

LENTIL ASCOCHYTA BLIGHT AND BREEDING FOR ITS RESISTANCE

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ABSTRACT

This paper summarises existing studies of the genetics of resistance of lentils to *Ascochyta* blight, and the genetic variation among pathogen populations with particular emphasis on the results from our programme. Breeding methods are discussed. Six pathotypes have been identified. Resistance is mainly under the control of major genes, but minor genes also play a role. Current breeding programs are based on crossing resistant cultivars with high yield cultivars and multi-location testing. Gene pyramiding, exploring slow blighting and partial resistance and the use of genes from wild relatives will be the methods used in future.

Key words: *Ascochyta* blight, lentil, resistance, breeding methods.

INTRODUCTION

Ascochyta blight of lentil was first identified in 1938 (Bondartzeva-Monteverde and Vassilievsky 1940) and is an important disease of cultivated lentil (*Lens culinaris*). It has been reported to be the major disease in many lentil production areas (Cromey *et al.* 1987; Erskine *et al.* 1994). Bondartzeva-Monteverde and Vassilievsky (1940) named *Ascochyta lentis* as the causal organism of *Ascochyta* blight of lentil. Gossen *et al.* (1986) suggested that *Ascochyta lentis* should be synonymised with *A. fabae*. They proposed two special forms: *A. fabae* f. sp. *fabae* for isolates from faba bean and *A. fabae* f. sp. *lentis* for isolates from lentil. This proposal has been widely accepted. However, Nasir and Bretag (1997) recently suggested that the causal pathogen of *Ascochyta* blight in lentil is a species that is distinct from *A. fabae*. Kaiser and Hellier (1993) found the sexual state and showed that *A. fabae* f. sp. *lentis* is heterothallic with two mating types, and that it is probably a species of *Didymella*.

Ascochyta blight considerably reduces seed quality and yield (Gossen and Morrall 1983; Cromey *et al.* 1987). Chemical, physical and cultural control is effective, but may be uneconomic (Russell *et al.* 1987; Morrall 1997). Disease resistance provides an additional means of control of the disease. This paper discusses genetics of resistance to *Ascochyta* blight, pathogenic variation and methods of breeding for resistance.

GENETIC VARIATION IN HOST AND PATHOGEN

Genetic resources of *Ascochyta* blight resistance

Genotypic differences for *Ascochyta* resistance are present in cultivated lentil. Disease symptoms on different accessions range from small flecks (resistant) to extensive lesions on both leaves and stems with the death of some plants (highly susceptible). Resistant lines and cultivars have been identified by extensive screening of germplasm collections and of breeding lines (Erskine and Bayaa 1993; Nasir and Bretag 1996, 1998). Resistance to blight has also been found in wild *Lens* species (Erskine and Bayaa 1993; Bayaa *et al.* 1994).

Inheritance of *Ascochyta* blight resistance

Several resistance genes have been proposed using segregation analysis and the genetic models suggested are summarised in Table 1. Using recombinant inbreds from two crosses between resistant (ILL 5684 and W 6 3241) and susceptible (Titore) lines, which are homozygous for the major resistance gene, Ye (2000) showed that minor genes contribute to the resistance, and may provide more durable resistance than major

genes. However, distinguishing minor gene effects requires a scoring system better than the 1 - 9 system which is frequently used (Ye 2000). The relatively small effects observed for minor genes may be due to masking effects of the major genes. Thus, minor genes may confer more resistance than has been discovered and sufficient resistance may still exist in lines containing the minor genes if major gene resistance is broken down.

Using generation-mean analysis with six basic generations of four crosses, Ye (2000) showed that genetic regulation of resistance is complicated with significant inter-loci interaction in three of four crosses. Dominant effects played an important role in all crosses. The genetic mechanisms underlying the amount of seed infection were more complicated than those for foliar resistance.

TABLE 1: Major gene(s) conferring inheritance of *Ascochyta* blight resistance in lentils.

