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# Screening techniques and sources of resistance to foliar diseases caused by fungi and bacteria in cool season food legumes

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**Abstract**

Screening techniques are an important component of the overall strategy of breeding for resistance to diseases in cool season food legumes. Suitable screening methods have been developed for several major foliar diseases of chickpea, pea, faba bean, and lentil, and sources of resistance have been identified. International cooperation plays an important role in promoting research and keeping collections of cultivated species and their wild relatives. New biotechnological approaches are promising for enhancing the practical use of genes for resistance.

## Introduction

Chickpea (*Cicer arietinum* L.), faba bean (*Vicia faba* L.), lentil (*Lens culinaris* Medik.), and pea (*Pisum sativum* L.) are very important sources of proteins for the human diet, or for animal feed, in several countries. Acreage and production are second only to cereals, although research efforts have been discontinuous and, globally, rather poor (Hawtin *et al.*, 1988). Considering the studies related to disease resistance in the four crops, a comparatively greater amount of research has been devoted to pea and to faba bean, probably because developed countries are more interested in these crops. In more recent years, national governments of developing countries and international organizations have put more impetus on research on chickpea, faba bean, and lentil. Also some countries in the northern hemisphere have been involved in such studies, in programs supporting less developed countries abroad, or marginal areas inside their own borders, and our knowledge of these crops is progressively increasing, along with their yield. Nevertheless, a lot remains to be done, and resistance to diseases has a major role to play in the improvement of food legumes, both in terms of increasing and stabilizing production.

The crops considered in this paper suffer from a number of diseases caused mainly by viruses, bacteria, fungi, and nematodes, that can affect one or more organs of the plant. Major diseases are systemic virus infections, fungal root rots, fungal wilts, and fungal and bacterial diseases of leaves and stems. This paper deals with diseases caused by fungi and bacteria that affect the above ground tissues of the plant.

Damages caused by foliar pathogens affecting supply and translocation of photosynthates are of primary importance. Nevertheless, other effects, e.g., on growth regulators and water relations, influence disease impact on yield (Griffiths, 1984). The yield reductions caused by bacterial and fungal diseases of the aerial parts of plants can be severe. For example, losses caused by Ascochyta blight in chickpea, reported by diverse authors in different years and locations, were more than 40% (Nene & Reddy, 1987). Most of the pathogens causing foliar diseases often attack

pods and infect or infest seeds; and, by this means, several pathogens are able to survive and disseminate, even through the small amount of seeds exchanged among scientists (Frison *et al.*, 1990). Although soilborne pathogens affecting roots or the vascular system can be, under some conditions, seed transmitted, transmission of foliar diseases by seed is far more common.

Resistant cultivars provide an efficient means of controlling foliar diseases. The judicious choice of appropriate methods of screening should make it possible to identify genotypes with suitable resistance in germplasm collections, in segregating populations, or in advanced breeding lines. This paper deals with the rationale behind this approach and gives practical examples of screening for resistance and known resistance sources to major foliar diseases of cool season food legumes.

## Strategies and methods of screening for resistance

The prerequisites to obtain resistant cultivars are: (1) the knowledge of the pathogenic variation of the disease incitant, (2) the development of a screening method able to mimic the conditions met by the plants when exposed to natural sources of inoculum in diverse field environments, and (3) the availability of usable sources of resistance.

Screening methods for disease resistance should be developed within the framework of a general strategy for resistance. The changes in the frequency of virulence genes among the populations of pathogens inciting disease of the above-ground parts of plants are very frequent. Populations of such pathogens vary in time and space because of the airborne or seedborne nature of inoculum which facilitates long distance dispersal of their variants. As a result of these situations, breeding for resistance to foliar pathogens is, in general, more difficult than in the case of less mobile pathogens, e.g., soilborne fungi which are, therefore, more stable.

Screening as part of a strategy for developing resistant cultivars requires good planning and understanding of the processes involved in resistance. A screening program should be initiated with a clear statement of the type of resistance which is sought, i.e., complete resistance or partial resistance, or both, and with at least some knowledge of pathogenicity and virulence patterns in the pathogen. Considerable progress has been made in the last decade regarding the nature and durability of resistance, and on effective methods of evaluating plant material for resistance to a number of pathogens. A clear understanding of the concepts and terminology involved is essential to the success of any screening program. *Host resistance* is defined as the ability of the host to hinder the growth and/or development of the pathogen, *complete resistance* is used when the sporulation of the pathogen is prevented. *Incomplete resistance* refers to resistance that allows some sporulation. *Partial resistance* is used when the host is susceptible to infection but spore production is reduced (Parlevliet, 1979; Bernier *et al.*, 1988). The term *durable resistance* has generated some confusion because of its sometimes inappropriate use. The term is descriptive and does not explain the underlying causes. The durability of resistance can be practically tested only when the resistant cultivar is widely used in space and time (Johnson, 1984). Multilocation cultivar testing or the challenge of cultivars with a large collection of pathogen variants can help to verify resistance and give timely warning of the possibility of resistance breakdown, but cannot actually be considered as a test for durability of resistance.

Care must be taken in interpreting results of glasshouse or laboratory tests, as the expression of resistance in the field may be considerably modified because of interaction between microorganisms and between pathogens and environmental conditions.

For foliar pathogens, the plant material must be adequately challenged with a single race or pathotype at a realistic inoculum dose to allow disease development, but at the same time, not obscure minor differences in host response required to identify partial resistance. Use of inoculum composed of a mixture of races or naturally infested crop debris of unknown pathotype composition will not be adequate to achieve this objective. The rationale behind this approach has been reviewed by Parlevliet (1979, 1983). To illustrate, three cultivars each having a single gene for complete resistance to a given race would be identified only when inoculated singly with each isolate but not if the isolates were used in a mixture. Parlevliet also concluded that using a single race provides the best conditions for the selection of partial resistance in the presence of complete resistance, and that the selected race should have the broadest possible virulence spectrum to suppress the expression of as many complete resistance genes as possible. Genotypes with resistance to one virulent race should then be systematically tested to a collection of other isolates.

