcript abundance indeed affects the defence responses in barley by

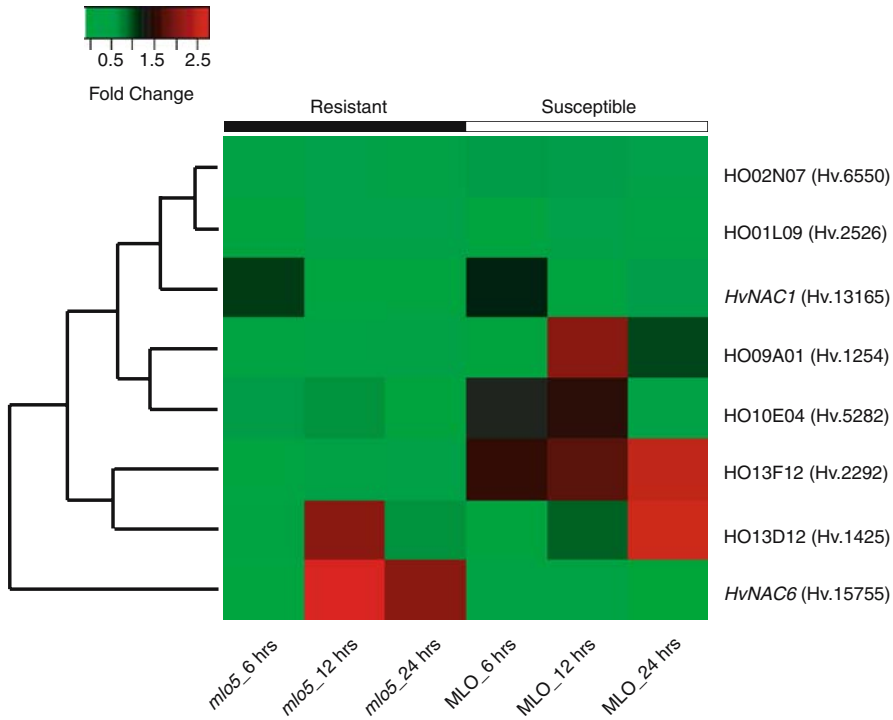

Fig. 1. Transcript accumulation of eight barley NAC genes and UniGene members upon *Bgh* inoculation and modulation of *Mlo*. An analysis of the epidermis-specific *Bgh*-inoculated cDNA library spotted onto nylon membranes (Zierold et al. 2005) reveals eight NAC encoding transcripts. The *bottom panel* displays the experimental conditions; genotype and hours after *Bgh* inoculation. Gene names and UniGene cluster gene-oriented names are given to the *right*. The *colours* refer to mean ratios of gene centred signal intensities of inoculated samples versus corresponding control samples (Eisen et al. 1998). Hierarchical clustering was performed using unscaled correlation and complete linkage clustering. *Colour key* displays correlation between colour and fold changes of *Bgh*-inoculated vs. control samples (non-inoculated)

positively regulating the formation of papilla and effective penetration resistance (Jensen et al. 2007).

To summarize, the wealth of data deposited in publicly available repositories provide a cost-effective tool for 'desk-top-to-bench-top' analyses of transcripts of interest (Fig. 3). Though data should be thoroughly inspected with respect to their origin and relevance to the research in question, it can accommodate new hypothesis to be tested in the laboratory or field. In the case of barley NAC transcription

