

Stylosanthes research in India: Prospects and challenges ahead

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Of many species of Stylosanthes introduced in India since early seventies, the recently added species, S. seabrana shows great promise in terms of seed vigour, establishment as well as nutritional parameters. High regeneration potential further makes this species superior to S. hamata which have been naturalized in India. Molecular marker analysis indicated low level of polymorphism in available accessions of S. seabrana. However, it harbours good seedling vigour and frost tolerance. Being one of the diploid progenitors, it provides an opportunity to artificially synthesize tetraploid S. scabra, a hardy species, using its pre-selected accession along with S. viscosa, a second progenitor. The species is better in checking soil erosion as it covers soil maximally. We take a look at the prospects of Stylosanthes improvement both for biotic and abiotic stresses initially by screening under different situations, through QTL analysis for disease and then by conventional approach using carbon isotope discrimination function analysis and water use efficiency for drought tolerance.

Keywords: Anthracnose, germplasm, research prospects, *Stylosanthes* sp., wastelands.

IN India, *Stylosanthes* is regarded as the most important range legume for the humid to semi-arid tropics. It is extensively utilized in pastoral, agropastoral and silvipastoral systems for animal production. Due to its ability to restore soil fertility, improve soil physical properties and provide permanent vegetation cover, it plays a vital role in the development of wastelands in India. It is also considered a nurse crop in plantation on degraded lands. Therefore, *Stylosanthes* has been identified as an important component of national programmes sponsored by the National Afforestation and Eco-Development Board, National Wastelands Development Board and State Departments of Agriculture, Animal Husbandry and Forestry and the Indian Grassland and Fodder Research Institute (IGFRI), Jhansi. The most specific problems associated with *Stylosanthes* are the limited variations of available germplasm and the susceptibility to anthracnose disease caused by the fungus *Colletotrichum gloeosporioides*. The disease was first recorded at Deodoro, Brazil in 1937 on *S. humilis*¹ and is now widespread in all countries where this legume is grown. The reliance on susceptible to moderately resistant cultivars poses a potentially serious threat from anthracnose. Identification and development of germplasm with increased resistance and wider geographical adaptations are important research priorities.

In the past, mainly five species of *Stylosanthes* (*S. hamata*, *S. scabra*, *S. humilis*, *S. viscosa* and *S. guianensis*) have been introduced primarily from Australia and evalu-

ated at different sites in India²⁻⁶. This was in addition to the native perennial *S. fruticosa* Alston, which is widely distributed throughout the southern peninsular regions⁷. Though the first record of *Stylosanthes* in transition dates back to early forties⁸, regional evaluations of introduced germplasm in India started in the 1970s at various regional and main research centres depicted a wide range of variability in plant height, number of main branches and days to flowering. In 1976, eight cultivars of *Stylosanthes* species evaluated under arid conditions of Rajasthan demonstrated low germination as well as low seed yield. However, *S. hamata* (CPI 38842) and *S. scabra* (CPI 40205) were superior over other accessions. During this period, evaluation in the eastern part of the country, i.e. at Ranchi (altitude 625 m) and Kalyani (altitude 975 m), both at 25°N with 1300–1500 mm annual rainfall respectively, revealed the establishment of *S. guianensis* (perennial) and *S. hamata* (annual) species⁴. Under dryland condition, some lines of *S. hamata* (CPI 55830) gave outstanding performance⁹. In addition, one or more accessions of *Stylosanthes* have been included in several regional trials by the All-India Coordinated Research Project on Forage Crops (AICRPFC) and during the same time, despite the major initiatives taken by IGFRI¹⁰, no cultivar has yet been formally released in India. Nevertheless, two Australian cultivars, *S. scabra* cv. Fitzroy and *S. hamata* cv. Verano are widely grown in India. Due to vulnerability of these two cultivars towards anthracnose, commercial seed production of these cultivars suffered seriously and farmers did not grow this species later¹¹. The recent upsurge and evaluation of a new species of *Stylosanthes*, namely *S. seabrana* under an ACIAR project at different

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centres as well as at IGRI indicated promise of this species for wider applications (Figure 1).