Res. parent	Sus. parent	Organ	Inheritance model	Reference
Indian head	PI 345635	Seed	Two duplicated recessive genes	Andrahennadi 1994
Indian head	Titore	Foliar	Two additive recessive genes	Ye 2000
W6 3241 (<i>L. orientalis</i>)	Invincible	Foliar	One dominant gene	Ahmad <i>et al.</i> 1997
W6 3241	Titore	Foliar	One dominant gene	Ye 2000
W6 3261 (<i>L. orientalis</i>)	Invincible	Foliar	One dominant gene	Ahmad <i>et al.</i> 1997
W6 3261	Titore	Foliar	One dominant gene	Ahmad <i>et al.</i> 1997
W6 3261	Titore	Foliar	One partial dominant gene with large effect and one dominant gene with less effect	Ye <i>et al.</i> 2000
W6 3261	Olympic	Foliar	One dominant gene	Ahmad <i>et al.</i> 1997
W6 3192 (<i>L. eroides</i>)	Titore	Foliar	Two dominant complementary genes	Ahmad <i>et al.</i> 1997
W6 3192	Olympic	Foliar	Two dominant complementary genes	Ye 2000
W6 3222 (<i>L. odemensis</i>)	Titore	Foliar	Two dominant complementary genes	Ahmad <i>et al.</i> 1997
Laird	Eston	Seed	Single recessive gene	Tay 1989
Laird	Titore	Foliar	Single recessive gene	Ye 2000
ILL5588	Eston	Foliar	Single dominant gene	Ford <i>et al.</i> 1999
ILL5588	Eston	Seed	Single dominant gene	Andrahennadi 1994
ILL5588	Eston	Seed	Two dominant and one recessive	Vakulabharanam <i>et al.</i> 1997
ILL5588	Eston	Seed	Two dominant and one recessive	Tay 1989
ILL5588	Eston	Seed	One dominant gene and one recessive gene	Sakr 1994

Genetic variation among isolates of *Ascochyta* blight

There is only limited information on variation in *A. fabae* f. sp. *Lentis* (Ahmed and Morrall 1996; Ahmed *et al.* 1996). Nasir and Bretag (1997) identified six pathotypes out of 39 isolates based on the different reactions of the six. Most isolates were virulent to some of the cultivars, three infected all the cultivars, and five were avirulent to all the cultivars. Thus resistance is likely to be pathotype-specific.

BREEDING FOR RESISTANCE

Current breeding method

Breeding for resistance to *Ascochyta* blight in lentil has been the objective of several international and national breeding programs (Erskine *et al.* 1994). The International Agricultural Research Institute for Drought Areas (ICARDA) has coordinated multi-location trials resulting in the registration of resistant cultivars in several countries (Russell 1994; Erskine *et al.* 1996). Currently, breeding for resistance uses the same method used for other traits except that several tests for resistance are added. Combined, bulk population and pedigree selection has been used successfully in both chickpea (*Cicer arietinum*) and lentil breeding at ICARDA (Singh 1993; Muehlbauer *et al.* 1995). Breeding for *Ascochyta* blight resistance in chickpea using a modified version of this procedure has had good success (Singh 1993) and it could be used in breeding for *Ascochyta* blight in lentil. This procedure, as described by Singh (1993), consists of seven steps.

1. Lines with *Ascochyta* blight resistance to multiple pathotypes are crossed to locally adapted superior cultivars. They are then backcrossed to the adapted parent (if necessary). F₁ plants are grown in optimal conditions to produce seed.
2. F₂ plants are tested for resistance in field in epiphytotic with artificial inoculation. Selected resistant (rating 1 - 4) and partially resistant (5 - 6) plants are bulk harvested.
3. F₃ and F₄ bulks are advanced in disease-free conditions and selected for growth habit, branch number, flowering time, maturity and seed size. Selected F₄ plants are harvested individually.
4. F₅/F₆ progenies are tested for resistance. The effects of minor genes are more obvious due to several generations of recombination.
5. Selected lines are evaluated in preliminary yield trials. High yielding lines are selected.
6. Selected lines are multi-location tested. The locations should cover the target cultivation region of the new cultivars. Tests for yield and other important traits and tests for *Ascochyta* blight resistance may need to be done separately. One or two superior lines with good combinations of traits can be selected.
7. On-farm test of the selected lines and cultivar registration.

The above breeding procedure relies on multi-location testing to obtain cultivars with broad resistance. This method is justified because only resistance to the prevalent pathotypes in the target region is required by producers. However, there is no guarantee that the resistant cultivars, which are identified, will be durably resistant.

FUTURE BREEDING METHODS

Multiple resistance by gene pyramiding

When there are different pathotypes and corresponding resistance genes, one method of breeding for resistance is to combine different resistance genes into a single cultivar (gene pyramiding). This allows the cultivar to be used in more diverse environments where different pathotypes are likely to be dominant. The presence of more genes implies that pathotypes have to be virulent to all of the genes before a resistant cultivar will lose its resistance (Crute 1998). Additionally, multiple resistance genes may have additive effects.

