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Screening techniques and sources of resistance against parasitic weeds in grain legumes

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Summary

A number of parasitic plants have become weeds, posing severe constraints to major crops including grain legumes. Breeding for resistance is acknowledged as the major component of an integrated control strategy. However, resistance against most parasitic weeds is difficult to access, scarce, of complex nature and of low heritability, making breeding for resistance a difficult task. As an exception, resistance against *Striga gesnerioides* based on a single gene has been identified in cowpea and widely exploited in breeding. In other crops, only moderate to low levels of incomplete resistance of complex inheritance against *Orobanche* species has been identified. This has made selection more difficult and has slowed down the breeding process, but the quantitative resistance resulting from tedious selection procedures has resulted in the release of cultivars with useful levels of incomplete resistance. Resistance is a multicomponent event, being the result of a battery of escape factors or resistance mechanisms acting at different levels of the infection process. Understanding these will help to detect existing genetic diversity for mechanisms that hamper infection. The combination of different resistance mechanisms into a single cultivar will provide durable resistance in the field. This can be facilitated by the use of *in vitro* screening methods that allow highly heritable resistance components to be identified, together with adoption of marker-assisted selection techniques.

Introduction

Parasitic flowering plants form a close connection to the vascular system of their host plants through a specialised structure known as a haustorium. There are about 3000 species, representing a fascinating group of plants of great interest for botanists and ecologists (Kuijt, 1969). Unfortunately for farmers, a small number of these species have become weeds, posing severe constraints to major crops including grain legumes. By far the most economically damaging are root parasites belonging to the genera *Orobanche* (broomrapes) and *Striga* (witchweeds). *Orobanche* species, found largely in Mediterranean and warm temperate areas of Europe, North Africa and the Middle East are holoparasites, devoid of chlorophyll and totally dependent on the host for organic carbon, water and nitrogen. *Striga* and the closely related genus *Alectra* are hemi-parasitic and a particular problem in sub-humid and semi-arid areas of Africa (Parker & Riches, 1993).

O. crenata (crenate broomrape) has been known to threaten legume crops since antiquity (Cubero et al., 1994). It is an important pest in faba bean (*Vicia faba*), pea (*Pisum sativum*), lentil (*Lens culinaris*), vetches (*Vicia* spp.), grass pea (*Lathyrus sativus*) and other grain and forage legumes in the Mediterranean basin

and Middle East (Rubiales, 2001). Yield loss can be severe with complete loss of pea crops in severe cases (Bernhard et al., 1998; Rubiales et al., 2003b). Losses from 5 to 95% have been reported in faba bean (Mesa-García & García-Torres, 1986) and lentil (Sauerborn, 1991; Bayaa et al., 2000) depending on the infestation level and the planting date.

O. aegyptiaca (Egyptian broomrape) is an important pest of many crops in the Middle East and Asia. Similar to *O. crenata* this species attacks faba bean, common vetch, chickpea and lentil, but in addition it can also severely attack peanut (*Arachis hypogea*). Cruciferous crops, particularly cabbage and oilseed rape, and several members of the families Solanaceae, Apiaceae and Asteraceae are also susceptible to *O. aegyptiaca* (Parker & Riches, 1993).

O. foetida is widely distributed in natural habitats in the Western Mediterranean area (Portugal, Spain, Morocco, Algeria, Tunisia) parasitising wild herbaceous leguminous plants of the genera *Anthyllis, Astragalus, Ebenus, Lotus, Medicago*, *Ononis, Scorpiurus* and *Trifolium* (Pujadas-Salvá, 1999, 2002). It is, however, considered an important agricultural parasite in faba bean in Beja region of Tunisia (Kharrat et al., 1992) with yield losses ranging 66–83% (Kharrat, 1999). This species seems to be more aggressive on faba bean and common vetch (*V. sativa*) than on other legumes (Kharrat, 2002a). Of the cool-season grain legumes only pea (*Pisum sativum*) escapes its attack (Kharrat, 1999). Infection by this broomrape has also been reported in Tunisia on *Lathyrus odoratus, L. sativus, Trifolium alexandrinum, Medicago truncatula* and *V. sativa* ssp. *amphicarpa* (Kharrat, 2002a). It has recently also been found in Taounate, Morocco infecting common vetch (Rubiales et al., 2005a).

Although other *Orobanche* species can infect leguminous plants, they are generally of little economic importance. *O. minor*, however, has a wide host range among forage legumes in temperate climates. It is of economic importance on clover that is grown for seed and has recently become a problem on red clover in Oregon, USA (Osterbauer & Rehms, 2002; Eizenberg et al., 2004). It has been introduced into South and West Australia where it can be seen in gardens and in crop and pasture paddocks (Hussey et al., 1997) but is reported as a potential problem only for *Vicia ervilia* (Carter et al., 1996).

