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BRIEF COMMUNICATION

Variation in drought tolerance of different *Stylosanthes* accessions

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Abstract

Twenty genotypes of *Stylosanthes* consisting four species were evaluated under rain fed condition employing biochemical and physiological attributes to select drought tolerant lines. Relative water content measured at 50 % flowering stage of the plants showed significant variations among the lines which ranged from 32.11 in *S. scabra* RRR94-86 to 83.33 % in *S. seabrana* 2539. The results indicated that *S. scabra* genotypes were more tolerant to drought over other lines as evidenced by high leaf thickness, proline accumulation, content of sugars and chlorophyll, and nitrate reductase activity.

Additional key words: chlorophyll, leaf thickness, nitrate reductase, osmotic potential, proline, proteins, relative water content, soil moisture, sugars.

Shortage of water limits plant growth and crop productivity in arid and semi arid regions more than any other single environmental factor. Genetic modification of plants by breeding and identification of germplasm for their growth and yield under unfavourable conditions is a solution to the problems of environmental stresses. Additionally, better understanding of the tolerance behaviour at morphological, physiological and biochemical levels leads to identify genotypes which could adapt to water deficit and maintains growth, development and productivity during stress period especially in arid and semi arid conditions.

In the semi arid tropics, where *Stylosanthes* spp. have their most significant role as pasture plants, water is the major environmental constraints to the plant growth (Williams and Gardener 1984). Therefore, a better understanding of the mechanism that enable plants to adapt to water deficit and maintain growth, development, and productivity during stress periods could help in screening and selection of tolerant genotypes which can be further utilized in breeding programmes. Apart from the activation of different antioxidant enzymes, accumulation of various osmoprotectants protecting the plant as a whole have been well documented in different crops (Chauhan *et al*. 1995, Chandra *et al*. 1998, Phutela *et al*. 2000).

As the kharif legumes are grown under rain fed conditions crops experience water stress of varying degree and durations at different growth stages, the screening of genotypes under such conditions assumes great importance. Though identification of the traits conferring to the drought resistance and incorporating them into plant improvement programs is a challenging task, the screening of different accessions and species in rain fed conditions utilizing established selection criteria could enrich the gene pool to be utilized under such situations. Keeping this in view, twenty accessions of *Stylosanthes* consisting of four species were evaluated for drought tolerance employing morphological, physiological and biochemical attributes.

Study was carried out in twenty predominantly self-pollinated cultivars and accessions belonging to four species of *Stylosanthes: S. scabra* (cvs. Fitzroy, Seca, CPIs Q10042, 36260, 93116, RRR94-97, RRR94-96, RRR94-86, RRR94-100, RRR94-93), *S. hamata* (CPIs 110123, 110135, 61670), *S. seabrana* (CPIs 2523, 110372, 104710, 2539, 2534, 105546B) and *S. viscosa* (CPI 33941). These were obtained from CSIRO, Australia.

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Abbreviations: Chl - chlorophyll; NR - nitrate reductase; RWC - relative water content.

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They were sown and established in three replications. At the time of germination, one irrigation was given so that seedlings in (3 × 4 m) plots could be established well. Relative water content and soil moisture denoted the status of water stress of each genotype. Third leaves from the top were taken from plants and used for experimental analyses. Relative water content (RWC) was determined at 50 % flowering stage of the plant using the method of Barrs and Weatherley (1962). Proline was extracted in 3 % sulphosalicylic acid and estimated by the colorimetric method of Bates *et al*. (1973). The 0.1 M phosphate buffer (pH 7.0) was used to ground the fresh leaves and supernatant was used to measure the soluble protein using Lowry's method (Lowry *et al*. 1951). The total soluble sugar in dry sample was measured using anthrone (Hedge and Hofreiter 1962).

