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# Screening techniques and sources of resistance to root diseases in cool season food legumes

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**Summary**

Soil-borne fungal diseases are among the most important factors, limiting the yield of grain legumes in many countries worldwide. Root rot, caused by *Aphanomyces euteiches*, *Rhizoctonia solani*, *Fusarium solani* and wilt, caused by several *formae speciales* of *Fusarium oxysporum* are the most destructive soil-borne diseases of pea, chickpea, lentil, fababean and lupin. The most effective control of these diseases is achieved through the use of resistant varieties. In this paper, recent advances in conventional and innovative screening methods for disease resistance are presented. Many grain legume accessions, which are maintained in national and international germplasm collections, have been evaluated for disease resistance and numerous resistant varieties have been released following incorporation of identified resistance genes from these sources. Recent identification of molecular markers tightly linked to resistance genes has greatly enhanced breeding programs by making marker assisted selection (MAS) possible and allowing the development of varieties with multiple disease resistance. Progress in the understanding of the biology of soil-borne fungal pathogens of grain legumes is also reviewed with particular reference to the genetic structure of their populations, diagnosis and host–pathogen interaction.

## Introduction

Legumes are a primary source of protein in human diets and animal feed worldwide but diseases caused by fungi and viruses are important factors that limit their yield and quality. Allen and Lenné (1998) published an exhaustive list of pathogens associated with the most important food and pasture legumes, including information on their aetiology, biology, symptomatology, epidemiology and management. Soil-borne fungal pathogens can cause disease at all stages of plant development under diverse climatic conditions (dry, temperate and humid). Severity of the disease varies with crop and pathogen species, geographic area, environmental conditions and cultural practices. Soil-borne diseases are more important on chickpeas, lentils and peas than on fababeans and lupins. Pulses are very important in developing countries, where low input agriculture is practised and only simple technologies are available.

Correct diagnosis of disease is fundamental to control and this has been facilitated by advances in pathogen identification. Control of diseases caused by soil-borne fungi, does not usually rely on the use of chemicals but is achieved mainly by integration of different disease management procedures. These are the use of resistant cultivars, sowing certified seed that is clean and choosing fields with low inoculum levels.

The use of resistant cultivars is widely recognized as the safest, most economical and most effective method for protecting crops from disease (Johnson & Jellis, 1992). Successful screening for disease resistance is based on (i) the availability of large and diverse germplasm collections, including wild species; (ii) the knowledge of both plant and pathogen biology, variability, host–pathogen interaction, genetic structure and geographic distribution; (iii) the availability of precise and accurate screening techniques. Many examples of durable resistance to soil-borne fungal pathogens have been reported for grain legumes (Lenné & Allen, 1998; Muehlbauer & Kaiser, 1994). Screening methods and strategies for disease resistance have been extensively reviewed in the past (Dhingra & Sinclair, 1985; Porta-Puglia et al., 1994; Porta-Puglia & Aragona, 1997). Nevertheless, there is still a need for the standardization of methods for the evaluation of resistance of grain legumes to many diseases, including those caused by soil-borne fungi.

Recently, increased emphasis on research of diseases affecting grain legumes has been promoted through the interaction among the institutes of the Consultative Group for International Agricultural Research (CGIAR), namely the International Institute for Agricultural Research in the Dry Areas (ICARDA), the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) and the International Institute of Tropical Agriculture (IITA) with Developing Countries. This cooperation has been effected by several national and international cooperative projects and has allowed the close interaction between geneticists, phytopathologists, physiologists and producers, as recommended by many authors (Jiménez-Díaz et al., 1998; Lenné & Allen, 1998; Porta-Puglia et al., 1994). Root rots and wilt diseases of grain legumes have been addressed at several international conferences and by specific reviews with emphasis on effective screening techniques and sources of resistance (Grünwald et al., 2004; Jiménez-Díaz et al., 1993b; Kraft & Kaiser, 1993; Kraft et al., 1994; Nene & Reddy, 1987). In the following sections, progress toward improved screening methods for resistance to the major fungal pathogens of grain legumes that are soil-borne and the development of new varieties that are resistant will be illustrated and discussed in detail.

## Germplasm collections

Germplasm collections maintained in gene banks (outside their area of growth) or *in situ* (in natural environments) are valuable resources for plant breeders (Innes, 1992). Many wild relatives of crop plants have co-evolved with their pathogens and have consequently developed several means of counteracting them: for this reason they represent an important source of resistance genes (Burdon & Jarosz, 1989; Shoen & Brown, 2001). With the establishment of the International Board for Plant Genetic Resources (IBPGR) in 1974, many grain legume species, including several wild species, have been stored, catalogued, characterized and provided freely to breeders worldwide. The larger grain legume collections are maintained at ICARDA (Aleppo, Syria) where there are more than 26,000 accessions of food legumes comprising 7827 lentils, 9116 kabuli chickpeas and 9074 fababeans and at ICRISAT (Patancheru, India) where there are about 16,961 chickpea accessions (http://www.cgiar.org/impact/accessions.html accessed July 27, 2005). A significant number of germplasm accessions of grain legumes are also maintained in national collections, including the USDA-ARS grain legume collection held at Pullman, WA, USA, comprising 2822 accessions of *Lens*, 4834 of *Cicer*, 3997 of *Pisum*, 743 of *Lupinus* and 565 of *Vicia faba*.

## Major soil-borne diseases of grain legumes

Several soil-borne diseases cause yield loss in grain legumes worldwide and are considered prime targets for control by genetic resistance through plant breeding (Lennè & Allen, 1998). The most important of these are listed in Table 1. Based on symptoms, they have been grouped into two main classes: wilts and root rots (Kraft et al., 1994). Several species within each group share common biological traits and environmental requirements. Knowledge and evaluation of these characteristics by efficient screening techniques is a prerequisite for the implementation of control strategies, particularly breeding for disease resistance.

## Disease assessment

The development of appropriate methods for the evaluation of plant reactions to infection by pathogens is of paramount importance for the breeding of disease resistant varieties. This topic has been extensively reviewed in the past, with particular reference to crop loss assessment (James, 1974; Seem, 1984; Strange,

*Table 1*. List of the most important soil-borne diseases affecting pea, chickpea and lentil worldwide

| Host | Disease | Causal agent | Distribution | Symptoms | References |
|---|---|---|---|---|---|
| Pea | *Fusarium* wilt | *Fusarium oxysporum* Schl. f. sp. *pisi* | Worldwide | Roots: superficially normal, yellow to orange discolouration of root vascular tissue. Plant: downward curling of leaves and stipules, basal internode thickened, leaves and stem brittle. | Haglund & Kraft (2001) |
| | *Aphanomyces* root rot | *Aphanomyces euteiches* Drechs. f. sp. *pisi* Pfender & Hagedorn | Worldwide | Roots: infected roots are initially honey brown, cortical tissue becomes soft and darker, discolouring continues up to epicotyl. Plants: severely stunted, shoots chlorotic, leaves progressively yellow upward on the plant. | Malvick et al. (2001) |
| | *Fusarium* root rot | *Nectria haematocca* Berk. and Broome; anamorph *Fusarium solani* (Mart.) Appel & Wr. f. sp. *pisi* (F.R. Jones) Snyd & Hans. | North America, Europe | Roots: primary and secondary roots consist of reddish brown to black streaks that coalesce during the season. Plants: stunted, yellowing, necrosis of basal foliage. | Kraft (2001) |
| Chickpea | *Fusarium* wilt | *Fusarium oxysporum* Schlecht. emend Snyder: Fr. f. sp. *ciceris* (Padwick) Matuo & K. Sato | Worldwide | Roots: discolouration of vascular tissues. | Nene & Reddy (1987); |
| | | | | Plants: progressive foliar yellowing with vascular discolouration followed by plant death within 40 days (yellowing pathotype). Severe chlorosis and flaccidity, vascular discolouration and plant death within 20 days after inoculation (wilting pathotype) | Trapero-Casas & Jiménez-Diaz (1985) |