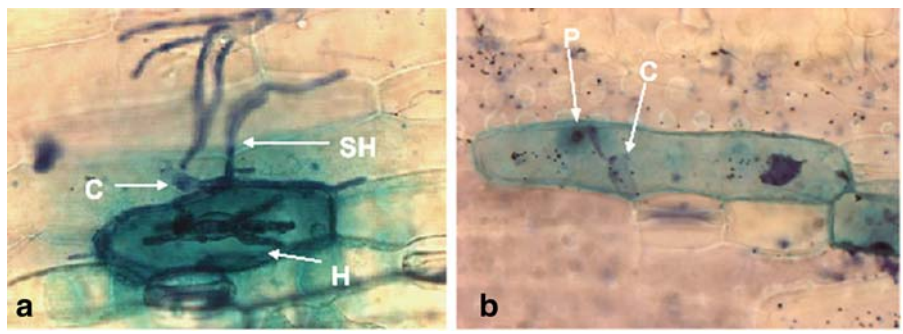

Fig. 2. Barley epidermal single-cell interaction outcomes with *Bgh* provides a well-established system for transient expression studies of genes of interest using GUS as a transformation control. (**a**) Susceptibility. An epidermal cell penetrated by a *Bgh* conidium (*C*) and subsequent development a feeding organ, known as an haustorium (*H*) and secondary hyphae (*SH*) elongation. (**b**) Race-non-specific resistance. A penetration resistant epidermal cell showing race non-specific resistance towards *Bgh* penetration attempts by formation of a papilla (*P*)

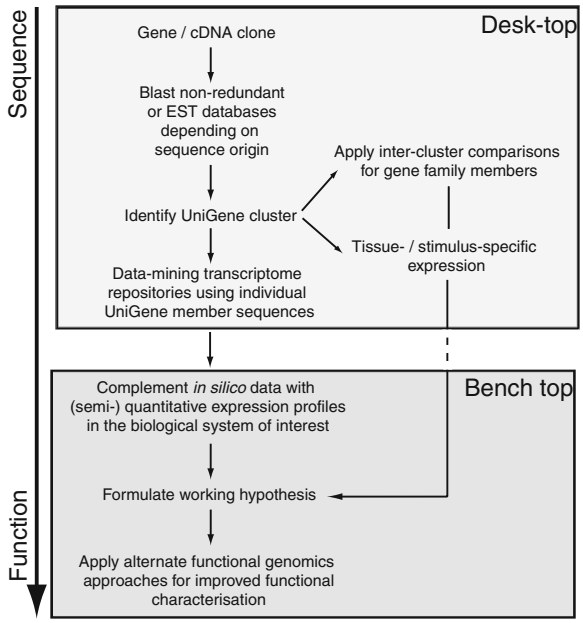

Fig. 3 Diagram showing a cost-effective strategy for the generation of working hypotheses aiming at functional characterisation of isolated genes or gene products

factors, interesting hypothesis have been tested and verified, partially based on publicly available EST resources and relatively simple analyses of expression platforms and reverse genetics approaches.

The role of individual genes in defence

Much of the effort to understand the defence mechanisms of plants concerns the identification of components of defences rather than understanding the role of the individual genes expressed in the defence response. It is abundantly clear from the literature that mutational approaches aimed at identifying genes necessary for disease resistance rarely lead to the identification of defence genes per se, i.e. those encoding antimicrobial proteins or enzymes involved in biosynthesis of antimicrobial phytoalexins (Field et al. 2006; Hammerschmidt 1999; van Loon et al. 2006). Instead, mutations affecting resistance are generally in genes involved in the regulation of defence mechanisms, including race-specific resistance genes themselves, and are often associated with signal transduction pathways (Glazebrook 2005; Panstruga and Schulze-Lefert 2002; Takken et al. 2008). They therefore fall outside the subject of this review. The lack of mutants in defence genes which exhibit compromised resistance implies that individual components of the defence response have an incremental, rather than determinative, role on the outcome of an interaction with a pathogen. The approach which to date has given the most extensive data set for understanding the impact that individual genes encoding components of defence mechanisms have on resistance to *Bgh*, is the use of transient RNA interference, itself a defence mechanism which operates against viruses (Lindbo and Dougherty 2005; MacDiarmid 2005). An inverse repeat RNA structure is produced in the host cell (Waterhouse et al. 2001). This RNA folds to make a double-stranded RNA molecule which stimulates the host cell's defence against viruses with the result that both extraneous and endogenous copies of the transcript are essentially eliminated, and, as a consequence, the host gene product is no longer produced. This can be achieved by direct particle bombardment using plasmid constructs which contain an inverted repeat of the sequence of interest, or by infecting with a virus containing the sequence, termed virus-induced gene silencing (VIGS). Most studies have used the former approach (Christensen et al. 2004; Douchkov et al. 2005; Jensen et al. 2007; Schweizer et al. 2000; Shen et al. 2007), and this includes the use of high-throughput technologies for studying large groups of genes (Douchkov et al. 2005). To date, few studies have used VIGS successfully to investigate the role of individual defence and disease resistance genes in the barley–*Bgh* interaction (Hein et al. 2005; Shen et al. 2007). A common feature of the results obtained is that the effect of silencing is usually partial. This is in accordance with results obtained with mutational studies where mutations in regulators provide the major phenotypic effects and no defence-related genes per se have been identified (Glazebrook 2005).

