Plant Resistance to Parasitic Plants: Molecular Approaches to an Old Foe

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Summary:

Parasitic weeds pose severe constraint on major agricultural crops. Varying levels of resistance have been identified and exploited in the breeding programmes of several crops. However, the level of protection achieved so far is either incomplete or ephemeral. Resistance is mainly determined by the coexistence of several mechanisms controlled by multigenic and quantitative systems. Efficient control of the parasite requires a better understanding of the interaction and their associated resistance mechanisms at the histological, genetic and molecular levels. Application of post-genomic technologies and the use of model plants should improve the understanding of the plant-parasitic plant interaction and drive not only breeding programs through either Marker-Assisted Selection (MAS) or transgenesis but also the development of alternative methods to control the parasite. This review presents the current approaches targeting the characterisation of resistance mechanisms and explores their potentiality to control parasitic plants.

Keywords: Biotechnology; Crop Improvement; Model Plant; *Orobanche* spp.; Parasitic Plant; Resistance Mechanism; *Striga* spp.

Introduction

About 3500 flowering plant species have lost their autotrophic way of life during evolution and have adapted to parasitize other plants in order to supply themselves with water and nutrients. Among these, the obligate root parasites *Striga* and *Orobanche* are

the two major economically damaging parasitic weed genera causing important losses in a large number of crops (Yoder, 2001; Rubiales, 2003).

Striga spp. are major problems in semiarid African regions and in parts of Asia affecting production of cowpea and many cereals. *Orobanche* spp. parasitize a large number of crops such as legumes, crucifers, tomato, sunflower and tobacco, and constitute one of the most important biotic constraints to the food crop production in Southern and Eastern Europe, North America, North and East Africa, the Middle East and the Indian subcontinent (Joel *et al.*, 2006). Most *Striga* and *Orobanche* species show a large genetic diversity and complexity as a result of co-evolution with its host (Botanga *et al.,* 2002; Román *et al.,* 2002a). Their lifecycle is highly specialised for parasitism. For instance, the tiny *Orobanche* seeds, following a period of conditioning that is required for the dormancy-germination transition and the responsiveness to external stimulants, germinate in response to a specific root host stimulus (Figure 1). The germination gives rise to the radicle that elongates toward the host root and adheres to it through the formation of an *attachment organ* (appresorium). Then, *Orobanche* develops the *haustorium* that penetrates through the cortex, grows to the vascular cylinder and acts as the bridge through which all transfer between host and parasite is achieved. The outer part of the seedling develops into a tubercle which gives rise to a flowering spike that emerges from the soil (Figure 1). Successful parasite establishment creates a strong sink of nutrient in detriment of the host leading to drastic growth and yield reductions (Keyes *et al.,* 2001; Joel *et al.*, 2006).

Many control strategies have been applied from agronomical practices to genetic improvement of crops. However, only marginal successes have been obtained so far (Joel *et al.,* 2006). Nowadays, studies on agronomical and chemical practices are still under investigation, although the major effort shifted to genetic improvement of crops

that appears as the most appropriate and cost-effective control practice. Alternatively, studies aiming at the molecular characterisation of the plant-parasitic weed interaction and its resistance through expression analysis of the genes, proteins and metabolites involved in these processes, are attracting an increasing interest (Dos Santos *et al.*, 2003a,b; Castillejo *et al.*, 2004). These studies by increasing the understanding of the molecular bases of the interaction attempt to identify new targets to confer resistance to important crops. In this review the latest knowledge on the molecular basis of the resistance against parasitic plants is presented with a particular emphasis on the *Orobanche* interaction.

Control methods

So far, the effectiveness of conventional control methods is limited due to numerous factors and in particular the complex nature of the parasites, which reproduce by tiny and long-living seeds and are difficult to diagnose until they irreversibly damage the crop. The intimate connection between host and parasite also hinders efficient control by herbicides. Chemical control is the most common approach to limit crop damage although herbicides are hitherto effective only as a prophylactic treatment. Herbicide-resistant cultivars are targeted through mutagenesis, transgenesis or screening of naturally existent variation in germplasm collections (Joel *et al.,* 2002; Kanampui *et al.,* 2003; Gressel *et al.,* 2004). Another approach being at present prospected is the use of herbicide-filled nano-particles to specifically target the parasite (Pérez-de-Luque, personal communication). These methods may help reducing parasite-induced crop damages, however, they are insufficient to address the long-term management of root parasitic weed that require the destruction of parasite seed bank. To this aim various