(*Continued on next page*)

*Table 1.* (*Continued*)

| Host | Disease | Causal agent | Distribution | Symptoms | References |
|---|---|---|---|---|---|
| | Dry root rot | *Macrophomina phaseolina* (Tassi) Goidanich | North and South America, Asia and Africa and some parts of Europe | Roots: generally dry, tap root is dark and rotted, absence of most lateral roots.<br>Plants: leaves and stems of affected plants are usually straw coloured, brown in the lower part. Drying of plants appears suddenly. Drooping of branches and leaves of the upper part of the plant. | Nene & Reddy (1987) |
| | *Fusarium* black root rot | *Fusarium solani* (Mart.) Sacc. f.sp. *pisi* (F.R. Jones) W.C. Snyder & H.N. Hans, | Worldwide | Roots: generally rotted, most fine roots disappear and the remainder turn black.<br>Plants: yellowing and wilting at any stage of plant growth. | Kraft (1969) |
| | Wet root rot | *Rhizoctonia solani* Kühn | Worldwide | Roots: rotting with dark brown discolouration extending above ground level, root tissues are soft and wet.<br>Plants: gradual wilting. | Nene & Reddy (1987) |
| | Seed rot and pre-emergence damping-off | *Pythium ultimum* Trow | Worldwide | Roots: larger roots are necrotic, discoloured and devoid of feeder rootlets. At seedling stage, roots become stunted.<br>Seed rotting common. | Kaiser & Hannan (1983) |
| Lentil | *Fusarium* wilt | *Fusarium oxysporum* Schlechtend.: em. Snyder & Hans. f.sp. *lentis* Vasudeva & Srinivasan | Worldwide | Seedling: sudden drooping.<br>Roots: appear healthy, with reduced proliferation.<br>Plants: sudden dropping of leaflets starting at the plant top and progressing basally; leaflets close, do not shed prematurely, and turn dull green. Vascular discolouration. | Bayaa & Erskine (1998) |
| | Wet root rot | *Rhizoctonia solani* Kühn | Worldwide | Roots: become reddish-brown with a clear constriction at the collar region or below.<br>Plants: show yellowing of leaves progressing from lower to upper plant part. | Beniwal et al. (1993) |
| | *Sclerotinia* stem rot | *Sclerotinia sclerotiorum* (Lib.) de Bary | Worldwide | Plants: rotting of stem above the collar region or of major branches. Yellowing and drooping of leaves above the infection point. | Beniwal et al. (1993) |
| | Collar rot | *Sclerotium rolfsii* Sacc. | Worldwide | Plants: rotting and discolouration near the collar region, and white strands of the fungus mycelia are characteristic; general yellowing, drooping of leaves and death of plants.<br>Seedling: collapse caused from collar rot. | Beniwal et al. (1993) |

2003; Tinline et al., 1989). Most principles of disease evaluation are shared between soil-borne and air-borne pathogens. Major constraints in the assessment of diseases caused by soil-borne pathogens are due to the possible interactions with other pathogens present in the soil that could alter symptoms expression and the destructive assessment of the germplasm. Direct assessment of disease on the plant is typically done by evaluating two parameters: incidence, expressed as the percentage of infected/dead plants within a sampling unit and severity, expressed as the percentage of diseased plant tissue in a given area.

Evaluation of incidence is particularly suited to the assessment of systemic infections leading to the death of the plants (i.e. wilt). Whenever possible, determination of the number of plants emerging before disease appearance and enumeration of wilted plants at each time of scoring is recommended.

Assessment of disease severity is necessary when resistance is inherited quantitatively giving a continuous gradient of symptom severity within a host plant population (Russell, 1978). Disease severity scales, often 0–5 or 1–9 corresponding to the extent of the damage in the infected plant, have been developed for many pathosystems. These are often illustrated with pictures or diagrams of each class of reaction (James, 1974; Strange et al., 2004). Keys for the rating of disease severity often use visual judgment of symptoms, rendering standardization difficult. Moreover, according to the Weber–Feckner law, visual acuity depends on the logarithm of the intensity of the stimulus, so the eye can accurately assess only very low or very high levels of disease (James, 1974). Percentage scales, which accommodate the full range of expression of symptoms, are preferred. The descriptors used must be clear, easily recorded and meaningful in the context in which a particular plant or crop is being considered (Williams, 1989). Depending on the disease and the accuracy required, scoring may be done on the above ground organs, on principal roots or on other plant tissues, i.e. crown region, secondary roots, fine lateral rootlets. Accuracy may be predicated by whether screening is performed on large populations in the field or smaller populations in the greenhouse or laboratory and whether the objective is determination of the race of the pathogen or the inheritance of resistance.

Aphanomyces root rot symptoms on above ground organs is rated on a 1–5 scale, where 1: healthy plants; 2: slight yellowing of lower leaves; 3: necrosis of the lower leaves up to the 3rd or 4th node, some stunting; 4: necrosis of at least half or more of the plants with stunting, more than half of plants in a row dead; 5: all plants dead or nearly so (Pilet-Nayel et al., 2002). Alternatively, plants can be dug, washed and rated on a root rot index of 1–5 (Rao et al., 1995), where 1: healthy root and epicotyl; 2: roots white, feeder roots pruned and epicotyl coloured; 3: taproot discoloured and epicotyl discoloured and shrunken; 4: roots discoloured and soft, epicotyl discoloured and soft; 5: root disintegrated and epicotyl completely rotted or nearly so – plant dead. Alternatively, the scale proposed by Davis et al. (1995) may be applied, where 0: no visible symptoms; 1: a few small discoloured lesions on the entire root system; 2: minor discolouration covering the root system; 3: brown discolouration on entire root system, no symptoms on epicotyl or hypocotyl; 4: brown discolouration on entire root system, shrivelled and brown epicotyl or hypocotyls and 5: plant dead.

A 0–5 scale is used for evaluation of pea roots after inoculation with *Fusarium solani*, where: 0: no symptoms; 1: slight hypocotyl lesions; 2: lesions coalescing around epicotyls and hypocotyls; 3: lesions starting to spread into the root system with root tips starting to be infected; 4: epicotyl, hypocotyl and root system almost completely infected and only slight amount of white, uninfected tissue left; 5: completely infected root (Grünwald et al., 2003a,b).