In the early 1970s, it was estimated that approximately 2 million ha colonized by sown and naturalized Townsville stylo (TS, *S. humilis*) in Australia was destroyed by the major disease of stylo, anthracnose, caused by *Colletotrichum gloeosporioides* and it is now of little significance in stylo pastures¹². The disappearance of TS was a devastating event in the historical development of stylo pastures in Australia. This led to the identification and characterization of suitable parents and selective breeding for the development of durable resistant cultivars. Currently, nearly one million ha of grazing land in Australia is under *Stylosanthes*¹³. Though a reasonable number of cultivars in different countries (Australia, Colombia, Brazil, China and USA) has been released, only a limited number of lines has so far been identified in India. When *S. scabra* cv. Seca¹⁴ was released in Australia in 1976, it was highly resistant to all known strains of *C. gloeosporioides*¹⁵ and by 1982 a new race virulent on Seca was identified¹⁶. The latest two cultivars of *S. seabrana*, Primar and Unica, have started showing disease as new races have evolved within five years of their release¹⁷. Since its introduction in India in 1999, at least four pathogen race clusters were observed to be aggressive to highly aggressive on the *S. seabrana* accession 105546B in India¹¹. However, widespread damage has not been recorded at field sites in India on this species¹¹. Thus careful and continued monitoring of the pathogen population is necessary to detect early signs of severe damage following the introduction of new cultivars.

Two genetically distinct biotypes of *C. gloeosporioides* infecting *Stylosanthes* spp. have been identified in Australia, which have clonally descended from two separate introductions^{15,18}. Biotype A causes disease on most of the species, whereas biotype B manifests as a blight symptom on leaf and stem of *S. guianensis*. Apart from these



Figure 1. A stand of *Stylosanthes seabrana*.

two biotypes, there are strains in South America and Africa that do not clearly belong to either biotype¹⁹. Despite the relatively short history of *Stylosanthes* in India, the anthracnose pathogen has diversified and needs regular monitoring of its population to detect early appearance of new races, especially when a new species, i.e. *S. seabrana* which is close to native *S. fruticosa*²⁰ has been introduced. Various forms of resistance to stylo anthracnose have been reported from breeding programmes conducted at CSIRO, Australia²¹ and in South America by CIAT²². Oligomeric resistance has been detected in some accessions of *S. guianensis*²³ and *S. scabra*²⁴; race specific is found in *S. viscosa* accession CPI 33941 (ref. 24); quantitative resistance believed to be due to more than one gene has been observed in some accessions of *S. guianensis*²⁵, *S. scabra*²⁶ and in *S. hamata*²⁷. Results employing eight parents suggested that the resistance is controlled by several genes and is quantitatively inherited²⁸.

The work on stylo in India was largely restricted to evaluation at different agro-ecological zones until it was emphasized through a collaborative Australian Centre for International Agricultural Research (ACIAR), Australia project where development of genetic linkage map and QTL identification for anthracnose resistance and epidemiology of disease at a larger scale were attempted. Part of the progress of the work has been presented in the form of a compendium (www.aciar.gov.au). Apart from India, Australia, China, Colombia and Brazil participated in the project. We present here an update on stylo research *vis-à-vis* a close look on the performance of newly introduced species *S. seabrana* and its possible role in various utilization systems.

Establishment and evaluation: Promise as nutritious fodder legume

Stylosanthes is currently used as cut and carry feed and for grazing cattle, buffalo, sheep, goats and pigs; wasteland reclamation and as a component of dryland mixed cropping⁶. In major areas where stylo is sown, cultivars Seca, Fitzroy and Verano have been most popular. The introduction of new species, *S. seabrana* in 1999 under the ACIAR–ICAR project has shown great promise in terms of both establishment and ability to perform better over other species in heavy soil. Research at IGRI demonstrated that once the crop is established, seeding is not required for another three years due to its regeneration ability. A managed and controlled access to grazing and fodder harvesting areas restored with *Stylosanthes* has allowed sustainable utilization of this legume to restore fertility, productivity and sustainability of the marginal lands. Intercropping with cereals and plantation crops, ley farming to add 80–100 kg N to the soil, leaf meal and agroforestry systems are among the other significant areas of potential utilization of *Stylosanthes* in India. As a

component of agroforestry and silvipastoral systems, *Stylosanthes* can play a significant role in the stabilization and sustainable utilization of degraded lands. *S. fruticosa* (wild lucerne), naturally occurring in dry localities of Orissa, Andhra Pradesh, Karnataka, Tamil Nadu and Kerala at altitudes up to 900 m and on the coastal areas, has been emphasized for reclamation of rangelands⁶.