approaches have been used from soil treatment, by fumigation or solarization, to biological control using the insect *Phytomyza orobanchia* or the pathogenic fungus *Fusarium oxysporum* f.sp. *orthoceras* as well as transgenic hypervirulent derivatives (for more details see Amsellem *et al.,* 2001, Cohen *et al.,* 2002 and Joel *et al.,* 2006). Although several potential control measures were developed in the past decades, any approach applied alone is often only partially effective, sometimes inconsistent and affected by environmental conditions (Joel *et al.*, 2006). The only way to cope with the weedy root parasites is through an integrated approach, harbouring a variety of measures in a concerted manner, starting with containment and sanitation, employing direct and indirect measures to prevent the damage caused by the parasites, and finalising with means to eradicate the parasite seed bank in soil as attempted in Kenya for *Striga* (Oswald, 2005).

Resistance sources, resistance mechanisms and its genetic bases

The development of improved cultivars with resistance to a single pathogen is often straight-forward if a good source of resistance is available and an efficient and practical screening procedure exists to provide sufficient selection pressure. Unfortunately, this is seldom the case with parasitic weeds. Resistance against most parasitic weeds is difficult to assess, scarce, of complex nature and of low heritability, making breeding for resistance a difficult task (Haussman *et al.*, 2000; Rubiales *et al.*, 2006). In a few instances, resistance of simple inheritance, acting after parasite penetration, has been identified and exploited in breeding. This has been particularly important for sunflower and cowpea breeding against *O. cumana* and *S. gesnerioides* respectively (Lane *et al.*, 1993; Fernández-Martínez *et al.*, 2000). However, breeding programs based on only a

few dominant genes are in serious risk of resistance breakdown. A number of single dominant genes for resistance named *Or1 – Or5* have been progressively identified and introduced in commercial sunflower hybrids as soon as new *O. cumana* races appeared. Resistance of complex inheritance has also been identified in sunflower (Pérez-Vich *et al.*, 2004) that were neglected in the past in favour of monogenic resistance. Sunflower breeders are currently starting to realise the need to accumulate levels of quantitative resistance together with qualitative resistance to avoid breakdown of resistance due to new races of the parasite (Pérez-Vich *et al.*, 2004).

In other crops, such as tomato (Qasem & Kasrawi, 1995), legumes (Rubiales *et al.,* 2006) or maize (Menkir, 2005), only moderate to low levels of incomplete resistance of complex inheritance has been identified against broomrape or witchweed. Screening of wild relatives for resistance to parasitic plant is also a promising approach to detect and transfer novel resistance mechanisms to crops such as those identified in *Tripsacum dactyloides* and in some *Viciae* species (Gurney *et al*., 2003; Sillero *et al.*, 2005). The quantitative resistance resulting from tedious selection procedures has resulted in the release of cultivars with useful levels of incomplete resistance combined with a degree of tolerance (Cubero *et al.*, 1994; Pierce *et al.,* 2003). The resulting resistance, which might be based on a combination of resistance mechanisms, is more likely to last longer than resistances based on a single gene. Dissecting the escape and resistance factors will help to detect existing genetic diversity for mechanisms that hamper infection (Pérez-de-Luque *et al.,* 2005a). New sources of resistance to parasitic weeds have been discovered and, to varying degrees, exploited in breeding programs. The best-characterised resistance phenotype is low germination stimulant production which is commonly found in *Striga*-resistant sorghum genotypes and begins to be recognised as an important mechanism against *Orobanche* (Hess *et al.*, 1992; Rubiales

et al., 2003). This mechanism has been successfully used for sorghum breeding (Haussmann *et al.*, 2000) and is assessed in transgenic maize impaired in terpenoid biosynthesis (Matusova *et al.*, 2005). Beyond low germination stimulant production by host plants, several other resistant phenotypes are being discovered. This includes pre-penetration mechanisms such as exudation of parasitic-seed germination inhibitor (Serghini *et al.*, 2001), low production of the *Striga* and *Tryphisaria* haustorium-inducer (Rich *et al.,* 2004; Gurney *et al.,* 2003), post-penetration through formation of chemical and physical barrier (Pérez-de-Luque *et al.*, 2005a,b; Pérez-de-Luque *et al.,* 2006a) and post-establishment by occlusion of vessels with mucilage (Pérez-de-Luque *et al*., 2006b). Hypersensitive-like responses have been evidenced in cowpea- *S. gesnerioides* (Lane *et al.*, 1994) and in the non-host interaction marigold-*S. asiatica* (Gowda *et al.*, 1999), however, its existence in some other pathosystem is still under debate (Pérez-de-Luque *et al.*, 2005b).

