

# INFLUENCE OF SOIL SALINITY ON MORPHOLOGY, RATE OF CARBON ASSIMILATION, PHOTOSYNTHETIC PRODUCTS AND ENZYME ACTIVITIES IN A SORGHUM HYBRID CSH-5

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## SUMMARY

Effect of different levels of soil salinity on growth, stomatal response,  $^{14}\text{CO}_2$  assimilation, products of steady state photosynthesis and activity of main photosynthetic enzymes in a sorghum hybrid were investigated. Soil salinity adversely affected photosynthetic area as well as rate of carbon assimilation. The lowered photosynthetic ability under salt stress conditions was due to stomatal closure inhibition of chlorophyll synthesis and the decreased activity of PEP and RuBP carboxylases. Excessive accumulation of malate during steady state photosynthesis could be correlated with the possible inhibition of malic enzyme. The reduction in yield was attributed to the low rate of photosynthesis and reduced synthesis of sugars. It is concluded that sorghum hybrid CSH-5 is salt sensitive and records negligible yield beyond soil salinity of 1.6 EC.

## INTRODUCTION

Sorghum hybrid CSH-5 is a popular cultivar of Maharashtra region but now exposed to problem of salinity, cropped up due to poor water management and indiscriminate use of chemical fertilizer. There are several reports about the decrease in net photosynthetic rate due to salt stress (Gale *et al.*, 1967; Gale and Poljakoff-Mayber, 1970; Chavan and Karadge, 1980). A  $\text{C}_3$  to CAM shift in salt stressed *Mesembryanthemum crystallinum* (Winter and Willert, 1972), a  $\text{C}_3$  to  $\text{C}_4$  shift of photosynthetic pathway in salt treated *Aeluropus littoralis* (Shomer-Ilan and Waisel, 1973) and *Parthenium hysterophorus* (Hegde and Patil, 1982) are well known. Salt stress brings an inhibition of carboxylating enzymes (Joshi and Naik, 1980), induction of glycolate pathway (Downton, 1977) and transamination reaction (Joshi, 1976; Kennedy, 1977) and depressed chloroplast metabolism (Reddy and Das, 1978).

In the present investigation, effect of natural salinity on crop growth, rate of  $^{14}\text{CO}_2$  assimilation, steady state products of photosynthesis and photosynthetic enzymes are assessed in sorghum hybrid CSH-5.

## MATERIALS AND METHODS

Sorghum cultivar CSH-5 was grown in a natural saline field situated at Digraj (50 km from Kolhapur). The situation of the field was such that it was having different salinity levels at different places. The plants were grown under different salinity conditions and analysed for their responses to salt stress. The salt concentration at the root zone level was 0.6 EC (treated as control) 1.6, 6.0 and 11.0 EC.

CO<sub>2</sub> assimilation rate was determined by feeding <sup>14</sup>CO<sub>2</sub> for 1 h to the detached leaves (Naik and Joshi, 1979) suspended in 50 mM Tris-HCl buffer pH 8.3 with NaH <sup>14</sup>CO<sub>3</sub> (spec. act. 1.80 MBq μs<sup>-1</sup> mol<sup>-1</sup>) at 1600 μEm<sup>-2</sup> s<sup>-1</sup> light intensity and at temperature not exceeding 28 C. The reaction was terminated with boiling ethanol (80% v/v). The leaves were homogenized and the insoluble material was successively extracted first with 80%, 60%, 40% ethanol (all v/v) and then twice with boiling water. The extracts were pooled and concentrated to a small volume by evaporating *in vacuo*. The amount of <sup>14</sup>CO<sub>2</sub> incorporated was determined by planting 100 μl aliquots of the combined extracts on planchet in a gas flow proportional counting system. The carbon assimilation rate was expressed as μmol CO<sub>2</sub> min<sup>-1</sup> mg<sup>-1</sup> chlorophyll. <sup>14</sup>C labelled compounds in ethanol extracts were separated and studied by two-dimensional paper chromatography and autoradiography (Benson *et al.*, 1950; Raghavendra and Das, 1977) on Whatman No. 1 chromatography paper. The radioactive spots were located by exposure to x-ray films and radioactivity on corresponding chromatographic paper bits was measured with a continuous gas flow proportional counter.