In order to overcome the problems of visual scoring, direct and indirect methods have been proposed. Non-destructive methods using thermography and chlorophyll-fluorescence imaging can demonstrate changes in photosynthetic efficiency and transpiration in plants challenged with biotic stresses and their use to accelerate the screening of plant populations for resistance has been proposed (Chaerle et al., 2004). The quantification of fungal biomass in infected tissues by means of molecular tools has been investigated as a method of identifying lines with improved resistance to the pathogens. Discrimination between resistant and susceptible cultivars was possible by measuring GUS activity in potato and wheat cultivars infected with GUS+ isolates of *Phytophthora infestans* and *Pseudocercosporella herpotrichioides*, respectively (De la Peña & Murray, 1994; Kamoun et al., 1998). Conversely, higher levels of GUS activity were observed in resistant tomato cultivars infected with GUS+ isolates of *Fusarium oxysporum* f. sp. *radicis-lycopersici*, as compared to susceptible ones (Papadopoulou et al., 2005). Real-time PCR methods are rapidly gaining interest also for the quantification of several plant pathogens both in infected tissues and in the soil (Okubara et al., 2005; Schaad & Frederick, 2002;

Schena et al., 2004; Ward et al., 2004). Highly significant correlations between the amount pathogen DNA and cultivar reaction were obtained by the adoption of real-time procedures in several pathosystems, e.g. in susceptible rice infected with *Magnaporthe grisea* (Qi & Yang, 2002) and alfalfa infected with *Aphanomyces euteiches* (Vandemark et al., 2002). When applied to the pea/*A. euteiches* system, this technique allowed a positive and significant Spearman rank correlation between the amount of the fungal DNA and the Disease Severity Index ($P < 0.05$) for three isolates, but the correlation for the other two isolates was not significant (Vandemark & Grunwald, 2005). Quantitative PCR detection methodology has been published for *F. solani* f. sp. *glycines* in soybean roots (Gao et al., 2004) and it is anticipated that this information will also facilitate the development of quantitative PCR methodology for Fusarium root rot of pea. All the above mentioned techniques have limited application as a selection tool for resistance, especially for mass-scale germplasm evaluation in the field, although they may make valuable contributions to the study of mechanisms underlying host–pathogen interactions.

**Inoculum characterization**

In many plant–pathogen systems, changes in virulence of fungal populations often render existing resistance ineffective. Variability of pathogenicity has been observed for many soil-borne fungi lacking sexual recombination (Taylor et al., 1999). Multiple mutation or transposition events, parasexuality and horizontal transfer of pathogenicity genes on conditionally dispensable chromosomes have been hypothesized for the high variability observed in many soil-borne fungi, e.g. several species of the *F. oxysporum* complex (Baayen et al., 2000; Kistler & Miao, 1992). Pathogenicity genes for the production of host-specific toxins of *Alternaria alternata* (Hatta et al., 2002), or for the ability to detoxify the pea phytoalexin pisatin by genes in the pea pathogenicity gene cluster (PEP genes) of *Nectria haematococca* (Temporini & VanEtten, 2002, 2004) have been shown to be located in supernumerary chromosomes, dispensable for the normal growth of the fungus in culture. In chickpea, despite the variability in symptom type, race and geographical distribution, recent molecular evidence proved *F. oxysporum* f. sp. *ciceris* (*Foc*) to be monophyletic (Jiménez-Gasco et al., 2002, 2004a). The origin and the stepwise evolution of the *Foc* races were demonstrated for the first time by means of molecular tools (Jiménez-Gasco et al., 2004b).

The phylogenetic structure observed within members of the *F. oxysporum* complex did not correlate with pathogenicity on pea, suggesting that some field isolates of this complex could have evolved recently from a non-pathogenic ancestor or may have simply lost their ability to cause rot root (Skovgaard et al., 2002). Thirty-three isolates of *F. oxysporum* f.sp. *lentis (Fol)* from different lentil-growing areas in north-western Algeria differed in aggressiveness on susceptible lines but belonged to the single vegetative compatibility group VCG 0471 (Belabid & Fortas, 2002). Following RAPD and AFLP analysis, the same set of isolates was grouped into two subpopulations. Little genetic variability among the two subpopulations and no apparent correlation with geographical origin or aggressiveness of the isolates was observed, suggesting that the *Fol* isolates were derived from two genetically distinct, clonal lineages (Belabid et al., 2004).

Variability in virulence in the pathogen populations and the prevalence of particular races or pathotypes in the target environment must be carefully assessed, when breeding for disease resistance. Such characterization allows breeders and plant pathologists to use defined pathogen isolates and defined resistant/susceptible cultivars in the development of new cultivars with true resistance against the most virulent isolates. Isolates are characterized by selecting a set of differential cultivars appropriate for the defined isolates in a given region. Pathogen characterization requires deep knowledge of the optimal requirements of both plant and fungus for disease establishment. The procedures are time-consuming and the results are often influenced by environmental (light, temperature, humidity) and biological (type and levels of inoculum, genetic purity of both host and pathogen) factors as well as variability in visual evaluation of symptoms and disease scoring. Lack of uniformity in these factors leads to conflicting results. The need for standardization in characterizing physiological races of both air-borne and soil-borne pathogens has been stressed by several authors for many diseases (Kraft, 1994; Porta-Puglia et al., 1994; Sharma et al., 2005). Uniform evaluation should involve standard inoculation of a common set of as few differential lines as possible which are characterized by unambiguous disease phenotypes. Lines with intermediate reactions should be avoided and growing conditions standardized. Resistance thresholds and disease criteria should be defined using uniform scoring procedures and disease criteria. Knowledge of the

genetic control of race-specific resistance is also critical for development of resistant cultivars. Advances in the recognition of the molecular variability within plant pathogens have been made possible by the adoption of PCR-based techniques (Correll, 1992; Henson & French, 1993; Porta-Puglia & Aragona, 1997). Molecular markers have been developed for rapid and accurate identification of many fungal species, both at the inter- and intra-specific level. The rationale in using molecular markers to study genetic variation in fungal populations has been reviewed by Milgroom (1997). The most important advantages in respect of traditional methods is that they are fast, reproducible, not influenced by environmental factors and allow the characterization of many isolates simultaneously.

Physiological specialization has been described for many soil-borne pathogens of grain legumes. Four specific races of Fusarium wilt of pea, *F. oxysporum* f. sp. *pisi* (*Fop*), have been identified and studied in the USA (Kraft, 2001) and Canada (Neumann & Xue, 2003). Races 1 and 2 were the only economically important ones in the United States until race 5 appeared in Northwestern Washington in 1963, followed by race 6 (Kraft, 2001). Races 1 and 2 occur worldwide, while races 5 and 6 are only important in western Washington State and their impact has lessened as fresh pea production has moved out of Western Washington (Debra Inglis, personal communication). A list of standard control differentials is listed in the Pea Compendium (Haglund & Kraft, 2001). Although these lines have been used successfully for many years, their reaction to race 2 has recently been questioned. Intermediate reactions of the standard differential lines confound results from individual experiments. In the Pea Compendium (Haglund & Kraft, 2001), a supplemental set of differential lines is proposed. These have distinct resistant and susceptible reactions to race 2 which will help in its recognition and contrasting morphological characters, each genotype having a unique combination of cotyledon colour, leaf and vine morphology (Table 2). Genetic variability within four races (1, 2, 5 and 6) of *Fop* was assessed by 14 random amplified polymorphic DNA (RAPD) bands. Banding patterns generated from isolates of race 2 were uniform relative to patterns generated from races 1, 5 and 6. Race-specific patterns were not found for races 1, 5 and 6 (Grajal-Martin et al., 1993).

Physiological specialization of *Foc* has been extensively studied in several countries in which chickpea is cultivated. Eight races have been described: four physiological races (1–4) were initially identified in India based on the reaction of isolates on a set of 10 chickpea differential lines (Haware & Nene, 1982). Different disease reactions based on a set of differential chickpea cultivars allowed the identification of three additional races (0, 5 and 6) in Spain (Jiménez-Diaz et al., 1989). Race 1 was then subdivided into race 1A and 1B/C (Jiménez-Díaz et al., 1993a). Races 0 and 1B/C caused yellowing, whereas the others induced wilting symptoms. The geographical distribution of the races is shown in Table 3. Race 1A is the most widespread and was found in India, California and in some countries of the Mediterranean region (Jiménez-Gasco et al., 2001). Recently, Sharma et al. (2005) proposed a new set of chickpea differential lines to differentiate the six races of *Foc* unambiguously. The differential set consists of eight RILs from of a cross between a resistant (WR 315) and a susceptible (C-104) chickpea accession which show extreme phenotype (0 or 100% wilt incidence) to different races. The standardization of procedures is particularly necessary for the monitoring of race distribution of this important pathogen of chickpea.

