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Review Paper

# Diversity among *Stylosanthes* species: Habitat, edaphic and agro-climatic affinities leading to cultivar development

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***Abstract:*** *Since the introduction of Stylosanthes, a range legume, in India in early seventies extensive efforts have been made for its evaluation and adaptation. However, limited germplasm and narrow genetic base were major impediments in its wider adaptations. Of late, introduction of several new improved germplasm including newly identified species, cultivars and bred materials from Australia, Colombia, Brazil and Ethiopia and their evaluation at selected centers under different agro-climatic conditions improved the existing scenario as many lines including S. scabra RRR as well as newly introduced species S. seabrana has shown great promise for diverse agro-climatic zones. Because of concerted efforts which was largely generated from the recently concluded Austrialian Centre for International Agricultural Research (ACIAR) stylo project and background study during the period of early eighties Stylosanthes has been considered as the most important tropical legume which not only improve the soil fertility but also provide nutritive forage. Two species namely S. hamata and S. scabra largely contribute to the supply of forages for cattle, buffalo, goats and sheep. As a nitrogen fixing legume, the plant helps replenish soil nutrients when used in ley farming, mixed and inter-cropping systems. The scenario has largely changed due to the better performance of newly introduced S. seabrana species which possessed high seedling vigour, high nutritional parameters and better adaptation under rain-fed situations in heavy clay and cracking soil types. It provides good foliage and being erect and low sticky in nature showed compatibility for mixed cropping. Results also demonstrated reasonable yield in first year by all four major species which ultimately geared up in second year of growth. This was stable in S. hamata and S. viscosa for another two years whereas other two species namely S. scabra and S. seabrana indicated enhanced yield in consecutive years.*

***Key words:*** *Agro-climatic condition, Diversity, DNA markers, Forage, Rain-fed environment, Stylosanthes species*
*PDF of full length paper is available online*

## International scenario

The *Stylosanthes* genus is a diverse group of species with a wide distribution in tropical, subtropical and temperate regions of the America, tropical Africa and south east Asia (Williams and Gardner, 1984). Geographic information systems (GIS) has tremendously helped in identifying regions with similar ecological profile to target new exploration and collection sites for new useful genetic resources (Burt *et al*., 1980). Sawkins (1999) studied four species using GIS and showed that *S. guinensis* has the widest geographic distribution as compared to *S. viscosa*, *S. humilis* and *S. capitata*. The diversity of different *Stylosanthes* species has been characterized through GIS mapping approaches (Sawkins, 1999) using germplasm accessions that are considered to adequately represent the known distribution of major species (Schultze-Kraft and Keller-Grein, 1994). Vander Stappen *et al*. (2000) investigated the diversity of Mexican accessions of *S. humilis* and concluded that Mexico may be rich in unique diversity that could be used for the conservation and utilization of *S. humilis* germplasm. Promising *S. scabra* introduction from northeastern Brazil emphasizes further collections of such lines from these regions (Burt *et al*., 1979). Four major species of stylo namely *S. scabra*, *S. hamata*, *S. humilis* and *S. guianensis* are widely used as forage legumes in tropical regions (Kazan *et al*., 1993). Over 600 accessions of *S. scabra* have been accumulated world wide. More than 90% of them were collected in Brazil, with Colombia and Venezuela each contributing about another four percent of the collections (Schultze Kraft *et al*., 1984). Clustering analysis based on morphological and agronomical (M-A) characteristics grouped *S. scabra* accessions into four varietal types, including continental, Brazilian Coastal, cf. *scabra* and aff. *scabra* types (Maass, 1989). Relationship between genetic diversity and geographical distribution of different species of stylo would lead in facilitating a systematic exploitation of the existing collections and identifying regions with high genetic diversity where there are good opportunities to collect novel genotypes. Recently concluded ACIAR stylo project where Australia, India, Brazil, Colombia and China participated, has highlighted the importance of regional evaluation of stylo germplasm in exploring new ecological niches that may be suitable for the more productive and anthracnose resistant germplasm. Being most damaging disease of stylo, a good collection of germplasm and their evaluation have been made against anthracnose (Cameron and Trevorrow, 1988). Nutritional evaluation, particularly the amino acid profile revealed that though *S. scabra* cv Seca is anthracnose resistant cultivar, it is poor in quality (low essential amino acids) in comparison to *S. guianensis* cultivars (Guodao *et al*., 2004). In *S. guianensis* the expression of SgNCED1 (9-cis-epoxycarotenoid dioxygenase) has been observed to be induced in both leaves and roots. Both dehydration and salt stress induced the expression of SgNCED1 strongly and rapidly (Yang and Guo, 2007). Results based on modeling using the available Indian data pertaining to *S. hamata*, *S. scabra* and *S. guianensis* indicated widespread suitability of soils for *S. hamata* and *S. guianensis*. Due to requirement of high rainfall *S. guianensis* is likely to be restricted to coastal regions of Southern India. *S. scabra* was shown to be moderately suitable over a wide area, but less suitable in central and western districts (White *et al*., 2001). Both transpiration efficiency and carbon isotope discrimination analysis in stylo indicated the importance of these characters along with specific leaf area in identifying the drought tolerant lines (Thumma *et al*., 1998; Chandra and Bhatt, 2008). The causal nature of relationships among these characters has been also substantiated using quantitative trait loci (QTLs) approach (Thumma *et al.,* 2001). Major work on disease epidemiology and diversity of the causing pathogens (*Colletotrichum gloeosporioides)* has been attempted under Austrialian Centre for International Agricultural Research (ACIAR) stylo project where apart from India, Australia, China, Brazil and Colombia have participated. Considerable pathogenic and genetic diversity has been reported in the pathogen population from centre of both diversity and utilization (Weeds *et al.,* 2003).