Areas like improving natural grazing lands, rejuvenating rainfed crops and livestock production system have been greatly influenced by the use of *Stylosanthes* species. It supports the livelihood of >70% of the nomadic and rural population, where livestock is dependent on these resources²⁹. It is also used as a pioneering colonizer to stabilize degraded and problem soils and wastelands in extensive areas spanning most of the Indian states. Both government and non-government sectors have actively participated in this work, often following participatory approaches. There are reports also that in India it has been emphasized to be used in the development and establishment of watershed and wastelands, including soil conservation and land stabilization practices²⁹⁻³¹. However, the difficulty associated with the development of wastelands, which are also common grazing lands, is the ability to control grazing. Consequently, many government-run programmes have been unsuccessful in maintaining a productive grazing resource, where land has been fenced-off for two to three years. Overexploitation of such community resources once they are opened up for grazing, has been the principal reason for the failure of many such schemes. Nevertheless, there are many case studies where success has been achieved and reported²⁹⁻³⁵. In addition to improving natural rangelands, *Stylosanthes* has been also emphasized to be used to conserve *in situ* moisture, as a nurse crop, intercropping with rainfed crops, revegetation of mine spoil sites and in large areas where there are wind mills in the southern part of the country³⁶.

The expansion of the Joint Forest Management (JFM) programme to about 28 million ha of degraded forests in the coming decade provides great scope for using stylo as a nurse crop to benefit the land and livestock²⁹. The recently added species *S. seabrana* with increased adaptation increases the potential areas and prospects for the use of stylo in India.

Molecular analysis and its prospects

Breeding programmes for improvement of stylo were initiated long back by CSIRO, Australia; CIAT, Colombia and EMBRAPA, Brazil in addition to their germplasm enrichment activities^{21,22}. Only two released cultivars, i.e. cv Siran³⁷ and cv. Estilosantes Campo Grande³⁸ have been bred to date. Classical taxonomic treatments of *Stylosanthes* have been mainly based on some aspects of the floral and fruit morphology³⁹. In 1838, Vogel established the main division of the genus into the sections *Styposanthes*

and *Stylosanthes*⁴⁰, based on the presence of rudimentary secondary floral axis and two inner bracteoles in the former and no such axis and only one inner bracteole in the latter^{39,41}. Phylogenetic studies, including inter- and intra-specific genetic diversity in *Stylosanthes* have been carried out⁴²⁻⁵⁷ utilizing both biochemical and molecular marker techniques.

Most species of *Stylosanthes* are diploid ($2n = 20$) but polyploid species ($2n = 40$ and $2n = 60$) also exist. The latter are exclusively allopolyploid. Sect. *Styposanthes* contains both diploid and polyploid species, while species in Sect. *Stylosanthes* are exclusively diploid. In most cases, molecular markers have proved that a tetraploid ($4n$) is a combination of a diploid ($2n$) species from Sect. *Stylosanthes* and a diploid ($2n$) species from Sect. *Styposanthes*^{43,58}. We have worked out *S. angustifolia* as the third putative diploid progenitor of the only one hexaploid species, i.e. *S. erecta* so far reported, utilizing STS and RFLP markers⁵⁹. With reference to species characterization as well as diversity analysis, different workers have largely used STS and RAPD markers^{42,43,47,48,50,54,60-62}. However, low specificity shown by STS markers reduces its value when used for species identification⁶³, as in many cases STS behaved similarly to most restriction fragment length polymorphism (RFLP) probes, i.e. generated products from a wide range of closely related species. However, the same property at the same time makes *Stylosanthes* STS more attractive for work, such as inter-specific gene transfer and comparative mapping experiments. The conservation between *Stylosanthes* species seems to be close enough to allow most PCR primers designed for use in one species to be used in another. This property of STS was employed by us in deciphering the progenitors of *S. erecta*. It is possible to search for the genetic origin of polyploids in *Stylosanthes* by STS analysis and combining it with cytogenetic analysis. RFLP, STS and RAPD markers elucidated the genetic origins of many natural tetraploids and hexaploids, which further helped in identification of the constituent genomes in allo-tetraploids. Recently, we have reported the number of protein bands (10-13) shared by diploid progenitors in allotetraploid species⁶⁴. Molecular analysis provides a useful basis upon which to assess suitability of potential parental lines in selective breeding programmes and should allow the transfer of useful genes such as those conferring disease resistance from diploid progenitors to commercially important tetraploids species such as *S. scabra* and *S. hamata*⁶⁵.