Striga gesnerioides and *Alectra vogelii* cause considerable yield reduction of grain legume crops, particularly cowpea, throughout semi-arid areas of sub-Saharan Africa (Parker & Riches, 1993). As crops of resource-poor households are affected by these parasites they impose an additional stress with which farmers, who have little capacity for investment in crop production, have to cope in an environment characterised by marginal rainfall for cropping and declining soil fertility. *S. gesnerioides* occurs in natural vegetation throughout the drier regions of Africa parasitising genera within the Leguminosae (e.g. *Indigofera* and *Tephrosia*) and Convolvulaceae (Mohamed et al., 2001). Strong host specificity has evolved among its populations and the *Vigna* strain causes extensive damage to cowpea in the Sudano-Sahelian belt of W. Africa from Senegal to Chad and to southern Togo, Benin, Nigeria and northern Cameroon. Yield losses of 30% or more are common while total crop loss, particularly in Nigeria, of the most susceptible cowpea cultivars is not uncommon (Aggarwal & Ouedraogo, 1989; Emerchebe et al., 1991).

A. vogelii infects a number of grain legume crops in a range extending from Northern Province of South Africa and Swaziland, through Central Africa to Burkina Faso and Mali in the west and Kenya in the east. Cowpea in Southern, East and West Africa and groundnut in East and West Africa are important hosts. Soyabean (*Glycine max*), bambara (*Vigna subterranea*), common bean (*Phaseolus vulgaris*), mung bean (*Phaseolus radiata*) and many legume fodder crops, including *Lablab purpureus*, Siratro (*Macroptilium atropurpureum*) and velvet bean (*Mucuna pruriens*) are also parasitized (Riches, 1989). Cowpea has traditionally been grown in multiple cropping systems in which low populations of landraces are planted in mixtures with cereals. An increase in the importance of *A. vogelii* during the past 30 years has often been associated with a change to sole cropping of introduced, potentially higher yielding susceptible cultivars, an increase in the area and frequency of cultivation. Yield loses of 50% are common in Tanzania (Mbwaga et al., 2000), total crop loss has been reported in recent years in parts of Kenya (Bagnall-Oakley et al., 1991) while in Botswana losses in the highly susceptible introduced cultivar Blackeye reach 80–100% (Riches, 1989). Up to 15% yield loss has been observed in groundnut in Nigeria (Salako, 1984), 30–50% reductions in yield of bambara in South Africa (Beck, 1987) while late-sown crops of soyabean may be completely destroyed by the parasite in northern Nigeria (Lagoke, 1989).

Several strategies have been developed for the control of parasitic weeds, from cultural practices to chemical control (Parker & Riches, 1993; Joel, 2000b;

Rubiales et al., 2003b,d; Pérez-de-Luque et al., 2004a), but all without unequivocal success. Breeding for resistance is the most economic, feasible and environmental friendly method of control. However, appropriate screening methods and effective selection indices are needed to ensure success. 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, easily controlled and practical screening procedure exists to provide good selection pressure. Unfortunately, this is seldom the case with parasitic weeds. Resistance against most parasitic weeds is difficult to access, scarce, of complex nature and of low heritability, making breeding for resistance a difficult task. In spite of these difficulties, significant success has been achieved in some crops. In a few instances, resistance of simple inheritance has been identified and widely exploited in breeding. This has been particularly important allowing rapid progress to develop cultivars of sunflower that are resistant to *O. cumana* and of cowpea resistant to *S. gesnerioides.* However, breeding programmes based on only a few dominant genes are in serious risk of breakdown of resistance. A well studied case is *O. cumana* attacking sunflower in which at least seven races have so far been described (Fernández-Martínez et al., 2000).

Only moderate to low levels of incomplete resistance of complex inheritance against parasitic weeds has been identified in other crops, such as in legumes against *O. crenata, O. foetida* and *O. aegyptiaca* (Cubero et al., 1994; Goldwasser et al., 1997; Khalil & Erskine, 1999; Kharrat, 2002b). This has made selection more difficult and has slowed down the breeding process, but 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; Khalil & Erskine, 1999). The resulting resistance, which might be based on a combination of resistance mechanisms, is more likely to last longer than resistances that are based on a single gene.

