

REVIEW

Disease Resistance in Pea (*Pisum sativum* L.) Types for Autumn Sowings in Mediterranean Environments

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Abstract: Pea is an important grain legume mainly grown as spring crop in temperate regions. However, in areas with mild winters and dry springs, like Mediterranean type environments, spring pea types are autumn sown. Unfortunately, little efforts have been made so far in pea breeding for constraints typical of these environments, such as crenate broomrape (*Orobanche crenata*), rust (*Uromyces pisi*), powdery mildew (*Erysiphe pisi*) and ascochyta blight (*Mycosphaerella pinodes*). In this paper we revise the present state of the art in pea breeding against these diseases and we will critically discuss present progress and future perspectives.

Keywords: ascochyta blight; broomrape; powdery mildew; rust

Pea (*Pisum sativum* L.) is a cool season legume grown worldwide as a source of protein both for human food and animal feed. Pea is the most widely grown grain legume in Europe and the second-most in the world (FAOSTAT 2008) and represents a versatile and inexpensive protein source for animal feeding. Significant efforts have been made in pea breeding for disease resistance in continental and oceanic conditions where it is mainly spring sown (COUSIN 1997). However, relative prevalence and importance of the various diseases varies with agroecological conditions. In areas with mild winters and dry springs, like Mediterranean type environments, spring pea types are autumn sown. Unfortunately, little efforts have been made so far in pea breeding for constraints typical of

these environments, such as crenate broomrape (*Orobanche crenata* Forsk.), rust (*Uromyces pisi* (Pers.) Wint.), powdery mildew (*Erysiphe pisi* DC) and ascochyta blight (*Mycosphaerella pinodes* (Berk & Blox) Vesterg). In this paper we will revise the present state of the art in pea breeding against these diseases and we will critically discuss present progress and future perspectives.

Crenate broomrape (*Orobanche crenata* Forsk.)

Pea cultivation is strongly hampered in Mediterranean and Middle East farming systems by the occurrence of crenate broomrape (RUBIALES *et al.* 2003, 2009a, b). The lack of resistance and

the absence of a suitable control method have relegated pea cultivation to uninfested areas. Rather than being controlled, the broomrape problem is increasing both in intensity and acreage (RUBIALES *et al.* 2008, 2009b). With the climate change, these invasive parasites are spreading further northward in Europe, and further southward in Africa. Recent studies (GRENZ & SAUERBORN 2007) have suggested that very large areas of new territories are at risk of invasion if care is not immediately taken to limit the introduction of broomrape seeds and to educate farmers and others to be on alert for new infestations. So far the effectiveness of conventional control methods has been limited due to numerous factors, in particular the complex nature of the parasites which reproduce by tiny and long-living seeds (RUBIALES *et al.* 2009b).

Breeding for broomrape resistance is difficult considering the scarce and complex nature of resistance in legumes in general (RUBIALES *et al.* 2006; RISPAIL *et al.* 2007) and in pea in particular. Only incomplete resistance to *O. crenata* was found in other grain legumes like faba bean that has been successfully accumulated by breeding, allowing the release of resistant cultivars (PÉREZ-DE-LUQUE *et al.* 2009). A similar effort in pea breeding for broomrape resistance has been started only recently. Various levels of resistance have been reported in accessions of *P. sativum* ssp. *sativum*, *abyssinicum*, *arvense* and *elatius* and in *P. fulvum* (RUBIALES *et al.* 2005). All these accessions have been successfully crossed with pea cultivars and a breeding program to introgress the resistance from these pea relatives into elite pea germplasm is underway (RUBIALES *et al.* 2009a).