The identification of cultivars with complete resistance is but a first step in the development of effective, durable resistance and genetic analysis of resis-

tant reactions is essential to reveal similarities and differences in the gene(s) that confer resistance in each genotype. The information is then used to recombine, in a single cultivar, several genes known to be effective against a given race and genes effective against all prevalent races in a production region.

In the last 20 years, there has been a tremendous increase in the number of countries that have established germplasm banks. Many of these countries maintain germplasm collections of varying sizes of one or more of the cool season food legumes (Van der Maesen *et al.*, 1988). Two of the international agriculture research centers, ICARDA in Syria and ICRISAT in India, maintain large and diverse collections of chickpea (ICARDA and ICRISAT), faba bean (ICARDA), and lentil (ICARDA). For several years, both centers have had excellent programs for distributing germplasm of their mandate crops to researchers worldwide. One particularly useful and valuable service of ICARDA and ICRISAT is the distribution of international yield, adaptation and disease and insect resistance nurseries to cooperators in different countries, i.e., ICARDA's Chickpea International Ascochyta Blight Nursery and Lentil Rust and Ascochyta Blight Nurseries. These and other multilocation tests, promoted by international centers help also in standardization of inoculation techniques and rating scales.

*Screening techniques in the field*

Screening for resistance in the field provides a comparatively cheap means for testing, during the whole plant cycle, a large number of individuals under conditions similar to those in which the resistant cultivars are expected to perform. On the other hand, the environmental conditions, the nature of the inoculum available in the area, and the interactions with other organisms, can affect the expression of resistance to such an extent that screening can be effective only during epidemic years. There is also some risks of confusing escape with resistance. Some of these constraints can be controlled by appropriate techniques. For example, relative humidity can be increased by sprinkler or perfo-irrigation. Artificial inoculation can be applied and epidemics can be encouraged by interplanting rows of susceptible genotypes (spreaders).

*Screening techniques in the glasshouse*

Screening germplasm of cool season food legumes for resistance to foliar diseases under glasshouse conditions has some advantages over screening in the field. It is often easier to perform than field screening because various environmental factors, such as temperature and relative humidity, can be regulated to favor infection and disease development. Plants can be inoculated at different stages of development and with varying concentrations of inoculum of one or more purified isolates of the pathogen. Additionally, the inoculum can be distributed in such a way that it will result in more uniform infection. When a high relative humidity is required for infection, inoculated plants can be incubated in a chamber covered by plastic or cloth where the relative humidity can be maintained at or near 100% by various methods. One major disadvantage of glasshouse screening is that space is often a limiting constraint. As cool season food legumes are field crops, results obtained in the greenhouse need to be validated in the field.

*Screening techniques in the laboratory*

In circumstances when conditions in the field are not favorable for infection and disease development and when it is not possible to increase the leaf wetness duration period by irrigating or misting the plots or by covering them with polyethylene sheets, detached leaves can be used to assess host reactions in the laboratory. The method has been used successfully to assess reactions of faba bean genotypes to chocolate spot and also to test a large number of isolates of the fungus for pathogenicity (Hanounik & Maliha, 1986; Hanounik & Robertson, 1988).

In several crops, culture filtrates or purified phytotoxins have been tested, particularly *in vitro*, as agents for selection. There is a lack of research regarding the possible use of such methods in cool season food legumes. The results obtained with other species indicate that these methods may be useful for selection only when a host-specific toxin is involved in pathogenesis. In the case of nonhost-specific toxins, the methods can be of some help in a few host-pathogen systems (Buiatti & Ingram, 1991; Van den Bulk, 1991).

*Rating scales*

For rapid evaluation of a large number of lines in the field, it is necessary to have a simple rating scale. But for more precise studies, such as components of resistance, genetics of resistance, and pathogenic variation, it is desirable to have a more detailed scale taking into consideration disease severity and sporulation of the

fungus. A 9-point scale based on visual judgement of disease severity in chickpea Ascochyta blight has been found to be very useful (Reddy *et al.*, 1984). The advantages of the scale are that it is rapid, repeatable, and covers a wider range of disease severities. The 9-point scale is suitable for scoring progeny rows and yield plots. Similar scales can be developed for other foliar diseases.

It is also important to establish relationships between disease severity scores and extent of yield loss. This information is essential in selecting lines in resistance breeding programs. In case of chickpea Ascochyta blight, the relationship between 1 to 9 scores and yield loss was estimated (Reddy & Singh, 1990). The loss in yield in lines scored 2 to 4 was less than 10% and in those with a 5 score, it was 16%. The yield loss in lines with a 6 to 7 score was 26 to 27%, while in those with an 8 to 9 score, 81 to 98%.

A more precise 9-point scale can be devised taking into consideration the extent of damage to the crop in the form of defoliation, stem blighting, pod infection, lesion size on stem and pods, and the extent of sporulation in the lesions. Generally, a correlation is observed between these characters. The major problem observed in devising a precise rating scale (e.g., for Ascochyta blight of chickpea) is variation in disease severity between the plants of the same line and variation in the lesion size and sporulation on the same plant. Diverse scales can be developed for evaluating material in the greenhouse and laboratory.

*Testing related and linked characters*

Many of the testing procedures for disease resistance are rather complex and time consuming, and so breeders have looked for easily selectable markers linked to the resistance.

Unfortunately, disease resistance is often associated with traits which the breeder is trying to select against, such as late maturity [e.g., resistance to *Mycosphaerella pinodes* (Berk. & Bloxam) Vestergr. in pea, see Lawyer, 1984], high content of phenolics in seed (e.g., resistance to various pathogens affecting establishment in pea, chickpea, and faba bean, see Muehlbauer & Kraft, 1978; Knights & Mailer, 1989; Pascual Villalobos & Jellis, 1990), and long straw (e.g., resistance to *Ascochyta fabae* Spegazzini in beans, see Jellis *et al.*, 1985).