Resistance is a multicomponent event, being the result of a battery of escape factors or resistance mechanisms acting at different levels of the infection process. Host plants might escape infection by reduced root biomass and by root architecture that avoids the soil layer in which the seeds of the parasite are more common. The host may limit damage (tolerance) by factors that influence source–sink relationships, such as osmotic pressure (Wegmann et al., 1991). Low germination stimulant production has been successfully exploited in breeding sorghum cultivars that are resistant to *Striga asiatica* (Ejeta et al., 2001). Although low induction of germination was considered to play little role in resistance to *Orobanche* in legumes (ter Borg, 1999), this trait has recently been found in some accessions of a range of legumes, including vetches, peas and grass peas (Sillero et al., 2005; Pérez-de-Luque et al., 2005a). Low production of *O. crenata* germination stimulant production is also very common in chickpea and wild *Cicer* species (Rubiales et al., 2003c, 2004). Necrosis and/or the development of protective layers that block the development or the intrusion of the haustorium inside host tissues has been reported in cowpea to *Striga* (Lane & Bailey, 1992), *Vicia athropurpurea* to *O. aegyptica* (Goldwasser et al., 1997), and common vetch (Pérez-de-Luque et al., 200la), faba bean (Zaitoun et al., 1991) and chickpea (Rubiales et al., 2003c) to *O. crenata*. In addition to those mechanisms, browning and death of attached *Orobanche* seedlings without the presence of a darkening reaction in the host root has been observed in *Pisum* spp., faba bean and vetch with *O. crenata* (Pérez-de-Luque, unpublished results).

There is variation for these traits in germplasm collections. Land races have been widely replaced by homogeneous cultivars that may not possess the combined tolerance and resistance already accumulated in land races or wild accessions by natural selection. Genetic resources remain highly unexplored and underused and might contain very valuable additional sources of resistance (Rubiales, 2003). Understanding the escape and resistance factors will help to detect existing genetic diversity for mechanisms that hamper infection (Labrousse et al., 2001; Rubiales et al., 2003c). Combining different resistance mechanisms into a single cultivar will provide a durable outcome. This can be facilitated by the use of *in vitro* screening methods that allow the identification of highly heritable resistance components, together with adoption of marker-assisted selection techniques (Ouedraogo et al., 2002; Román et al., 2002a; Boukar et al., 2004; Pérez-Vich et al., 2004; Valderrama et al., 2004).

Techniques of screening

Resistance indices

One of the problems in breeding for broomrape resistance is the lack of an effective selection criterion and of a suitable screening method. Several indices have been used by different authors to measure the levels

of resistance to parasitic weeds, such as height of the parasitic flowering shoots, total weight of shoots per host plant, number of emerged shoots per unit area, rate of reproduction, etc., but the most widely used index for resistance to *Orobanche, Alectra* and *Striga* is the total number of emerged shoots per host plant (Gil et al., 1987; Cubero, 1991). Although this index has some drawbacks, as we will mention below, it is simple to measure while other indices are less accurate. *Orobanche* attack is related to the growth vigour of the host and there is a competition for resources among attachments (Aalders & Pieters, 1987), thus, indices based on size and weight of broomrapes can be misleading. The lower the amount of attachments, the bigger they are, resulting in similar weights of broomrape collected on susceptible and resistant plants (ter Borg et al., 1994).

Some host lines might have levels of rate reducing resistance, delaying establishment and/or emergence, that could slightly affect the final number of emerged shoots in favourable springs (fresh and rainy), but could notably reduce it in some unfavourably short springs (hot and dry) as found in pea (Rubiales et al., 1999). This type of rate reducing resistance might be overlooked in standard screenings based only on final counts. This rate reducing resistance has commonly been underestimated, as breeders prefer for their convenience, a complete clear cut resistance. However, its value in increasing durability of the resistance has been proved in other pathosystems, including infection of cereals by rusts (*Puccinia* spp.) (Niks & Rubiales, 2002). Sequential counts of emerged parasite stems can be used to build the "area under parasite severity progress curve", a technique that has been used in the search for resistance to *Striga* in sorghum (Omanya et al., 2001). Parasite severity values are calculated on a plot basis by multiplication of parasite counts and a vigour score based on parasite height and degree of branching for successive assessment dates using a formula developed for the "area under the disease progress curve" (Shaner & Finney, 1977).

The opposite situation also occurs. In faba bean, some lines are so susceptible, that many tubercles are established but remain small due to competition for nutrients. Damage occurs early in the development of the host so that the majority of *Orobanche* shoots fail to emerge (Sillero et al., 1996). This extreme susceptibility could be confounded with resistance if only numerical counts on emergence are recorded, so it is necessary to pay attention also to the general host plant health condition and vigour.

