

RESPONSES OF *SPOROBOLUS ARABICUS* AND *SESBANIA AEGYPTICA* AS AFFECTED BY DENSITY, SALINITY OF IRRIGATION WATER AND INTER-CROPPING

RAFIQ AHMAD AND SYED HASAN ZAHEER

Department of Botany,
University of Karachi, Karachi-75270, Pakistan.

Abstract

Sporobolus arabicus and *Sesbania aegyptica* grown in sandy soil as mono- and mix-culture using saline water (EC_{iw} : 6 & 12 $dS.m^{-1}$) for irrigation showed that the productivity of grass - legume mix-culture per unit area was much higher irrespective of the salinity of irrigation water. Reduction in growth of *S. aegyptica* at higher salinity (EC_{iw} : 12 $dS.m^{-1}$) was more as compared to *S. arabicus*. No significant reduction in growth of *S. aegyptica* was observed at low salinity (EC_{iw} : 6 $dS.m^{-1}$), whereas growth in *S. arabicus* was promoted. Increase in proline content at higher salinity was evident in *S. aegyptica* with no effect in *S. arabicus*. Alteration in the absorption of radiant energy (quantum yield) were non-significant in *Sporobolus* in response to salinity and cropping system, while *Sesbania* plants showed a positive response with respect to salinity and inter-cropping with *Sporobolus*. The same trend was evident for stomatal conductance. Transpiration rate of *Sporobolus* was low when grown as mix-culture, while that of *Sesbania* was highly reduced by salinity under both cropping systems.

Introduction

Salinization of soil and water is a factor responsible for desertification in arid and semi-arid regions of the world (Szabolcs, 1992), which leads to reduction in yield, consequent degradation of livestock and reduction of available wood biomass. Existence of forage shortage, especially that of winter forage is a major problem for livestock farming in Pakistan. The "Biosaline Technology", where the exploitation of underground saline water for irrigation at sandy soil has been found suitable for cultivation of salt tolerant grasses and shrubs (Ahmad *et al.*, 1986), may be useful to feed the livestock and also stabilize the sand dunes. The grasses and shrub species although have great potential to tolerate highly saline conditions and produce economically feasible grazable biomass, but are of low quality in respect to animal nutrition, due to low protein content. There is therefore a need to develop a system of mixed cropping of such grass species with forage legumes having high protein content which can improve the feed value of pasture lands.

There are evidences of improved productivity and net income per hectare through intercropping [or] multiple cropping as compared to mono-culture (Santa-Cecilia & Viera, 1978). However, a compatibility of all components is of great importance. Kass (1978) has emphasized the need to consider the effect of each crop component on the other(s) to determine the plant types that are compatible for multiple cropping/intercropping, especially under saline environment since the leguminous plants generally cannot withstand high salinity at which certain grasses grow luxuriantly.

Experiments were therefore conducted to study the performance of a grass-legume intercropping system under saline water irrigation. *Sporobolus arabicus*, a salt tolerant grass species and *Sesbania aegyptica* (Janter), a leguminous forage species, usually recommended for growing on salt affected soils were selected and grown in mono- and mix-culture(s) under different salinity levels.

Materials and Methods

The study was conducted at the Department of Botany, University of Karachi. Plastic drum pots of 0.6 m² diameter installed on RCC platform were filled with 200 Kg of coastal sand, provided with a basal outlet for drainage. Experimental design was a completely randomized design with three replicates as per following treatments.

(A) **Salinity Treatments:** Three salinities of irrigation water used were i) Control [Tap water] (EC_{iw} : 0.28 dS.m⁻¹); ii) 10% sea water (EC_{iw} : 5.87 dS.m⁻¹); and iii) 20% sea water (EC_{iw} : 11.92 dS.m⁻¹).

(B) **Cropping System:** (i) *Sporobolus arabicus* (mono-culture); (ii) *Sesbania aegyptica* (mono-culture) with 2 plants/pot; (iii) *S. aegyptica* (mono-culture) with 4 plants/pot; (iv) *S. arabicus* + *S. aegyptica* [2 plants/pot] (mix-culture); (v) *S. arabicus* + *S. aegyptica* [4 plants/pot] (mix-culture).

Seedling establishment and pre-conditioning: Seeds of *S. aegyptica* were sown in small plastic bags filled with sand, frequently flushed with tap water. On emergence the seedlings were thinned to retain one seedling per bag, which were allowed to establish for a period of two weeks.