Combination of these different resistance mechanisms into a single cultivar should provide a more durable resistance. This can be facilitated by the adoption of MAS techniques (Haussmann *et al.,* 2000; Román *et al.*, 2002b; Pérez-Vich *et al.*, 2004), together with the use of in vitro screening methods that allow dissecting parasitic weed resistance into highly heritable components (Rubiales *et al.*, 2003; Pérez-de-Luque *et al.*, 2005a).

QTL mapping and MAS breeding

The development of MAS techniques for parasitic plant resistance is a promising approach to rapidly improve crop resistance since screening for resistance is often difficult, expensive and sometimes unreliable. These techniques are particularly useful

for the *O. cumana*-sunflower interaction where race-specific dominant genes seem to be responsible for resistance since the transfer of resistance to desired genotypes only require single-cross hybrid breeding (Lu *et al.*, 2000). However since this type of resistance can be rapidly overcome by new parasite races, pyramiding of allelic and non-allelic resistance genes in a single hybrid genotype must be performed (Pérez-Vich *et al.*, 2004). Apart from this interaction, host resistance to broomrape is generally multigenic as illustrated by the purely quantitative genetic system with strong additive effects controlling faba bean resistance to *O. crenata* (Cubero *et al.*, 1994; Román *et al.*, 2002b).

Different studies have identified and located resistance genes / Quantitative Trait Loci (QTLs) to parasitic plants in host molecular maps. The identification of molecular markers such as the cowpea AFLP (Amplified Fragment Length Polymorphism)-derived SCAR (Sequenced Characterized Amplified Region) marker linked to *Rsg3* that confers resistance to race 1 of *S. gesnerioides,* should help breeding for resistance through MAS in this system (Ouedraogo *et al.*, 2002). Similarly, the SCAR and SSR (Simple Sequence Repeat) markers, linked to the *Or5* gene conferring sunflower resistance to race *E* of *O. cumana,* have been proposed to assist breeding for sunflower resistance (Lu *et al.*, 2000). However, the closest mapped marker still remains 5.6 cM distal to the gene. In legumes, *Orobanche* resistance QTLs have been detected in pea and faba bean (Román *et al.*, 2002b; Valderrama *et al.*, 2004). However, the saturation of the maps used to locate these regions, are still insufficient for an efficient MAS breeding.

With the emergence of large-scale genomic tools, the combination of genetic mapping with gene expression studies, can offer an integrated approach to study resistance to parasitic plants. In this sense, testing the role of candidate genes selected

from expression experiments, can simplify the search of sequence polymorphisms for a more efficient and rapid MAS for both monogenic (*O. cumana*-sunflower, *S. gesnerioides*-cowpea) and quantitative traits (all other *Orobanche*-crop pathosystems). The expression level of "switched on" genes can also be treated as a quantitative trait determining the eQTLs (expression quantitative trait loci) or loci that can account for variation in the levels of gene expression (Schadt *et al.*, 2003). Thus, the combination of genetic information and gene expression should clearly help to understand the molecular bases of parasitic plant resistance and contribute to more effective breeding in the next future (Figure 2).

Molecular bases of the parasitic interaction and its resistance

A thorough knowledge of the molecular bases of resistance to stresses is essential to provide the fundamental information necessary to drive not only crop improvement but also the development of alternative control methods (Tuberosa & Salvi, 2006; Figure 2). To address this point, several tools have been developed to analyse the expression or accumulation of genes, proteins or metabolites individually, the so-called targeted approach, or as a whole, that are, the transcriptomic, proteomic and metabolomic approaches (Dita *et al.*, 2006). The emergence of these post-genomic tools have already allowed valuable breakthroughs in our understanding of plant responses to abiotic stresses, pathogen attacks or symbiotic interactions (for review see Dita *et al.,* 2006, Stacey *et al.,* 2006, Tuberosa & Salvi, 2006 and references therein). Indeed, it indicated that the plant response to stresses was a highly complex event involving the coordinated regulation of thousands of genes of different cellular process that lead to reorganisation of the metabolic fluxes (Kreps *et al.,* 2002; Castillejo *et al.*,

2004; Colebatch *et al.*, 2004). Interestingly, these approaches also revealed the existence of a general adaptative pathway in response to stresses since some genes and proteins such as specific Pathogenesis-Related (PR) proteins, peroxidases and phytoalexin biosynthetic enzymes were activated in most cases. To date, the molecular bases of the plant-parasitic plant interaction remain mostly unknown. Thus, applying these approaches in the context of plant-parasitic plant interaction should give invaluable data on the molecular responses involved in the resistance to parasitic plants and have useful applications to control the parasitic weeds. This section will focus on the recent advance in the understanding of the molecular bases of the plant-parasitic plant interaction with an emphasis on the undergoing projects.