Field testing

Field trials with a large number of accessions are generally used by breeding programmes to select potentially resistant genotypes from a germplasm collection (Rubiales et al., 2002, 2004b). Trials are undertaken under field conditions using heavily infested plots. It is crucial to ensure uniform distribution of the parasite seeds in the soil to prevent selection of genotypes that merely remain unchallenged. It is recommended to sow a susceptible cultivar the year before the experimentation. The upper layer of the soil should be cultivated several times after harvest in order to ensure a more uniform distribution of seeds across the whole field (Rubiales et al., 2002). For small scale tests, the plots can be artificially inoculated mixing parasite seeds with sand and applying them to the row with the crop seeds when sowing. However, escapes cannot be precluded, and each test row should be surrounded by rows of a susceptible and vigorous check to be used as a reference for each accession (Rubiales et al., 2003a).

Pot testing

Despite these safeguards field screens are probably most efficient for discarding susceptible accessions. Carefully conducted pot trials are needed to confirm that accessions remaining uninfected in the field are truly resistant. Pot methods allow control over the environment, the inoculum density and its origin. Pots were used successfully to identify sources of resistance to *A. vogelii* in the Botswana cowpea collection (Riches, 1987). Several methodologies (substrate, pot size, etc.) can be used. Linke et al. (1991) suggested the use of plastic pot (5 l) with steam sterilized soil-sand (3:1 v:v) mixed with 7500 (about 30 mg) *O. crenata* seeds/kg substrate to screen chickpea for broomrape resistance. A mixture of clay, silt sand and organic matter (58:22:18:2%) with 40 mg seeds/kg substrate in 2 l pots has been suggested by Goldwasser et al. (1997). A mixture of vermiculite-sand (3:1 v:v) and some fertilizer, and adding 25–30 mg seeds in 1 l pots also gives good results (Pérez-de-Luque et al., 2004b). It is possible to use other substrates like soil or peat instead of vermiculite, but they can hinder the later observation of the broomrape tubercles. The main point is to get a substrate that allows a good plant growth and broomrape infection, and can be easily removed and washed from the roots, facilitating observation of parasite development. Pot trials are usually located in a glasshouse. To overcome the problem of variability of infestation, but

to allow screening under field temperature and moisture conditions when germplasm is in limited supply, Riches (1989) developed the use of pots filled with artificially infested soil sunk into the ground.

In vitro testing

Various techniques have been used to allow close observation of the germination, attachment and early development of parasitic weeds. These so called *in vitro* methods can be used to characterise the resistance of lines selected under field conditions. Use of petri dishes was described by Sauerborn et al. (1987) for mass screenings of lentils under controlled conditions. This method, or slight modifications of it, have been successfully applied by Rubiales et al. (2003c, 2004), Sillero et al. (2001b), Pérez-de-Luque et al. (2005a) to characterise the resistance of several grain legumes, and by Linke et al. (1993) and Sillero et al. (2005) to study the resistance of forage legumes. It has also been used to characterise early stages of interaction of several *Orobanche* species with the model legume *Medicago truncatula* (Rodríguez-Conde et al., 2004). However these petri dish methods unfortunately do not work nicely for faba bean, perhaps due to susceptibility of the roots to oxygen depletion.

Plants (a minimum of 10 per entry) are grown individually in 15 cm diameter petri dishes with 8 mg of surface-sterilized and conditioned *Orobanche* seeds spread over glass microfibre filter discs. The petri dishes are sealed with parafilm, wrapped in aluminium foil and placed vertically, the hole with the germinating plant seed up and uncovered to allow seedling emergence and growth, in a growth chamber at 20 °C. Forty-five days later broomrape attachments per petri dish are counted. Root length should be estimated in order to exclude escapes due to differing amount of roots produced per genotype. The intercept method of Tennant (1975) can be used. Number of broomrape attachments per plant should be referred to as number of attachments per root length unit.

Petri dish methods also allow more detailed studies on induction of germination and resistance to penetration. Germination can be monitored about 30 days after preparing the plates, by studying 500 seeds that are close (<3 mm) to the host roots per petri dish under a binocular microscope at 30× magnification to determine the percentage of germination. The method also allows exogenous applications of the germination stimulant GR24 to bypass the germination step and concentrate on observations of attachment or post-attachment events. The experimental procedure is the same as above, but 10 days after preparation 5 ml of GR24 (10 ppm) are uniformly distributed on the filter disc. Percentage of germination is studied 7 days after GR24 application. Ten days later the broomrape radicles contacting the host roots can also be studied and the percentage of those causing a necrosis of the host tissue surrounding the contact point determined. Five days later the final number of attachments is counted and root length estimated as above.