*Table 2.* Supplemental set of differentials for Fusarium wilt of pea

| Differential | Accession[a] | *Fw*[b] | *Fnw*[b] | *Le*[b] | *Af*[b] | *I*[b] | Reference |
|---|---|---|---|---|---|---|---|
| PRIL12-65 | W6 26199 | − | − | − | + | + | McPhee K.E., personal communication |
| Stirling | PI 634571 | + | − | − | − | − | McPhee and Muehlbauer and 2004 |
| PRIL12-126 | W6 26200 | − | + | + | + | + | McPhee K.E., personal communication |
| Joel | PI 619080 | + | + | + | + | − | Muehlbauer (2002) |

Presence of the dominant allele for each race of Fusarium wilt resistance and morphological markers is represented by (+) and the alternate allele is represented by (−).

[a]The lines may be ordered online from USDA-ARS http://www.ars-grin.gov/npgs/orders.html using the PI accession number or from K.E. McPhee.

[b]*Fw*: Fusarium wilt race 1; *Fnw*: Fusarium near wilt race 2; *Le*: long internode; *Af*: afila and *I*: cotyledon colour.

*Table 3.* Geographical distribution of races of *Fusarium oxysporum* f.sp. *ciceris* (Haware & Nene, 1982; Jiménez-Diaz et al., 1993a; Halila & Strange, 1996; Jiménez-Gasco et al., 2001)

| Races | Countries |
|---|---|
| 1(A), 2, 3, 4 | India |
| 0, 1A, 1B/C, 5, 6 | Spain, USA (California) |
| 0, 1A, 6 | Israel |
| 0, 1B/C | Syria, Turkey, Tunisia |
| 1A, 6 | Morocco |
| 0 | Lebanon |

In the past there has been considerable variation in inoculation procedures (pot screening, water culture, sick plot), disease assessment (wilt incidence, disease severity based on percentage of foliage yellowing or necrosis affected), sets of differentials lines, and criteria used to assign plants to susceptible or resistant categories.

The use of the random amplified polymorphic DNA (RAPD) technique has allowed the separation of isolates of *Foc* into two clusters corresponding to the two pathotypes which cause "yellowing" and "wilting" (Kelly et al., 1994). Race-specific RAPD bands were identified (Jiménez-Gasco et al., 2001) and used for the design of sequence-characterized amplified region (SCAR) primers specific for races 0, 1B/C, 5 and 6 (Jiménez-Gasco & Jiménez-Diaz, 2003). Chakrabarti et al. (2001) reported that amplification of intergenic spacer (IGS) regions and digestion with restriction enzymes could be used to study polymorphism in *Foc* and proposed that races 1 and 4 are similar. Three VCGs were identified among 15 Iranian isolates of *Foc*; no correlation between RAPD patterns and virulence or geographic origin was observed (Zamani et al., 2004). Pathotype-specific primer pairs based on a sequence-characterized amplified region (SCAR) derived from a RAPD sequence which was unique for "wilting" *Foc* isolates was designed and used for *in planta* detection (Kelly et al., 1998). Two additional primer pairs were designed, based on the sequences of the same RAPD fragment, and used for the detection of wilting isolates of *Foc* in the soil in a nested PCR procedure (García-Pedrajas et al., 1999).

No physiologic specialization was observed in *A. euteiches.* Resistance is quantitatively inherited (Marx et al., 1972; Pilet-Nayel et al., 2002). A series of publications from Malvick and Percich (1998a,b), Malvick et al. (1998), Wicker and Rouxel (2001), Wicker et al. (2001, 2003), Levenfors et al. (2003), and Grünwald (2003a,b) have given researchers clear ranges of plant host specificity of *A. euteiches*, the range of virulence found in pea–*A. euteiches* interactions, sets of cultivars to differentiate the plant resistance/pathogen virulence interactions, and sets of molecular markers to classify *A. euteiches* all identified from careful assays of worldwide collections of isolates. Microbial population dynamics of *A. euteiches* were thoroughly studied using virulence, host range and molecular characterizations in the USA, France and Sweden. SCAR primer pairs specific for *A. euteiches* of pea were designed for detection of the fungus in soil organic debris previously assessed as conducive to the disease (Vandemark et al., 2000).

Although physiologic specialization has not been determined for *Fol*, the presence of strains with different aggressiveness (Abbas, 1995; Belabid & Fortas, 2002; Kannaiyann & Nene, 1978), could limit the effectiveness of screening using only a local isolate. Multilocation tests are thus necessary for confirmation of resistance under different conditions.

The availability of powerful neutral molecular genetic markers and the adoption of hierarchical sampling procedures have allowed new advances to be made in studies of the population genetics of phythopathogenic fungi (Leung et al., 1993; McDonald, 1997). Populations of fungal species for which extensive genetic studies were available, could be ranked in increasing risk categories based on their evolutionary potential. According to this model, populations of many soilborne pathogens with no known sexual reproduction (e.g. Fusarium wilt), often composed of clonal lineages with little potential for genotype flow and small effective population size, are considered to be in the lower risk category. For low risk pathogens, the use of major gene resistance is probably the most appropriate as it is likely to have reasonable durability (McDonald & Linde, 2002).

## Screening techniques

Effective screening for disease resistance requires accurate simulation of natural environmental conditions where plants are exposed to the inoculum (Porta-Puglia & Aragona, 1997). Optimum inoculation and incubation conditions should be established so that susceptible and resistant genotypes can be easily differentiated. Screening can be performed directly in the field, in the greenhouse or in the laboratory. Each of these methods

has positive and negative aspects that will be discussed in detail with examples in grain legumes.

*Field screening*

Evaluation of breeding material can be accomplished easily and directly in the field with little expense by the use of naturally or artificially infested fields or plots ('sick plots', SP). The main advantage of SP is that they allow simultaneous screening of a large amount of genetic material under environmental conditions similar to those of cultivated plants. Several methods have been described for the development of wilt sick-plots (WSP) for grain legumes (Bayaa et al., 1994; Nene et al., 1981). WSPs should be established based on the presence of the disease as indicated by visual symptoms and reisolation of the causal fungus.

In contrast to diseases caused by foliar fungi where natural epidemics are unpredictable, incidence of disease caused by soil-borne fungal pathogens in a SP is much more reliable. Artificial infestation is accomplished by incorporating chopped wilted plants into the soil at the end of the growing season for at least three seasons followed by cultivation of a susceptible cultivar. To increase the inoculum density, the fungus may be grown on sterilized substrates, e.g. lentil seeds, and uniformly incorporated into the field (Kraft et al., 1994). Test material is generally sown in 2-m rows with a susceptible control repeated every two/four test-entry rows as an indicator of the spatial distribution of the pathogen. If the WSP is not uniformly infected, a covariance analysis of systematically arranged control plants is suggested (Bayaa et al., 1997). A 2 year crop rotation is recommended for chickpea WSPs in order to homogenize and uniformly spread the fungus across the field and to maintain usefulness of the WSP for longer periods. As the optimum temperature for Fusarium wilt is 25 °C, date of sowing is very important. Disease reaction is rated periodically as the percentage of wilted/dead plants per plot, starting when a uniform and high level of disease incidence is observed on the susceptible check and continuing until late pod fill. In preliminary screening, scoring *Foc* on chickpea in the field should be done at least twice. First when the susceptible check (early wilting) shows 90–100% mortality and secondly at the seed filling stage, or when physiologic maturity is observed for the earliest entry. Resistant and moderately resistant lines identified in a preliminary screen should be re-tested in the field to confirm their reactions. The reduced number of lines tested in the confirmation test allows more frequent scoring, approximately every 10 days, until plants reach physiologic maturity. At least two replications should be used for preliminary screening of genetic material in rows 2–4 m in length (25–50 seeds per row) alternating with appropriate susceptible checks ('ILC 482' for race 0 and 'JG 62' for the other races) after every second test row. It is important to consider sowing a highly resistant line after every 10th row and the susceptible check should not be highly susceptible to others diseases prevalent in the region in order to minimize confounding of results. Almost the same method is followed for screening lentil germplasm for resistance to *F. oxysporum* f. sp. *lentis* (*Fol*). The different patterns of disease progression among lentil genotypes and the temporal variation in disease reaction to Fusarium wilt emphasizes the need for repeated scoring in order to avoid missing 'late-wilters'. Test material is considered highly resistant when percentage of wilted/dead plants is $\leq$5%, and resistant when it ranges from 5 to 20% (Bayaa et al., 1997).