S. arabicus was propagated vegetatively. Bunch of the stalk with 2-3 nodes were planted in fine sand irrigated with tap water and allowed to establish for a month. The established seedlings/saplings of *S. aegyptica* and *S. arabicus* were divided into three uniform groups representing three irrigation levels. Control plants were irrigated with tap water, while others were irrigated with saline water gradually increased by an EC_{iw} of 2.0 dS.m⁻¹, at three days intervals till the desired salinity levels (EC_{iw} : 6 and 12 dS.m⁻¹) of irrigation water was achieved.

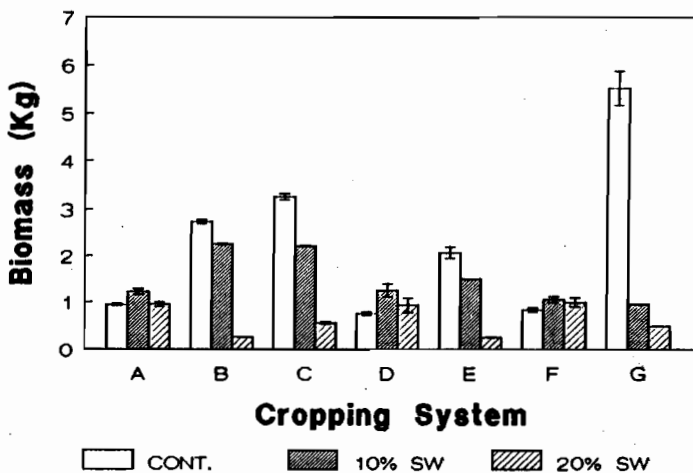


Fig.1. Biomass of *Sporobolus arabicus* and *Sesbania aegyptica* grown as mono-culture and mix-culture, with saline water irrigation.

A) *Sporobolus arabicus* = [Mono-culture], B) *Sesbania aegyptica* (2 Pl.) = [Mono-culture]

C) *Sesbania aegyptica* (4 Pl.) = [Mono-culture], D) *S. arabicus*, = [Mix-culture with 2 *Sesbania*]

E) *S. aegyptica* (2 Plants) = [Mix-culture with *Sporobolus*], F) *S. arabicus* = [Mix-culture with 4 *Sesbania*]

G) *S. aegyptica* (4 Plants) = [Mix-culture with *Sporobolus*]

Transplantation: Pre-conditioned plants of uniform size were transplanted in drum pots as per above mentioned experimental design in August, 1991 for a period of one year.

Harvest: Due to fast growth rate of *S. aegyptica*, the plants were clipped twice (November, 91 and March, 92) at a height of 45 cm from ground surface during the growth of one year. Final harvest was taken in August, 1992.

All the plants were up-rooted, separated into roots and shoot and weighed for fresh weight. In mix-cultures, both the grass and legume components were weighed separately to determine their respective contribution in the biomass production. Weight of the clipped components of the forage legume (*Sesbania*) were added to the fresh biomass obtained at the time of harvest, to obtain the cumulative fresh biomass produced over a period of one year.

Physiological studies: Quantum yield, stomatal conductance and transpiration rate was measured using a Li-1600 steady-state porometer (Li-Cor, Inc. USA).

Biochemical Estimations: Leaf samples were collected from experimental plants after 60 days for biochemical analyses. Chlorophyll was extracted and estimated as described by Maclachlan & Zalik (1963) using Spectronic-20 spectrophotometer. Sugar content was determined by Anthrone's method (Yemm & Willis, 1956) and protein was extracted and estimated by Lowry's method (Lowry *et al.*, 1951).

