Legume Breeding for Broomrape Resistance

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Abstract

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Legume cultivation is hampered in Mediterranean regions by the occurrence of the root parasitic weeds *Orobanche crenata* (crenate broomrape) and *Orobanche foetida* (foetida broomrape). Strategies of control have been developed but only marginal successes have been achieved. Most control methods are unfeasible, uneconomical, and hard to achieve or result in incomplete protection. Breeding for resistance is possible, but is hampered by the lack of sufficient levels of resistance, the complexity of its inheritance and the unreliability of available screening methods. Recent achievements in the identification of resistance levels and their deployment in breeding programmes will be presented and critically discussed.

Keywords: biotechnology; broomrape; crop management; faba bean; Lathyrus; *Orobanche*; pea; resistance; vetches

Broomrape problem on legumes

Annual grain and forage legumes such as faba bean (*Vicia faba* L.), vetches (*Vicia* spp.), lentil (*Lens culinaris* Medik.), pea (*Pisum sativum* L.), grass pea (*Lathyrus sativus* L.) and chickling vetch (*Lathyrus cicera* L.) are important crops grown worldwide as a source of protein both for human food and animal feed. However, their cultivation is strongly hampered in Mediterranean and Middle East farming systems by the occurrence of broomrape causing important yield losses (RUBIALES *et al.* 2006; JOEL *et al.* 2007; PARKER 2009). Legumes are parasitized mainly by two different species of broomrapes, namely crenate broomrape (*Orobanche crenata* Forsk.) and foetida broomrape (*Orobanche foetida* Poir). *O. crenata* has threatened the legume cultivation in the Mediterranean Basin and Middle East crops since antiquity. On the contrary, *O. foetida* has been reported to damage faba bean in Tunisia only (KHARRAT *et al.* 1992). However, it is common in native habitats in the western Mediterranean (VAZ PATTO *et al.* 2008) and has recently been found also in Morocco infecting common vetch (*Vicia sativa* L.) (RUBIALES *et al.* 2005b).

A number of strategies of root-parasitic weed control have been developed including cultural practices and biological and chemical control (JOEL *et al.* 2007; RUBIALES *et al.* 2009b; FERNÁNDEZ-APARICIO *et al.* 2011b). However, only marginal success have been achieved, with most control methods being unfeasible, uneconomical, hard to realize or resulting in incomplete protection. The integration of several control measures seems to be the most desirable strategy. The only way to cope with the weedy root parasites is through an integrated approach, employing a variety of measures in a concerted manner, starting with containment and sanitation, direct and indirect measures to prevent damage caused by the parasites, and finally eradicating the parasite seedbank in soil (RUBIALES & FERNÁNDEZ-APARICIO 2012).

Sources of resistance

Only moderate to low levels of incomplete resistance of complex inheritance against *O. crenata* have been identified in legumes (RUBIALES *et al.* 2006; PÉREZ-DE-LUQUE *et al.* 2009; SILLERO *et al.* 2010) making selection more difficult and slowing down the breeding process. Resistance to broomrape appears to have multiple components and to be based on a chain of escape and resistance mechanisms that either act alone or in combination and at different stages of the infection process (RUBIALES 2003).

Escape due to early flowering is known in some legumes (GRENZ *et al.* 2005; RUBIALES *et al.* 2005a; FERNÁNDEZ-APARICIO *et al.* 2008b, 2009a, 2011a).

This escape in the early pod forming genotypes may be explained by a competition between the parasite and the pods limiting infection (Grenz *et al.* 2005). Not only very early, but also very late accessions could escape from broomrape infection by growing and developing most roots late when conditions are less favourable for broomrape establishment (Fernández-Aparicio *et al.* 2009a, 2011a). Escape due to low root biomass has also been reported in some legumes (Aalders & Pieters 1987; Rubiales *et al.* 2003a, b; Pérez-de-Luque *et al.* 2005a; Fernández-Aparicio *et al.* 2008b).

Orobanche spp. seeds germinate in response to chemical signals exuded from host roots (Fernández-Aparicio *et al.* 2009b, 2011c) (Figure 1A). Subsequently, the seedling of the parasite develops into an appressorium, a specialised structure that penetrates the host root, and then into a haustorium, which forms a connection between the host vascular tissue and the parasite (Figure 1B). Then, the parasite develops into a tubercle, a bulbous structure from which a shoot arises to emerge from the soil to flower and set seeds (Joel *et al.* 2007) (Figure 1C–E).