Recently, there has been much interest in using isozymes and restriction fragment length polymorphisms (RFLPs) as selectable markers. These can identify the presence of resistance genes without depending on phenotypic testing.

The use of isozymes depends on a close association between resistance and a specific enzyme banding pattern. An example of a case where this has been used very successfully is in wheat, where an endopeptinase gene *Ep-D1b* is tightly linked to the gene *Pch1* which confers high resistance to eyespot [*Pseudocercosporella herpotrichoides* (Fron) Deighton] (Summers *et al.*, 1988). Gaur & Slinkard (1991) have recently described an isozyme gene map for chickpea. Linkage of morphological markers and isozymes has also been studied in lentil (Vaillancourt, 1989). In pea, a considerable amount of work has been done on linkage relationships of isozyme loci (Mahmoud *et al.*, 1984; Weeden & Marx, 1987). Recently, Weeden *et al.* (1992) have shown a close linkage between the peroxidase gene *Prx–3* and the gene conferring resistance to strain P1 of pea seedborne mosaic virus. Such developments may lead to the successful development of isozyme marker assisted selection for resistance to pea diseases in the future.

With the development of molecular biology, there is currently considerable interest in using RFLPs as markers (see Tanksley *et al.*, 1989, for a general review). RFLP linkage maps are currently being constructed for pea (Davies, 1990) and other legumes and we can expect rapid developments in this field.

## Screening for resistance in the major cool season food legume crops

*Chickpea*

One of the major reasons for slow progress of foliar disease resistant work in chickpea in the past has been the lack of efficient field inoculation techniques. Techniques have been developed only recently for Ascochyta blight caused by *Ascochyta rabiei* (Pass.) Labrousse.

*Ascochyta blight*

In view of the polycyclic nature of Ascochyta blight (and other foliar diseases) and association between age of the plant and disease susceptibility, field evaluation of the lines exposing all stages of crop to the disease is necessary. It has been found to be necessary to expose the materials to disease even after the susceptible checks were killed as the disease progresses with time in the resistant materials until maturity. At ICAR-

DA, efficient techniques to evaluate large amounts of chickpea germplasm against Ascochyta blight in up to 8.5 ha fields have been developed (Reddy *et al.*, 1984).

Temperature and humidity are critical factors for blight development. Planting the crop in a period when the average minimum and maximum temperatures are between 10 and 20°C is essential. Relative humidity can be increased by sprinkler or perfo-irrigation, if needed. Inoculations are done either by spraying plants with spore suspensions of the fungus multiplied in the laboratory or by scattering diseased debris in the field. The advantage of the diseased debris method is that inoculations can be done any time and blight develops when conditions become favorable for the disease. With the spore suspension method, it is necessary to inoculate plants when natural conditions are favorable for disease development or by providing favorable conditions after inoculation. The success rate of this method is low and requires repeated inoculations. Instead of using diseased debris, the fungus can be multiplied on chickpea seed or chickpea dextrose broth and the dried seed or mycelial mats can be spread in the field. In the Mediterranean region, planting the crop in the winter season exposes it to high disease pressure. The other advantages of the diseased debris method are that it is closer to the natural mode of spread of the disease and it can be used where laboratory facilities are lacking.

Systematic evaluation of chickpea for Ascochyta blight resistance started with the initiation of the ICRISAT-ICARDA kabuli chickpea program in 1978. The entire world collection of germplasm of over 15,000 accessions comprising both desi and kabuli types was evaluated at ICARDA in Syria and the accessions resistant at this site (Table 1) were evaluated at other blight endemic locations in the world (Reddy & Singh, 1984). A few accessions of wild *Cicer* species were also found promising against blight at ICARDA (Table 2) (K. B. Singh & M. V. Reddy, unpublished). Many cultivated chickpea lines that were resistant in the vegetative stage showed severe infection in the pod stage. The reaction of the lines varied with the location. In general, the lines showed higher disease severity in India and Pakistan than in West Asia, North Africa, and Southern European countries. The variable reaction of the lines was attributed to physiologic specialization in the blight pathogen (Reddy & Kabbabeh, 1985). On the other hand, other workers obtained results suggesting that the isolates differ only in the degree of virulence (Gowen *et al.*, 1989). More information is needed about these aspects to improve the efficacy of screening methods for disease resistance.

A few lines, such as ILC 72, ILC 182, ILC 201, ILC 202, ILC 2380, ILC 2956, ILC 3279, ILC 3868, ILC 3870, and ILC 4421 showed resistance across locations (Singh *et al.*, 1984). Most of these were found to be either kabuli or intermediate types. They were also found to be tall and late maturing. A few breeding lines, such as FLIP 82–191C, FLIP 83–46C, FLIP 83–49C, FLIP 83–72C, FLIP 83–97C, FLIP 84–83C, FLIP 84–93C that were recently developed at ICARDA also showed resistance to blight at multiple locations (Reddy *et al.*, 1992).

*Other diseases*

Satisfactory screening techniques for the other foliar diseases have not been developed (Reddy *et al.*, 1990). "Hot-spot" locations for Botrytis gray mold, Stemphylium blight, and rust are known. For gray mold, Northeast India, Nepal, and Bangladesh are endemic areas. Pantnagar in India, Rampur in Nepal, and Ishurdi in Bangladesh are gray mold hot-spot locations. Closer spacing, early sowing, establishing a good stand and good canopy through good agronomic practices, and increasing humidity by perfo- or flood irrigation helps in obtaining higher disease pressure.