Results

Growth responses of *S. arabicus* and *S. aegyptica* were variable when grown as mono- or mix-culture (Fig. 1). *Sporobolus* when grown as mono-culture, showed promotion in biomass production at 10% sea water [EC_{iw} : 6 $dS.m^{-1}$] with no reduction in biomass production at 20% sea water [EC_{iw} : 12 $dS.m^{-1}$]. Analysis of variance (Table 1) showed that variations among treatments (T), were statistically significant ($P < 0.05$), while cropping system (S) irrespective to the density of inter-cropped *Sesbania* plants was non-significant, when analyzed separately or in interaction to salinity treatment (SxT). No significant reduction in the biomass of *Sesbania* was observed at low salinity - low density, when grown as mono-culture, whereas, at high salinity growth was significantly reduced. At high density, biomass of mono-cultured *Sesbania* plants was significantly reduced at both salinity levels. However, the net productivity (grass + legume) per unit area was much higher in mix-culture(s), irrespective to the salinity of the irrigation water (Fig. 2). Statistical analysis (Table 1) confirms the influence of density (D) and treatment (T) and their interaction (DxT) for biomass production. Cropping system (S) showed no significant effect on growth of *Sesbania* plants, but its interaction with density (DxS), treatment (TxS) and their combined interactions (DxSxT) were highly significant ($P < 0.001$).

The contents of chlorophyll "a" and "b" of salt stressed *Sporobolus* remained unchanged when grown as mono-culture, but exhibited a gradual reduction when grown as a component of mix-culture(s) (Fig.3). However, these differences were statistically non-significant (Table 1). At high density mixculture (grass:legume ratio of 1:4), the chlorophyll content in *Sesbania* plants showed a consistent negative response with respect to salinity. The influence of cropping system (S) over chlorophyll "a" content of *Sesbania* plants was highly significant ($P < 0.001$).

Table 1. Pooled ANOVA for growth, physiological and biochemical responses of *Sesbania acgyptica* and *Sporobolus arabicus* grown as mono-culture and mix-culture, with saline water irrigation.

Parameter	Plant species	ANOVA	Density (D)	System (S)	Treatment (T)	SxT	DxS	DxT	DxSxT
Biomass	S. acgyptica	F. Ratio Probability	34.87 0.001	0.59 n.s	247.61 0.001	22.54 0.001	12.77 0.01	38.40 0.001	23.90 0.001
	S. arabicus	F. Ratio Probability		0.21 n.s	3.84 0.05	0.35 n.s			
Chl-a	S. acgyptica	F. Ratio Probability	0.92 n.s	17.36 0.001	3.20 n.s	1.54 n.s	0.39 n.s	2.68 n.s	1.23 n.s
	S. arabicus	F. Ratio Probability		0.40 n.s	1.36 n.s	0.42 n.s			
Chl-b	S. acgyptica	F. Ratio Probability	2.75 n.s	0.49 n.s	0.78 n.s	1.63 n.s	1.13 n.s	1.74 n.s	0.51 n.s
	S. arabicus	F. Ratio Probability		0.13 n.s	1.43 n.s	0.56 n.s			
Soluble sugars	S. acgyptica	F. Ratio Probability	4.24 n.s	0.94 n.s	3.82 0.05	1.91 n.s	1.39 n.s	0.36 n.s	0.60 n.s
	S. arabicus	F. Ratio Probability		0.86 n.s	0.55 n.s	1.56 n.s			
Total proteins	S. acgyptica	F. ratio Probability	0.16 n.s	1.24 n.s	3.25 n.s	11.93 0.001	22.78 0.001	0.69 n.s	1.80 n.s
	S. arabicus	F. Ratio Probability		0.15 n.s	1.99 n.s	0.80 n.s			
Quantum yield	S. acgyptica	F.Ratio Probability	0.60 n.s	16.73 0.001	16.05 0.001	0.42 n.s	0.00 n.s	0.66 n.s	1.59 n.s
	S. arabicus	F. Ratio Probability		3.04 n.s	0.11 n.s	2.33 n.s			
Stomatal conductance	S. acgyptica	F. Ratio Probability	2.35 n.s	0.07 n.s	9.39 0.001	1.60 n.s	59.15 0.001	0.50 n.s	7.77 0.01
	S. arabicus	F. Ratio Probability		1.13 n.s	0.13 n.s	0.67 n.s			
Transpiration	S. acgyptica	F. Ratio Probability	0.03 n.s	0.92 n.s	12.25 0.001	0.58 n.s	5.04 0.05	0.30 n.s	0.21 n.s
	S. arabicus	F. Ratio Probability		2.44 n.s	0.79 n.s	2.40 n.s			

D = Density of *Sesbania* plants ; S = System of cropping (Mono/Mix) ; T = Salinity treatments