Leaf area/leaf dry mass ratio was measured by randomly harvesting six trifoliate leaves. Leaf area was measured using *LI 3000* (*Licor*, Lincoln, USA) portable leaf area meter and the leaves were oven-dried at 70 °C for 48 h for dry mass measurement. Osmotic potential was measured using vapor pressure osmometer model *5500* (*Wescor*, Logan, USA). Chlorophylls (Chl) *a* and *b* were extracted in dimethyl sulphoxide solution following the method of Hiscox and Israelstam (1978) and absorbance was recorded using *ATi Unicam* (Cambridge, UK) spectrophotometer. Concentrations of pigments were calculated using Arnon's formulas (Arnon 1949). *In vivo* nitrate reductase (NR) activity was carried out in both root and leaves by following the method of Srivastava (1975). All morphological attributes were recorded at 50 % flowering stage following standard procedure. Statistical analyses were performed by following the standard statistical methods (Gomez and Gomez 1984).

Twenty accessions of *Stylosanthes* consisting of four major species namely *S. scabra*, *S. hamata*, *S. seabrana* and *S. viscosa* used for the present investigation indicated wide variations in RWC. The minimum RWC was noticed in *S. scabra* RRR94-86 and maximum in *S. seabrana* 2534 (Table 1). Soil moisture measured from each plots varied from 1.6 to 4.2 % indicated the operation of stress in the plants. Except *S. seabrana* 2534 genotype, the RWC was between 50 to 60 % in most of the genotypes. The soil moisture and RWC were not found complementary to each other as *S. scabra* RRR94-86 showed 32 % RWC and 1.6 % soil moisture whereas *S. seabrana* 2539 showed 83 % RWC and 1.98 % soil moisture indicated different RWC by genotypes although growing in similar soil moisture. The content of total soluble proteins and soluble sugar also varied among different species as well as between the genotypes of the same species (Table 2). Maximum content of soluble sugars [35.75 mg g^{-1}(d.m.)] was observed in *S. scabra* cv. Seca and proteins [301.2 mg g^{-1}(d.m.)] in *S. scabra* RRR94-93.

The osmotic potential varied from -2.90 to -1.46 MPa among the genotypes and did not show any relationship with RWC. However, when proline content was

Table 1. Physiological attributes of 20 *Stylosanthes* genotypes grown under rain-fed condition and measured at 50 % flowering. Means ± SE, $n = 3$.

Genotypes	Soil moisture [%]	RWC [%]	Osmotic potential [-MPa]	Leaf area/mass ratio [cm^2 g^{-1}]	Chl *a* [mg g^{-1}(d.m.)]	Chl *b* [mg g^{-1}(d.m.)]
S. scabra RRR94-97	1.63±0.37	56.98±0.05	2.90±0.39	129.00±11.00	1.44±0.09	0.564±0.02
S. scabra RRR94-96	3.35±0.29	70.28±2.89	1.82±0.09	102.96±13.71	2.85±0.10	0.618±0.03
S. scabra RRR94-86	1.60±0.52	32.11±1.53	1.69±0.18	113.59±15.33	3.72±0.08	1.182±0.03
S. scabra RRR94-100	3.26±0.24	64.46±1.80	1.76±0.07	112.50±11.64	3.42±0.08	0.385±0.04
S. scabra RRR94-93	3.00±0.27	68.19±5.09	1.57±0.09	115.62±14.07	3.03±0.08	0.378±0.04
S. scabra cv. Fitzroy	2.74±0.18	54.84±1.50	1.94±0.08	119.98±16.94	2.61±0.08	0.885±0.05
S. scabra Q10042	3.06±0.21	55.19±2.76	1.71±0.11	148.62±19.61	3.09±0.06	1.023±0.04
S. scabra 36260	2.13±0.26	55.97±2.28	1.77±0.11	102.14±5.27	3.45±0.11	1.293±0.06
S. scabra cv. Seca	3.49±0.28	66.69±0.24	1.89±0.25	114.57±13.00	2.43±0.16	0.289±0.08
S. scabra 93116	3.10±0.41	66.96±6.50	1.46±0.08	131.17±12.84	1.40±0.06	0.198±0.03
S. seabrana 2523	2.72±0.52	65.20±5.17	2.08±0.06	155.36±7.89	2.42±0.07	0.730±0.01
S. seabrana 2539	1.98±0.46	83.33±1.74	2.16±0.25	124.65±10.50	2.18±0.22	0.383±0.06
S. seabrana 2534	2.29±0.35	67.76±3.81	2.17±0.22	125.28±13.97	1.48±0.04	0.296±0.03
S. seabrana 110372	3.82±0.25	68.21±0.81	2.08±0.04	97.09±13.6	0.74±0.06	0.392±0.02
S. seabrana 104710	1.80±0.23	59.59±4.70	1.54±0.05	154.69±20.7	2.76±0.58	0.514±0.22
S. seabrana 105546B	2.13±0.26	69.16±4.80	2.04±0.08	121.27±12.00	1.68±0.05	0.231±0.02
S. hamata 110123	2.30±0.24	54.57±2.30	1.59±0.03	119.34±12.18	2.02±0.05	0.610±0.00
S. hamata 110135	4.18±0.27	61.80±3.97	1.99±0.09	136.75±17.00	2.30±0.12	0.752±0.05
S. hamata 61670	2.57±0.32	70.50±3.70	1.64±0.06	111.32±17.03	2.00±0.06	0.328±0.04
S. viscosa 33941	2.67±0.33	60.77±2.41	1.66±0.16	118.53±10.47	0.94±0.16	0.283±0.03