### National scenario

Since the introduction of *Stylosanthes* in India, Indian Grassland and Fodder Research Institute (IGFRI), Jhansi provided a strong platform for the evaluation of five species namely *S. scabra, S. hamata, S. viscosa, S. humilis* and *S. guianensis* (Chandra *et al*., 2006). Genotypes of *S. scabra* was more tolerant to drought over lines of other species as evidenced by high leaf thickness, more proline accumulation, contents of malondialdehyde (MDA), sugars, starch and chlorophyll and low carbon isotope discrimination (CID) values (Chandra and Bhatt, 2008). Rate reducing resistant lines of *S. scabra* showed better drought tolerance in comparison to other accessions (Chandra *et al.,* 2004). Morphological and agronomical evaluation at IGFRI and its regional center as well as institute like Central Arid Zone Research Institute (CAZRI) also generated information pertaining to the adaptation and utilization. Though initial success has been limited in the < 400 mm rainfall zones of CAZRI , however, the performance of *S. hamata* and *S. scabra* was significant in IGFRI, Jhansi zones. Evaluation in west Bengal showed *S. humilis* as the best species for marginal lands. In contrast, *S. guianensis* was the most suitable species for the fodder production in areas of Kerala and Manipur (Gupta *et al*., 1989). Multifaceted use of *Stylosanthes* has been advocated since the time of its evaluation. In drought prone areas to mitigate the fodder shortage the dry matter yield from 2-6 t $ha^{-1}$ have been reported for *S. hamata* and *S. scabra* (Rai and Pathak, 1985). Under 1300-1500 mm rainfall in Ranchi and Kalyani, yields of 7.5 to 10 t $ha^{-1}$ have been recorded for *S. humilis* (Chatterjee *et al.,* 1985). In combination with range grasses the productivity and nutritive value of herbage have also been achieved (Das, 1984; Rai and Pathak, 1985).

It has been also used a feed for pigs (Yadav *et al*., 1990) and trials conducted on broilers have shown that fish meal in rations can be replaced with fresh green *Stylosanthes* for economic poultry production (Gupta *et al*., 1992). For rabbits, dried and ground *S. hamata* can be used to substitute for up to 25% of the dry matter in the diet (Gupta *et al*., 1993). The recent introduction of *S. seabrana* species has provided better scope as a leaf meal for poultry due to better nutritional availability in this species (Changjun *et al*., 2004). Recent upsurge particularly in the utilization of *Stylosanthes* in form of whole meal, leaf meal and leaf meal block and stylo block has tremendously improved the utilization and conservation of this legume for lean period (Mojumdar *et al*., 2005).

*Stylosanthes* species are being grown as an intercrop with food and fodder crops to improve the soil fertility and soil conservation and to provide additional forage (Ramesh *et al*., 1997). Successful intercropping of *S. hamata* between rows of sorghum has been demonstrated in different locations (Singh and Hazara, 1988). It has also been used as a ley in various cropping and forage production schemes. For example, growing of *S. hamata* for three years as a ley supplied an estimated 80-100 kg $ha^{-1}$ N to the soil (Reddy *et al*., 1989). It has been also used as a component crop of 11 different cropping systems on upland, low land, valley and foothill areas of Nagaland (Dwivedi, 1988).