## Photosynthetic Enzymes

**Extraction.** Pre-chilled leaf material (2 g) was homogenised using pestle and mortar in a buffer containing 200 μM Bicine, 10 mM MgCl<sub>2</sub>, 170 mM 2-mercaptoethanol and 16 g/L PVP (Mw 10,000) at pH 8.3. The homogenate was squeezed through four layers of cheese cloth. An aliquot was set aside for chlorophyll estimation (Arnon, 1949). For enzyme assays the extract was centrifuged at 15,000 g for 20 min in a refrigerated centrifuge. The cleared sample was passed through Sephadex G 25 columns (1 × 6 cm), which had been pre-equilibrated with the above extraction medium. All the operations were carried at 0 to 4 C and the enzymes were assayed spectrophotometrically at 340 nm and 30 C (Kluge and Osmond, 1972).

## PEP Carboxylase (EC 4.1.1.31)

Carboxylation of PEP was coupled with reduction of the resulting oxaloacetate to malate via malate dehydrogenase. The assay contained in a final volume of 1 ml : 50 μM MES, 50 μM Bicine, 10 μM mercaptoethanol, 1 μM PEP, 0.083 μM NADH, 2 μM NaHCO<sub>3</sub>, 1 unit malate dehydrogenase, pH 6.5 and 0.1 ml enzyme.

**RuBP Carboxylase (EC 4.1.1.39)**

This was measured in a medium of 50  $\mu\text{M}$  MES, 50  $\mu\text{M}$  Bicine, 10  $\mu\text{M}$   $\text{MgCl}_2$ , 50  $\mu\text{M}$   $\text{NaHCO}_3$ , 1  $\mu\text{M}$  mercaptoethanol, 0.25  $\mu\text{M}$  NADH, 0.2  $\mu\text{M}$  RuDP, 6  $\mu\text{M}$  ATP, containing in 1 ml 4.5 units GAP dehydrogenase, pH 8.5 and 0.1 ml enzyme.

**NADP-malic Enzyme (EC 1.1.1.40)**

The assay contained in final volume of 1 ml : 50  $\mu\text{M}$  MES, 50  $\mu\text{M}$  Bicine, 10  $\mu\text{M}$   $\text{MgCl}_2$ , 10  $\mu\text{M}$  mercaptoethanol, 0.37  $\mu\text{M}$  NADP, 6  $\mu\text{M}$   $\alpha$ -malate and 0.1 ml enzyme.

Stomatal aperture was measured by taking nail paint impression from the leaf surface (Stoddard, 1965) and the chlorophylls were estimated by the method of Arnon (1949).

**RESULTS AND DISCUSSION**

Under salinity regime sorghum hybrid cultivar CSH-5 has shown a significant drop in height, total leaf area and dry matter production (Table I). The yield in terms of earhead weight is negligible beyond  $\text{EC}_e$  1.6  $\text{dS m}^{-1}$  (Fig. 1) and the productivity in the form of biomass has little use in terms of yield.

