Recent advances in studies of anthracnose of *Stylosanthes*. III. *Stylosanthes* breeding approaches in South America

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Abstract

Evaluation of natural *Stylosanthes* germplasm leading to the release of 8 commercial cultivars began in South America in the late 1960s and early 1970s. Perceived deficiencies in existing germplasm, including anthracnose susceptibility, led to the initiation of *Stylosanthes* breeding projects as early as 1979. Four of these programs are described. While each breeding project has made demonstrable progress towards stated objectives, to date, no bred *Stylosanthes* cultivar has been commercialised in South America. The reasons for this lack of commercial success are a lack of interest or demand for forage legume technology in the region, and deficiencies in the products of *Stylosanthes* breeding projects such as lack of persistence and poor seed yield under grazing. Recent breeding and selection work in Brazil, which builds upon lessons learned from past experiences, may lead to success in the near future.

Introduction

The great majority of efforts to develop commercial *Stylosanthes* cultivars in South America have directly exploited natural germplasm (Edye and Grof 1983). These programs have led to the naming and release of 8 cultivars in 3 species: *S. guianensis* cvv. Deodora I, Deodora II, IRI-1022, Bandeirante (1983), Pucallpa (1985) and Mineirâo (1993); *S. macrocephala* cv. Pioneiro (1983); and *S. capitata* cv. Capica (1983) (Miles and Lascano 1997). All commercial cultivars have deficiencies, and those released more than 10 years ago had, at best, a limited and brief commercial existence. While the fate of cv. Mineirâo remains to be assessed, it has several recognised deficiencies which warrant a continuing search for more suitable *S. guianensis* cutivars (Miles and Lascano 1997).

In light of the perceived attributes in successful *Stylosanthes* cultivars, breeding programs involving artificial hybridisation and selection were initiated in South America as early as 1979 (Cameron *et al.* 1984). These have been justified universally, at least in part, on the basis of resistance to the anthracnose disease which is seen as the major limitation to commercial use of *Stylosanthes* in tropical America (Lenné and Calderón 1984; Lenné 1994; Trutmann 1994).

While a rigorous analysis of pathogenic diversity in native *Colletotrichum* spp. populations and their interactions with genetic diversity in the *Stylosanthes* host is only beginning, a common assumption in breeding programs has been that *Colletotrichum* population diversity in tropical America is immense (*e.g.* Lenné and Calderón 1984; Lenné 1994; Trutmann 1994). Genetic diversity for host resistance has been documented in presumably stable, natural populations of *S. guianensis* (Miles and Lenné 1984) and *S. capitata* (Lenné 1988). While genetic segregation of resistance was detected in several of the *S. guianensis* progenies evaluated, no evidence of major resistance genes was found in this material (Miles and Lenné 1984).

Deployment of major resistance genes, even if they had been found, was considered to have little chance of success. Faced with an apparently highly diverse pathogen population, the aim of all breeding programs has been to exploit genetic diversity for resistance, either through polygenic resistance in more-or-less pure lines, or by the development of heterogeneous populations as cultivars.

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Four South American *Stylosanthes* breeding projects are reviewed. Two of these were conducted at Centro Internacional de Agricultura Tropical (CIAT) with field work being carried out at the Carimagua research station in the Colombian Eastern Plains region. The *S. capitata* project initiated at CIAT was concluded in Brazil at the Cerrados Agricultural Research Center (CPAC) of the Brazilian Corporation for Agricultural Research (EMBRAPA) in Planaltina, Distrito Federal. Two more recent *Stylosanthes* breeding projects are being carried out at EMBRAPA's National Center for Beef Cattle Research (CNPGC) in Campo Grande, Mato Grosso do Sul.