Resistance to *O. crenata* associated with low induction of parasite seed germination has been reported in chickpea, faba bean, lentil, pea, vetches and *Medicago truncatula* Gaertn. (Rubiales *et al.* 2003c, 2004; Rodríguez-Conde *et al.* 2004; Pérez-de-Luque *et al.* 2005a; Rubiales *et al.* 2005a; Sillero *et al.* 2005a; Fernández-Aparicio *et al.* 2008a, b; Abbes *et al.* 2009). Once germinated, additional prehaustorial mechanisms of resistance such as reinforcement of cortical host cell walls by protein cross-linking, suberisation or callose deposition as well as lignification of endodermal cells could operate preventing the penetration of the parasite through the cortex and into the central cylinder and formation of the haustorium (Goldwasser *et al.* 1999; Pérez-de-Luque *et al.* 2005b, 2006a, 2007, 2008; Echevarría-Zomeño *et al.* 2006; Fernández-Aparicio *et al.* 2008a). These physical mechanisms can also be associated with the expression of pathogenesis related (PR) proteins such as peroxidase and β-1,3-glucanase or increase in phenolic content (Lozano-Baena *et al.* 2007).

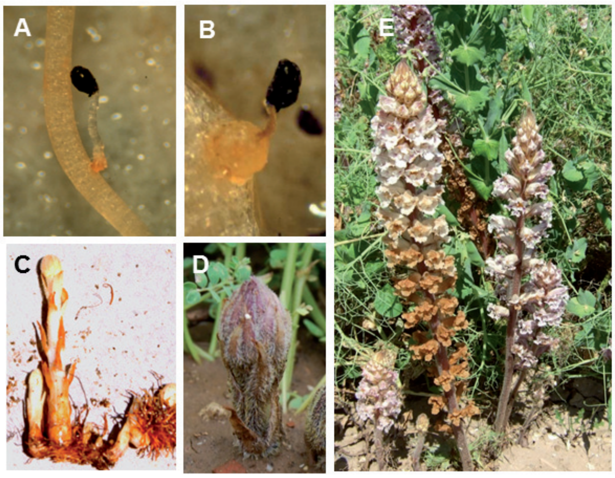

Figure 1. Life cycle of *Orobanche crenata*: (A) germinating seedlings contacting with host roots, (B) detail of an anchoring radicle forming an haustorium in a host root, (C) tubercles at various stages of growth formed still under the surface, (D) emerging shoot, (E) flowering shoots

Additional resistance mechanisms might operate after haustoria formation preventing or retarding further development into a broomrape shoot. This can result in the darkening and necrosis of tubercles as seen in vetch, faba bean, pea, chickpea, lentil and chickling vetch (Goldwasser *et al.* 1997; Rubiales *et al.* 2003c, 2004; Pérez-de-Luque *et al.* 2005b; Fernández-Aparicio *et al.* 2008b, c, 2009c; Fernández-Aparicio & Rubiales 2010, 2012). The darkening and subsequent death of *O. crenata* tubercles have been ascribed to the accumulation of substances inside host xylem vessels (Pérez-de-Luque *et al.* 2005b, 2006b). These substances seem to block the normal flux of water and nutrients between the host and the parasite and the tubercles die after exhausting their reserves. However, other mechanisms should not be discarded, such as the production by the host and delivery into the parasite of toxic metabolites (phenolics), as described in *M. truncatula*–*O. crenata* interaction (Lozano *et al.* 2007).

Resistance breeding

Legume breeding for broomrape resistance is difficult considering the scarce and complex nature of resistance in legumes in general (Rubiales *et al.* 2006). This contrasts with the success experienced in other crops such as sunflower (*Helianthus annuus* L.), in which single genes governing resistance against *Orobanche cumana* Wallr. have been identified and exploited (Fernández-Martínez *et al.* 2008). This limitation has made selection more difficult and slowed down the legume breeding process.

Nonetheless, progress has been made in accumulating the available quantitative resistance by breeding, allowing the release of resistant faba bean cultivars with various levels of resistance (Cubero & Hernández 1991; Kharrat *et al.* 2010; Pérez-de-Luque *et*

Figure 2. Faba bean breeding line resistant (right) versus susceptible (left) to *Orobanche crenata*

al. 2010; Maalouf *et al.* 2011) (Figure 2). However, all these arose from programs using Egyptian line F402 as the major donor of resistance. This reinforces the need to find new sources of resistance, to study its stability and to understand the responsible resistance mechanisms in order to facilitate the development of resistant cultivars. Recently identified resistance (Fernández-Aparicio *et al.* 2012) based on low induction of broomrape seed germination is the most relevant in this respect. This low induction of germination is operative also against other *Orobanche* species. Relevance of this low germination induction is shown by its successful use in sorghum breeding for resistance to *Striga hermonthica* (Del.) Benth. (Ejeta 2007). Similarly, tomato mutants with reduced exudation of strigolactones (Dor *et al.* 2010) have shown to be resistant to *Orobanche aegyptiaca* Pers. Pea mutants deficient for strigolactone production are therefore being explored for their potential in *O. crenata* resistance breeding (unpublished).