*Stylosanthes* has been maximally used as cover crop in agroforestry and silvipastoral systems. Areas that are open to grazing in forest are maximally used by *Stylosanthes*. Therefore many government agencies have identified this crop to be used in such areas and major seed produced are going to these sectors. *Stylosanthes* has been used to suppress weeds, enrich soil and provide fodder in plantation crops and orchards. The most significant one is the use of *S. guianensis* with *Alnus nepalensis* and pineapple on hilly terrain of Meghalaya (Chauhan *et al*., 1993) and with coconut in Kerala (Pillai *et al*., 1995).

Wasteland development and soil conservation is one of the important sectors where *Stylosanthes* has played a major role in ameliorating and increasing them respectively (Pathak *et al*., 2004). As a component of agroforestry and silvipastoral systems, it has played a significant role in stabilization and sustainable utilization of degraded lands. A number of central and state government organizations have been active in the restoration of wastelands for sustainable development for forage, fuel wood, forest products and horticultural resources. Among many species of *Stylosanthes*, *S. hamata* and *S. scabra* have shown promise for such areas.

Molecular evaluation of genotypes of different species of *Stylosanthes* utilizing different sets of molecular markers and other associated molecular work namely development of genetic map of *Stylosanthes* has been initiated at IGFRI in recent past. A partial genetic map of *Stylosanthes* has been made using F2 population of an interspecific cross (*S. scabra* cv Seca x *S. fruticosa*) (Chandra, 2006). Random amplified polymorphic DNA (RAPD) and sequence-tagged-sites (STS) markers have indicated more closeness of 41117A accession of *S. fruticosa* to the population used for the development of genetic map (Chandra, 2007). The STS markers of *Stylosanthes* have been attempted in studying the genetic diversity of genus *Cenchrus* (Chandra and Dubey, 2007). Assessment of genetic variations of two progenitors of *S. scabra* was investigated employing isozyme, RAPD and STS markers. High frequencies of isozyme band sharing between *S. seabrana* and *S. scabra* (0.125 to 0.30) as well as high genetic similarities (59%) of it with *S. seabrana* as reveled from dendrogram analysis indicated impact of close proximity of the occurrence of *S. seabrana* in the development of *S. scabra*. The proportion of bands shared by RAPD (40%) and STS (40%) markers were also observed high between *S. seabrana* and *S. scabra*. However, the numbers of unique bands were less in all these species. More correspondence and sharing of bands by *S. seabrana* towards *S. scabra* suggested using selected lines of *S. seabrana* the genetic base of allo-tetraploid, drought tolerant *S. scabra* lines can be broaden (Tewari and Chandra, 2008).

### Distribution and evaluation of species

Prominent accessions constituting four major species of *Stylosanthes* evaluated under complete rain fed regime indicated second year was the best year in terms of both green and dry fodder yield. In the first year as the establishment year the germination of *S. seabrana* was superior in comparison to other species (Fig. 1). The weather data recorded also indicated below average rainfall during these four years of evaluation. The results presented below constitute the habitat of the species and suitability of the crops for the regions and also characteristic features of species helps in identifying their prominent utilization systems.

***Stylosanthes scabra*:** This is most important species for drier regions as this is hardy erect woody type and get well adapted in low rainfall areas. It has been also found suitable for semi arid areas of Maharastra, Andhra Pradesh, Karnataka and Tamil Nadu. It has been successfully grown on degraded soil with low fertility, shallow sandy clay loam to black cotton and red chalaka soils. It is suitable for hill slopes, sate lands and degraded areas and can establish in areas with about 325 mm rainfall. Four years (2001-2004) of evaluation at IGFRI main center under complete rain-fed conditions indicated variations in yield in four years. Maximum yield was obtained in second year in most of the accessions. In the first year (establishment year) the plant population was improved if the seed was properly scarified. The first emergence of seedlings appeared just at the onset of monsoon. The most prominent difference of this species in comparison to the other was the time of flowering which is too late and plant are usually green till the month of march. The erect and woody nature of this species along with the drought tolerance behaviors suited better for dry areas. Rate reducing resistance (RRR) lines received under ACIAR project showed promise in terms of different biochemical characters for drought tolerance (Chandra *et al*., 2004). *S. scabra* having forage value is allo-tetraploid with basic chromosome number X=10. The two progenitors of this species have been worked out as *S. seabrana* and S. *viscosa* (Liu and Musial, 1997). This species was also recognized with the presence of sticky leaves. Some of the cultivars like *S. scabra* cv Seca were prominently identified by the parallel branching; CPI 93116 with red or pinkish stem and cv Fitzroy with the presence of small leaves and highly susceptible to disease anthracnose. Two cultivars namely *S. scabra* cv Seca and CPI 93116, resistant to anthracnose have shown some level of damage caused by *C. gloeosporioides* causing anthracnose.