For pea, a screening nursery for race 1 of Fusarium wilt was established at the WSU Spillman Research farm near Pullman, WA, by depositing several truck loads of soil infested with *Fop* in a defined area. Test entries including breeding lines or germplasm accessions are sown in single rows 150 cm length with a susceptible check line included at regular intervals in order to monitor the uniformity of disease development. Disease reactions are usually clear and easily scored allowing unequivocal discrimination between resistant and susceptible entries. A similar nursery for race 2 is not currently available.

Assessing the microbial composition and inoculum potential in different parts of a field is important prior to using a given field for screening. Results could be compromised by differences in soil texture, uneven distribution of inoculum and differences in time of exposure of roots to the infested soil. Classical methods for the direct assay of phytopathogens in soil were reviewed by Menzies (1963) and Dhingra and Sinclair (1985). Several bioassays for quantification of *A. euteiches* inoculum density in naturally infested soil have been compared (Kraft et al., 1990; Malvick et al., 1994; Williams-Woodward et al., 1998). In chickpea, an average concentration of about 3,000 propagules/g soil (ppg) induced 100% incidence of wilt in the susceptible chickpea line ICC 4951 in a WSP in Pakistan (Ali et al., 1994). Similarly 3283 ppg of race 1 of *Foc* killed the chickpea line 'JG-62' and affected the late wilting line 'K850' but did not affect the resistant line 'WR315' (Kraft et al., 1994), while at lower inoculum

levels (483 ppg), 100% wilt incidence was observed only on 'JG-62'. At Béja, Tunisia, a WSP infested with race 0 of *Foc* presented a level of inoculum equivalent to 1795 cfu $g^{-1}$ of soil and resulted in a uniform wilting reaction of the susceptible check 'ILC 482' across the plot (Halila & Strange, 1997). Inoculum density of *Fol* in natural fields in Syria ranged from $2 \times 10^4$ to $10^5$ cfu $g^{-1}$ of soil in 1990 and from 0 to $10^3$ cfu $g^{-1}$ of soil in 1992. No significant correlations were observed between inoculum density and wilt incidence in the field in either year, possibly owing to the presence of a diverse microflora potentially with antagonistic effects and varying environmental conditions (Erskine & Bayaa, 1996).

Successful application of both naturally and artificially infested fields has been reported for the major soil-borne pathogens of legumes. In pea SPs have been used to increase the level of resistance to Aphanomyces root rot in breeding lines in the USA (Table 4; Pilet-Nayel et al., 2002), France (Moussart et al., 2003) and New Zealand (Timmerman-Vaughan et al., 2003).

Wilt sick-plots (WSP) have been developed and used in many countries with serious disease problems and active improvement programmes have been established for chickpea (Ahmed et al., 1990; Gupta, 1995; Haq & Jamil, 1995; Halila & Strange, 1996; Hunde et al., 1992; ICARDA, 1994; Jimenez-Diaz & Trapero-Casas, 1990; Kraft et al., 1994; Nene & Haware, 1980; Pawar et al., 1992; Reddy et al., 1990) and lentil (Bayaa & Erskine, 1990; Bayaa et al., 1994, 1995, 1997; Sarker et al., 2001, 2005).

Field studies for Fusarium root rot of pea require a plot heavily infested with *F. solani* f. sp. *pisi* and as other diseases such as Pythium damping off, Fusarium wilt or Aphanomyces root rot can occur, these studies need to be supplemented with controlled greenhouse experiments in order to verify disease reactions.

At the Spillman Farm, Washington State (USA), 15 cultivars and one lentil breeding line were tested in a plot artificially infected twice with the *Sclerotinia sclerotiorum*, the causal agent of white mold: the first with cold-treated sclerotia (4 °C for 9 weeks), the second with colonized oat kernels spread over the plot area. Disease severity ratings were recorded twice, according to a 0–8 scale, based on the percentage infection. Although disease severity was generally low owing to a dry summer, none of the test entries were immune to white mold; six cultivars and the breeding line appeared relatively resistant (Chen et al., 2003). The reaction of several fababean accessions and cultivars to *Rhizoctonia solani* Kühn (AG-4) was evaluated under field conditions by adding inoculum grown on autoclaved proso millet (*Panicum miliaceum* L.) along with the seeds at a rate of 40 ml per row (Rashid & Bernier, 1993).

One major disadvantage of the use of SPs is the risk that multiple soil-borne diseases could be present at the same time and interfere with disease assessment. In this case, evaluation of resistance to a root rot complex might be more realistic than evaluation for resistance to a single disease. Infection of chickpea by root-knot nematodes (*Meloidogyne artiellia, M. incognita* or *M. javanica*) may negatively impact resistance to *Foc* (Castillo et al., 2003; Krishna Rao & Krishnappa, 1996; Mani & Sethi, 1987; Uma Maheshwari et al., 1995, 1997). In lentil, the highest wilt incidence was observed in pot experiments with simultaneous inoculation of both *Meloidogyne javanica* and *Fol* (De et al., 2001). Repeated annual use of the same field for screening against *Foc* could also modify the microbial composition of the WSP by selection for pathotype/race populations of the pathogen under pressure exercised by continuously planting resistant cultivars (Jiménez-Diaz et al., 1991; Kraft et al., 1994).

*Greenhouse and laboratory screening*

Evaluation of germplasm in controlled environments is an important tool in many breeding programs focused on disease resistance. Screening in the greenhouse or growth chamber allows breeding material to be challenged with well characterized isolates without interaction with other phytopathogenic organisms throughout the year. Environmental factors such as humidity, light and temperature can be easily managed to establish optimum conditions for disease development. Limited availability of space is often the major constraint to screening in controlled environments. Disease evaluation in controlled conditions is often used to identify resistant breeding material during non-crop periods, but may also be used to confirm the reaction of resistant genotypes identified in the field or for characterization of pathogen variability.