Parker and Dixon (1983) demonstrated that hosts infected by either *Orobanche* or *Striga* spp. can be maintained in culture on sterilised glass fibre paper sheets held in clear polyethylene bags suspended in nutrient solution. Goldwasser et al. (1997) used this system to screen vetch for resistance to *O. aegyptiaca*. While this system is helpful for most of the legume crops, and allows numerous plants to be maintained in a small space, only the very early stages of *Orobanche* infection can be followed in some crops including faba bean.

The "sandwich" assay involves growing host and parasite in sand or vermiculite held in the gap between two glass sheets. Two cork strips of 0.5 cm thickness are placed between both glasses in left and right sides, and the lower side is sealed with a porous material (foam rubber, sponge) that allows nutrient solution to penetrate. Seeds are placed on the sand in the upper side of the plates. This method allows large plants like faba bean or chickpea to be grown between glass sheets of 50 cm × 30 cm, and also to follow the roots spatial distribution and the development of the broomrapes at different depths.

Lane et al. (1991) devised a technique for growing cowpeas on filter paper mounted on a plastic tray which allows pre-germinated parasite seeds to be placed directly onto the roots of test plants. The development of the parasite can then be easily examined via a binocular microscope allowing the identification of germplasm with *Striga* resistance that operates post-germination or post-attachment of the parasite.

Pot and *in vitro* methods (either petri dishes, polyethylene bags, glasses, etc) are faster and cheaper than the field screening, prevent the escapes due to an uneven distribution of the *Orobanche* seeds in the soil and reduce the environmental influence. Also the effect of plant vigour and root length can be more easily studied. These methods also allow more detailed studies on both the inheritance and the mechanisms of resistance. A further advantage is the feasibility of testing with different *Orobanche* populations and species.

Molecular techniques

Apart from the conventional screening methods described above, further laboratory assays which allow the non-destructive, rapid and inexpensive evaluation of individual plants for resistance are desirable. In the *Orobanche* genus, the only description of a marker useful for such purposes has been reported for the *O. cumana*-sunflower system. In this case five SCARs (Sequence Characterized Amplified Regions) linked to *Or5* gene confering resistance to race E of *O. cumana* were developed (Lu et al., 2000). A genetic linkage map for cowpea based on the segregation of various molecular markers and biological resistance traits in a population of recombinant inbred lines includes AFLP markers for resistance to two of five known strains of *S. gesnerioides* found in West Africa (Ouedraogo et al., 2002). An AFLP fragment from a marker combination linked to *Rsg1*, the gene conferring resistance to strain 3 from Nigeria has been converted into a SCAR marker which will be useful in future breeding programmes (Boukar et al., 2004).

O. crenata resistance identified in legumes so far is of polygenic nature (Cubero & Moreno, 1999; Román et al., 2002a; Valderrama et al., 2004; Rubiales et al., 2003b). The development of marker-assisted selection (MAS) techniques for broad-based polygenic resistance is a particularly promising approach since *Orobanche* resistance tests are difficult, expensive and sometimes unreliable. A further advantage is that parasite seed is not needed during the initial stages of selection overcoming the need to work under quarantine when studying a number of races of a species collected from diverse geographic sources. The development of markers tightly linked to QTLs previously detected in a map and the conversion into co-dominant SCARs, by sequencing a polymorphic fragment linked to the trait, is the most immediate need. The only *Orobanche* resistance QTLs detected so far in legumes are those reported for faba bean and pea (Román et al., 2002a; Valderrama et al., 2004). Nevertheless, in both cases the saturation of the maps is still inadequate to allow the required precision for a good marker/QTL association. These markers located near to the resistance QTLs would enable the saturation of relevant genome regions in order to obtain good candidate markers to be exploited in MAS. With improved genetic maps and the validation of the QTLs detected so far across environments and locations, a better accuracy in the selection of the markers to assist breeding can be expected.

New molecular strategies such as the candidate gene (CGs) approach could offer new possibilities for screening of *Orobanche* resistance in legumes. Validated CG for QTL will provide efficient molecular markers since recombination between markers and QTLs would be absent (Pflieger et al., 2001). Testing the role of a candidate gene can be carried out by a conventional co-segregation analysis in structured segregating populations, in which the gene is used as a marker in order to relate the sequence polymorphism in the gene with variation in the quantitative trait. Association-based approaches represent a second strategy to test and validate candidate genes by looking for phenotype associations in germplasm collections or in natural populations with contrasting phenotypes. The final objective is to correlate the distribution of candidate gene genotypes in the form of DNA sequences with relevant phenotypes.