Table 2. Biochemical attributes of 20 *Stylosanthes* genotypes grown under rain-fed condition and measured at 50 % flowering. Means ± SE, $n = 3$.

Genotypes	Soluble proteins [mg g^{-1}(d.m.)]	Soluble sugars [mg g^{-1}(d.m.)]	Proline content [mg g^{-1}(d.m.)]	Leaf NR activity [mmol g^{-1}(d.m.) h^{-1}]	Root NR activity [mmol g^{-1}(d.m.) h^{-1}]
S. scabra RRR94-97	249.6±2.35	16.73±0.64	125.55±0.40	4.22±0.05	1.17±0.15
S. scabra RRR94-96	262.2±1.50	26.24±0.93	41.04±1.42	4.33±0.32	1.99±0.03
S. scabra RRR94-86	265.8±7.45	15.87±0.43	1296.78±7.65	5.29±1.05	2.65±0.28
S. scabra RRR94-100	178.8±2.04	13.87±0.57	87.51±1.11	1.30±0.10	1.93±0.91
S. scabra RRR94-93	301.2±4.38	14.44±1.29	125.31±1.42	3.90±0.40	3.10±0.70
S. scabra cv. Fitzroy	170.7±5.29	24.31±1.43	135.93±0.77	2.47±0.18	2.85±0.32
S. scabra Q10042	268.2±2.50	14.73±0.57	178.41±0.08	2.92±0.55	1.48±0.04
S. scabra 36260	265.8±2.40	12.29±0.57	273.84±2.50	1.94±0.03	1.33±0.21
S. scabra cv. Seca	238.8±2.95	35.75±1.28	157.11±1.60	3.86±1.59	2.27±0.42
S. scabra 93116	176.4±7.80	16.80±0.50	41.52±1.24	1.82±0.42	2.27±0.01
S. seabrana 2523	183.2±3.20	14.30±0.64	49.82±1.30	2.50±0.35	1.09±0.14
S. seabrana 2539	176.4±6.52	15.01±0.86	22.10±0.95	1.70±0.24	1.60±0.07
S. seabrana 2534	146.4±3.72	26.31±0.85	52.66±2.14	5.17±0.34	1.55±0.01
S. seabrana 110372	110.4±2.20	18.01±0.71	66.64±1.38	1.32±0.03	1.09±0.22
S. seabrana 104710	182.0±0.90	32.74±1.25	42.16±1.08	2.86±0.04	0.69±0.04
S. seabrana 105546B	158.8±3.90	15.01±0.86	28.80±0.61	3.27±0.44	1.81±0.14
S. hamata 110123	172.8±1.30	18.59±0.71	255.46±1.47	2.12±0.14	0.73±0.03
S. hamata 110135	174.4±3.18	18.08±0.79	189.38±1.45	0.75±0.12	1.63±0.40
S. hamata 61670	178.4±0.60	8.93±0.78	96.46±1.67	2.44±0.83	2.32±0.63
S. viscosa 33941	105.6±2.23	20.87±0.71	29.74±0.70	1.65±0.10	1.18±0.36