Transcriptomic bases of the plant defence to parasite

Initially, gene expression studies with parasitic plants were performed with the targeted gene approach mainly based on the knowledge already gained from other plant stress studies. This allowed the identification of a handful of genes involved in plant defence against parasitic plants. Promoter fusion experiments in tobacco showed the activation of the isoprenoid and phenylpropanoid pathways, two defence-related metabolic pathways, in response to *O. aegyptiaca* infection (Griffitts *et al.*, 2004). These investigations also targeted the Pathogenesis-Related (PR) genes (PR-1) that are linked to the systemic-acquired resistance (SAR) showing the induction of PRB-1b (Joel & Portnoy, 1998) but not PR-1a (Griffitts *et al.*, 2004) during the tobacco-*O. aegyptiaca* interaction. Application of the differential display approach also indicated the induction of other PR genes with plant-parasitic plant interaction such as PPRG2, a PR-10 homologue, isolated from *Cuscuta trifolii*-infected alfalfa (Borsics & Lados,

2002). Interestingly, the same authors also identified a calmodulin-related protein, PPRG1, indicating that defence signalling pathways to dodder is linked to calcium (Borsics & Lados, 2001). In addition, a Suppression Substractive Hybridisation (SSH) strategy interaction also indicated that an aquaporin gene, *Leaqp2*, and a xyloglucan endotransglycosylase / hydrolase, *Lexth1*, two genes involved in cell and cell-wall elongation respectively was rapidly induced at parasite infection site during incompatible tomato-*C. reflexa* (Werner *et al.*, 2001; Albert *et al.*, 2004). Interestingly, the cloning of NRSA-1 that have homology to the well-characterised disease resistance gene *RPP5* and *N* of tobacco during the aborted *Striga* invasion of Marigold may prove very useful to circumvent *S. asiatica* infection in other host species (Gowda *et al.*, 1999).

These studies primed the description of the molecular dialogue involved in the plant-parasitic plant interaction. Following the example of other plant pathogen-interaction, the use of model plants such as *Arabidopsis thaliana, Medicago truncatula* and *Oryza sativa,* may improve our understanding of the plant-parasitic plant interaction. *A. thaliana* and *M. truncatula* are suitable hosts of some *Orobanche* species (Westwood, 2000; Rodríguez-Conde *et al.*, 2004), while *O. sativa* may be used to study *Striga* (Gurney *et al.*, 2006). Further studies should thus take advantage of these models to get insight more rapidly into the molecular bases of plant resistance, which should improve the efficiency of both MAS and transgenic approaches for crop improvement toward resistance to parasitic plants.

To date, the use of model plants to study the parasitic plant resistance have been limited. The more comprehensive study of gene expression induced in response to parasitic plants has been performed in the *A. thaliana-O. ramosa* system. Monitoring the activity of all well-characterised defence pathways in response to parasite through

the candidate gene approach reveals a rapid and transient induction of most monitored genes indicating the activation of the ethylene- and jasmonate- defence pathways in addition to the phenylpropanoid and isoprenoid pathways (Dos Santos *et al.*, 2003a). Furthermore, using a SSH strategy, these authors also identified 13 differentially expressed genes in this system including calmodulin, peroxidase and jamonate and ethylene responsive genes confirming their previous study (Dos Santos *et al.*, 2003b).

No other large-scale analyses of gene expression have been published to date. However, our group, in the frame of the "Grain Legumes" European Union FP6 Integrated Project, is now performing a microarray analysis of *M. truncatula* genes regulated in response to *O. crenata* using the recently developed M16kOLI1 microarray (M-A. Dita , unpublished). Preliminary analysis of the comparison of the transcriptome of two *M. truncatula* genotypes with different resistance mechanisms indicated significant changes in the steady-state levels of many transcripts belonging to several functional categories, including pathogen-induced genes, such as PR genes, hormone-associated genes and transcription factors. These analyses also revealed the activation of both the Salicylic acid (SA) and Jasmonate defence-pathways (M-A. Dita, unpublished). In parallel, our group is also developing a SSH library in *M. truncatula* to identify gene specifically induced during this interaction. Preliminary results suggested the presence of more than 300 candidate genes that are now under further investigation (J. Die, unpublished). Although these experiments are still undergoing, these preliminary results supports the previously established results and should prove useful to identify potential candidate genes for crop improvement.