For Stemphylium blight, Dholi, India and Ishurdi, Bangladesh are hot-spot locations and the conditions that favor Botrytis grey mold are also favorable for Stemphylium blight. Chickpeas sown in the summer (July to October) at Terbol, Lebanon were found to develop severe rust infections. The evaluation of chickpea for resistance to Botrytis gray mold, Alternaria blight, Stemphylium blight, and rust has been very limited, and mainly confined to field tests. The experience with Botrytis gray mold at ICRISAT indicates that it may be difficult to get high levels of resistance to the disease. A few lines that showed some promise with moderate disease pressure at Pantnagar, India were susceptible at Rampur, Nepal, a hot-spot location for the disease. Again, the tall types with compact canopy were found to be more resistant to the disease than the conventional spreading types (Reddy *et al.*, 1990). The work on the host-plant resistance to foliar diseases in chickpea other than Ascochyta blight is very limited. Frequent loss of resistance and lack of stability across locations was observed.

*Table 1.* Desi and kabuli chickpea germplasm accessions resistant or moderately resistant in field and greenhouse evaluation trials (on a 1 to 9 scale) to *Ascochyta rabiei* in Syria (ICARDA)

| Field evaluation (1979 to 1991)[b] | | | Greenhouse evaluation |
|---|---|---|---|
| Accession No.[a] | Average blight score (range) | No. of years of evaluation | Blight score (1990) |
| Desi | | | |
| ICC 3606 | 3.0 (2-4) | 2 | 5.0 |
| ICC 4286 | 4.0 (4-5) | 2 | 5.0 |
| ICC 4475 | 3.5 (3-4) | 4 | 4.0 |
| ICC 4828 | 4.0 (3-5) | 2 | 5.0 |
| ICC 6328 | 3.5 (3-4) | 2 | 4.0 |
| ICC 8540 | 4.0 (4-4) | 2 | 5.0 |
| ICC 8566 | 4.0 (4-4) | 2 | 5.0 |
| ICC 9584 | 4.0 (4-4) | 2 | 5.0 |
| ICC 12004 | 3.0 (3-3) | 3 | 4.0 |
| Kabuli | | | |
| ILC 187 | 3.0 (2-4) | 9 | 5.0 |
| ILC 200 | 3.0 (2-4) | 9 | 3.5 |
| ILC 3856 | 3.0 (3-4) | 6 | 5.0 |
| ILC 5913 | 3.0 (2-4) | 2 | 5.0 |
| ILC 6482 | 4.0 (4-4) | 2 | 2.0 |

[a] ICC = ICRISAT Chickpea assigned to ICRISAT germplasm accessions and ILC = International Legume Chickpea assigned to ICARDA germplasm accessions.

[b] Evaluations during 1985, 1987 and 1990 were not effective.

*Faba bean*

Faba bean germplasm and breeding lines have been evaluated for resistance to three major diseases, namely rust [(*Uromyces fabae* (Grev.) Fuckel = *U. viciae-fabae* Pers.:Pers.) J. Schröt], Ascochyta spot (blight) (*Ascochyta fabae*), and chocolate spot (*Botrytis fabae* Sardiña).

*Rust*

In the case of rust, evaluations were first conducted indoors to identify resistance and to provide information on the race composition of rust isolates from cultivated and wild legume hosts in Manitoba, Canada (Conner & Bernier, 1982a). Single pustule isolates were used as inoculum, and single plants of four cultivars were assessed for reaction on the basis of infection type (IT) of 0 to 4, where 0 = highly resistant (no sporulation), 1 and 2 = resistant (small pustules, about 0.5 mm in diameter), and 3 and 4 = susceptible. The four cultivars were heterogeneous for rust reactions but a few plants in each were found to be resistant to each of two rust isolates. When seven faba bean inbred lines (some derived from single resistant plants), and 12 pea cultivars were used to test 17 rust isolates, 11 races were identified. The rust reactions remained the same when the lines and cultivars were tested in the field, indicating that as with other rust fungi, evaluations can be conducted in the greenhouse as well as in the field.

Crosses involving 11 faba bean inbred lines revealed the presence of several genes conditioning resistance to two rust races (Rashid & Bernier, 1986a). The study also showed that IT 2 was controlled by a single gene in several inbred lines, and that gene(s) controlling resistance to race 3 appear to condition IT 0 in line 1, IT 1 in line 3, and IT 3 in line 4. These

*Table 2.* Accessions of wild annual *Cicer* species resistant or moderately resistant to Ascochyta blight, Tel Hadya, Syria, 1988 to 1991

| ICARDA legume wild accession No. | Ascochyta blight score on 1 to 9 scale | | | | | |
|---|---|---|---|---|---|---|
| | Field tests | | | Greenhouse tests | | |
| | 1988 | 1989 | 1991 | 1989 | 1990 | 1991 |
| *C. jaduacum* | | | | | | |
| ILWC 4-2 | 3 | 2 | 3 | 5 | 5 | 4 |
| ILWC 29/S-9 | 3 | 2 | 3 | 5 | 4 | 4 |
| ILWC 31-2 | 3 | 2 | 4 | 4 | 4 | 2 |
| ILWC 47/2 | 3 | 2 | 3 | 5 | 5 | 5 |
| *C. pinnatifidum* | | | | | | |
| ILWC 7-5 | 3 | 3 | 4 | 5 | 5 | 4 |
| ILWC 9/2 | 3 | 2 | 3 | 4 | 5 | 5 |
| ILWC 29/S-10 | 3 | 2 | 4 | 5 | 5 | 5 |
| ILWC 30-1 | 2 | 2 | 3 | 5 | 5 | 4 |
| ILWC 30-3 | 3 | 2 | 3 | 5 | 5 | 4 |
| ILWC 30/S-2 | 3 | 2 | 3 | 4 | 4 | 2 |
| ILWC 49/1 | 3 | 2 | 5 | 5 | 5 | 5 |
| *C. arietinum* | | | | | | |
| ILC 1929 (Susceptible check) | 9 | 9 | 9 | 9 | 9 | 9 |

results indicate that selection for intermediate IT would not lead to quantitative resistance in this host-pathogen system, and confirm results obtained with rust on cereals.