In the near future, these new approaches could serve to assist legume breeders and geneticists in identifying promising resistant genotypes and should facilitate the efficient transfer of the resistance genes among breeding lines. Furthermore, molecular markers linked to broomrape resistance genes would permit a better understanding of the genetic basis of resistance against parasitic weeds.

Pathogenic variation

Knowledge on the existence of host specialisation and parasite races is vital for any breeding programme. So far, there is only a single report on race identification in *O. crenata*. Joel (2000a) described a virulent population of *O. crenata* that successfully attacked previously resistant vetch plants in Israel. This race seems to have been selected by the frequent culture of the resistant vetch cultivar in the area. Otherwise, there is no clear evidence for the existence of any other race of *O. crenata* (Cubero et al., 1979; Radwan et al., 1988; Cubero, 1991). This might be due to the lack of a selection pressure as there is little resistance in commercial cultivars of most legume hosts. Differences in the level of aggressiveness among populations of *O. crenata* have, however, been detected (Verkleij & Pieterse, 1994). Molecular analysis suggest that most of the intraspecific variation in *O. crenata* is among individuals, and not related to host preference or geographic distribution (Paran et al., 1997; Zeid et al., 1997; Román et al., 2001). It would appear at least possible that new parasitic biotypes could originate, however, as *O. crenata*

populations are very heterogeneous chromosomically (Cubero et al., 1979) as well as genetically (Román et al., 2001, 2002b). There is the risk that diverse *O. crenata* populations could be selected for virulence when challenged by the widespread use of highly resistant cultivars.

The use of molecular markers may be a suitable method for the identification of pathogenic groups within parasite populations (Gagne et al., 1998). Román et al. (2002b) compared *O. crenata* populations from two distant zones of the Mediterranean area (Spain and Israel) to explore the genetic differentiation among them, using inter simple sequence repeat (ISSR) markers. The analysis of molecular variance in Spain and Israel indicated that most of the genetic diversity can be attributable to differences between individuals within populations although significant divergences were also found between regions. The results clearly divided six populations by region, with the Spanish populations being more similar to each other than the Israeli populations. These results are consistent with the predominantly allogamous behaviour of *O. crenata* and the extremely efficient dispersal of its numerous seeds.

Molecular markers can also assist diagnostic studies in the *Orobanche* genus. *Orobanche* species identification is particularly needed because of the differences in the host preferences of the various species. Because of its miniscule seed size, contamination of fields and legume seed lots by broomrape seeds is difficult to detect and confirm via conventional methods. As *Orobanche* seeds will only germinate and grow in the presence of a susceptible host, the early identification of the parasite species in the field by soil sampling is of great importance to farmers. Joel et al. (1996, 1998b) developed RAPD and SCAR markers for the identification of single tiny seeds of the various *Orobanche* species, and Portnoy et al. (1997) applied these methods for the identification of soil-borne *Orobanche* seeds. Single seeds of *O. minor* infecting alfalfa, red and white clover, can similarly be detected by a rapid and reliable PCR-assay consisting of the development of primers based upon unique sequences in the internal transcribed spacers (ITS) regions of the nuclear ribosomal DNA (Osterbauer & Rehms, 2002).

Host preference may vary between regions and should be taken into consideration in breeding programmes. Based on RAPD analysis Joel et al. (1998a) have shown an increase in genetic distance between *Orobanche* populations that correlates with geographical distance even within small regions. The genetic distance may allow variation in host preference, which in turn would allow changes in the host range of different populations. Geographic variation in host preference is seen in both *A. vogelii* and *S. gesnerioides. A. vogelii* populations from West Africa and Cameroon attack cowpea and groundnut. Those from Eastern Botswana and northern areas of Northern Province of South Africa also attack mung bean, while populations from eastern areas of Northern Province, Kenya, Malawi and Zimbabwe parasitize bambara in addition to the crops that are susceptible elsewhere (Riches et al., 1992). Beyond West Africa, Chad and northern Cameroon, populations of *S. gesnerioides* occurring in natural vegetation are unable to attack cowpea and extreme host specificity has been demonstrated by a number of authors. Musselman and Parker (1981) showed that four strains of the parasite would only attack and emerge on the host species, cowpea, *Indigofera* spp., *Tephrosia* spp. or *Jacquemontia* spp. from which they had been collected. A strain from South Africa and Zimbabwe can attack tobacco (*Nicotiana tabacum*). Although it is stimulated to germinate by root exudates from cowpea it is unable to develop on the roots of the crop (Wild, 1948). Accordingly, *S. gesnerioides* is commonly seen in fields in eastern Botswana growing on *Indigofera daleoides* but never on cowpea (Parker & Riches, 1993).