In parallel to the host-based analyses, some studies targeted the parasite genes required for pathogenicity. Most of them targeted the hemi-parasite *Triphysaria versicolor* allowing the identification of 137 genes induced in response to the

haustorial-inducing signal (Matvienko *et al.*, 2001a). In particular, they identified a quinone oxidoreductase, which may be required for haustorium formation (Matvienko *et al.*, 2001b). Three peroxidase-encoding genes, PoxA and PoxB from *S. asiatica* and a *O. ramosa* homologue *prx1* were also shown to be important for haustorium formation presumably through a crucial pre-infection function (Kim *et al.*, 1998; González-Verdejo *et al.*, 2006). In addition, M6PR, a gene involved in mannitol biosynthesis, a polyol responsible for the high sink strength of the parasite, has been isolated in *O. ramosa* (Delavault *et al.*, 2002). The identification of these genes provided a better understanding of the parasite development and may allow developing alternative method to control the parasite.

Proteomic bases of the plant defence to parasite

In addition to gene expression analysis, the study of host root protein accumulation in response to parasite infection should be undertaken to get more insight into the molecular bases of resistance since it takes into account post-transcriptional regulation. To this goal, proteomics, understood as protein biochemistry on an unprecedented and high-throughput scale, is becoming a promising and active approach. Such an approach was applied to compare the proteome of two pea genotypes differing in their sensitivity to *O. crenata* at different stages of the infection (Castillejo *et al.*, 2004). This allowed the detection of 79 proteins differentially regulated of which only 20 were identified hampered by the low level of pea sequences available in databases. The identified proteins belonged to different functional groups such as defence and carbohydrate metabolism. Interestingly, defence- and stress-related proteins either accumulated at higher amount or were only present in the resistant genotype (Castillejo

et al., 2004). This set of proteins includes: PRs proteins, cystein proteinase, and ABA responsive proteins supporting the existence of similar defence strategies against different pathogens, including bacteria, fungi, and parasitic plants. For the most susceptible pea genotype, inoculation also decreased proteins of the carbohydrate oxidation pathway (Castillejo *et al.*, 2004). This metabolic change could reflect either a decrease in the photosynthetic activity occurring in parasite-infected plants and/or a decrease in the availability of the translocated sucrose to the host cells. By contrast, an increase in glutamine synthase protein was observed in the resistant pea genotype upon inoculation. The fact that both carbon and nitrogen metabolism appeared affected in the susceptibility/resistance of pea genotypes opens up new possibilities to better understand the re-direction of host assimilates from host sinks to parasite.

This study highlighted the usefulness of this approach by giving the first clues of the plant response to the parasite and its resistance at the protein level. However, the low level of pea protein sequences in database hampered a more comprehensive analysis of the proteomic changes induced in response to the parasite. The use of model plant should improve our understanding of this interaction by allowing the identification of much more of the interesting protein spots observed. Thus our group is now looking at the proteomic changes induced in *M. truncatula* roots in response to *O. crenata* infection to further characterise legume responses to parasitic plants (Castillejo, 2005). In parallel, we are initiating a comparative proteomic approach during the sunflower-*O. cumana* interaction to determine the protein changes induced in other *Orobanche* host (S. Echevarría, unpublished). These two studies are expected to improve further our understanding of the resistance/defense of host plant against *Orobanche spp* in the near future. However, proteomic analysis targeting other parasitic plant genera such as *Striga*, *Cuscuta* or *Tryphisaria*, as well as other model plants would be also required in

order to get a more comprehensive view of the plant-parasitic plant interaction at the protein level.

The secondary metabolites in the parasitic interaction

Studies targeting the characterisation of metabolite changes during particular plant processes are essential to complement the gene and protein expression experiments. This requirement is even more pressing when targeting the interactions between plants and other organisms that require a complex molecular dialogue with extensive signalling between both partners. A good example of this requirement is provided by the extensive study of the early stages of the nitrogen-fixing symbiosis occurring between most legumes and rhizobia. Indeed it showed that the first step of the interaction was the perception by the rhizobium of specific secondary metabolites exuded by host roots which induce the synthesis and secretion a polymer of chitin, the *Nod* factor by the bacterium that in turn induce most physiological changes required for the establishment of this symbiont within the host (Stougaard, 2000).