S. capitata project, CIAT and EMBRAPA/CPAC

The first *Stylosanthes* breeding project, based on artificial hybrids between *S. capitata* accessions, was initiated at CIAT in 1979 (Hutton and Grof 1993). The major goal of this breeding work was to improve forage and seed yields. Stable resistance to anthracnose disease and to a stem-boring insect (*Caloptilia* sp.) were also sought. A 13-parent half-diallel was attempted originally and largely achieved (CIAT 1979). However, pedigree selection was concentrated on only one of the crosses — CIAT 1019 × CIAT 1097 (Hutton and Grof 1993). CIAT 1019 is early flowering and hence not as productive as the late flowering CIAT 1097. CIAT 1097 was considered susceptible to stem borer and anthracnose, whereas CIAT 1019 was resistant to both pests in the Colombian Llanos. The aim of the breeding work was to retain disease and insect resistance in selected lines by advancing progenies of only those plants with little or no disease symptoms. Assessment of disease (and insect) resistance was on spaced plants in field trials conducted at Carimagua. Eighty-nine F_5 lines were assessed for anthracnose reaction by artificial inoculation of seedlings in the glasshouse with "virulent anthracnose composite of Colombian and Brazilian strains" (Hutton and Grof 1993).

No information on the genetic control of the observed differences in reaction to anthracnose was available at any point in the breeding program.

Lines with high forage and seed yields were isolated from this breeding project. In addition, it was claimed (Hutton and Grof 1993) that the resistance to anthracnose (and to stem borer) of the resistant parent (CIAT 1019) was maintained over cycles of pedigree selection. However, supporting data from a rigorous evaluation of the disease or insect resistance of the resulting F_6 and F_7 lines is not presented. The authors note that anthracnose resistance can be "unstable" owing to the occurrence of new races "at any time". One highly promising line succumbed to anthracnose only after being advanced to large-scale, pre-release seed multiplication at CPAC (B. Grof, unpublished data).

S. guianensis project, CIAT

This breeding project began with the 45 hybrids from a half-diallel set of crosses among 10 *S. guianensis* accessions selected on superior survival (probably largely, but not exclusively related to anthracnose resistance) from a field agronomic evaluation of a large set of accessions conducted during 1978 and 1979 at Carimagua. When the project was initiated, the major limiting disease of *S. guianensis* was anthracnose. More recently a wilting/die-back disease, whose causal organism has not been identified unequivocally (Lenné 1990; CIAT 1995), appears to be equally as important as, or more important than anthracnose. This may be because generally higher levels of resistance to *Colletotrichum* have been achieved in the breeding populations.

The aims of the exercise were to combine high seed yield with high levels of resistance to anthracnose. The resulting F_2 populations were advanced to the F_4 generation by pedigree selection, and selected families have been propagated in bulk.

All selections were based upon observations of field-grown plants under conditions of natural inoculation at Carimagua. No artificial inoculation, either in the field or in the glasshouse, was used.

The program was based upon a supposition of multigene, "quantitative" resistance to anthracnose. No evidence of segregation of major disease-resistance genes has been observed in the materials included in the breeding program (Miles and Lenné 1984; CIAT 1985).

Pedigree selection successfully isolated 2 "lines" (bulk advanced from the F_4 generation) which are disease-resistant. While they produce

2–3 times more seed than the most disease-resistant germplasm accessions (CIAT 1991; 1995), yield is still only of the order of 100–150 kg/ha at best, with insecticide control of bud worm (*Stegasta bosquella*) and hand harvest. Both lines are of the var. *pauciflora* type, which, in general, is more disease-resistant than the earlier flowering var. *vulgaris*.

One cycle of recurrent selection was attempted, but was ineffective in improving either disease resistance or seed yield over that of selections made from the initial cycle of pedigree selection (CIAT 1991).

Starting with the same 45 original diallel hybrids, a second selection program was initiated by submitting a mixture of F_2 seed to a bulk generation advance scheme (Casali and Tigchelaar 1975; Simmonds 1979). Six sub-populations, differentiated by bulk seed harvest date, have been advanced over 13 generations under severe natural anthracnose pressure at Carimagua (CIAT n.d.; J.W. Miles, unpublished data). A rapid shift in flowering time of the sub-populations, associated with bulk seed harvest date, has been documented (CIAT 1985). There has been a significant increase in seed yield over cycles of selection. In spite of large year-to-year fluctuations, yield of hand-harvested seed was correlated positively with cycle number for each population (r^2 between 0.53 and 0.67; $P < 0.01$). Whether this response is owing to enhancement of genetic seed yield potential, resistance to bud worm, or some other factor(s) such as seed retention, has not been determined.