In contrast to the above-mentioned efforts in faba bean where broomrape resistance has been a priority in most faba bean breeding programs for decades (Cubero & Hernández 1991), a similar effort on pea was started only recently. Little resistance is available within the pea germplasm against *O. crenata* (Rubiales *et al.* 2003b), but promising sources of resistance have been identified in wild relatives within the genus *Pisum* (Rubiales *et al.* 2005a; Pérez-de-Luque *et al.* 2005a). These have been successfully hybridised with cultivated pea and submitted to breeding (Rubiales *et al.* 2009a) resulting in the submission of the first resistant cultivars to the European catalogue (unpublished) (Figure 3).

Moderate levels of resistance have been reported in lentil only recently (Fernández-Aparicio *et al.* 2008b, 2009c). Resistance is also very limited in *L. sativus* (Figure 4) and *L. cicera* (Fernández-Aparicio *et al.* 2009a, 2011a; Fernández-Aparicio & Rubiales 2010). Higher levels of resistance are available in related *Lathyrus* species (Sillero *et al.* 2005a). However, resistance is frequent in common vetch and chickpea germplasm and cultivars (Gil *et al.* 1987; Rubiales *et al.* 2003a, c; Fernández-Aparicio *et al.* 2008c), as well as in their wild relatives (Rubiales *et al.* 2004, 2005a; Sillero *et al.* 2005b).

In contrast with *O. cumana* in which races have been identified and the new ones are continuously evolving defeating newly introduced resistance genes (Fernández-Martínez *et al.* 2008), there is no clear evidence for the existence of races of *O. crenata.* This might be due to the lack of a selection pressure as there is little resistance in commercial cultivars of most legume hosts (Rubiales *et al.* 2006). However, *O. crenata* populations are known to be very heterogeneous (Román *et al.* 2001, 2002a) and the risk exists that they can be selected for virulence when challenged by the widespread use of highly resistant cultivars. In fact, a virulent population has already been selected by the frequent culture of the resistant vetch cultivar in Israel (Joel 2000).

Figure 3. Pea breeding line resistant (left) versus susceptible (right) to *Orobanche crenata*

Figure 4. Grasspea breeding line resistant (left) versus susceptible (right) to *Orobanche crenata*

Potential applications of biotechnology in broomrape resistance breeding

The recent emergence of biotechnology techniques currently enables to use molecular markers in plant breeding and heterologous gene transfer. In faba bean, a number of quantitative trait loci (QTLs) linked to *O. crenata* resistance under field conditions have been reported using various segregating populations (named from *Oc1* to *Oc13*) (Román *et al.* 2002b; Díaz-Ruiz *et al.* 2010; Gutiérrez *et al.* 2013), however, they were frequently instable across environments and explained little phenotypic variation and therefore they were of low value in marker-assisted selection (MAS). It now seems that *Oc7* might be a promising candidate for MAS as it is located within a narrow genomic region on chromosome VI, explains a substantial part of the variation for this trait and was consistently detected over three seasons (Gutiérrez *et al.* 2013).

Similarly, QTLs conferring resistance to *O. crenata* under field conditions have been identified in pea (Valderrama *et al.* 2004) explaining also little phenotypic variation. However, a more accurate phenotyping complementing field screenings with *in vitro* screenings in minirhizotrons enabled the identification of QTLs governing specific mechanisms of resistance that explained a high proportion of phenotypic variation (Fondevilla *et al.* 2010). Thus, QTLs for the low induction of *O. crenata* seed germination, lower numbers of established tubercles per host root length unit, and slower development of tubercles were identified (Fondevilla *et al.* 2010). It should therefore be remarked that the accuracy of phenotypic evaluation is of the utmost importance for the accuracy of QTL mapping. Phenotypings performed under field conditions are needed but they lack the sufficient control of crucial environmental factors and inoculum homogeneity in the soil (Rubiales *et al.* 2006; Fernández-Aparicio *et al.* 2009a, 2011b; Pérez-de-Luque *et al.* 2010). The identification of QTLs involved in specific mechanisms of resistance could be useful for combining different escape and resistance mechanisms in a single cultivar. That may provide increased resistance while at the same time being more difficult to lose through the evolution of the parasite, compared with resistance based on a single mechanism.

However, before this can be effectively used in MAS, the genomic regions containing the QTLs should be further saturated, the position of QTLs should be further refined and molecular markers should be more closely linked to resistance (Cobos *et al.* 2013). The integration of information obtained from QTL analysis with gene and protein expression analysis currently performed in pea or in the model plant *M. truncatula* in response to *O. crenata* infection (Castillejo *et al.* 2004, 2009, 2012; Die *et al.* 2007; Dita *et al.* 2009) can shortcut conventional breeding or marker-assisted selection in identifying candidate genes. Also, sequence information obtained from different parasitic plant species (Westwood *et al.* 2012) will help in understanding parasite virulence and host resistance mechanisms. Therefore, increased efforts in delivering the control by resistant cultivars can be more effectively made, and the tools of modern plant breeding and of heterologous gene transfer (Dita *et al.* 2006; Rispail *et al.* 2007; Yoder *et al.* 2009) will be valuable.

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