Several excellent growth chamber/greenhouse methodologies for Aphanomyces root rot have been published recently which provide procedures for identifying lines with improved resistance unambiguously (Moussart et al., 2001). All controlled environment procedures use the zoospore production technique of Mitchell and Yang (1966). Test seedlings are grown in vermiculite (five seeds in each pot, four pots per line) and inoculated with 25 ml zoospore suspension

*Table 4.* Sources of resistant germplasm and cultivars released with improved tolerance/resistance to pea soil-inhabiting fungi, all with white flowers (a allele) in the last years

| Name | Accession[a] | Status | Other resistances reported | Reference |
|---|---|---|---|---|
| MN 144 | W6 26201 | Germplasm | Aph[b], *Fnw*[c] | Davis et al. (1995) |
| MN 313 | W6 26202 | Germplasm | Aph, *Fnw* | Davis et al. (1995) |
| MN 314 | W6 26203 | Germplasm | Aph, *Fnw* | Davis et al. (1995) |
| Wis 8901 | PI 538355 | Germplasm | Aph, *Fw*[d] | Gritton (1990) |
| Wis 8902 | PI 538356 | Germplasm | Aph, *Fw* | Gritton (1990) |
| Wis 8903 | PI 538357 | Germplasm | Aph, *Fw* | Gritton (1990) |
| Wis 8904 | PI 538358 | Germplasm | Aph, *Fw*, *er*[e] | Gritton (1990) |
| Wis 8905 | PI 538359 | Germplasm | Aph, *Fw* | Gritton (1990) |
| 96-2052 | PI 606694 | Germplasm | Aph, Frr[f], *Fw*, *Fnw*, *er* | Kraft and Coffman (2000a) |
| 96-2058 | PI 606695 | Germplasm | Aph, Frr, *Fw*, *Fnw*, *er* | Kraft and Coffman (2000a) |
| 90-2068 | PI 606696 | Germplasm | Aph, Frr, *Fw*, *Fnw*, *er* | Kraft and Coffman, 2000a |
| 96-2198 | PI 606697 | Germplasm | Aph, Frr, *Fw*, *Fnw*, *er* | Kraft and Coffman (2000a) |
| 96-2222 | PI 606698 | Germplasm | Aph, Frr, *Fw*, *Fnw*, *er* | Kraft and Coffman (2000a) |
| 97-261 | PI 606702 | Germplasm | Aph, Frr, *Fw*, *Fnw*, *Fwf*[g], PSbMV[h] | Kraft and Coffman (2000b) |
| 97-2154 | PI 606703 | Germplasm | Aph, Frr, *Fw*, *Fnw*, *Fwf*, PSbMV | Kraft and Coffman (2000b) |
| 97-263 | PI 606699 | Germplasm | Frr, *Fw*, *Fnw*, *Fwf*, Fws | Kraft and Coffman (2000c) |
| 97-2170 | PI 606700 | Germplasm | Aph, *Fw*, *Fnw*, *Fwf*, Fws | Kraft and Coffman (2000c) |
| 97-2162 | PI 606701 | Germplasm | Aph, *Fw*, *Fnw* | Kraft and Coffman (2000c) |
| Stirling | PI 634571 | Cultivar | *Fw*, *er* | McPhee and Muehlbauer (2004) |
| Lifter | PI 628276 | Cultivar | *Fw*, Frr, *er*, PSbMV, PEMV | McPhee and Muehlbauer (2002)a |
| Franklin | PI 628275 | Cultivar | *Fw*, Frr, *er*, PSbMV, PEMV[i] | McPhee and Muehlbauer (2002b) |
| RIL 846-31, RIL 847-28 | W6 27367, W6 27368 | Germplasm | Aph, *Fw* | Coyne et al. (2006a) |
| RIL 846-39, RIL 847-66 | W6 26742, W6 26746 | Germplasm | Frr, *Fw* | Coyne et al. (2006b) |

Other important pea disease resistances listed in germplasm/cultivar releases are noted.
[a]The lines may be ordered online from USDA-ARS http://www.ars-grin.gov/npgs/orders.html using the PI accession number or from the developer.
[b]Aph: improved resistance to *Aphanomyces.*
[c]*Fnw*: resistance to Fusarium near wilt, race 2.
[d]*Fw*: resistance to Fusarium wilt, race 1.
[e]*er*: resistance to powdery mildew.
[f]Frr: resistance to Fusarium root rot.
[g]*Fwf*: resistance to Fusarium wilt race 5.
[h]PSbMV: resistance to pea seed-borne mosaic virus.
[i]PEMV: resistance to pea enation mosaic virus.

giving $10^3$ zoospores per plant. Plants are well watered for 3 days and grown for 170 degree days, before assessing disease severity. Correlations between disease ratings in growth chambers and field assessments using the same genotypes were positive (Moussart et al., 2001). Additionally, a soil-free disease screening method was successful in evaluating lines for resistance to Aphanomyces root rot and also correlated well with field disease assessments (Rao et al., 1995). Seven-day-old pea seedlings were transplanted into aeroponic chambers, inoculated with zoospores and rated for disease development 14 days later. A third controlled-environment screening technique commonly used is to inoculate 10-day-old seedlings sown in vermiculite. Seedlings are harvested 14 days post-inoculation and roots and shoots are weighed separately and compared with uninoculated controls (Malvick & Percich, 1999).

Inoculum of *F. solani* for greenhouse screening is prepared by inoculating individual 250 ml Erlenmeyer flasks containing 120 ml of Kerr's medium with 2 mm agar plugs of the isolates to be tested. Flasks are placed on an orbital shaker under continuous light, at room temperature, for 6 days. Conidia are collected by

straining cultures through sterile cheesecloth and the concentration of conidia is adjusted to $10^6$ ml$^{-1}$. Inoculum can be kept chilled at 4 °C until use on the same day. Pea seeds are inoculated by soaking in 50–60 ml of the conidial suspension in 100 ml beakers at room temperature overnight. After inoculation, 10 seeds of each genotype are planted in single rows in plastic trays (Landmarks, Akron, Ohio; 10 3/4 in. × 20 1/8 in. × 2 1/2 in.) of perlite in 2–3 replications per experiment (Grünwald et al., 2003a,b). Three accessions can be planted per tray. Experiments are repeated at least twice. A susceptible cultivar such as 'Dark Skin Perfection' should be included as a control in each experiment. Plants are harvested after 20 days and roots scored for resistance.

Evaluations under controlled conditions using single spore pure cultures are also used routinely to evaluate a range of pea entries for resistance to individual races of Fusarium wilt. Techniques for inoculation have been reviewed previously (Haglund, 1989) and involve removing approximately one-third of the root system from 7–10-day-old seedlings and submerging the rest of the root system in inoculum adjusted to $1 \times 10^6$ spores ml$^{-1}$. Methods for inoculum production can be found in Haglund (1989) and McPhee et al. (1999). The difference in techniques is based on whether the roots are trimmed while submerged in the inoculum (dip-and-cut) or prior to submersion (cut-and-dip). Growth conditions should be 25–27 °C with 50% humidity and a 16:8 h, light:dark photoperiod. Ratings should be made at 7–10 day intervals beginning 14 days post-inoculation and typically continuing for 6–8 weeks. Inclusion of control cultivars with known reaction to each of the wilt races is recommended in each experiment to ensure accurate results.

Screening chickpea in controlled conditions is usually done in pots as described by Nene and Haware (1980) and Jiménez-Gasco et al. (2001). Tekeoglu et al. (2000) reported a technique for evaluating chickpea for resistance to *Foc*: chickpea seeds are immersed in metalaxyl to control seed-borne Oomycetes and incubated in a germination chamber at 22 °C and 100% relative humidity for 3 days. Seedlings are transferred to plastic flats containing sterile, coarse perlite and placed in a greenhouse maintained at 21–26 °C until plants reach the third to fourth node stage. Seedlings are then removed from the perlite and approximately one-third of the root system pruned. The remainder of the seedlings' roots are submerged in spore suspension for 5 min prepared from potato dextrose broth ($10^6$ spores ml$^{-1}$) and replanted in the perlite. A noninoculated control submerged in sterilised distilled water should be included in each experiment. At least 10 plants of each entry are recommended. Scoring should be done every 2 days by counting the percentage of dead plants for 6–8 weeks. The water-culture technique described by Nene & Haware (1980) was used for screening wild *Cicer* species for resistance to *Foc* (Infantino et al., 1996). In screening for resistance to *F. solani* f. sp. *pisi* and *Chalara elegans* under greenhouse conditions, Bhatti and Kraft (1992) failed to identify chickpea lines with complete resistance to *F. solani* f. sp. *pisi* but identified 10 desi accessions resistant (mean disease index less or equal to 3, on a 1–9 scale) to root rot caused by *C. elegans.*

Laboratory and glasshouse screening techniques for resistance to wilt of lentil have been described in previous reviews (Bayaa et al., 1994; Khare et al., 1993; Kraft et al., 1994). The use of appropriate inoculum density in greenhouse screening is of primary importance in order to avoid the risk of discarding promising genetic material with acceptable level of resistance. An inoculum density of $10^5$ microconidia ml$^{-1}$ caused 100% mortality in the susceptible line 'ILL 4605' but the same concentration had no effect on the resistant line 'ILL 5588' while $10^6$ microconidia ml$^{-1}$ caused 75% mortality (Erskine & Bayaa, 1996).