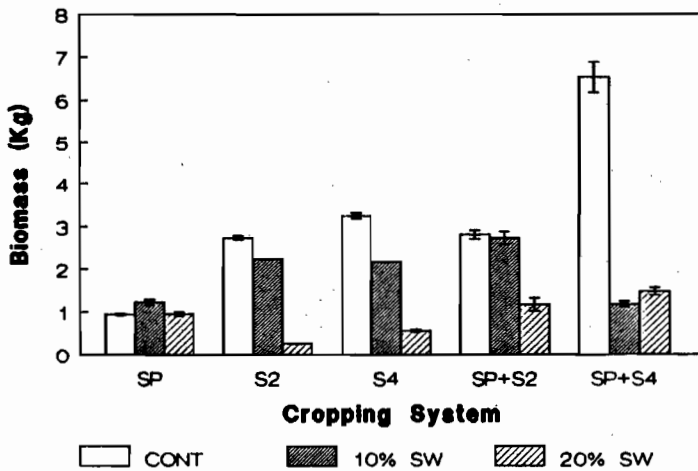


Fig.2. Cumulative biomass per unit area of *Sporobolus arabicus* and *Sesbania aegyptica* grown as mono-culture and mix-culture, with saline water irrigation.

SP = *Sporobolus arabicus* = [Mono-culture], S2 = *Sesbania aegyptica* (2 Plants) = [Mono-culture]
 S4 = *Sesbania aegyptica* (4 Plants) = [Mono-culture], SP + S2 = *S. arabicus* + *S. aegyptica* = [Mix-culture]
 SP + S4 = *S. arabicus* + *S. aegyptica* = [Mix-culture]

No significant reduction in the sugar content of *Sporobolus* was evident in response to salinity as compared to control when grown as mono- or mix-culture (Fig. 4A). *Sesbania* when planted as mono-culture, showed a proportional increase in its sugar content in response to salinity. Increase in the density of *Sesbania* plants (4 plants/0.6 m²) showed a 25.75% increase in sugar content when grown under controlled condition, as compared to low density (2 plants/0.6 m²). Sugar content of *Sesbania* plants when grown as an inter-crop remained high under control conditions, irrespective to their density per unit area with a marked reduction in response to low salinity. Analysis of variance (Table 1) confirms the validity of difference ($P < 0.05$) within treatments (T) only.

An increase of 8.57% in the protein content of *Sporobolus* in mono-culture was observed, when irrigation water of low salinity was used while at high salt level, it significantly decreased (Fig. 4B). In mix-culture, the protein content of *Sporobolus* proportionately decreased under control condition with increase in the density of *Sesbania* plants. At low density - low salinity, no change in protein content of *Sporobolus* was found, while at low density - high salinity, a reduction of 7.31% was observed. In contrast, at high density, an increase in protein content of salt stressed *Sporobolus* was observed, both at low and high salinity levels. Statistically these effects were found to be non-significant (Table 1). An increase in protein content of *Sesbania* plants in mono-culture was observed in response to increasing salinity and density. The inter-cropped *Sesbania* plants showed a proportional decrease in protein content with increase in salinity level as well as the density per unit area. Analysis of variance (Table 1) shows that interaction of cropping system (S) with density (DxS) and treatment (SxT) were highly significant ($P < 0.001$).