Concluding remarks

In this review, we have demonstrated the use of the genomics resources available in barley, a species where the genomic sequence is not yet available, for the identification and validation of the roles of individual genes involved in the regulation of, or encoding components of, specific defence mechanisms, and have illustrated the process with the NAC transcription factor HvNAC6. *Arabidopsis*, poplar and rice have

the advantage of full sequenced genomes and many tools have been developed which are not available in barley. For example, T-DNA insertion lines (i.e. tagged mutant collections) are available for many *Arabidopsis* genes, as are whole genome (TILLING) arrays. Furthermore, well-characterised mutants are available in barley, and several alleles of key (regulatory) genes have been described in *Arabidopsis*. A large body of knowledge about genes and encoded proteins and regulatory networks have been elucidated. Nevertheless, there is a wealth of well-documented natural genetic variation in barley, especially with respect to disease resistance, and this material is supplemented with many good mutants (Jørgensen 1994; Panstruga and Schulze-Lefert 2002).

Although microarrays are available for many species, it is a major undertaking to design and prepare microarrays carrying a significant part of a genome. What do you do if there is no microarray in your biological system? In these cases, the best approach is to use more classical gene discovery techniques such as the use of subtractive libraries (van den Berg et al. 2004) or differential display techniques, such as cDNA-amplified fragment length polymorphism (Liang and Pardee 1997; Ramonell and Somerville 2002). An alternative, though more costly approach would be to prepare a customized array, designed from the cDNA library of interest, for example from subtractive libraries, reflecting the transcriptome of the interaction of interest or for specific families of genes. One technology uses the Affymetrix GeneChip® platform, for example, through the collaboration between NimbleGen Systems and Affymetrix. Another very promising platform is the Agilent® multiplex custom arrays, which brings down the price for 15,000 features to roughly €100. Moreover laboratories worldwide are set up to make dotted oligonucleotide arrays, which may be a cheaper option. However, with a price tag of up to €800 per replicate, depending on platform, the microarray approach is still prohibitively expensive for many laboratories which instead make a more limited array experiment and support this with more detailed expression studies of individual candidate genes using northern blotting or quantitative real-time PCR.

Finally, for what can the knowledge gained from these studies be used for in the context of developing sustainable agriculture? The answer is currently not much–yet! Studies made to date have made it clear that the idea of taking an antimicrobial protein and using it to make a transgenic plant which has gained effective disease resistance is now largely discredited as the effect observed is at best, partial resistance (Collinge et al. 2008). This, however, is in itself valuable knowledge. A second achievement from the study of defence mechanisms is the realisation that the regulation of plant defence mechanisms is more complex than previously imagined. It is now becoming clear that antagonistic regulatory and interlinked signalling pathways are involved in both biotic and abiotic stress signalling. This may mean that the dream of making a universally disease resistant plant through the manipulation of single genes may remain that for the foreseeable future. The real challenge, and therefore our efforts, needs to be concentrated on understanding the nature of the regulatory networks underlying host defence responses against biotic and abiotic stress and through this be able to manipulate them to achieve resistance.

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