Liu *et al.*⁴³ and Ma *et al.*⁵⁹ identified ten basal genomes, named A to J in stylo. The genus *Stylosanthes* has a monophyletic origin^{44,55} and is reported to be closely related to *Arachis*⁶⁶, with the *S. guianensis* species complex as the most ancient group. Genomes A and C have been identified as natural donor for tetraploid with AABB and AACC genome respectively. As more than one species have the same ancestral genomes, it seems most likely that more than one hybridization event has taken place⁵⁴.

Because of the disjunction of the natural distribution of several *Stylosanthes* species, independent evolution of similar tetraploid combination has also been suggested⁶⁷. This has led to restriction of the *Stylosanthes* species to isolated areas and only few species are widely distributed. This scenario proposes utilization of species having good quality attributes to artificially synthesize the species possessing similar level of genome structure for easy and stable survival.

Attempts have been made to increase digestibility and resistance to anthracnose through genetic engineering approach by inserting anti-fungal protein in stylo⁶⁸. Lignin is one of the major components of fibre in tropical forages and its high level has been found to be associated with poor digestibility and low nutritional value. Efforts have been made in suppressing one of the enzymes of lignin biosynthesis in *S. humilis* and a difference in concentration of lignin levels has been reported⁶⁸.

Low level of species divergence in *Stylosanthes* was evident as STS has indicated low level of genome specificity. This along with low polymorphism as indicated by RAPD and other marker systems makes it difficult in developing a good linkage map for this crop. Hitherto two mapping populations, viz. *S. scabra* 93116 × *S. scabra* cv Fitzroy and *S. scabra* cv Seca × *S. fruticosa* were used to develop a map along with phenotypic data for anthracnose disease to identify QTLs. With the first population, a map was constructed using 151 RAPD markers^{69,70}. Six QTLs linked with anthracnose resistance have been identified at different levels of LOD score located on six chromosomes. The markers located along the QTLs are now the important source to be utilized to screen the breeding materials/accessions with the objective of selecting genotypes containing several major genes conditioning anthracnose resistance. With the second population also a tentative map has been developed^{71,72}. The same population is being used to score more markers so to place enough number of markers to get a better map. One of the major hurdles in achieving good genetic map is the low polymorphism observed between the parents as in case of the cross of *S. scabra* cv Fitzroy and *S. hamata* cv Verano with only 44 and 47% RAPD and RFLP polymorphism respectively, and only 12 and 16% RAPD and RFLP respectively, in the diploid cross of *S. viscosa* 33941 and *S. viscosa* 34904 (ref. 73). A partial genetic map based on a cross between *S. scabra* × *S. hamata* reported earlier revealed several putative regions conferring anthracnose resistance⁷⁴. Some of the RAPD markers located in these QTL regions have been successfully converted into STS markers.

Perennial stylo like *S. scabra* has various adaptive strategies to cope with severe water deficits⁷⁵. They exhibit substantial osmotic adjustment that contributes to the maintenance of turgor at low tissue water potentials; and their tissue is insensitive to considerable desiccation⁷⁶. They are also able to root deeply and extract water at water potentials considerably lower than the -1.5 MPa normally