Similarly to this symbiotic interaction, the plant-parasitic plant interaction is dependent on complex molecular dialogues between both partners (Keyes *et al.*, 2001; Yoder, 2001). As a result, host and parasite synthesised numerous secondary metabolites to mediate this communication at the various stages of the parasite development and establishment. These metabolites have adverse or advantageous effects on the parasite development, which determine the overall susceptibility and resistance level of the host plant. To date, no large-scale analysis of the secondary metabolite recruited during parasitic infection have been performed but targeted

metabolic and cytological analyses have shown that numerous secondary metabolites are involved in this interaction or its resistance (Bouwmeester *et al.*, 2003).

It is well-documented that the parasite can use specific secondary metabolites secreted by host roots as inducers of its germination (Bouwmeester *et al.*, 2003; Akiyama *et al.*, 2005). The active compounds mainly belong to the strigolactones group of isoprenoid, although other compounds may also act as germination stimulant such as the benzoquinone sorgoleone or some anthocyanidins (Albrecht *et al.*, 1999; Bouwmeester *et al.*, 2003). Interestingly, these molecules are often considered as phytoalexins and some may also act as hyphae-branching factor of arbuscular mycorrhiza fungi required to initiate the mycorrhizal symbiosis indicating that the parasite have taken advantage of other plant signalling pathways as an adaptative evolution (Akiyama *et al.*, 2005). In some cases, specific host root exuded molecules have also been involved in the parasite attraction toward host root and its attachment (Bouwmeester *et al.,* 2003). Apart from these molecules, some volatiles, such as β-myrcene, have been recently shown to selectively chemo-attract the above-ground parasite *Cuscuta pentagona* toward its host (Runyon *et al.,* 2006). Due to their importance in the parasitic infection, these compounds and their production are subject to intensive studies to find either analogous chemicals to induce suicidal parasite germination or genotypes with reduced induction levels (Bouwmeester *et al.*, 2003).

Later, synthesis and accumulation of specific secondary metabolites may participate in the developmental arrest and necrosis of the parasite (Serghini *et al.*, 2001). Evidence indicated accumulation of uncharacterised coloured or fluorescent compounds at the host-parasite interface in resistant host associated with parasite necrosis (Goldwasser *et al.*, 2000). Soluble and cell wall-bond phenolics have been shown to accumulate in pea-*O. crenata* and tomato-*C. reflexa* interactions which was

more intense in resistant interaction (Sahm *et al.*, 1995; Pérez-de-Luque *et al.*, 2005b). This suggested that some of the accumulated fluorogens corresponded to phenolics. This hypothesis was supported by the accumulation of coumarin phytoalexins in sunflower in response to *O. cumana* that limit both germination and development of the parasite (Serghini *et al.*, 2001).

Altogether these targeted analyses showed that various secondary metabolites, mainly of terpenoid and phenolic nature, play important roles in the parasitic infection process and host resistance. However, these results have been so far unexploited to confer more efficient parasite resistances in crops. The potential of combining metabolic with transgenic approaches to confer resistance against pathogenic fungus in alfalfa has already been demonstrated (He & Dixon, 2000). The breeding for high level of these compounds in roots either by classical breeding or by transgenesis is thus a promising approach that would be worth exploring to increase host resistance to the parasite. On the other hand, these analyses only considered a limited number of specific classes of compounds so that other important defence molecules that do not belong to these classes may have been missed. The recent technical improvements in metabolomic approaches make possible large-scale metabolite profiling in order to monitor simultaneously most cell metabolites. Such approach has recently been applied to study the metabolic changes induced during nodulation of the model legume *Lotus japonicus* and alfalfa and largely improved the understanding of nodule metabolism (Barsch *et al.,* 2006; Colebatch *et al.,* 2004). The application of these techniques to study the plant-parasitic plant interactions would allow for the observation of the metabolic networks involved in this complex interaction and its resistance and would be essential in the future. By this way, a better understanding of the parasitic interaction should be gained complementing and confirming the results obtained by transcriptomic and proteomic

approaches. In addition, it should also help identifying new potential targets to improve crop resistance or parasite control.