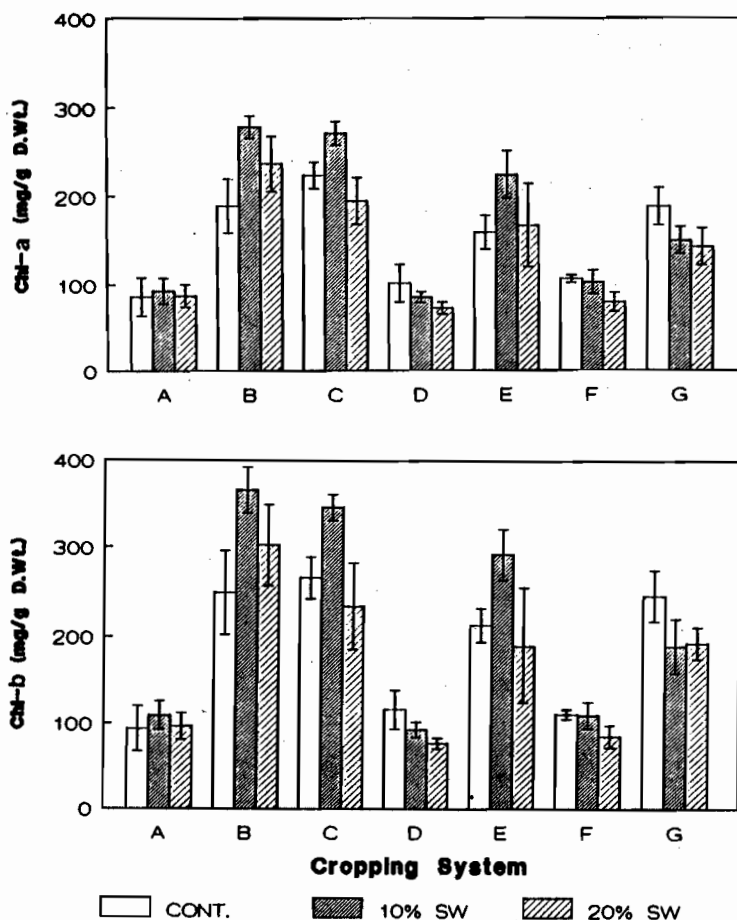


Fig.3. Chlorophyll "a" and "b" content of *Sporobolus arabicus* and *Sesbania aegyptica*, grown as mono-culture and mix-culture, with saline water irrigation. A-G, as figure 1.

Quantum yield of *Sporobolus* remained unchanged irrespective of salinity and cropping system (Fig. 5A). Increasing density of *Sesbania* plants showed no effect on quantum yield, irrespective to the cropping system, while inter-cropping and salinity showed a proportional increase in case of *Sesbania*.

No significant change in stomatal conductance was observed, when *Sporobolus* was grown as mono-culture under salt stress (Fig. 5B). *Sesbania* plants however, exhibited differential responses to increasing density and inter-cropping. In mono-culture at low density, there was a significant difference in the stomatal conductance at low salinity while at high salinity no change was observed. With increase in density of *Sesbania* plants, the rate of stomatal conductance also increased both under control and saline conditions. In mix-culture, *Sesbania* plants grown at low density, showed similar response to that of high-density monoculture. At high density, the stomatal conductance of intercropped *Sesbania* plants showed a similar trend to those grown as

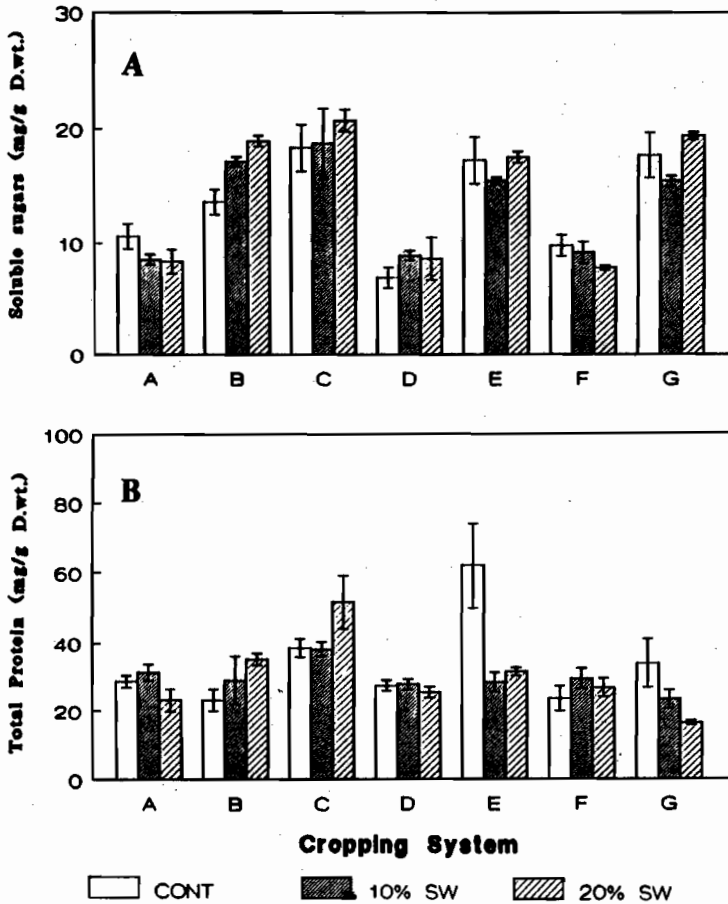


Fig.4. (A) Soluble sugars and (B) Total protein content of *Sporobolus arabicus* and *Sesbania aegyptica*, grown as mono-culture and mix-culture, with saline water irrigation. A-G as figure 1.

mono-culture and at low density. The responses of *Sesbania* to density (D) and cropping system (S) were non-significant (Table 1), however showing significant interactions ($P < 0.001$) for DxS and DxSxT. In the present study no significant effect on transpiration rate of *Sporobolus* in mono- and mix-culture was observed (Fig. 5C). However, transpiration rate of *Sesbania* significantly reduced with an increase in salinity of irrigation water in both cropping system and density of plants.