**Fig. 1:** A stand of newly introduced species of stylo (*Stylosanthes seabrana*)

***Stylosanthes hamata*:** This is one of the most adaptable species in India due to its intrinsic nature of fast growth, soft stem and leafy in nature. In comparison to all other species introduced in India, *S. hamata* have been observed highly diversified species in terms of both adaptation and yield performance including seed production. The main advantage of this species is its land covering capacity to maintain and conserve moisture and that leads to fast growth. Both diploid and tetraploid lines have been reported in this species. However, tetraploid species is more prominently used as forage. Diploid *S. hamata* and *S. humilis* are the two progenitors of this species (Curtis *et al*., 1995). This species is short perennial in nature and flower mostly in month of September to October. It has been found highly palatable and preferred by animals over other species. The minimum level of stickiness of leaves present in this species is also one of the reasons for its preference by animals. In comparison to *S. scabra,* it is less drought–hardy, however it is grazing tolerant. Not much difference in production potential among different lines evaluated at IGFRI was observed. Some of the lines were peculiarly identified due to their different habit. However majority of the lines were observed as herbaceous procumbent. The height reached to the level of 60 cm and normally grows very fast and spread like dense mat. Being highly adaptable to this country this species have been extensively used by different government and semi-government agencies for different purposes. This species has been identified as suitable for reclaiming hill slopes.

***Stylosanthes viscosa*:** This species is marked by early emergence and highly stickiness of the leaves and stems. This is also characterized by the whitish leaves and grows in a very sparse manner. The height of this species usually do not cross 50 cm. Being one of the diploid progenitor of *S. scabra*, the potential lines of this species can be used in synthesizing artificial *S. scabra* lines. This species has got less preference however browsers chew it as and when made available.

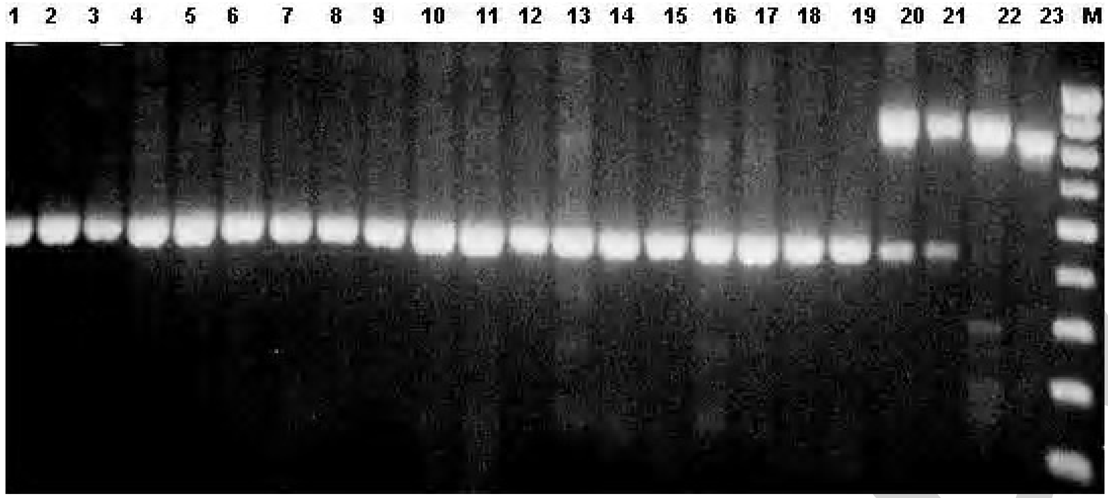


**Fig. 2:** STS profiles with SHST1F3/R3 of all *S. seabrana* lines and two accessions of *S. scabra* and S. viscosa showing sharing of three bands amplified by *S. scabra*. Lanes 1-19 *S. seabrana* accessions, lanes 20-21 *S. scabra* and lanes 22-23 *S. viscosa*. M 100 bp DNA marker