In view of the existence of numerous races in faba bean rust, open-pollinated faba bean accessions were evaluated for their ability to retard rust development in order to identify more durable and quantitative resistance. Some 252 accessions were tested in the field over four years as single or double-row plots 2.5 m long (Rashid & Bernier, 1986b). Two rows of a rust susceptible accession were planted at right angles to the plots, at both ends, to act as rust-spreader rows. The spreader rows were inoculated 3 to 4 weeks after emergence with a mixture of two virulent races. Since the accessions were generally heterogeneous in their disease reactions, mass selection (MS) was performed every year by eliminating the resistant plants (IT 0 to 2) from accessions with a predominance of IT 3 and 4. Rust development was assessed using keys of 1, 5, and 10% leaf area with sporulating pustules. The values of leaf area infected over time were summarized as area under the disease progress curve (AUDPC) values. Eight populations consistently had low AUDPC values and were considered slow rusters. Rust development and spread were also evaluated in isolated test plots using one slow-, one moderate- and one fast-rusting MS population. Populations were found to be similarly ranked in both adjacent and isolated plots, confirming the adequacy of using small adjacent plots in evaluating large number of accessions for slow-rusting.

More recently, it was shown that rust caused yield losses of only 1 to 2% in three slow-rusting populations, whereas in other populations with equal AUDPC values, losses ranged from 6 to 43% (Rashid & Bernier, 1991). The results of this work agree with the conclusions reached by Buddenhagen (1981) that it is essential to assess yielding ability as well as disease severity in order to identify populations with tolerance. Clearly, slow-rusting capability may not reflect the yield potential under epidemic conditions. Evaluation of genotypes for slow-rusting and tolerance must be done concurrently over several years of field testing to ensure that genotypes with both traits are identified.

*Leaf spots*

Two necrotrophic diseases of faba bean, chocolate spot, and Ascochyta spot, provide good examples of successful screening for resistance where the work was conducted first in the field and then confirmed in green-

house tests (Table 3). For both diseases, genotypes were evaluated by artificially inoculating micro plots in the evening and covering them with polyethylene sheets supported by metal or wood frames to maintain leaf wetness overnight (Hanounik & Robertson, 1988; Rashid & Bernier, 1991).

*Chocolate spot*

Since the extent of pathogenic variability in *Ascochyta fabae* was not known, a mixture of 20 isolates was used as inoculum in a first evaluation (Hanounik & Robertson, 1988). Some of the plants in this test developed a few susceptible lesions which were believed to be due to highly virulent forms of the fungus. To provide more rigorous testing, 20 isolates of the fungus were obtained from such lesions and used to retest 53 resistant progenies from the first season. Fourteen of the 53 progenies remained resistant in the second testing. These were also resistant when tested on detached leaves in the laboratory. Further comparisons of the efficiency of the resistant populations were made by growing 19 lines in Syria, Egypt, England, and the Netherlands. Only three lines remained resistant at all locations, and 19 and 16 were resistant in Egypt and Syria, respectively (Hanounik & Maliha, 1986). The results indicate that more virulent pathotypes of the fungus exist in Europe than in the Middle East, or that the environmental conditions in the former were more favorable for disease development.

Finally, it is worth mentioning that some hybrid bulk populations tested in Egypt under field conditions showed multi-resistance to chocolate spot, rust, and Alternaria leaf spot (Khalil *et al.*, 1986).

*Ascochyta spot*

A slightly different approach was used to evaluate faba bean lines for resistance to Ascochyta spot in Manitoba since isolates of known virulence were available (Kharbanda & Bernier, 1980). Open-pollinated and mass-selected populations were tested in the field over a 3-year period using two virulent isolates (Isolate A and Y1) each at a separate location (Rashid *et al.*, 1991a). The plots were inoculated and covered with polyethylene as described above for rust, and rated for reaction on a 0 to 5 scale where 0 = no infection, 1 = flecking and 2 = localized lesions without pycnidia, i.e., no sporulation, 3 = localized lesions with pycnidia, 4 = spreading lesions with pycnidia, and 5 = coalescing lesions with pycnidia. Classes 0 to 2 were resistant and 3 to 5 susceptible. To improve the homogeneity for disease reaction, all susceptible plants were removed from a given population. The number of populations tested was reduced from 370 in the first year to 50 and 23 in subsequent years. In the last 2 years, plants were sequentially inoculated with each isolate. Isolate A was applied first and isolate Y1 applied to new growth 8 days later.

After three cycles of testing and mass-selection, the level of heterogeneity was reduced but no population was homogenous for resistance. A total of 18 and 11 populations were identified with > 80% plants resistant to isolates A and Y1, respectively, and two populations had > 80% plants resistant to both isolates. These populations were then tested and selfed in the growth room where, after four cycles, seven and eight inbred lines were homogenous to isolates A and Y1, respectively, and five were homogenous for resistance to both isolates. The inbred lines were then used to differentiate 10 isolates originating from various regions of the world into seven races. Such a high number of races can now be explained by the recent first report of the teleomorph (sexual stage) of *A. fabae* in the UK (Jellis and Punithalingam, 1991). In Europe, selection has not been made within partially inbred lines in the hope that a more heterogeneous population may provide a more durable resistance.

In a recent study, the genetics of resistance to five isolates of *A. fabae* was investigated in 19 faba bean inbred lines (Rashid *et al.*, 1991b). Seven genes for resistance to specific isolates were identified. Single genes, or in some cases two genes, controlled resistance to a given isolate. Some genes appear to confer resistance to more than one isolate of the fungus.

It is noteworthy that both leaf spot disease resistant reactions were effectively scored on the basis of infection types, that high levels of resistance (no sporulation) were identified, and that considerable variation was observed in pathogenicity and virulence of each pathogen.

*Pea*

The pea crop is attacked by a number of fungal and bacterial pathogens that affect foliar growth, seed quality, and yield. Resistance to many of these diseases has been sought and found, and breeding programs have been developed to incorporate these resistances. Many of the testing procedures described in the literature rely on glasshouse inoculation techniques and not all have been verified by field performance.