Discussion

In the present study a slight increase in biomass of *S. arabicus* at low salinity and no change at high salinity confirms its halophytic nature. Low density of *Sesbania* intercropped with *Sporobolus* showed no influence on the productivity of *Sporobolus*, which is an evidence of success of inter-cropping system under salt stress thus ensuring full production of grass component of the mix-culture. In contrast, high density of

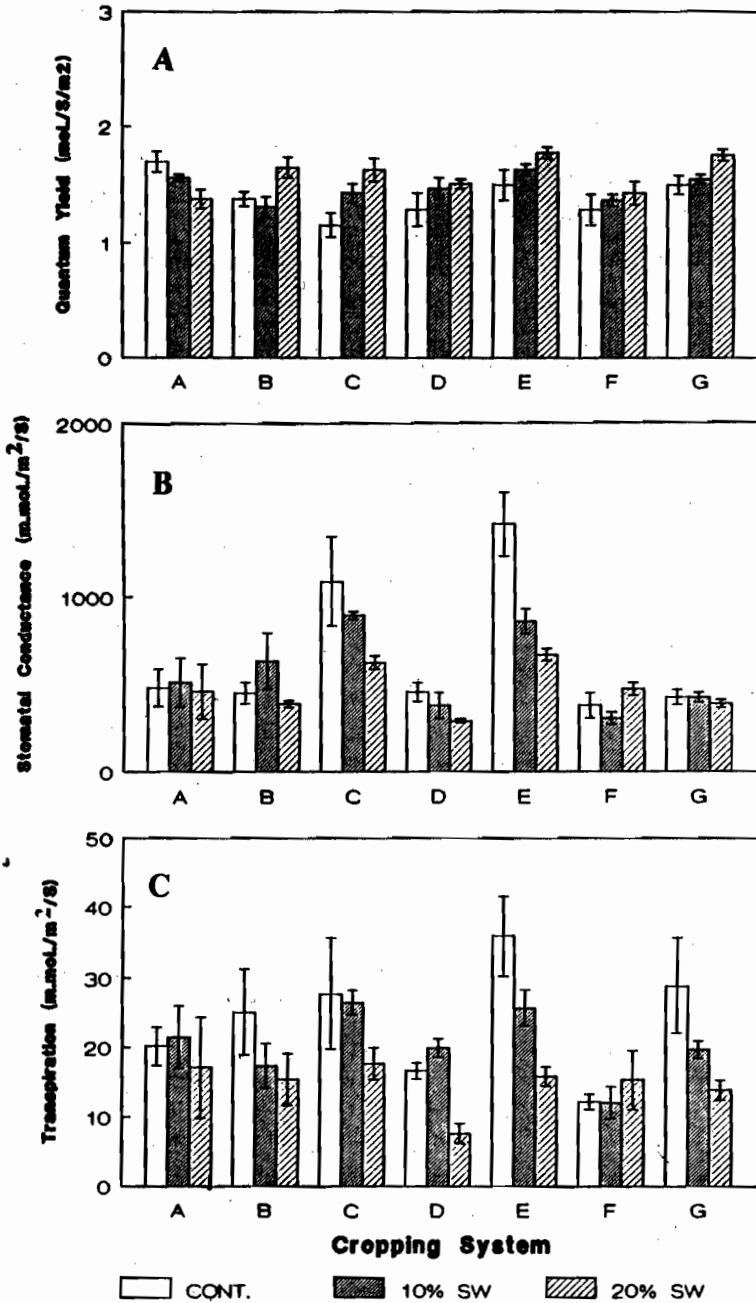


Fig. 5. (A) Quantum yield, (B) stomatal conductance and (C) transpiration rate of *Sporobolus arabicus* and *Sesbania aegyptica* grown as mono-culture and mix-culture, with saline water irrigation. A-G as figure 1.