In small-plot trials, 2 late-flowering bulks are highly persistent at Carimagua and Caquetá in Colombia, and Planaltina and Campo Grande in Brazil (S. Kelemu, personal communication). Presumably, these are highly resistant to prevalent *Colletotrichum* races, as well as other diseases and insects.

A rigorous assessment of the effect of bulk advance on anthracnose resistance and other attributes is in progress.

S. guianensis project, EMBRAPA/CNPGC

This on-going project addresses the deficiencies recognized in cv. Mineirâo by seeking anthracnose-resistant genotypes that flower and mature early, produce commercially acceptable seed yields, are tolerant of drought, and retain green leaves during the dry season. It is based upon the bulk populations developed at Carimagua, which appear to offer the best opportunity for improved *S. guianensis* cultivars.

Selection was carried out over 4 generations. Two generations were grown in Campo Grande, Mato Grosso do Sul, Brazil, and an additional 2 generations at 2 different sites in the Philippines, under conditions of 2000 mm or 4000 mm annual rainfall. Anthracnose severity was less in the Philippines than in Brazil. Foliar blight, caused by *Rhizoctonia* sp., was, however, more severe at the high rainfall site in the Philippines than in Brazil (B. Grof, unpublished data).

Four bulk populations have been formed by physically mixing seed from single-plant selections of similar phenology, seed yield, and morphological characters. Early- and mid-flowering selections evaluated in 3 separate experiments at Campo Grande matured seed from the first week of June (shortening photoperiod) and prior to the period of severe moisture stress normally experienced during July and August. In the same experiments, seed of cv. Mineirâo was harvested on 28 August, well into the dry season and approximately 2 months after seed ripening on the early- and mid-season hybrid selections (B. Grof, unpublished data).

Twenty selections from this program were evaluated for seed production potential on a dark red Latosol (Oxisol) at Campo Grande in an experiment containing cv. Mineirâo and a local *S. guianensis* accession (GC348). Seed yield of the 20 hybrid-derived selections ranged between 10 and 200 kg/ha. Checks yielded 10.4 kg (Mineirâo) or 19 kg (GC348) (B. Grof, unpublished data).

Basal leaf area remaining after defoliation, an attribute associated with rate of regrowth, was higher for 5 of 22 hybrid selections than for Mineirâo (B. Grof, unpublished data).

Stability of disease resistance of superior selections and adaptation to a wider range of conditions will be assessed in multi-locational trials and grazing trials to determine animal production to identify a superior cultivar for the acid-soil savannas.

S. capitata-S. macrocephala project, EMBRAPA/CNPGC

A bulk composite population has been developed from a mixture of *S. capitata* and *S. macrocephala* germplasm sampled in 1990 near an abandoned forage species agronomic evaluation trial site on an acid (pH 5), infertile, quartz sand. *S. capitata* and *S. macrocephala* were the only 2 of 21 legume species, which had survived under conditions of uncontrolled, continuous, heavy grazing. All *S. capitata* accessions included in the original agronomic trial were of Brazilian origin. It is assumed that at least some natural outcrossing among accessions occurred (Miles 1983), and that subsequent natural selection over the years following the abandonment of the agronomic trial positively affected the genetic structure of the surviving population, for both anthracnose resistance and persistence under heavy grazing.

Subsequently, 8 productive, anthracnose-resistant *S. capitata* accessions of Venezuelan origin were introgressed into the original composite population by natural outcrossing. The resulting population, which included natural hybrids, was advanced through 4 generations of bulk seed production to improve its stability, to synchronise harvest maturity, and primarily to facilitate natural intercrossing among *S. capitata* genotypes. Seed of 4 selected *S. macrocephala* accessions was added to the final composite. The composite contains approximately 20% by weight of *S. macrocephala* seed, which includes *S. macrocephala* from the originally sampled population as well as the 4 accessions added subsequently.