*Table 3.* Inventory of genetic resistance to major faba bean diseases available in germplasm and breeding lines

| Disease | Pathogen | Resistance | | Patho-types | References |
|---|---|---|---|---|---|
| | | Type | Nature of inheritance | | |
| Rust | *Uromyces viciae-fabae* | SR | Monogenic dominant | yes | Conner & Bernier (1982a,b); Rashid & Bernier (1984, 1986a); Khalil *et al.* (1985); Rashid & Bernier (1991) |
| | | rr | – | | |
| | | tol. | – | | |
| Ascochyta spot (blight) | *Ascochyta fabae* | SR | Monogenic dominant | yes | Kharanda & Bernier (1980); Hanounik & Maliha (1984); Rashid *et al.* (1991a,b) |
| | | rr | – | | |
| Chocolate spot | *Botrytis fabae* | SR | Monogenic? | yes? | Hanounik (1983); Hanounik & Maliha (1986); Elliott & Whittington (1979); Khalil *et al.* (1984); Hanounik & Maliha (1986); Hanounik & Robertson (1988) |

SR = strong resistance
rr = rate reducing resistance
tol. = tolerance
– = Data not available

Some of the foliar diseases, particularly those caused by *Mycosphaerella pinodes* and *Phoma medicaginis* Malbr. & Roum. var. *pinodella* (L. K. Jones) Boerema affect the base of the stem as well as the foliage. Tests for resistance involve inoculating seed (e.g., Sakar *et al.*, 1982), or infesting the growing medium with inoculum either after planting or after plant establishment (e.g., Kraft & Roberts, 1970).

Assessment of resistance in the foliage to these two species and *Ascochyta pisi* Lib. has been performed in the glasshouse by inoculating with conidia, incubating for 48 h in a mist chamber and then placing the plants on a glasshouse bench and assessing symptoms after 7 to 14 days (Ali *et al.*, 1978). No single sources of resistance to all three species was found. Furthermore, there is no correlation between resistance to the footrot and foliage phases of the disease caused by *P. medicaginis* var. *pinodella* according to Sakar *et al.* (1982). Resistance to *A. pisi* is complicated by the existence of distinct races (Darby *et al.*, 1986) and differential interactions have also been reported for *M. pinodes* (Clulow *et al.*, 1991). The situation is further complicated by a strong correlation between maturity and infection by *M. pinodes*, so care must be taken in interpreting results.

Screening for resistance to downy mildew [*Peronospora viciae* (Berk.) Casp.] in the glasshouse or growthroom can be done either by inoculating seed with oospores or pregerminated seed with sporangia before planting, when plants become systemically infected (Ryan, 1971; Hubbeling, 1975), or by spraying seedlings with a sporangial suspension. Differential interactions between host and pathogen have been reported (Hubbeling, 1975).

Selection for resistance to powdery mildew [*Erysiphe pisi* Syd. (= E. polygoni DC.)] normally relies on natural attacks in the field in areas where the disease is known to be severe. Infections are most damaging on late maturing crops (Thomas & Sweet, 1990), so sowing trials later than usual can be an advantage.

Resistance to bacterial blight (*Pseudomonas syringae* Van Hall pv. *pisi*) is race specific. Six races have been characterized and one partially characterized at present (Taylor *et al.*, 1989). Pathogenicity tests are usually made on the stem of pea seedlings. Bacteria are scraped from the surface of 24 to 48 h cultures

and stabbed into the main stem at the junction with the stipules at the two youngest nodes and plant reaction recorded after 5 to 10 days. Resistant lines show a localized necrotic reaction and susceptible ones, an area of water soaking that spreads from the site of inoculation.

These techniques have proved very useful in identifying sources of resistance to specific pathogens. Details of resistant lines can be found in the references cited in Table 4.

*Lentil*

Two of the most important and devastating foliar diseases of lentil in many countries are rust caused by *Uromyces fabae* (= *U. viciae-fabae*) and Ascochyta blight incited by *Ascochyta fabae* Speg. f. sp. *lentis* Gossen *et al.* (= *A. lentis* Vassiljevsky). Rust and blight affect all aerial parts of the lentil plant (Khare, 1981). Infection and disease development and spread are favored by cool, wet weather (Khare, 1981; Nene *et al.*, 1988). Sources of resistance in lentil germplasm to rust and blight have been identified.

*Rust*

The rust pathogen, which is autoecious, infects several legumes, including species of *Lathyrus*, *Lens*, *Pisum*, and *Vicia* (Laundon & Waterston, 1965). In India, pathotypes of *U. fabae* from pea were identified which varied in their virulence on a set of differential lentil hosts (Singh & Sokhi, 1980).

Most screening of lentils for resistance to rust has been done in the field (Khare, 1981; Khare *et al.*, 1990). The results of field screening often vary from season to season and location to location depending on environmental conditions and source of viable inoculum. Field screening needs to be done in conjunction with screening in the glasshouse where environmental conditions and inoculation with known isolates of the pathogen can be regulated more precisely. Kramm & Tay (1984) refined glasshouse techniques for inoculating lentils with rust. However, information is lacking on the pathogenic variability within the lentil rust population. Additional information in this area would help to improve the effectiveness and reliability of screening in the glasshouse and field, and in selection of more durable and improved sources of resistance.

*Ascochyta blight*

The blight fungus is a seedborne pathogen with a host range confined to lentils. The teleomorph (sexual stage) of the lentil blight fungus was identified as *Didymella* sp. (W.J. Kaiser, unpublished). The pathogen survives from one season to the next in infected seeds and infested crop residues (Nene *et al.*, 1988).

