An Integrated Program to Improve Anthracnose Resistance in Stylosanthes - A Review

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Summary. Following the destruction of S. humilis and S. guianensis cultivars by anthracnose caused by C. gloeosporioides, an integrated research effort has been developed to achieve disease control. The improvement program has been based on a strong plant pathology input to identify fungal races, test germplasm and characterise host responses. A wide-ranging introduction and evaluation program has commercialised species new to agriculture and provided elite germplasm for different breeding strategies. Advances in molecular biology have improved knowledge of stylo defense mechanisms and offer scope for producing further cultivars with durable resistance through the use of novel genes for resistance.

Introduction

Following the accidental introduction of Stylosanthes humilis to northern Australia at the beginning of the century, commercial development of this pasture legume genus was based on S. humilis and cultivars of S. guianensis adapted to the wet tropics. With the advent of damaging strains of the fungus Colletotrichum gloeosporioides in 1973, pastures of these two species were largely destroyed by anthracnose and stylo development shifted to two species new to agriculture, S. hamata and S. scabra. About one million hectares of stylo pasture have now been sown, primarily to S. hamata cv. Verano and S. scabra cv. Seca.

Research on the reaction of cultivars and accessions of Stylosanthes to C. gloeosporioides soon identified significant genetic variation in the virulence of fungal populations (1). In order to develop cultivars with durable resistance, a range of improvement strategies has been developed based on continuing research on host/pathogen interactions and disease epidemiology. In this paper we shall briefly discuss epidemiology research and improvement strategies ranging from plant selection among naturally occurring genotypes to the development of novel disease resistance genes by genetic engineering.

Host/pathogen interactions and epidemiology of anthracnose

Australian populations of the fungus have been classified into two types, A and B, based on host range and disease symptoms (1). Different races have also been recognised within each type and race variation is being monitored in the Australian populations by regular field surveys and testing of isolates of C. gloeosporioides. Artificial inoculation techniques, based on temperature and leaf wetness requirements for disease development, are routinely used to screen new plant collections and breeding populations for disease resistance. Germling assays have been developed to improve efficiency (2) and further attempts are being made to improve the reliability of the assay for pathogenic variation in Type A. Recent applications of multivariate analysis have improved the capacity to distinguish new virulence groupings in the Type A population (3).

Effects of host diversity on disease development have been studied in small plots of mixed genotypes, in grazed paddocks and in natural populations in South America. Whereas there has been little evidence for protection of susceptible genotypes in the small plot studies (4), disease development over the long term in grazed paddocks (5) or in natural populations (6) suggests that host diversity for resistance has a stabilising effect on the evolution of pathogen virulence. Repatterning of host resistance genes through natural outcrossing may account for these different results.

Cultivar development by selection from plant collections

Broad coverage of the natural distribution range of Stylosanthes in Latin America by plant collectors has provided substantial numbers of accessions for S. scabra and related taxa,and for S. hamata , S. guianensis , S. viscosa, S. fruticosa. Following the early work which led to the commercial release of S. scabra cvv. Seca and Fitzroy (7), subsequent evaluation has documented the agronomic performance and anthracnose resistance of more than 300 accessions of S. scabra, but none of these has performed better than Seca over a wide range of tropical and subtropical locations. Seca is now the most widely sown stylo cultivar with annual seed production of 100-150 t. Evaluation of S. hamata accessions has resulted in the commercial release of cv. Amiga, which has shown better production than cv.Verano in drier and cooler environments (8). Collections of S. hamata derived from elevated tropical areas have been screened without success in a search for a cultivar with better adaptation to subtropical conditions. High yielding accessions with good field resistance to anthracnose have recently been identified as potential replacement cultivars for Verano and Amiga in case these latter cultivars suffer serious disease damage from more virulent races of the pathogen.

An unidentified species of Stylosanthes, S. sp. aff. scabra, has shown considerable promise as a new legume for clay soils in semi-arid regions of northern Australia. The potential of this species was first noticed in plants growing as a contaminant in a spaced plant trial of accessions of S. hamata. Careful searching through the collection of Stylosanthes accessions has identified 17 accessions of this new species, and a wide network of evaluation trials has been conducted with collaborators over the clay soils region of Queensland. Elite accessions of this perennial species have yielded well despite a series of very low rainfall years, and have good anthracnose resistance. Uninoculated seed nodulates only sparsely with native strains of root nodule bacteria, which are poorly effective in nitrogen fixation.

Strains collected recently in Brazil have proved effective in soil pot experimentation in the glasshouse (9). Field sowings with these strains have shown improved nodulation over uninoculated controls in the year of sowing. Current work is directed at serotyping to confirm that these nodules were formed by the introduced strains. Release of commercial cultivars of S. sp. aff. scabra is dependant on demonstration of persistence of these effective strains in the field.