Concluding Remarks

Over the years, many researches tackled the problem caused by parasitic plant in infested regions. Although advances have been gained in the understanding of the interaction, complete solutions remain to be found. Lack of efficient control methods is rooted to the high complexity of the interaction and the nature of the parasite. The detection of partial resistance within genotype of crop germplasm collections oriented further development of control methods toward genetic crop improvement. However, the multigenic and quantitative system generally controlling the resistance dramatically slows down breeding. It appears now evident that efficient control of the parasite requires a more comprehensive understanding of the molecular bases of the interaction and its transfer to breeders. As such, the few studies targeting the analysis of gene expression and accumulation of protein and metabolite done so far initiated to reveal the molecular dialogue involved in resistance. However, it is only a beginning that requires to be further exploited. On the other hand, many biotechnological tools that have been developed can be used to solve problem caused by pathogens (Dita *et al.*, 2006). Application of some of these biotechnological tools have been already initiated to solve plant parasite problems such as MAS or the “omic” technology but to obtain resistant crop the inclusion of other tools such as genetic transformation and functional genomics will be needed. The most efficient approach to crop improvement would be the integration of these different tools from fundamental to applied biology (Figure 2). Indeed, the comprehensive understanding of the interaction obtained from molecular

studies of the host responses to parasite in model plant, including transcriptomic, proteomic and metabolomic, should provide candidate genes to improve resistance in crop that will require to be validated through functional analysis. These validated candidates may then be used for genetic improvement of crop either directly through genetic transformation or indirectly by MAS (Figure 2). In addition, the better understanding of the interaction and the parasite biology gained by these molecular methods may also allow the development of new methods of control. Although many works still remain to be done, the different approaches presented in this review should allow to reduce or to solve the problem caused by parasitic plant in infected region in a near future.

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Legend:

Figure 1: Life cycle of *Orobanche* species.

Figure 2: Integrated scheme outlining key steps for plant molecular breeding for parasite resistance using model plant and biotechnology.