Resistance against root parasitic weeds is a multi-component event, being the result of a number of avoidance factors and/or resistance mechanisms acting at different levels of the infection process (RUBIALES 2003; FERNÁNDEZ-APARICIO *et al.* 2008b). Avoidance due to precocity is known in legumes (FERNÁNDEZ-APARICIO *et al.* 2009), including pea (RUBIALES *et al.* 2005), early flowering genotypes having an advantage limiting the *O. crenata* infection. Avoidance due to low root biomass has also been reported in pea (PÉREZ-DE-LUQUE *et al.* 2005a). This indicates that such accessions having low root biomass avoid the broomrape attack reducing the chance of contact between host and parasite. Resistance to *O. crenata* associated with a low induction of parasite seed germination was reported in several legumes

and is also present in *Pisum* germplasm (PÉREZ-DE-LUQUE *et al.* 2005a; RUBIALES *et al.* 2005). Pre-haustorial mechanisms of resistance have also been identified in *Pisum* germplasm. The protein cross-linking of the host cell walls in contact with parasite intrusive cells prevents the penetration into the central cylinder in these accessions. This protein cross-linking reinforces the pea cell walls, and is also associated with the expression of two pathogenesis related (PR) proteins: peroxidase and β -1,3-glucanase (CASTILLEJO *et al.* 2004; PÉREZ-DE-LUQUE *et al.* 2006). There may also occur a failure of attached parasites to further develop into a broomrape shoot. In pea, a high percentage of necrotic tubercles can be observed in some accessions, reaching circa 60% in a mini-rhizotron. The host vessels at the infection point are filled with mucilage-like substances (PÉREZ-DE-LUQUE *et al.* 2005b, 2006). 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 delivering into the parasite of toxic metabolites such as phenolics, as described in *Medicago truncatula*-*O. crenata* interaction (LOZANO-BAENA *et al.* 2007).

QTL conferring resistance to *O. crenata* have been detected in pea using a map developed from the cross *P. sativum* ssp. *syriacum* (= *P. humile*) \times *P. sativum* cv. Messire (VALDERRAMA *et al.* 2004; FONDEVILLA *et al.* 2009a). Four genomic regions associated with field resistance, assessed as the number of emerged broomrape shoots per pea plant under field conditions, were identified but they also explained a low to moderate proportion of phenotypic variation. A more accurate screening of this RIL assaying different phases of the parasite cycle using a Petri dish technique enabled the identification of QTL governing specific mechanisms of resistance. Thus, QTL for a low induction of *O. crenata* seed germination, lower numbers of established tubercles per host root length unit, and slower development of tubercles were identified. The identification of QTL 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 being at the same time more difficult to lose through the evolution of the parasite, compared with resistance based on a single mechanism. However, before using the available QTL

in MAS, the genomic regions containing the QTL should be further saturated in order to refine the position of the QTL and identify molecular markers more closely linked to the resistance genes.

An alternative strategy for the broomrape control would be the development of pea cultivars resistant to herbicides. Target site herbicide resistance might be a promising solution for controlling the broomrape, which is being explored in some crops (GRESSEL 2009), particularly in non-transgenic imidazolinone target-site resistant sunflowers which are now being released in Europe (TAN *et al.* 2004). This is therefore a promising solution for controlling *Orobanche* in pea. Resistance could be transferred into pea following the existing protocols, allowing an efficient broomrape control.

Ascochyta blight

(*Mycosphaerella pinodes* Berkeley & Bloxam)

Aschochyta blight, caused by *Mycosphaerella pinodes*, the teleomorph of *Ascochyta pinodes* (Berk & Blox) Jones, is a widespread pea disease. It constitutes the second major constraint for the crop after broomrape in the Mediterranean basin (RUBIALES *et al.* 2003). The existence of pathotypes for *M. pinodes* is still a matter of controversy (TIVOLI *et al.* 2006a). Although extensive searches have been carried out, only moderate resistance is available in pea cultivars and this has been inadequate to control the disease (FONDEVILLA *et al.* 2007b). Higher levels of resistance have been identified in wild species of *Pisum* (WROTH 1999; FONDEVILLA *et al.* 2005), but they have not been used efficiently in breeding programmes yet. Four major genes were described (CLULOW *et al.* 1991) that have not however been verified by any other ulterior study. In contrast, QTL mapping studies have resulted in the identification of numerous genomic regions involved in the control of resistance, confirming the polygenic nature of resistance. TIMMERMAN-VAUGHAN *et al.* (2002, 2004) identified 19 QTL associated with resistance, six of which were common to two crosses. TAR'AN *et al.* (2003) identified three QTL specific to resistance to *M. pinodes* under field conditions. PRIOUL *et al.* (2004) reported six QTL associated with seedling resistance to *M. pinodes* under controlled conditions and ten for adult plant field resistance, with four being common to both stages. More recently 6 QTL (*mp1–mp6*) have been as-