The bulk composite population has shown resistance to anthracnose in field trials conducted over the years since its synthesis in 1990. This resistance is presumably owing to the inherent genetic resistance of component genotypes as well as to inter- and intraspecific genetic heterogeneity.

Conclusions

Looking back

While a substantial effort has been invested in *Stylosanthes* breeding projects in South America, no bred cultivar has been released. This is not owing to a failure of conventional plant breeding procedures to effect the desired genetic changes; the stated objectives of the different breeding projects generally were achieved. The reasons for the lack of commercial release and adoption need to be sought elsewhere. Part of the lack of commercial adoption may be due to a poor choice of species in which to invest a plant-breeding effort, or the concentration of breeding objectives on ill-chosen or irrelevant attributes.

However, it seems more likely that the lack of commercial exploitation of the products of *Stylosanthes* breeding projects is related, in large part, to a general reluctance of South American ranchers to adopt legume-based pasture technology, at least that which has been developed to date. Only limited commercial success can be claimed to date for forage legume technology developed in tropical America from a substantial investment in germplasm collection and selection in a number of forage legume genera, including *Stylosanthes*, where at least 8 commercial cultivars have been released in 3 South American countries. Recent developments in the incipient commercialisation of *Arachis pintoi* may be a possible exception.

Observations over many years of field evaluations of *Stylosanthes* germplasm and breeding populations suggest that "breakdown" of anthracnose resistance has not occurred, at least for *S. guianensis*. Relative ranking of resistance is observed to be essentially constant both spatially and temporally. CIAT 184 is generally more resistant than the Australian cultivars, while the var. *pauciflora* CIAT 10136 is always among the least affected host genotypes. We would, of course, like to believe that the failure of resistance to "break down" is owing to inherent genetic durability of the resistance selected. However, observed "durability" may simply be due to lack of sufficient selection pressure on the pathogen since large areas of resistant *S. guianensis* have never been planted.

The reasons for the failure of several *S. capitata* lines to withstand anthracnose attack at the stage of large-scale, "pre-release" seed multiplication are not entirely clear, but may be the result of a shift in pathogen race population composition. Other possible causes include year-to-year weather differences or differences between the small-plot agronomic trial and the seed multiplication field. Current studies of temporal and spatial changes in *Colletotrichum* population structure should shed valuable light on the potential for resistance "breakdown" in future cultivars.

Persistence of *S. guianensis* in grazed, grass-legume pastures has been poor, with the legume component generally lasting only a year or 2. However, the cause of the poor persistence does not seem to be entirely anthracnose, as legume decline occurs even in areas, such as Colombia's Amazonian piedmont, where lines known to be very susceptible are only slightly affected by the disease in ungrazed agronomic trials. An experiment at Carimagua sought to assess one possible component of *Stylosanthes* persistence in grazed pastures: seedling survival. Results suggest that 2 *S. guianensis* bred lines differ in survival and that this difference is manifest regardless of the associated grass (CIAT 1995). Much remains to be learned about the dynamics of grass-*Stylosanthes* pasture persistence and productivity if relevant selection criteria and breeding objectives are to be formulated reliably.

Looking forward

Most of the bases for successful *Stylosanthes* breeding programs exist. Excellent collections of *Stylosanthes* germplasm are held at various institutions, such as EMBRAPA, CSIRO and CIAT. Previous *Stylosanthes* breeding work has produced a substantial body of scientific literature on reproductive biology, genetics and breeding methods for a genus that was practically unknown as a cultivated plant 30 years ago. It has been demonstrated in past breeding programs that directed genetic modification of *Stylosanthes* by conventional breeding techniques presents no particular difficulties. Current efforts to understand pathogenic variability of *Colletotrichum* in tropical America are expanding with the use of new molecular technologies. These studies will put future attempts at genetic management of host resistance on a much sounder footing.

The critical missing element appears to be a clear definition of the factors which constrain the commercial usefulness of *Stylosanthes* in South America. Plant breeding programs can be designed much more rationally and objectives more clearly formulated when a cultivar is grown on a large area and its deficiencies become more obvious. Recent advances in the development of *Stylosanthes* germplasm in Brazil give us hope of significant commercial exploitation and adoption in the near future.

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