1 Figure 1:

2

3

4

5

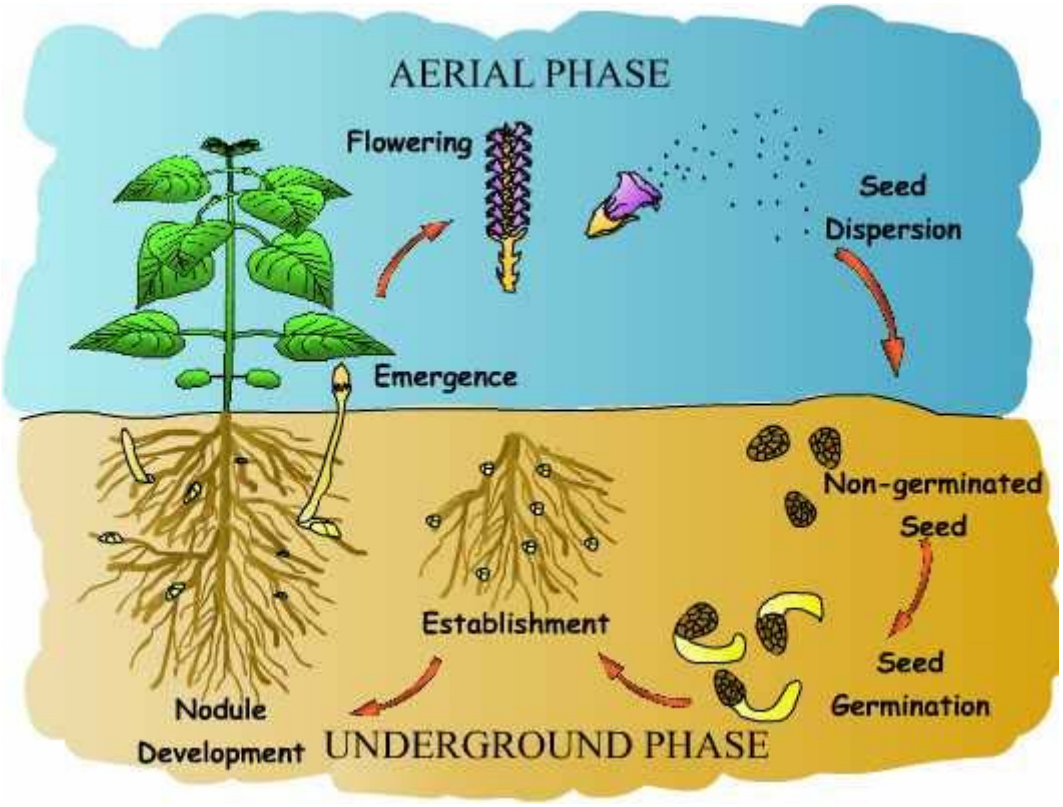
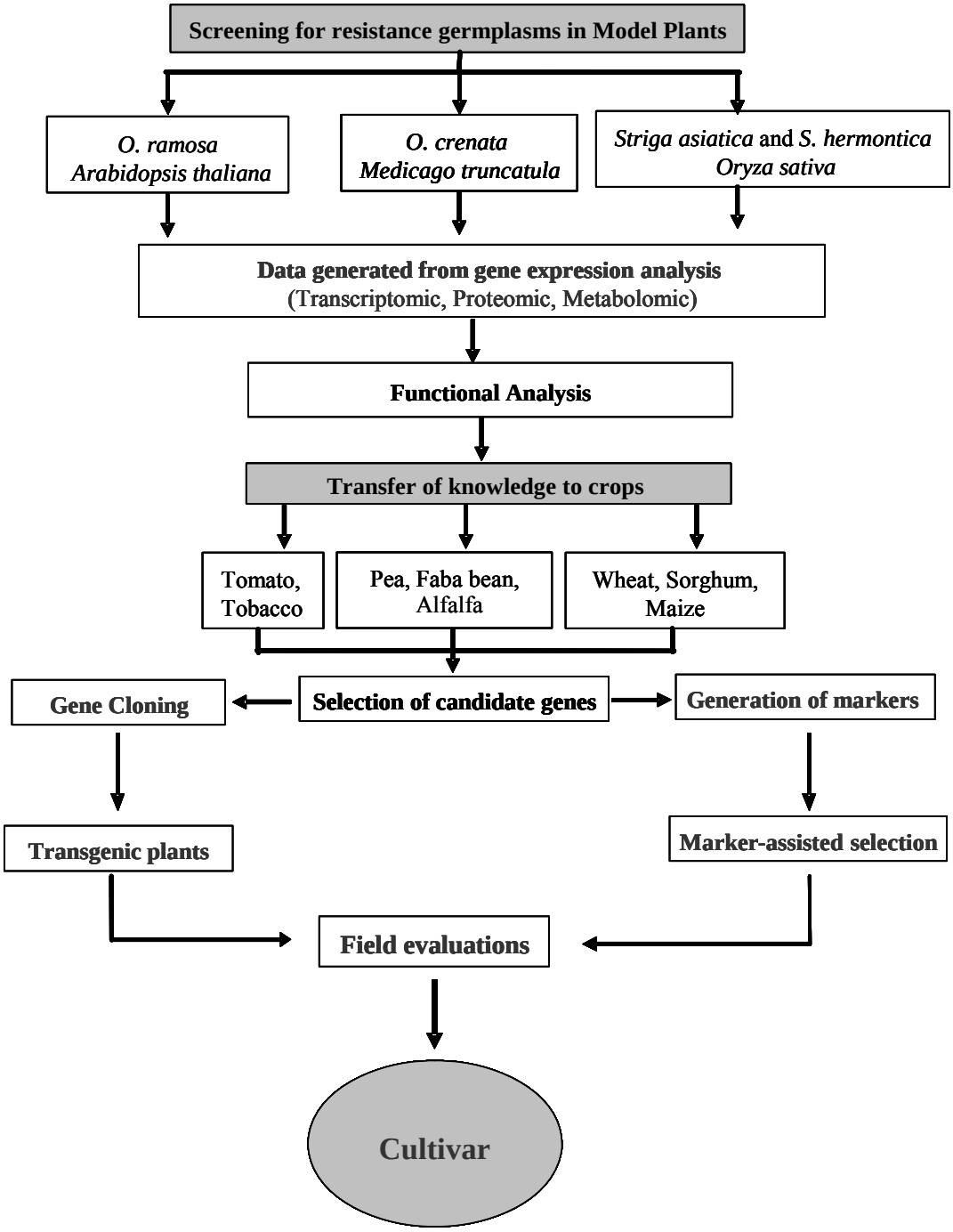


Figure 2


Screening for resistance germplasms in Model Plants
O. ramosa
Arabidopsis thaliana
O. crenata
Medicago truncatula
Striga asiatica and S. hermontica
Oryza sativa
Data generated from gene expression analysis
(Transcriptomic, Proteomic, Metabolomic)
Functional Analysis
Transfer of knowledge to crops
Tomato, Tobacco
Pea, Faba bean, Alfalfa
Wheat, Sorghum, Maize
Gene Cloning
Selection of candidate genes
Generation of markers
Transgenic plants
Marker-assisted selection
Field evaluations
Cultivar