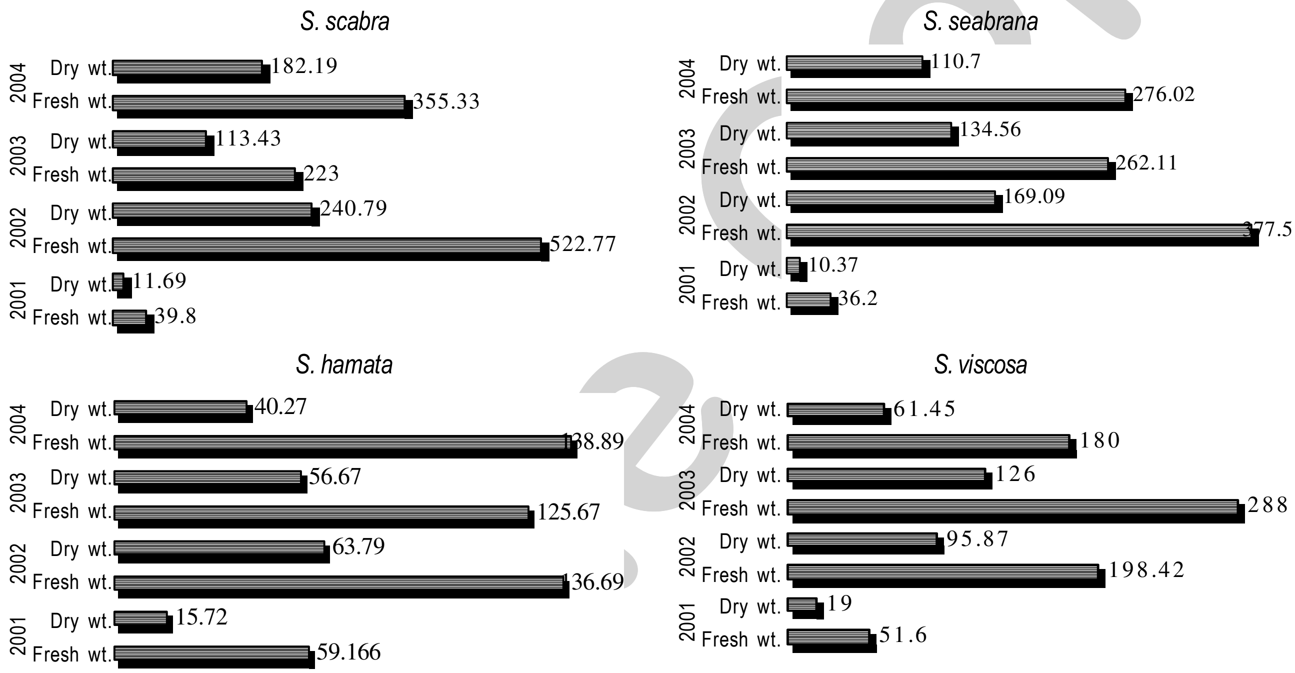


**Fig. 3**: Fresh and dry matter yield (q $ha^{-1}$) in four consecutive years of growth under complete rainfed conditions at IGFRI, Jhansi of four *Stylosanthes* species

***Stylosanthes humilis***: This species was once the major stylo species in Australia but due to susceptibility to anthracnose disease much of the areas was destroyed in 1970s and now it has little significance in stylo based pasture in Australia (Chakraborty *et al*., 1996). It is herbaceous and short in height in comparison to *S. hamata* and flowers very early in comparison to other species of *Stylosanthes*. It is also normal in feel and annual in nature. The seed of this species is recognized by the presence of large hook and small size of the seed in comparison to *S. hamata*. Stem are also thin and leaves are pale green in colour. Results at IGFRI indicated least production potential but better tolerance for salinity of this species in comparison to other. Presence of hairs on stems and leaves are some of the important features helpful in identifying the species.

***Stylosanthes guianensis***: This species has been found suitable for humid and higher rainfall regions. Because of this reason this species has not performed well at Jhansi. It has been found well suited to high rainfall areas of Assam, west Bengal, Maharstra and Andaman and Nicobar islands. It is leafy and provides good quality forage. Due to larger leaves it has been identified as a suitable for the production of leaf meal. In China and Brazil this species is largely used for such purposes. Flowering is also late in this species.

***Stylosanthes fruticosa***: *Stylosanthes fruticosa* Alston synonyms *Stylosanthes flavicans* Baker, *Stylosanthes mucronata* Wild, *Stylosanthes bojeri* Vogel and *Arachis fruticosa* Tetz. This is commonly known as African stylo and amenable to stable mixture with perennial