sociated with resistance to *M. pinodes* in a cross of the cultivar Messire with *P. sativum* subsp. *syriacum* (FONDEVILLA *et al.* 2008b). Candidate genes co-locating with QTL previously described for resistance to *M. pinodes* have been reported (PRIOUL-GERVAIS *et al.* 2007).

Powdery mildew (*Erysiphe pisi* Boerema & Verh.)

Pea powdery mildew, caused by *Erysiphe pisi*, is an air-borne disease with a worldwide distribution, being particularly important in climates with warm dry days and cool nights. Although varying levels of resistance to *E. pisi* have been observed in pea (HERINGA *et al.* 1969; FONDEVILLA *et al.* 2007a), only three genes for resistance named *er1*, *er2* and *Er3* (HERINGA *et al.* 1969; FONDEVILLA *et al.* 2007c) have been described so far.

Gene *er1* is widely used in pea breeding programmes and provides complete or incomplete resistance depending on the locations (HERINGA *et al.* 1969; TIWARI *et al.* 1997; FONDEVILLA *et al.* 2006). Resistance conferred by this gene has been proved to be stable and is caused by a barrier to the pathogen penetration (FONDEVILLA *et al.* 2006). RFLP, RAPD/SCAR and SSR markers linked to the *er1* locus have been identified (DIRLEWANGER *et al.* 1994; TIMMERMAN-VAUGHAN *et al.* 1994; TIWARI *et al.* 1998; EK *et al.* 2005; PEREIRA *et al.* 2009).

Gene *er2* (HERINGA *et al.* 1969) is not used commercially. The gene confers a high level of resistance in some locations but is ineffective in others (TIWARI *et al.* 1997; FONDEVILLA *et al.* 2006). The expression of *er2* is influenced by temperature and leaf age. Gene *er2* governed resistance is based mainly on post-penetration cell death complemented by a reduction of percentage penetration success in mature leaves (FONDEVILLA *et al.* 2006). AFLP, RAPD and SCAR markers linked to *er2* are available (TIWARI *et al.* 1999; KATOCH *et al.* 2009).

Gene *Er3* was recently identified in *P. fulvum* and has successfully been introduced into the adapted *P. sativum* material by sexual crossing (FONDEVILLA *et al.* 2007c). Resistance conferred by the gene *Er3* is due to a high frequency of cell death that occurs both as a rapid response to attempted infection and a delayed response that follows the colony establishment (FONDEVILLA *et al.* 2007a, c). RAPD markers tightly linked to *Er3* have been identified and converted into SCARs (FONDEVILLA *et al.* 2008a).

Rusts (*Uromyces pisi* and *U. viciae-fabae* Pers.)

Pea rust has become an important pathogen of dry pea from the mid-1980s particularly in regions with warm, humid weather (EPPO 2009). Pea rust has been reported to be caused either by the fungus *Uromyces viciae-fabae* (syn. *U. fabae*) or *U. pisi*. *U. viciae-fabae* is the principal causal agent of pea rust in tropical and subtropical regions such as India and China, where warm humid weather is suitable for the appearance of both the uredial and the aecidial stage (KUSHWAHA *et al.* 2006). However, it has been observed in temperate regions that although pea seedlings can be infected by *U. viciae-fabae*, it hardly gets established and progresses under field conditions (EMERAN *et al.* 2005; BARILLI *et al.* 2007, 2009c).

Several sources of incomplete resistance against *U. viciae-fabae* have been reported (PAL *et al.* 1980; XUE & WARKETIN 2001; VIJAYALAKSHMI *et al.* 2005; CHAND *et al.* 2006; KUSHWAHA *et al.* 2006). A single major gene (*Ruf*) has been reported as responsible for this partial resistance. Two RAPD markers have been detected flanking the gene *Ruf*, but both markers were not close enough to the gene to allow a dependable marker-assisted selection for rust resistance (VIJAYALAKSHMI *et al.* 2005).