Breeding S. scabra for durable resistance to anthracnose

In 1980, the appearance of anthracnose lesions on S. scabra cv Seca signalled early specialisation of Colletotrichum gloeosporioides towards this cultivar (10). By this time, susceptibility to anthracnose had already terminated the commercial life of Townsville stylo and two cultivars of S. guianensis. This ability of the fungus to attack previously resistant cultivars and accessions suggested that successful cultivars would need to have broadly based resistance for effective disease control.

The simplest approach to developing a S. scabra cultivar with durable resistance was to make a physical mixture of lines which carried different resistance genes. Putative sources of resistance were selected by glasshouse and field screening of the S. scabra collection, and genetic studies of a diallel cross among eight selected accessions identified five different resistances. Progeny selection within these hybrid populations and within a variable population of Seca produced a set of 52 lines which were evaluated individually and as composites to select three high-performing lines which incorporated four of the original resources of resistance. Lines carrying the fifth resistance were discarded because of susceptibility to another race of anthracnose which appeared during the course of the breeding program. The composite of three lines was released in 1990 as cv. Siran. Contrary to expectations, the anthracnose race which is specialised on Seca has not become highly damaging on that cultivar, so at present Seca and Siran show similar low levels of field disease and similar yields. It is recommended that graziers use a mixture of the cultivars, with the three additional sources of resistance in Siran acting as insurance against any evolution of the Seca race towards greater virulence.

To test the prospects for a second strategy for development of broadly based resistance in S. scabra, genetic studies were undertaken to determine whether lines with higher levels of resistance could be developed by intercrossing lines with partial resistance. Confirmation of significant genetic variation among lines with partial resistance prompted the execution of a recurrent selection program in which three cycles of crossing and selection were undertaken to produce segregates with levels of resistance

comparable to Seca and Siran (11). Selections are now being tested in regional trials and are also being exposed to C. gloeosporioides populations in Brazil, the centre of origin for S. scabra and its fungal pathogen.

A third approach to the development of S. scabra with broadly based resistance would be to pyramid genes with major effects on resistance into a single cultivar. Until recently, this approach has not been feasible with stylo anthracnose, because Australian races of the fungus do not carry a number of virulence genes which are needed to detect segregates with more than one major gene for resistance. With the development of DNA marker systems, we now have techniques which can be used to detect resistance genes through their genetic linkage to DNA markers. Thus indirect selection for linked DNA markers can allow us to accumulate major resistance genes into a single cultivar. This approach is described in another paper at this conference (12).

Genetic engineering for novel resistance to anthracnose

Rapid advances in the understanding of the structure and functions of genes now allow molecular biologists to transfer genes to host plants which may be remote in evolutionary terms from the donor plant species. Transfer of genes responsible for disease resistance to hosts which are unrelated to the donor species might be expected to confer durable resistance since pathogens co-evolve with their hosts and may be unable to readily adapt to novel resistances. This approach is being researched in our laboratories. With the advantage of established routines for tissue culture, rapid progress has been made in developing a transformation system to achieve expression of foreign genes in the target host species which is an essential prerequisite to genetic engineering, . Agrobacterium-mediated transformation of S. humilis has been achieved for A. tumefaciens and optimised for A. rhizogenes systems (13). S. humilis is used as a model system and further research is needed to adapt the transformation system for S. scabra and S. hamata.

Biochemical investigations of responses by S. humilis to infection with C. gloeosporioides have suggested involvement of peroxidases in plant defence mechanisms. Research has focused on the cloning of peroxidase genes and studies of their mode of action in transgenic plants. The very early induction of peroxidases in response to fungal infection (14) has led also to studies of gene regulation, since successful plant defence against pathogens requires rapid biochemical responses to prevent proliferation of the pathogen.

Whereas enhancement of plant defense by peroxidases represents one pathway to improved disease resistance, an alternative approach is to couple regulatory genes inducing rapid biochemical responses with genes coding for proteins which have strong antifungal properties. Plants from the native Australian flora are being screened for novel antifungal peptides. The peptides are being characterised, their genes cloned, and the genes inserted into constructs with appropriate regulatory elements.Their effectiveness in transformed plants will be compared with the antifungal protein isolated from radish (15).

Conclusions

Plant introduction and evaluation has been extremely effective in the commercialisation of species of Stylosanthes new to agriculture. A strong plant pathology input to the improvement program has played a central role in identification of fungal races, testing of germplasm against defined races, and characterisation of host responses. Accessions from the evaluation program have either been directly commercialised as cultivars or used as sources of resistance for the breeding program. Considerable progress in the development of cultivars with a diversity of plant resistances has been made from the use of three different plant breeding strategies. Advances in molecular biology now offer prospects for the development of durable resistance from the use of novel or enhanced resistances.

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