measured a relationship with RWC was observed. *S. scabra* RRR94-86 which showed 32 % RWC showed maximum content of proline [1296.78 µmol g^{-1}(d.m.)] whereas genotypes showing 83 % RWC indicated proline level of [22.10 µmol g^{-1}(d.m.)] and rest of the genotypes indicated level in between them. The content of proline increases with the decrease in RWC. The proline as osmoprotectant is well recognized at least in some crops (Jain *et al.* 1991, Chauhan *et al*. 1995, Phutela *et al*. 2000). There were many reasons of increase of proline content under stress. It could be due to prevention of feedback inhibition of all biosynthetic enzymes caused by sequestering of proline away from its site of synthesis, feed back inhibition of the regulatory step enzyme, gene amplification, up regulation of transcription of genes involved in proline biosynthesis (Kavikishore *et al*. 1995), reduction in rate of catabolism or decreased activity of enzymes involved in degradation of proline (Nayyar and Walia 2003).

Leaf area/mass ratio significantly varied among the accessions. The maximum and minimum leaf area/mass ratio was observed in different genotypes of the same species, *i.e*., *S. seabrana*. The leaf area/mass ratio denotes the thickness of leaves and the hardiness of plants. Some of the species and accessions of *Stylosanthes* namely *S. viscosa*, *S. seabrana* 110372, *S. hamata* 61670 and 110123, and all *S. scabra* RRR lines indicated low leaf area/mass ratio and thus more hardiness than that of others (Table 1). The decrease in leaf area/mass ratio has been reported in *Stylosanthes* under stress condition and emphasized to be used as one of the parameters in screening large number of segregating lines in early phase of breeding programs (Thumma *et al*. 1998). Lines with low leaf area/mass ratio or tend to have low leaf area/mass ratio could be identified as better lines to withstand stress.

Water stress in general decreased the chlorophyll content in leaves at all the growth stages (Vyas *et al*. 2001). Comparison among the ten *S. scabra* lines indicated variation from 1.60 to 4.80 mg g^{-1}(d.m.). *S. seabrana* indicated maximum chlorophyll content among the all genotypes. The variation among three lines of *S. hamata* was less in comparison to other species. The activities of nitrate reductase (NR) was high in leaves in comparison to roots and *S. seabrana* 2534 showed maximum activity in leaves whereas *S. scabra* 93116 in roots. The NR activity decreased due to water stress in clusterbean at all growth stages (Vyas *et al*. 1996). Though *S. scabra* RRR94-86 genotype possessed low level of RWC showed NR activity in both root and leaves close to the other *S. scabra* lines having high RWC indicated low impact of stress on nitrate metabolism of the plant (Table 2). Identification of traits like response of nitrate reductase along with germination, accumulation of proline and betaine and change in hormone concentration under moisture stress has been well discussed (Khanna-Chopra and Sinha 1996).

Thicker leaves and ability to high osmotic adjustment

under water stress makes most of the *S. scabra* RRR lines suitable for such conditions. Of these, *S. scabra* RRR94-97 and RRR94-86 were most promising as they showed better response in terms of NR activity, content of soluble protein and soluble sugar as well as other physiological characteristics.

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