Recently, two glasshouse inoculation methods were used to characterize *Fol* isolates in Italy: (i) direct sowing of surface disinfected lentil seeds in pasteurised soil infected with the fungus grown on autoclaved millet grains (10%, w/w) and (ii) inoculation by pouring a homogenate of the fungus grown on PDA near the roots of 15-day-old lentil seedlings in pots. Both methods caused wilting with comparable results (Riccioni et al., 2003).

Screening lentil germplasm for resistance to wet rot caused by *R. solani* has been done in the laboratory, in the greenhouse and in the field but little progress has been made in identification of sources of resistance (Khare et al., 1993; Kraft et al., 1994). In a recent pot experiment carried out under plastic greenhouse conditions in order to identify germplasm resistant to vascular wilt and other soil-borne diseases, 45 wilt resistant lines of lentil were evaluated for their reaction to wet root rot using the following method: mycelial mat of *R. solani* (2 g fresh weight) was incorporated in the upper 2 cm of soil in pots, followed immediately by sowing sterilized lentil seeds (10 seeds/pot). Pre- and post-emergence damping-off was recorded for 45 days after planting. The five most promising lines showed 7–17% incidence of wet root rot (Akem et al., 1998).

In screening lentil for resistance to *S. sclerotiorum*, seedlings (20-days-old) of 13 lines were inoculated individually at the base with 5 mm mycelial plugs of the fungus and covered with plastic bags for 72 h; disease incidence was recorded 5 days after inoculation. Low levels of resistance was observed (Akem et al., 1998).

Greenhouse evaluation of fababean lines for resistance to *R. solani* was done by placing 5-mm PDA discs from actively grown colonies of the fungus in contact with the basal-stem/root area of 7-day-old seedlings at 1 cm below soil surface (Rashid & Bernier, 1993).

Four species of *Fusarium* (*F. avenaceum, F. equiseti* (Corda) Sacc., *F. culmorum* (W.G. Smith) Sacc., and *F. oxysporum*) were among the fungi most frequently isolated from infected fababean plants in Poland (Zakrzewska & Oleksiak, 1993a). Different methods of inoculation (spraying of plants, injection of plants and soil inoculation) with three *Fusarium* species (*F. avenaceum*, *F. culmorum* and *F. oxysporum*) were compared. *F. oxysporum* caused greater reduction in pod number and seed yield irrespective of the inoculation method used (Zakrzewska & Oleksiak, 1993b).

**Sources and type of resistance**

Much progress has been made in releasing germplasm and cultivars of food legumes with resistance to soilborne pathogens in recent years. New sources of genetic resistance and greater understanding of the types of resistance have been made possible through advances in the mapping of resistance genes and identification of QTL for resistance.

Resistance to races 1, 2, 5 and 6 of *Fop* is conferred by single dominant genes that are inherited independently (Kraft & Pfleger, 2001) and which are present in many releases of germplasm (Kraft et al., 1998; Table 4). Resistance to race 2 was first discovered in 1945 by researchers in Wisconsin, USA in an adapted breeding line and has been attributed to a single dominant gene. However, segregation patterns have been difficult to explain and are skewed towards susceptibility (Hare et al., 1949). Recent genetic mapping has also demonstrated segregation ratios skewed towards susceptibility and several individual recombinant inbred lines showed an intermediate reaction indicating that additional genetic factors may be involved (McPhee et al., 2004). Plants categorized as intermediate wilted more slowly (2–4 weeks post-inoculation) than those categorized as susceptible which wilted within 2 weeks post-inoculation. A third category of lines was completely healthy after four weeks and were considered resistant. Additional studies are required to characterize resistance to race 2 definitively.

The genetics of resistance to Fusarium root rot in pea was first reported by Muehlbauer and Kraft (1973) and confirmed as quantitatively inherited (Coyne et al., 2004a). Currently, no commercial cultivars with complete resistance to Fusarium root rot are available (Grünwald et al., 2003a,b). Nevertheless, tolerant cultivars and improved germplasm have been identified and released (Table 4). More recently, accessions from the USDA-ARS *Pisum* core collection were evaluated for resistance to Fusarium root rot (Grünwald et al., 2003a,b). Total immunity to Fusarium root rot was not detected in over 300 accessions tested. Forty-four PI lines with a disease severity rating of 2.5 or less on a 0–5 scale (5: completely rotted) were selected as being partially resistant to root rot. These 44 accessions showing promise under greenhouse conditions have so far been evaluated in two growing seasons under field conditions; only a few accessions retained high levels of resistance including PI180693, PI197990, PI505122, PI196877 and PI197450 (Grünwald & Coyne, personal communication). A complete listing of the data for the partial resistance of all accessions tested can be found at the National Plant Germplasm System website, USDA-ARS (http://www.ars-grin.gov/npgs/orders.html). Comparison of disease resistance data for Aphanomyces root rot and Fusarium root rot showed a weak, but significant and positive correlation.

Given the quantitative inheritance of resistance (Marx et al., 1972), breeding for resistance to *Aphanomyces* has been best accomplished by combining both field and growth chamber results from ratings of above ground symptoms and root rot index (Moussart et al., 2001; Pilet-Nayel et al., 2005).

In chickpea, resistance to Fusarium wilt is governed by major resistance genes. In particular, resistance to race 1A, 2 and 4 is either under control of two or three genes, while resistance to race 3 and 5 is monogenic (Sharma et al., 2005). Rubio et al. (2003) found two genes responsible for resistance to race 0 of *Foc* in a cross between 2 resistant chickpea cultivars CA1938 and JG-62 with resistance that can be conferred by the presence of one of them. Several chickpea accessions, advanced lines and cultivars of both desi and kabuli types have been identified in recent years and selected for their resistance to *Foc* using the techniques described earlier (Halila & Strange, 1997; Yu & Su, 1997; Table 5). Evaluation of 100 advanced lines of kabuli chickpea (FLIP) from three

*Table 5.* Sources of chickpea germplasm and cultivars resistant to the most important soil-borne diseases described in the last 10 years

| Accessions/cultivars | Disease resistances | Reference |
|---|---|---|
| Hermosillo 93, Pitic 93 | FW | Morales-Gomez et al. (1994) |
| CCV89402, ICCV90902, ICCV90254, HG209, H88-2, RSG180, GF88426 | FW, DRR | Gupta (1995) |
| CC6045 | FW, DRR | Tadesse (1995) |
| PDG4 | FW, FR, DRR | Singh et al. (2002) |
| ICCV 2, UC 15, FLIP 85-20C, FLIP 85-29C, FLIP 85-30C | FW | Ali et al. (2002) |
| FLIP90-131C, FLIP96-152C, FLIP96-153C, FLIP96-155C, FLIP96-158C, ICCV95503 | FW | Khan et al. (2002) |
| Gujarat Gram 1 | FW | Pithia et al. (2003) |
| PBG 5 | FW, FR, DRR | Sandhu et al. (2004) |
| CA2954 | FW (races 0 and 5) | Rubio et al. (2004) |
| JGK 1 | FW | Gaur et al. (2004) |
| Virat | FW | Deshmukh et al. (2004) |
| Vihar | FW | Jamadagni et al. (2005) |
| H92-67, H00-256, H97-93, H00-216, H01-07, H01-08, H01-09, H01-10, H01-67, H01-74, H01-79 | FW | Waldia et al. (2005) |

FW: *Fusarium* wilt; DRR: dry root rot; FR: foot rot.