**TABLE I.** Effect of salinity on growth, total chlorophyll and carbon assimilation rate in sorghum hybrid CSH-5

Salinity level at root zone $\text{EC}_e$ $\text{dS m}^{-1}$	Height (cm)	Total leaf area ( $\text{cm}^2$ )	Dry matter (g/plant)	Total chlorophylls (mg/100 g fresh tissue)	Rate of $^{14}\text{CO}_2$ assimilation # mole $\text{min}^{-1}$ $\text{mg}^{-1}$ chlorophyll
0.6 (Control)	137.1 ( $\pm 15.2$ )	1642.7 ( $\pm 225.0$ )	21.55 ( $\pm 7.2$ )	247.7 ( $\pm 18.78$ )	42.43 ( $\pm 3.32$ )
1.6	67.86 ( $\pm 8.9$ )	618.30 ( $\pm 123.0$ )	7.89 ( $\pm 1.7$ )	205.4 ( $\pm 11.90$ )	37.44 ( $\pm 1.51$ )
6.0	30.64 ( $\pm 5.3$ )	115.18 ( $\pm 17.8$ )	0.91 ( $\pm 0.2$ )	160.63 ( $\pm 9.25$ )	19.2 ( $\pm 1.64$ )
11.0	19.02 ( $\pm 1.4$ )	30.35 ( $\pm 2.4$ )	0.33 ( $\pm 0.2$ )	87.5 ( $\pm 11.79$ )	8.31 ( $\pm 1.43$ )

Total chlorophyll content and the rate of  $^{14}\text{CO}_2$  fixation during 1 h of photosynthesis per unit chlorophyll was reduced by salinity (Table I). The low rate of fixation can be attributed to the possible accumulation of salt in photosynthesizing tissue causing incomplete opening of stomata (Fig. 2) and thereby bring constraints in the events. Salt-induced succulence might also decrease the photosynthetic rate, impeding the photosynthetic enzymes (Joshi and Karadge, 1979). As suggested by Ludlow (1980) that the osmotic adjustment forestalls stomatal closure in plants subjected to stress so also here stomatal closure is a possible adjustment of osmoticum of the cell (Fig. 2).

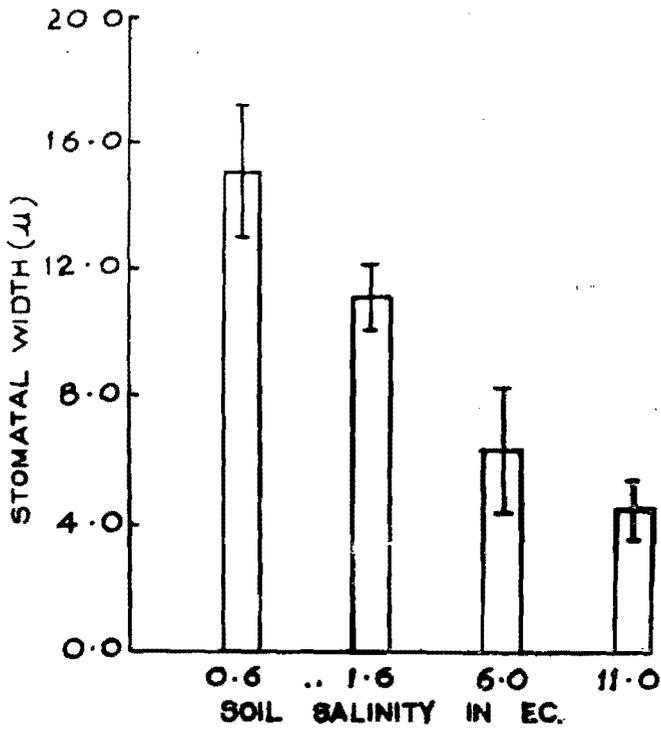


Fig. 1. Sorghum yield affected by soil salinity (0.6 ECe treated as a control).