A number of sources of resistance to the blight pathogen have been identified in different countries. Most of the screening has been done under field conditions (Khare *et al.*, 1993), but there is a need to corroborate the results of field screening with those in the glasshouse where controlled inoculations can be done with single or combined isolates of the pathogen. Isolates of *A. fabae* f. sp. *lentis* from different countries vary greatly in cultural characteristics (W. J. Kaiser, unpublished). However, little is known concerning the existence of races or pathotypes of the blight pathogen (Nene *et al.*, 1988). Information on pathogenic variation of the fungus could lead to a more efficient screening of lentil germplasm in the field and glasshouse and in development of lentil cultivars with resistance to one or more diseases.

Researchers in different countries have identified lentil germplasm with resistance or tolerance to lentil rust and Ascochyta blight. Some lentil accessions have been identified that have resistance or tolerance to more than one disease, such as Fusarium wilt and rust (Nene *et al.*, 1975), Ascochyta blight and rust (W. Erskine, personal communication), Ascochyta blight, rust, and wilt (Pandya *et al.*, 1980), and powdery mildew and rust (Khare *et al.*, 1993). Recent screening studies at ICARDA have identified several sources of resistance to Ascochyta blight in wild lentil germplasm (Bayaa *et al.*, 1991). Observations on the reaction of wild lentil germplasm is needed for other diseases of lentil, including rust. Lentil germplasm accessions, breeding lines, and cultivars reported to be resistant or tolerant to rust and Ascochyta blight are listed in Table 5.

## Future research needs and prospects

The increase in international exchanges of plant germplasm and the involvement of researchers in different countries in the breeding efforts to improve food legume resistance demand the standardization of the techniques utilized in different centers devoted to research and development of resistant plants. Standardization is particularly needed in the study of pathogenic

*Table 4.* Genes reported to control resistance to some pea diseases

| Disease | Pathogen | | Resistance gene(s) (where known) | Some key of references to sources of resistance |
|---|---|---|---|---|
| Ascochyta complex | | | | |
| Leaf and pod spot | *Ascochyta pisi* race | 1 | *rap-1* | Ali *et al.* (1978) |
| | | 2 | *rap-2* | |
| | | 3 | *rap-3* | Darby *et al.* (1986) |
| | | 4 | *rap-4* | |
| Mycosphaerella blight | *Mycosphaerella pinodes* | | *rmps-1* | Ali *et al.* (1978) |
| | | | *rmps-2* | Clulow *et al.* (1991) |
| | | | *rmps-3* | |
| | | | *rmps-4* | |
| | | | *rmpf-1* | |
| | | | *rmpf-2* | |
| | | | *rmpf-3* | |
| | | | *rmpf-4* | |
| Root rot | *Phoma medicaginis* var. *pinodella* | | polygenic | Ali *et al.* (1978)<br>Sakar *et al.* (1982) |
| Downy mildew | *Peronospora pisi* | | *rpv-1* | Hubbeling (1975) |
| | | | *rpv-2* | Stegmark (1988) |
| Powdery mildew | *Erysiphe pisi* | | *er-1* | Gritton & Hagedorn |
| | | | *er-2* | (1971) |
| Bacterial blight | *Pseudomonas syringae* pv. *pisi* | | 5 dominant genes | Taylor *et al.* (1989) |

variability of the pathogens and in laboratory tests to evaluate germplasm for resistance. The testing methods must be standardized to give reproducible results and produce disease intensity sufficiently high to allow appropriate selection, but not so severe that plants with some resistance are graded as susceptible (Dhingra & Sinclair, 1985). In fact, the nature of inoculum, its concentration and distribution on the host plants, the age of the host to be tested, and the environmental conditions under which the testing is performed are of paramount importance. They should be accurately defined, if possible as a result of comparative international testing, and then constantly used. This approach implies a coordinated effort to be shared by diverse institutions and specialists. Cooperation between plant pathologists and breeders should be a must from the very beginning of a screening program.

Sources of resistance from different institutions need to be pooled and evaluated in different disease endemic locations. This helps in both distribution of resistant materials to all those interested and in obtaining information on stability of resistance. The activities of international organizations in this field should be continuously supported. Furthermore, international institutions can contribute in coordinating the work on variability in the major pathogens by identifying suitable institutes and financially supporting research directly or through donors. The source of some newly released disease resistant cultivars in several countries has been from these international nurseries, such as rust and Ascochyta blight resistant lentil accession ILL 4605 that was released recently in Pakistan as "Manserha 89" (W. Erskine, personal communication).

Support given to gene banks worldwide by the International Board of Plant Genetic Resources (IBPGR) in Rome, Italy, is to be encouraged. IBPGR is also instrumental in organizing and supporting germplasm collecting expeditions to different regions of the world.

*Table 5.* Lentil germplasm accessions, breeding lines, and cultivars with resistance or tolerance to rust *(Uromyces viciae-fabae)* and Ascochyta blight *(Ascochyta fabae* f. sp. *lentis)*