A further narrowing of the host ranges of both *A. vogelii* and *S. gesnerioides* is seen among populations which attack cowpea but are only able to attack some cultivars but not others that are susceptible elsewhere (Parker & Polniaszek, 1990; Riches et al., 1992). In the case of *S. gesnerioides* five strains have been identified in West and Central Africa, classified on the basis of their ability to develop on seven differential cowpea lines (Lane et al., 1994, 1997). Strain 1 is found in Burkina Faso, strain 2 in Mali, strain 3 in Nigeria and Niger, strain 4 in Benin Republic and strain 5 in Cameroon. Research has shown that lines that are resistant to strain 3 from Nigeria confer resistance to all other strains except strain 4 from Benin. Testing of cowpeas in Nigeria and Benin is, therefore, adequate to identify cultivars with combined resistance to all five strains of the parasite. Although less systematic research has been completed with *A. vogelii* it is clear that there is variability in the virulence of populations of the parasite collected from different areas of Africa. The landrace B301, collected from Botswana, has been used as a "differential" line to distinguish between parasite populations. Although B301 is resistant in the field in Kenya and to *A. vogelii* from West Africa, pot trials have indicated that it is susceptible to strains of the parasite from Malawi,

Botswana and some areas of South Africa (Riches et al., 1992). Unfortunately lines selected as resistant in Nigeria, where there have been considerable efforts to develop cowpeas with dual resistance to both *A. vogelii* and *S. gesnerioides* will not necessarily be resistant in East and Southern Africa (Riches, 2001).

This aspect of variability within *S. gesnerioides* and *A. vogelii* has important implications for choice of resistant parents and management of breeding programmes.

Sources of resistance

Breeding of legume crop cultivars resistant to *Orobanche* was initiated with faba bean improvement in the early 1960s (Elia, 1964). However, little resistance to *O. crenata* was available until the appearance of the Egyptian line F402 (Nassib et al., 1982). This allowed the development of several resistant cultivars (Cubero et al., 1994; Khalil & Erskine, 1999). Little resistance is available within field pea germplasm (Rubiales et al., 2003b), but promising sources of resistance have been identified in *Pisum* spp. wild relatives that have successfully been hybridised with cultivated pea (Rubiales et al., 1999, 2004b; Pérez-de-Luque et al., 2005b). Resistance is also very limited in cultivated grass pea (*L. sativus*) and chickling pea (*L. cicera*) but is available in related *Lathyrus* species (Linke et al., 1993; Sillero et al., 2005). No resistance has been identified in lentils (Sauerborn et al., 1987; Khalil & Erskine, 1999). However, resistance is frequent in common vetch and chickpea germplasm and cultivars (Gil et al., 1987; Rubiales et al., 2003a) as well as in their wild relatives (Linke et al., 1993; Rubiales et al., 2004; Sillero et al., 2005). Resistance to *O. aegyptiaca* has been found in *V. athropurpurea* (Goldwasser et al., 1997).

Breeding for broomrape resistance in faba bean is a difficult task, but significant successes have been achieved (Cubero & Hernández, 1991; Cubero & Moreno, 1999; Khalil & Erskine, 1999). Some accessions with moderate to low levels of resistance and/or tolerance had been reported, such as VF172, cv. Express, BPL2210 or Locale di Castellano) (see Cubero et al., 1994, for a review), but the first significant finding of resistance was the identification of the family 402 derived from 3-year cycle of individual plant selection in an F7 from the cross Rebaya 40 × F216. Several accessions have been developed, either by individual plant selection from the open pollinated varieties and germplasm accessions or through segregating populations of targeted crosses. Giza 402, Giza 429, Giza 674, Giza 843 (with resistance derived from F402) have been released in Egypt. Baraca has been released in Spain with resistance derived from VF1071, that is itself a selection out of Giza 402 (Cubero & Moreno, 1999). A number of lines (ILB437, ILB4357, ILB4360) as well as some selections from crosses involving Giza 402 have been identified and are being utilized in crosses to breed for *Orobanche* resistance (Khalil et al., 2004). All these sources for *Orobanche* resistance in faba bean are minor or medium seeded types, of early maturity but susceptible to chocolate spot, rust and Ascochyta blight diseases, and frost. Recently, improved populations with gene pools adapted to West Asia, North Africa and Latin America were developed at ICARDA and shared with cooperating countries for recurrent selections (Khalil et al., 2004).