Sesbania plants exhibited a negative influence over *Sporobolus* biomass at low salinity level. This decrease could be attributed to competition of plants for sunlight as observed in inter-cropping of sunhemp (*Crotalaria juncea*) with mungbean and cowpea as compared to inter-cropping with sitaro (*Macroptilum atropurpureum*), due to faster growth of sunhemp inducing a shade effect (Tengco, 1992).

There are reports that intercropping of *Sesbania grandiflora* with *Panicum maximum* exhibited high mortality of the legume with increase in cutting frequency (Ella & Gowa, 1992). In the present study, though no mortality of *S. aegyptica* was observed after cutting, greater reduction in growth at high salinity might be the result of cutting in addition to salt stress. Still, the net productivity per unit area remained higher in mix-culture(s). Such similar observations have been made by Tengco (1992), where total dry matter production per unit area significantly improved due to integration of food and forage crops as compared to their mono-cultures.

Variations in biochemical compounds is reported under all types of stress due to interference in plant metabolism. Slight reduction in chlorophyll content of both component crops was observed in response to salinity. The same trend have been noted for *Pisum sativum* and *Vigna mungo* (Garg & Garg, 1980; Ashraf, 1989). According to Strogonov (1964) the binding between chlorophyll's and chloroplast proteins depends upon the ion content of cells. Under high salinity such links are loosened and results in the destruction of chlorophyll molecules. Reduction in chlorophyll content of high density *Sesbania* plants could be considered as shade effect.

S. arabicus exhibited no change in sugar content of the leaf while *S. aegyptica* showed an accumulation of sugar with increase in salinity and density of plants per unit area, which is generally considered a character strategy of salt excluding plants (Shannon & Qualset, 1984). As a component of mix-culture, sugar content of *Sesbania* plants remained high under control, while at low salinity level, slight reduction in sugar content of leaf was evident, which could be attributed to breakdown of complex molecules for supply of metabolic energy for the maintenance of growth under moderate salt stress.

A change in protein content of salt stressed *Sporobolus* was observed when grown as mono- or mix-culture. Increase in protein content at low salinity may be due to the salinity induced enhancement of protein synthesis as observed for cereals (Langdale *et al.*, 1973) and/or excessive incorporation of N into proteins (Helal *et al.*, 1975). A reduction in protein content under high salt stress could be attributed to decreased synthesis and/or increase proteolysis as also reported by Eder *et al.*, (1977), Garg & Garg (1980) and lesser availability of amino acids as well as denaturation of enzymes involved in the synthesis of protein and amino acids.

Salinity is reported to inhibit photosynthesis in plants (Cheesman, 1988) due to stomatal closure, inhibition of biochemical reactions and/or feedback inhibition of carbon metabolism (Seeman & Critchley, 1985). The result of the present study would suggest that light reaction of photosynthesis was not significantly altered in *Sporobolus*, in response to salinity and cropping system, while *Sesbania* plants showed a positive response to salinity and inter-cropping with *Sporobolus*. This could be attributed to variations in chlorophyll content of two plant species under prevailing conditions.

Though a number of factors contribute to reduced growth of plants under salt stress, a reduction in stomatal conductance could be a possibility which may be due to partial stomatal closure or reduced size and density of stomata per unit leaf area (Warne *et al.*, 1990). It is interesting to note that *Sporobolus* showed no significant change in stomatal conductance in response to salinity and/or cropping system while *Sesbania* plants were found quite sensitive.

As a component of mix-culture, *Sporobolus* showed a marked reduction in transpiration with increase in salinity and density of *Sesbania* plants. This can be attributed to its competition with *Sesbania* for light, which directly affects the stomatal aperture as well the rate of transpiration. *Sesbania* plants showed marked decrease in transpiration rate with increase in salinity. Low transpiration may limit the delivery of excess salt present in soil solution to the shoot and thereby promote the survival of plants under stress (Flanagan & Jefferies, 1988). An increase in density of mono-cultured *Sesbania* showed marked increase in transpiration, thus water is in short supply with increase in density per unit area of *Sesbania* plants. An indirect evidence appeared to be reduced transpiration of *Sesbania* in mixculture with *Sporobolus*, where extensive network of grass roots may enhance the retention of water in sand medium. Reduced transpiration with no major loss in photosynthesis results in high water use efficiency (Flanagan & Jefferies, 1988; Warne *et al.*, 1990).