Only recently has a pea germplasm collection been screened to identify sources of resistance to *U. pisi* both under field and growth chamber conditions (BARILLI *et al.* 2009b). No complete resistance has been identified so far, however, incomplete resistance was common in the collection. All the accessions displayed a compatible interaction (high infection type) both in adult plants under field conditions and in seedlings under growth chamber conditions, but with varying levels of disease reduction (BARILLI *et al.* 2009b). This resistance was not associated with host cell death (BARILLI *et al.* 2009a, b). A segregating population derived from the cross between a resistant and a susceptible accession of *P. fulvum* has recently been developed to study the resistance against *U. pisi* (BARILLI unpublished data). Preliminary results performed on F2:3 revealed two QTL for resistance to *U. pisi* in the field and controlled conditions, respectively, which explained a high percentage of the phenotypic variance (BARILLI *et al.* 2007). A RIL population derived from this cross is being developed at present in order to perform the required replications of field tests, characterize their effects and validate the stability of QTL across environments.

CONCLUSION

The current focus in applied breeding is leveraging biotechnological tools to develop more and better markers to allow marker-assisted selection with the hope that this will speed up the delivery of improved cultivars to the farmer. To date, however, progress in marker development and delivery of useful markers has been slow. Now we are also facing an accelerated progress in genomic and biotechnological research, which should soon provide the important understanding of some crucial developmental mechanisms both in the parasites and their host plants. The application of knowledge acquired from basic genomic research and genetic engineering will contribute to more rapid pea improvement.

In general, the scarce genomic resources developed for cool season legumes and the limited saturation of the genomic regions bearing putative QTL make it difficult to identify the most tightly linked markers and to determine the accurate position of QTL (RISPAIL *et al.* 2009; RUBIALES *et al.* 2009a). The effectiveness of MAS might soon increase with the adoption of new improvements in marker technology together with the integration of comparative mapping and functional genomics. It could be a key to saturate genetic maps with strong markers located in active genetic regions and genes that should allow us to detect expression QTL (eQTL) for reliable MAS selection.

M. truncatula is already being studied to unravel resistance to a large number of pathogens, from parasitic plants (LOZANO-BAENA *et al.* 2007; FERNÁNDEZ-APARICIO *et al.* 2008a), bacterial pathogens (VAILLEAU *et al.* 2007), nematodes (MOUSSART *et al.* 2007) to fungal and oomycete pathogens (RUBIALES & MORAL 2004; ELLWOOD *et al.* 2006; TIVOLI *et al.* 2006b; MOUSSART *et al.* 2007; PRATS *et al.* 2007). The transcriptomic and proteomic approaches developed for this model legume can be used to understand the molecular components and identify candidate genes involved in *M. truncatula* defence against these pathogens. Transcriptomic or proteomic studies have been performed to determine genes involved in defence mechanisms against *Aphanomyces euteiches* (NYAMSUREN *et al.* 2003; COLDITZ *et al.* 2005), *Erysiphe pisi* (CURTO *et al.* 2006, 2008; FOSTER-HARTNETT *et al.* 2007), *Orobanche crenata* (DIÉ *et al.* 2007; CASTILLEJO *et al.* 2009; DITA *et al.* 2009), *U. striatus* (CASTILLEJO *et al.* 2003) or *Mycosphaerella pinodes* (FONDEVILLA *et al.* 2009b).

Pea resistance breeding is slow not only due to the difficulty and the relatively low investment on genetics, genomics and biotechnology of the crop, but also, and mainly because of little knowledge on the biology of causal agents and on the pea/disease interactions. Comprehensive studies on the host status and virulence of causal agents are often missing, and in most of the examples listed above, there is a low agreement on the existence of races and on their distribution. This is a major limitation for any breeding programme. Also, available information on levels of resistance and on the responsible mechanisms is often incomplete. Only after a significant input to improve the existing knowledge on the biology of causal agents as well as on the plant, resistance breeding will be accelerated efficiently.

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