| | | | | | |
|---|---|---|---|---|---|
| Rust | | | | | |
| *India* | | | | *Ecuador* | *Morocco* |
| Bombay 18 | LG 8 | LWS 43 | 10465 | INIAP 406 | ILL 215 |
| BC 10 | LG 12 | LWS 81 | 10475 | (FLIP 84- | ILL 234 |
| C 31 | LG 41 | NP 47 | 10495 | 94L) | ILL 255 |
| EC 10 | LG 60 | Pant L 406 | 10502 | | ILL 275 |
| HPL 5 | LL 3 | Pant L 620 | 10506 | *Ethiopia* | ILL 277 |
| HY1-1 | LL 48 | Pant L 638 | 10507 | ILL 358 | ILL 857 |
| JL 599 | LL 56 | Pant L 639 | 10511 | ILL 857 | ILL 4605 |
| JL 632 | LL 71 | PL 5 | 10526 | | (Precoz) |
| JL 642 | LL 82 | PL 8 | | *Pakistan* | ILL 5883 |
| JL 648 | LL 83 | PL 538 | *Chile* | Manserha 89 | ILL 6002 |
| JL 673 | LL 103 | PL 539 | Araucana- | (ILL 4605) | ILL 6209 |
| JL 674 | LL 133 | PL 620 | INIA | | ILL 6212 |
| JL 676 | LL 178 | PL 640 | Centinela- | | ILL 6471 |
| JL 688 | LP 338 | PL 642 | INIA | | |
| JL 1004 | LP 409 | PLMA 183 | Laird | | |
| JL 1005 | LP 846 | Pusa 10 | Tekoa | | |
| K 75 | LWS 30 | RR 25 | | | |
| L 9-12 | LWS 38 | T 36 | | | |
| L 1278 | LWS 39 | UPL 172 | | | |
| L 2895 | LWS 42 | UPL 175 | | | |
| Ascochyta blight | | | | | |
| *India* | | *Pakistan* | *Morocco* | *Canada* | |
| HPL 5 | LG 201 | FLIP 84-27L | ILL 5698 | ILL 358 | |
| L 442 | LG 209 | FLIP 84-43L | ILL 5700 | ILL 5588 | |
| L 448 | LG 217 | FLIP 84-55L | ILL 5883 | ILL 5684 | |
| LG 169 | LG 218 | FLIP 84-85L | ILL 6212 | Laird | |
| LG 170 | LG 219 | FLIP 86-9L | | | |
| LG 171 | LG 221 | FLIP 86-12L | *Syria* | *Chile* | |
| LG 172 | LG 223 | ILL 358 | ILL 857 | ILL 358 | |
| LG 173 | LG 225 | ILL 858 | ILL 4605 | ILL 4605 | |
| LG 174 | LG 231 | ILL 5588 | ILL 5244 | | |
| LG 176 | LG 232 | ILL 5684 | ILL 5588 | *Ethiopia* | |
| LG 177 | LG 236 | ILL 6024 | ILL 5562 | ILL 358 | |
| LG 179 | 89 S 26013 | 78 S 26018 | ILL 5590 | ILL 857 | |
| LG 195 | Pant L 406 | 78 S 26052 | ILL 5593 | | |
| | | Manserha 89 (ILL 4605) | ILL 5684 | | |

References:

Rust: Bascur & Sepúlveda (1989); W. Erskine (personal communication, 1991); Khare (1981); Khare *et al.* (1979); Khare *et al.* (1990); Mishra *et al.* (1985); Nene *et al.* (1988); Pandya *et al.* (1980); Singh & Sandhu (1988).

Ascochyta blight: W. Erskinde (personal communication, 1991); Iqbal *et al.* (1990); Kapoor *et al.* (1990); Khare *et al.* (1990); Pandya *et al.* (1980); Singh *et al.* (1982); Slinkard *et al.* (1983); Tay (1989); Tay *et al.* (1981).

Wild relatives of the cool season food legumes are often poorly represented in most gene banks. There is a continuing need to collect additional germplasm of the wild species of these crops in the centers of diversity. Little is known concerning the resistance of the wild species of cool season food legumes to different diseases. An important step in this direction is the recent report by Bayaa *et al.* (1991) on the resistance of wild lentil germplasm to Fusarium wilt and Ascochyta blight.

Screening techniques can benefit from innovative approaches. At present, there is considerable interest in the development of techniques facilitating indirect selection for disease resistance using isozymes and RFLP markers, and linkage maps for pea, lentil, faba bean, and chickpea are being developed. As the maps become more complete, the opportunities for using linkage in effective screening programs will increase. A major goal will be the marking of specific chromosome segments involved in quantitative traits.

Quantitative assessment of pathogens in host tissue using enzyme-linked immunosorbent assay (ELISA) systems is another area where rapid progress is being made. Both polyclonal and monoclonal antisera have been produced to many fungal pathogens and these have been used to quantify mycelial growth. In host-pathogen systems where assessment is largely subjective, applications of the ELISA system may play a valuable role in the future (Harrison *et al.*, 1991).

The introduction of foreign genes by genetic engineering is another innovative approach commanding much attention at the present time. In order to be successful, transformation requires a system for delivering foreign DNA into rapidly dividing cells. *Agrobacterium*-mediated transformation is often the favored system in dicotyledonous crops but regeneration of transformed tissues has proved to be problematical in legumes. Success has been achieved in transforming soybean (Hinchee *et al.*, 1988), but the technique is dependent on a large-scale tissue culture effort and regeneration of many elite lines may prove difficult (Chee *et al.*, 1989). Other techniques, designed to overcome the regeneration problem, which have been successfully employed are the infection of germinating seeds with *Agrobacterium tumefaciens* (Smith and Towsend) Conn containing a binary vector (Chee *et al.*, 1989) and the penetration of meristematic cells with DNA-coated microprojectiles (McCabe *et al.*, 1988).

In pea, Nauerby *et al.* (1991) have described a successful system using nodal thin cell layer segments as explants. Furthermore, preliminary experiments demonstrated that transformation of pea with *A. tumefaciens* was possible using this system. With further refinement, transgenic legumes may soon be a commonplace as transgenic tobacco and potato plants are today and future developments in gene construction and expression will be available to these important crops.

## Concluding remarks

Methods suitable for screening for resistance to foliar diseases in cool season food legumes have been developed for a number of major diseases. In the last decade, international organizations and national governments and institutions have augmented and coordinated their efforts aimed at improving the efficiency of research projects and other initiatives concerning breeding for resistance in the four crops taken into account in this paper. The results are encouraging, nevertheless more impetus should be given to improve the exchange of knowledge and – with due quarantine precautions – plant materials among scientists and professionals involved in breeding for resistance. Collections of germplasm should also be increased and properly characterized to find sources of resistance suitable for breeding programs.

Future achievements in screening techniques and practical use of sources of resistance also can be expected from biotechnological approaches for cool season food legumes. Nevertheless, as it appears difficult to obtain suitable levels of resistance to the most important diseases of each species, the importance of an integrated disease management program should not be underestimated.

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