Legume Breeding for Broomrape Resistance

**Diego Rubiales**

Institute for Sustainable Agriculture, Spanish National Research Council (CSIC), Córdoba, Spain

**Abstract**


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 & de Baena 2005b, 2006b). These substances due to low root biomass have been identified and set seeds (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).

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
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 Lathyris 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).
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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**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


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Corresponding author:
Prof Diego Rubiales, Spanish National Research Council (CSIC), Institute for Sustainable Agriculture, Apdo. de Correos 4084, 14080 Córdoba, Spain; e-mail: diego.rubiales@ias.csic.es