ICARDA international nurseries (CIEN-W-03, CIEN-SP-03, CIABN-03) done in 2003 in a WSP infested with race 0 of *Foc* at Béja, Tunisia, showed that 23 FLIP lines and one Tunisian resistant variety ('Béja 1') had mortality less than or equal to 10% and were considered highly resistant. The susceptible check ('ILC 482') wilted uniformly and showed 100% mortality. Most resistant lines also showed some resistance to Ascochyta blight and were high-yielding (Kharrat, personal communication). Sources of resistance to wilt have been found in several wild *Cicer* species (Kaiser et al., 1994; Infantino et al., 1996). Unfortunately, the barriers to interspecific hybridization have confined the use of good sources of resistance to species belonging only to the primary gene pool. Chickpea accessions or varieties resistant to Dry Root Rot (DRR) are scarce compared to that for wilt. In Ethiopia, 46 entries out of 211 promising chickpea lines expressed resistance to wilt and DRR diseases and showed less than 20% mortality in field screening in wilt and DRR sick plots (Ahmed et al., 1990). Gupta (1995) identified in India seven desi lines resistant to wilt and DRR from 300 chickpea entries. Using the paper towel technique of Nene et al. (1981), Pande et al. (2004) found no lines immune to DRR out of 47 chickpea lines tested; however, one germplasm accession, 'ICC 14395', a cultivar 'ICCV 2' and an advanced breeding line, ICCX830203-BH-BH-11H, were resistant (score $>1$ and $\leq 3$).

Kamboj et al. (1990) reported that in India inheritance of resistance to vascular wilt of lentil is controlled by five independently segregating genes based on the reaction of individual plants. In these studies, two dominant genes with duplicate interactions were identified in 'L234', two dominant genes with complementary effects were found in 'IL446' and 'LP286', and a fifth gene complementary to the genes in 'IL446' and 'LP286' was identified in two susceptible lines. Abbas (1995) and Eujayl et al. (1998) found that resistance was governed by a single dominant gene. Sources of resistance to *Fol* have been identified in a core collection of 577 germplasm accessions from 34 countries that were screened in a well-developed WSP at ICARDA: 'ILL-422' and 'ILL-2313' from Chile, 'ILL-813' from Egypt, 'ILL-1220' and 'ILL-1462' from Iran and 'ILL-2684' from India showed high levels of resistance (Sarker et al., 2001). More recently, 1500 accessions from a core lentil collection, 892 lines with genes for resistance to wilt introduced in previous crossing programs and 467 accessions of four genera of lentil wild relatives (*Lens culinaris*, *L. erviodes*, *L. nigricans* and *L. lamottei*) were screened at ICARDA. Thirty-four accessions from 14 countries with stable resistance as well as breeding lines showing high wilt resistance and accessions belonging to all the wild lentil genera were selected (Sarker, personal communication). Three improved varieties resistant to vascular wilt were registered in Syria by ICARDA. 'Idlib-2' was derived from a single-plant selection from a Jordanian landrace (El-Ashkar et al., 2003) while 'Idlib-3' and 'Idlib-4' were developed through crossing programs (El-Ashkar et al., 2004a,b). Three wilt-resistant varieties, 'Talya-2', 'Rachayya'

and 'Hala' have also been released in Lebanon. The ICARDA-derived variety 'IPA-98' is widely cultivated in Iraq. Wilt-resistant cultivars released by the Southeast Anatolian Regional Agricultural Research Institute (SARARI) at Diyarbakir in southeast Anatolia, Turkey, are being widely cultivated in lentil-growing areas. The cultivars 'Adaa', 'Alemaya' and 'Assano' are resistant to both wilt and root rot and are largely cultivated in Ethiopia. Six promising lentil lines with high levels of resistance to wilt and to root-rot have been identified in Nepal (ICARDA, 2004). A better understanding of the inheritance of resistance to Fusarium wilt will provide information to set up DNA markers useful for marker-assisted screening and selection for vascular wilt in lentil (Eujayl et al., 1998).

In Canada, five fababean genotypes (2N114, 2N134, 2N487, 2N519 and N-2-2-2) showed a higher level of resistance to *R. solani* isolates compared with the commercial cultivars (Rashid & Bernier, 1993). Field screening and greenhouse test of 15 landraces and five cultivars of white lupin against Fusarium root rot, major disease in Egypt on this legume, revealed that varieties Giza 1 and Giza 2 were the most resistant (Raza et al., 2000).

## Conclusions and future developments

The use of well established tests for both field and greenhouse screening has allowed the recognition of useful sources of resistance to soil-borne diseases in several germplasm collections of cultivated and wild legume species which are now available for exploitation. However, efforts still have to be made to standardize evaluation and scoring criteria for some disease. Availability of accurate screening methods have resulted in the development of molecular markers linked to resistance genes for some diseases and these can now be used in marker assisted selection. These advances have facilitated the development of genetic maps and the introgression of many qualitative and quantitative resistance genes into commercial cultivars. The recent development in the analysis of genetic diversity of wild grain legume species bodes well for their exploitation in breeding programs (Muehlbauer et al., 1994; Rajesh et al., 2003).

Progress in the knowledge of host–pathogen interaction will be accelerated with candidate gene approaches and comparative mapping between the model legume *Medicago truncatula* and pea (Choi et al., 2004a,b), *Medicago sativa* and pea (Kalo et al., 2004), and *M. truncatula* and pea, chickpea and fababean (Gutierrez et al., 2005). The identity of many candidate genes which contribute to resistance to soil inhabiting fungal pathogens affecting pea, particularly *F. solani* and *A. euteiches*, have been published. These are beginning to give specific information on how resistance is conferred at the molecular level (Nyamsuren et al., 2003; Colditz et al., 2004). The candidate gene DDR206 (pI 206) is under study for its role in resistance and is most likely an important regulator of quantitative resistance known to function for Fusarium root rot and Aphanomyces root rot resistance in pea (Choi et al., 2004a,b; Ruiz-Lozano et al., 1999). Another is the DRR230 defensin family (PI230, PI39), which was identified as a response gene to *F. solani* (Lai et al., 2002). Progress has also been reported in understanding the pathogen genes involved in infection of pea by *F. solani,* such as an extracellular lipase (Nasser Eddine et al., 2001) and the ability to detoxify the phytoalexin pisatin (Temporini & VanEtten, 2004). For the single gene resistance of Fusarium wilt races, resistant gene analogs (RGAs) were identified for chickpea (Huettel et al., 2002) and pea (Timmerman-Vaughan et al., 2000); recently, seven of the nine pea RGAs were identified in a pea BAC library (Coyne et al., 2004b). Once the candidate genes are confirmed, primers can be designed to develop polymorphic 'perfect markers' to the resistance genes themselves which may then be used in marker assisted breeding. 'Model species', like *Magnaporthe grisea* and *Ustilago maydis* for air-borne fungi, do not currently exist for soil-borne pathogens. Nevertheless, much experimental evidence suggests that *Fusarium* could be a suitable model for advancing our knowledge of many aspects of fungal infection specific for soil-borne pathogens (Roncero et al., 2003).

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