Resistance against *O. foetida* has been identified in faba bean germplam in Tunisia (LPF58, LPF72, LPF89, LPF132, LPF138, LPF155, LPF170, LPF173, LPF179, BPL502, BPL503, BPL645, BPL714, BPL717, BPL726, BPL737, BPL769, BPL1181, BPL1295, BPL1326). A resistant cultivar (cv. Bader) is in process of registration by INRAT in Tunisia. One of the parents of this variety was a selection from an advanced breeding line provided by ICARDA with resistance derived from Giza 402. Some lines (674/154/85 L3-4 and 402/29/84) selected against *O. crenata* had shown also a high level of resistance to *O. foetida* and showed a high yield potential (Kharrat & Halila, 1994). Resistance to *O. crenata* does not seem to protect for *O. foetida* infection, but it has been possible to combine resistance to both species by selecting for *O. foetida* resistance in *O. crenata* resistant germplasm.

O. crenata can be a problem in winter sown chickpea in Mediterranean countries in some particularly conducive years. In contrast to the situation found on faba bean, peas and lentils where resistance is extremely scarce, useful levels of resistance are common in chickpea accessions and breeding lines that can be exploited in resistance breeding (Rubiales et al., 2003a,c, 2004) and be complemented with other control strategies such as an intermediate sowing date like December or herbicide treatment.

A comprehensive account of breeding for resistance to multiple strains of *S. gesnerioides* and of combined resistance to *Alectra* and *Striga* is provided by Singh (2002). Initially a systematic breeding programme in West Africa used the Botswana landrace B301 as a source of resistance to *S. gesnerioides*.

Following extensive field evaluation a number of new resistant cultivars have been released. These include IT89KD-374-57 (Sangaraka) and IT89KD-245 (Korobalen) released in Mali. IT90K-76 and IT90K-82-2 having combined resistance to aphids, bruchids, thrips, *Striga* and *Alectra* have been released in Nigeria. B310 and a second line, IT82D-849, are resistant to all strains of *S. gesnerioides* with the exception of strain 4 in Benin. Subsequent breeding work using these as parents and cultivar 58-57 that is resistant in Benin, resulted in a number of multiple strain resistant lines, including IT97K-819-154 which is also resistant to *Alectra* in Nigeria.

In Southern Africa resistance to *Alectra* was discovered during screening of more than 650 local and exotic accessions in the Botswana cowpea collection. The local landrace accessions B301 and B359 appeared to be particularly useful (Riches, 1987, 1989). An initial attempt to combine *A. vogelii* resistance from the late-maturing B359 with early maturity from the improved line TVX3836 proved successful in breeding work in Botswana although the progenies were not taken through to the stage of a resistant variety suitable for release to farmers (Riches, 1989). B359 has been shown in pot trials to be resistant to collections of *A. vogelii* from a number of countries in East, Southern and West Africa including Botswana, Cameroon, Mali, Malawi, Nigeria and South Africa (Riches et al., 1992). Mainjeni (1999) demonstrated the superiority of B359 as a source of resistance for southern Africa. B359 remained completely resistant in pot trials while B301 along with IT90K-59 and IT90K-76, two lines with B301 as parent, all supported the emergence of parasites of a population from Malawi. Breeding work is now needed to incorporate durable *Alectra* resistance into early maturing, disease and insect resistant cultivars for East and Southern Africa.

Resistance in cowpea B301 to *S. gesnerioides* and *A. vogelii* are conditioned by the single dominant Rsg_1 gene and duplicate genes Rav_2 and Rav_2 respectively (Singh & Emechebe, 1990; Singh et al., 1993) that are not linked (Atokple et al., 1993). As different dominant genes for resistance have been identified in lines resistant to different *S. gesnerioides* strains identified in West Africa these have been used as complimentary parents when breeding for all strains (Singh, 2002).

Conclusion

Resistance is a multicomponent event, being the result of a battery of escape factors or resistance mechanisms acting at different levels of the infection process. There is variation for these traits in germplasm collections. Landraces have been widely replaced by homogeneous cultivars, that may not possess the combined tolerance and resistance already accumulated in landraces or wild accessions by natural selection. Genetic resources remain highly unexplored and underused and might contain very valuable additional sources of resistance. Fully understanding the escape and resistance factors will help to detect existing genetic diversity for mechanisms that hamper infection (Labrousse et al., 2001; Rubiales et al., 2003c). Combination of different resistance mechanisms into a single cultivar will provide a durable outcome. This can be facilitated by the use of *in vitro* screening methods that allow dissecting parasitic weed resistance into highly heritable components, together with adoption of marker-assisted selection techniques (Ouedraogo et al., 2002; Román et al., 2002a; Boukar et al., 2004; Pérez-Vich et al., 2004; Valderrama et al., 2004).

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