Exploring slow blighting and partial resistance

Some cultivars/lines may only be highly resistant at early growth stages. The relatively slow development of disease has been identified in rust diseases in cereals where it has been used successfully in breeding for resistance (Wilcoxson 1981). Slow blighting has been explored in breeding for *Ascochyta* blight resistance in chickpea resulting in the release of a cultivar with partial but durable resistance (Singh 1993). Though there are no reports on the existence of slow blighting in lentils, Ye (2000) has demonstrated differences in seedling and adult resistance.

Use of wild relatives

Genes for *Ascochyta* blight resistance have been identified in wild lentil species, and it is likely that more resistance sources are present in the gene pool of wild

relatives. Therefore, the transfer of resistance genes from wild species into elite cultivars will be an important method of breeding for resistance. Viable hybrids between the cultivated and wild species are essential for the utilisation of genes from wild relatives by conventional breeding. The only wild species that can be easily intercrossed with cultivated lentil is *Lens orientalis*, the progenitor of the cultivated lentil (Ladizinsky 1993). However, viable hybrids have been obtained for all wild species crosses with cultivated lentil combinations by applying GA₃ after pollination (Ahmad *et al.* 1995). This technique may lead to an efficient method to transfer useful genes, including resistance genes, from all wild species into cultivated species. *In vitro* culture has been used in two ways to promote the utilisation of wild relatives in lentil. One is to rescue interspecific hybrids via embryo culture (Cohen *et al.* 1984). The other is to enlarge the F₁ population via micropropagation of limited F₁ materials (Ye 2000) to obtain enough hybrid material for further genetic study and breeding. Recently, a system was developed for producing genetically true-to-type hybrids, and two systems were developed to produce a large amount of breeding materials for future selection (Ye 2000).

Marker assisted introgression (MAI)

Genes conferring resistance are conventionally introgressed into an elite background by repeated backcrossing. Alternatively, instead of tracking the gene itself, a marker tightly linked to the gene can be used to trace the desirable gene's segregation in the selection process (marker-assisted introgression, MAI). MAI has several advantages.

1. Tedious and troublesome tests for resistance are not required and selection can be done in normal environments.
2. Selection can be done at an earlier stage and hence generation intervals may be reduced.
3. The transfer of both dominant and recessive genes is feasible.

The results from both theoretical study and practical application have shown that MAI reduces the necessary population size and the number of generations required for developing commercial varieties (Lee 1995; Brent and Yu 1999). Using bulk segregation analysis Ford *et al.* (1999) identified 7 RAPD markers (5 < 30 cM) linked to the resistance locus conferring *Ascochyta* blight resistance in ILL 5588. The closest flanking markers were approximately 6 and 14 cM away from the resistance locus.

Transgenic technology

Transgenic technology provides plant breeders with new tools in breeding for resistance. Its main advantages are:

1. Linkage drag, which is the inheritance of undesirable genes with desirable gene, can be avoided and consequently repeated backcrossing to the elite parent is not required and considerable time is saved.
2. Resistance genes from different sources including artificial constructs can be used. This should make plant breeders less reliant on natural variation for resistance genes.
3. By inducing or enhancing plant protective mechanisms, durable and/or multiple resistance may be achieved by a single engineering effort.

Because of its great potential in improving disease resistance, extensive studies using plant species that can be easily transformed have been conducted. As a result, several promising procedures have been demonstrated and used in economically important crops (Bushnell *et al.* 1998; Brent and Yu 1999). In view of the results achieved in other species, several authors have tried genetic transformation in lentil (Warkentin and McHughen 1992; Chowrira *et al.* 1995). These studies clearly showed that transformation could be easily achieved as confirmed by GUS assay, but regeneration of transgenic plants is difficult. Electroporation of DNA into intact nodal meristems has resulted in the production of transgenic plants (Chowrira *et al.* 1995).

Correlated selection response

Selection for resistance may result in a correlated selection response in other traits (Falconer 1989). When traits are negatively correlated, care must be taken to reduce the unfavourable response in the other traits. A recent study showed that the major gene for resistance in ILL 5588 and W6 3241 has adverse effects on seed yield/plant

(Ye 2000). Experience in breeding for resistance in other legume species indicates the effects of selection for resistance on the content of anti-nutritional factors, digestibility and maturity need to be determined.

CONCLUSION

Considerable progress has been made in the last two decades in understanding the Ascochyta blight pathogen and the resistance of its host. However, many challenges still remain both for plant pathologists and breeders. More resistance resources need to be identified to back up breeding programs. The host-pathogen interaction system needs to be carefully characterised to efficiently utilise resistance genes. New techniques for transferring genes from wild relatives and/or other sources, for shortening the breeding process, and for increasing breeding efficiency are required.

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