associated with wilting point. This makes the perennial species of stylo survive in dry seasons in the tropics. Variation in drought tolerance observed on the basis of different biochemical attributes in different *Stylosanthes* accessions indicated some of the scabra lines, especially RRR (Rate Reducing Resistance) as a good source of materials for drier regions⁷⁷. Additionally, transpiration efficiency (TE) or water use efficiency and carbon isotope discrimination (Δ) study indicated negative relationship between TE and Δ ($r = -0.71$). In this case also, perennial *S. scabra* cv Seca maintains the highest TE under both control and stress treatments⁷⁸. Using *S. scabra* 93116 × *S. scabra* cv Fitzroy F₂ population, the causal nature of different drought-responsive traits as well as relationship between TE and Δ were confirmed through QTL analysis⁷⁰. Genetic linkage map developed with intra-specific population (*S. scabra* cv Fitzroy × CPI 93116) used with traits for QTL a significant correlation of Δ was observed with TE and biomass production. Most of the QTLs for TE and Δ were observed on linkage groups 5 and 11. Similarly, QTLs for specific leaf area (SLA), transpiration and biomass productivity traits were clustered on linkage groups 13 and 14. The causal nature of relationship between TE and Δ was observed at the coincident markers between TE and Δ , high alleles of TE were associated with low alleles of Δ (ref. 70).

Targetted approach for improvement: Future outlook

Research on this crop has gained momentum; however, improvement of stylo utilizing molecular tools has not yet made any dent primarily due to non-availability of linkage map and not enough co-dominant markers *vis-à-vis* low level of polymorphism exhibited with different sets of markers. Unlike SSR, which targets highly variable sequences of a genome, STS is non-selective and amplifies random sequences with respect to the level of polymorphism. Further compared with RFLP, STS generates much shorter fragments and therefore would have less chance of detecting polymorphism caused by insertion/deletion events. Therefore, there is a need to develop enough SSR markers to be used for linkage map development and also in variation study to identify lines and for allele mining for abiotic stress adaptation, as *S. scabra* has been reported as a hardy species.

Testing and evaluation of wide germplasm, especially newly introduced lines on acid and saline soil which contribute major part of the soil of this country, has to be strengthened. The preliminary results carried out at IGFR indicated better adaptation of *S. hamata* and *S. seabrana* lines over other species in salinity. Surprisingly, the hardy and shrubby stylo (*S. scabra*) did not show much promise for such areas. However, the finding that *S. seabrana* is one of the progenitors of *S. scabra*

provided a possibility to artificially synthesize *S. scabra* using pre-selected *S. viscosa* and *S. seabrana* accessions^{79,80}. The nutritional parameters, especially the level of essential amino acids have been reported high in *S. seabrana* over other species⁸¹. These artificial *S. scabra* genotypes could be used directly, or more likely be used in breeding programmes.

The importance of this crop can be truly assessed when gradual decline in cultivated lands is taken into account. The scenario of limited grasslands in India is alarming which further deteriorated due to ecological retrogression and increased soil erosion. Potentially, the vast areas under wastelands, degraded forests, etc. can be transformed by introducing pasture and forage species in agropastoral, agroforestry and hortipastoral systems. In some of these areas, *Stylosanthes* especially the newly introduced species (*S. seabrana* and RRR lines of *S. scabra*) have the potential to become the dominant forage legume in improving soil fertility, in restoring degraded lands through biological nitrogen fixation and in providing a feed base for livestock production. Greater effort should be driven towards the seed industry which will play a major role in any future expansion of *Stylosanthes* utilization schemes.

Lastly, the major clientele of *Stylosanthes* seed in India are still government departments. Further research and transfer of technology must be aimed at the private sector as well as NGO-linked utilization schemes. There is a need to improve the contribution of *Stylosanthes* to small and marginal land-holder production systems and also its incorporation into mixed farming, which hitherto has been highly effective in improving the living standard of such peasants. Great opportunity lies ahead in research and development to suggest its utilization for cut and carry, *in situ* grazing, leaf meal production under intensive and extensive systems of livestock rearing and livelihood. Each species of *Stylosanthes* has its role in different agro-ecozones for its optimum production and use. In the larger area of the Central Indian plateau region and rainfed dry zones, *S. seabrana* has a great potential followed by *S. scabra*. The availability of newly added germplasm from ILRI, Ethiopia as well as under the recently concluded ACIAR project, opens a gate to emphasize on participatory research approach which offers a new paradigm for successful adoption of *Stylosanthes* in diverse production systems, including rehabilitation of degraded lands.

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