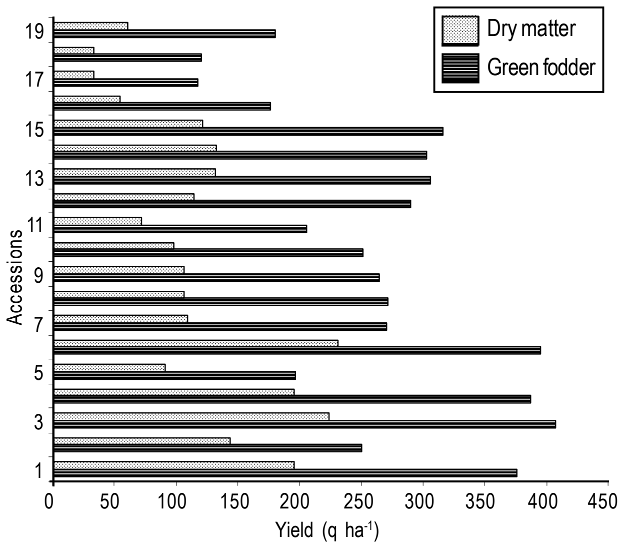


**Fig. 4:** Dry matter and green fodder yield of *Stylosanthes scabra* (1.RRR94-97, 2. Fitzroy 40205, 3. RRR94-96, 4. q10042, 5. 36260, 6. Seca*), Stylosanthes seabrana* (7. 408405, 8. 408404, 9. 408403, 10. 2523, 11. 110372, 12. 104710, 13. 2539, 14. 2534, 15. 105546B), *Stylosanthes hamata* (16. 110123, 17. 110135, 18. 61670*)* and *S. viscosa* (19. 33940) germplasm

grasses such as *Andropogon gayanus* and *Heteropogon contortus*. In areas protected from grazing and intermittently mown, it generally forms dense sward. Collections have been made from 36 sites of Tamil Nadu, 7 sites of Pondicherry, 29 sites of Andhra Pradesh and 38 sites of Karnataka state of India. Materials collected have shown morphological distinctness as some of them have been found growing to the level of 1800 m from sea level. The largest collections of *S. fruticosa* accessions is maintained at Indian Grassland and Fodder Research Institute (IGFRI), Regional Research Station (RRS), Dharwad, however the molecular characterization to maintain identity and purity for proper conservation and management for better use in breeding and proprietary reasons have not been attempted.

***Stylosanthes seabrana*****:** This is a new species introduced in India in the year 1998 through ACIAR project. This is one of the progenitor of hardy *S. scabra* and have shown great promise for this country. Evaluation at IGFRI and different locations has shown great potential and future of this species. It is highly nutritious and showed better establishment in different types of soils. It is diploid in nature and has been selected from the original field of *S. scabra*. It is erect in nature but stem are not hardy can be used for leaf meal preparations. It has been observed as highly palatable. In the very first year of establishment this species was superior in both green biomass and seed production. Apart from materials received from ACIAR project many lines were selected from the plots of *S. scabra* received from International Livestock Research Institute (ILRI), Ethiopia. Molecular characterization of 19 lines of *S. seabrana* showed low level of polymorphism among lines. The number of DNA fragments shared by *S. seabrana* to *S. scabra* was higher in comparison to *S. viscosa* (Fig. 2). The availability of such lines provided an opportunity for the improvement of *S. scabra* by selecting nutritious *S. seabrana* lines. It is also superior in characteristics like frost tolerance, high seedling vigour and essential amino acid level. At IGFRI this species has performed well under rainfed conditions during four years of evaluation. The regeneration potential of this species was also found better in terms of both yield and protein content. Species has performed well when it was sown in watershed areas where moisture is better. Animals especially browsers has preferred it over other species. The flowering of this species is almost synchronized and thus helps in collection of seeds as compared to other species. Preliminary results indicated the better germination of this species under mild level of salinity.

### Characteristics attributes

The genus *Stylosanthes* has a monophyletic origin (Gillies and Abbott, 1996) and is closely related to Arachis (Lavin *et al*., 2001), with the *S. guianensis* species complex as the most ancient group, clearly distinct from the rest of the genus. The species of this genus are grouped in two subgeneric sections, sect. *Styposanthes* and *Stylosanthes*, based on the presence or absence, respectively, of an axis rudiment, a small appendage at the base of the pod or loment (Mannetje, 1984). The progenitor analysis of *S. scabra* clearly indicated that one of the progenitor of this species is *S. viscosa* belongs to sect. *Stylosanthes* since it lacks the axis rudiment whereas other diploid progenitor of *S. scabra i.e. S. seabrana* belongs to sect. *Styposanthes* and possessed the axis rudiment. Such characteristic features have tremendously helped in solving the problems (Kirkbride and de Kirkbride, 1985). Additionally, characters like the presence of prominent hook in *S. hamata* and *S. humilis* seeds also helped in identification of these species. However, as such species identification in *Stylosanthes* based on morphological characteristics is notoriously difficult. There are different views on the species concept and little agreement exists on characteristics most suitable for species identification (Mannetje, 1984; Sousa Costa and Ferreira, 1984). As a result of these difficulties, there have been disagreements over the species status of a large proportion of the *Stylosanthes* genus (Mannetje, 1984; Williams and Gardner, 1984). This situation has, however, changed dramatically over the last few years due to the development and applications of molecular markers. In addition, the application of chloroplast DNA markers has allowed the identification of maternal donors of majority of the polyploid *Stylosanthes* species (Liu and Musial, 2001; Ma *et al*., 2004). There are reports that a group of accessions of *Stylosanthes,* of special interest for their adaptation to the heavy clay soils, were assessed on the basis of morphological characters and were grouped in three categories and main attributes contributed to the separation of the groups were the presence or absence of a stipule horn bristles, stipule horn lateral bristles, inflorescence bristles and leaf hairs either absent, all over leaf or back of leaf only.