Acknowledgement

Funds provided by National Scientific Research and Development Board (NSRDB) vide grant No. BSC (121)/KUK/90, for the research work is gratefully acknowledged.

References

- Ahmad, R., S. Ismail and D. Khan. 1986. *Saline agriculture at coastal sandy belt* (Final Research Report), Coordinated Research Programme on Saline Agriculture. University of Karachi/PARC, pp. 103.
- Ashraf, M. 1989. The effect of NaCl on water relations, chlorophyll, and protein and proline contents of two cultivars of black gram (*Vigna mungo* L.). *Plant and Soil*, 119: 205-210.
- Cheeseman, J.M. 1988. Mechanisms of salinity tolerance in plants. *Plant Physiol.*, 87: 547-550.
- Eder, A., W. Huber and N. Sankhla. 1977. Interaction between salinity and ethylene in nitrogen metabolism of *Pennisetum typhoides* seedlings. *Biochem. Physiol. Pflanz.*, 171: 93-100.
- Ella, A. and B.P.T. Gowa. 1992. Effect of plant density and cutting frequency on the yield of four tree legumes and interplanted *Panicum maximum* cv. Riversdale. *ACIAR Forage Newsletter*, 21: 7-8.
- Flanagan, F.B. and R.L. Jefferies. 1988. Stomatal limitation of photosynthesis and reduced growth of the halophytic, *Plantago maritima* L., at high salinity. *Plant Cell Environ.*, 11: 239-246.
- Garg, B.K. and O.P. Garg. 1980. Sodium carbonate and bicarbonate induced changes in growth, chlorophyll, nucleic acids and protein contents in leaves of *Pisum sativum*. *Photosynthetica*, 14: 594-598.
- Helal, M., K. Koch and K. Mengel. 1975. Effect of salinity and potassium on the uptake of nitrogen and on nitrogen metabolism in young barley plants. *Physiol. Plant.*, 35: 310-313.
- Kass, D. C. L. 1978. *Polyculture cropping systems: Review and Analysis*. Cornell Int. Agric. Bull., 32: 1-69.
- Langdale, G.W., J.R. Thomas and T.G. Lettleton. 1973. Nitrogen metabolism of stargrass as affected by nitrogen and soil salinity. *Agron. J.*, 65: 468-470.
- Lowry, O. H., N.J. Rosebrough., A.L. Farr and R.S. Randall. 1951. Protein measurement with folin reagent. *J. Biol. Chem.*, 193: 265-275.
- MacLachlan, S. and S. Zalik. 1963. Plastids structure, chlorophyll concentration and free amino acid composition of chlorophyll mutant of barley. *Can. J. Bot.*, 41: 1053-1062.

- Santa-Cecilia, F. C. and C. Vieira. 1978. Associated cropping of beans and maize. I. Effects of beans cultivated with different growth habits. *Turrialba*, 28: 19-23.
- Seemann, J. and C. Critchley. 1985. Effects of salt stress on the growth, ion content, stomatal behaviour and photosynthetic capacity of a salt sensitive species, *Phaseolus vulgaris* L. *Planta*, 164: 151-161.
- Shannon, M.C. and C.O. Qualset. 1984. Benefits and limitations in breeding salt tolerant crop. *Calif. Agric.*, 38: 33-84.
- Strogonov, B.P. 1964. Physiological basis of salt tolerance of plants (as affected by various types of salinity). *Israel Progr. Sci. Transl.*, Jerusalem.
- Szabolcs, I. 1992. Salinization of soil and water and its relation to desertification. *Desertification Control Bulletin*, 21: 32-37
- Tengco, P.L. 1992. Increasing food and animal feed productions through integration of forage legumes in rice-based farming systems. *ACIAR Forage News Letter*, 22: 11-12.
- Warne, P., R.D. Guy, L. Rallins and D.M. Reid. 1990. The effect of sodium sulphate and sodium chloride on growth, morphology, photosynthesis and water use efficiency of *Chenopodium rubrum*. *Can. J. Bot.*, 68: 999-1006.
- Yemm, E. W. and J. Willis. 1956. The estimation of carbohydrate in plant extract by Anthrone. *Biochem. J.*, 57: 508.

(Received for Publication 8 March 1993)