### Yield potential and impact of edaphic conditions

Newly added germplasm largely consisting of *S. hamata*, *S. scabra*, *S. guianensis* and *S. seabrana* was evaluated at different locations possessing different agro-climatic conditions. The evaluation carried out at IGFRI, Jhansi in four consecutive years demonstrated significant interspecific and intraspecific variations in fresh and dry weight yield (Fig. 3, 4).Variations in many quantitative traits were visible in different years of crop. In the first year, considered as establishment year the percentage dry matter was highest in S. *scabra* cv Seca. Three *S. hamata* accessions behaved similar in terms of % dry matter. Fresh weight yield on per plant basis was highest in *S. scabra* q 10042 during first year, *S. scabra* RRR 94-96 in second year and in third year the maximum yield was observed in *S. scabra* q 10042. In the fourth year, over all production in terms of q/ha, *S. scabra* q10042 stands first while *S. scabra* cv Seca second (Fig. 4). Among *S. seabrana* lines maximum yield was observed in 408403 during first year, 104710 in second year and 2534 in third year. Finally in fourth year *S. seabrana* 105546B gave maximum yield. The trend of overall production indicated variation in yield potential of species in different years. However at the same time second year was observed as the best year for production of fresh and dry matter. The variation in yield potential of different accessions of stylo indicated location specific differences. Earlier study has indicated the season wise difference in production of stylo species as *S. scabra* found more suitable in summer season and *S. hamata* in rainy season (Rai and Patil, 1985). With grass legume mixture, the compatibility for higher production in the mixture indicated interference by *Cenchrus* to *S. hamata*, *S. scabra* and *S. guianensis* while ideal association was observed with *Heteropogon* and *Dichanthium* with *S. hamata* (Rai and Patil, 1985). Nevertheless, under tropical situation the grass-legume mixture out yield monocrop (Rai *et al*., 1980; Singh *et al*., 1983).

### Regeneration potential of *S. seabrana* species

Regeneration potential of six lines of *S. seabrana* was assessed by employing three cutting schedules at one month intervals. Yield at two cuts of one month intervals as well as protein and total sugar content estimated at different cuts indicated variation in regeneration ability of the accessions. The difference in biomass accumulation at different cuts in the accessions indicated the regeneration potential of *S. seabrana* 104710 line was better over other lines, as it has maintained similar green biomass production if taken for two cuts at one month intervals. No difference in total biomass production was recorded when it was cut or uncut.

### Genetic diversity and progenitor analysis

The accessions of major stylo species have been characterized and reported using both biochemical and molecular markers. When large number of *S. scabra* accessions was characterized some of the accessions though acquired as *S. scabra* found more close to *S. seabrana*. The average dissimilarity among Brazilian accessions was much lower than those among Colombian and Venezuelan. The variation among Brazilian materials was more in comparison to other accessions and this was may be due to long distances introductions/dispersions of *S. scabra* accessions with Brazilian genotypes (Liu, 1997). Four agronomically important species have been together analyzed using RAPD markers. Relatively low level of polymorphism (0-16% of total bands in pair wise comparison) were found within each species, while polymorphism between the species were much higher (up to 46%) (Kazan *et al*., 1993; Liu *et al*., 1999). Low polymorphism (0-2%) were detected between the individuals of the same cultivar or accessions. The allotetraploid species *S. hamata* and its putative progenitor, *S. humilis* were more akin to each other than *S. scabra* and *S. guianensis*. No variation in RAPD markers was found between the two commercial *S. hamata* cvs Verano and Amiga. Low variations in seed proteins of *S. seabrana* have been also observed (Chandra *et al*., 2005). Suitable RAPD and STS markers have been also reported for genetic estimation of *S. fruticosa*, only species endemic to southern parts of the country (Chandra, 2007). Cultivar Oxley in *S. guianensis* was considerably different from the other cultivars and accessions of this species. The low level of polymorphism as reported within each species suggested that interspecific crosses may be better approach for the construction of linkage map in *Stylosanthes* (Chandra, 2006).

Sequence tagged sites (STS) markers also revealed similar type of inter-species relationships as shown by RAPD. Such type of markers have been found having added advantage of identifying the species, subspecies and genotypes in *Stylosanthes*, with a view to plant conservation and breeding (Vander Stappen *et al*., 1999). Such markers have been also successfully used in identifying the diploid progenitors of *S. scabra*, *S. fruticosa, S. erecta*, *S. hamata* allotetraploid and hexaploid species. Using this markers two diploid progenitor (*S. viscosa* and *S. seabrana*) of *S. scabra* have been identified (Liu and Musial, 1997). Recently, our efforts have made success in identifying the third putative progenitor of only hexaploid species (*S. erecta*) (Ma *et al*., 2004). Similarly the progenitors of *S. hamata* have been also identified using same markers (Curtis *et al*., 1995). Amplified fragment length polymorphism (AFLP) markers have been also used in studying the variability in *S. humilis* accessions of south American origin and these markers have differentiated all Mexican accessions from the accessions of South American origin. Although most Mexican accessions formed one major group, one accessions clustered with south American gene pool, indicated that Mexico may contain unique sources of *S. humilis* and therefore would merit attention for conservation and maintenance of *S. humilis* germplasm (Vander Stappen *et al*., 2000). At IGFRI nineteen accessions of *S. seabrana* have been evaluated using 30 RAPD markers and seed protein markers indicated sharing of more than 95 % bands among accessions (Tewari and Chandra, 2008). In 12 accessions of *S. seabrana* the dissimilarity values have been reported to the level of 0.078. These 19 seabrana lines were evaluated along with *S. scabra* and *S. viscosa* indicated many bands which were not present in *S. seabrana* though being one of the progenitors of *S. scabra*. Similarly *S. viscosa* has also not shown all bands present in *S. scabra*. The sharing of bands among *S. seabrana* to *S. viscosa* was also not to high level. Many bands have been observed in *S. scabra* not found either in *S. seabrana* and *S. viscosa*. Very low level of polymorphism have been also observed among the commercial Seca individuals (Chakraborty, 2004).

### Breeding strategies

Apart from the anthracnose threat, the limited number of potential germplasm availability necessitated adaptations of the new breeding strategies to increase the overall production of stylo. The potential of *S. seabrana* for tropical and subtropical regions of the country with clay and heavy soils, cool winters and distinct wet-dry seasonal conditions directed the use of this species in developing new breeding approach. The one could be based on the finding that it is the second progenitor of *S. scabra* which in turn elucidated the evolution of one of the most important *Stylosanthes* species, *S. scabra* may lead to important impacts on the efforts of improving *S. scabra* (Tewari and Chandra, 2008). It may be possible to artificially synthesize *S. scabra* using pre-selected *S. viscosa* and *S. seabrana* accessions (Liu and Musial, 1997). These artificial *S. scabra* genotypes could be used directly or more likely, be used in breeding programs. By doing so the genetic variation existing in the two diploid progenitor species would become available in improving the allotetraploid *S. scabra*. So far developed map and linked markers with anthracnose resistance also provide the opportunity to use them after converting them in sequence tagged sites (STS) or sequence characterized amplified region (SCAR) and then using them in direct breeding programs. The phylogenetic relationships among different *Stylosanthes* species also provided sufficient clue to use them in making new breeding strategies. The resistance available in some elite lines of *S. scabra* as well as further evaluation of these and some additional lime is required to select putative replacement cultivars for use in different parts of the country where *S. scabra* is a productive pasture component. The molecular genetic approaches have provided the means to broaden the resistance base of elite lines. This also enriches our genetic resources and gene pool. The new resistance source identified through molecular markers can be backcrossed into elite lines so that the back cross selection would have more broad based resistance to anthracnose.

## Future prospects

The combining of elite lines not only provide the suitable materials having durable resistance genetic resource but also make available good back cultivars if new pathogen race seriously damage such elite lines. Therefore, such efforts should continue. The screening of materials under different agro-climatic zones are required to be continued so that the genetic vigour that is existing can be adapted easily under diverse environment. Use and exploitation of weather data and GIS should be promoted for targeting new ecological niches. More and enough number of germplasm may be required for evaluation at different established stylo sites in country so that the suitable genotypes can be identified for problem soils *i.e.*, salt affected and acidic soils. Therefore, a program has to be initiated and implemented. The efficiency and number of polymorphic molecular markers has to be geared up and employed in targeted breeding programs as the crop in general is less divergent. The development and use of simple sequence repeats (SSR) markers would be one step ahead in this direction.

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