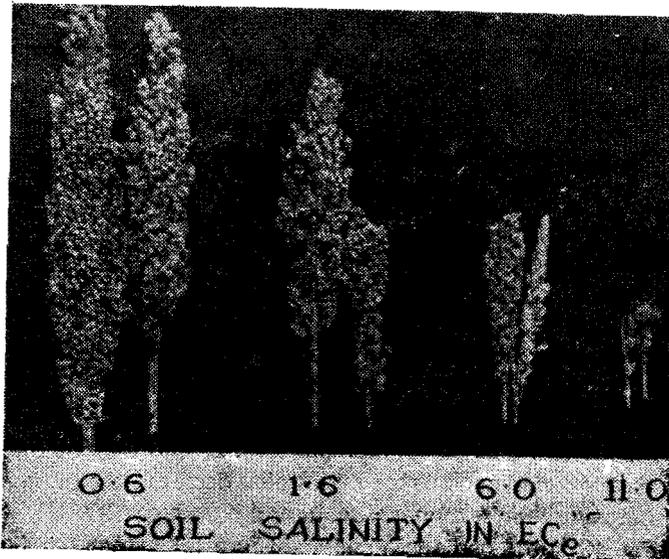


Fig. 2. Relation between stomatal aperture and salinity present at root zone level of sorghum hybrid CSH-5 (0.6 ECe treated as a control).

TABLE II. Effect of salinity on distribution of radioactivity in ethanol soluble compounds after one h light in the leaves of hybrid sorghum CSH-5

Compounds	Control (0.6)	Salt concentration in EC <sub>e</sub> at root zone level		
		1.6	6.0	11.0
PEP + PGA	1.99	2.1	3.87	10.89
SMP	4.20	5.12	—	—
SDP	1.25	3.81	—	2.19
<b>Total sugar phosphates</b>	<b>7.44</b>	<b>10.94</b>	<b>3.87</b>	<b>13.08</b>
Sucrose	22.43	10.94	7.32	7.80
Glucose	24.58	2.05	2.18	—
Fructose	1.07	1.00	—	—
<b>Total sugars</b>	<b>50.08</b>	<b>13.99</b>	<b>9.50</b>	<b>7.80</b>
Citrate	6.32	15.55	2.77	2.00
Malate	19.01	43.97	43.65	14.22
Glycolate	0.12	0.21	3.45	—
Glyoxylate	0.48	1.16	2.24	5.47
Glycerate	0.43	—	1.74	2.45
Fumarate	4.54	1.09	5.22	6.96
Ascorbate	0.17	—	1.34	1.22
Succinate	1.99	0.35	5.25	1.67
<b>Total organic acids</b>	<b>33.06</b>	<b>62.33</b>	<b>65.66</b>	<b>33.99</b>
Aspartate	0.35	2.12	3.79	2.93
Glutamate	0.63	1.76	1.65	3.22
Glutamine	—	—	—	4.48
Gly + Ser	3.82	0.31	4.63	3.93
Alanine	0.95	2.36	1.12	0.93
P. alanine	0.43	0.24	2.24	4.00
Valine	0.17	1.41	1.51	7.09
Leucine	0.32	0.42	2.36	5.61
<b>Total amino acids</b>	<b>6.87</b>	<b>8.62</b>	<b>17.30</b>	<b>40.29</b>
Unknown	2.00	3.67	2.00	—
Origin	0.54	0.35	1.54	4.29

Values are expressed as % of radioactivity counted on chromatogram.

The distribution of radioactivity in various compounds after steady state (1 h) of photosynthesis is presented in Table II. In control plant after one h of photosynthesis, the initial products of carbon assimilation, malate, aspartate and alanine are metabolically utilized for the synthesis of sugars and sugarphosphates. As the concentration of salt increases the accumulation of malate takes place and hence there is an increase in organic acid pool. This indicates inhibition of decarboxylating machinery which can be attributed to inhibition of malic enzyme (Table III). Similar observations have been reported by Joshi and Karadge (1979) in *Portulaca oleracea* and Hegde and Patil (1982) in *Parthenium hysterophorus*. It is also evident (Table II) that higher salt concentration inhibits the utilization of PEP + PGA.

TABLE III. Effect of salinity on the activities of PEPCase, RuBPCase and NADP malic enzyme in the leaves of sorghum hybrid CSH-5

Salinity level at root zone EC <sub>e</sub> dS m <sup>-1</sup>	PEPCase	RuBPCase	NADP-ME
0.6 (Control)	250.0 (±5.2)	40.5 (±2.75)	143.2 (±4.2)
1.6	209.0 (±3.7)	19.23 (±1.08)	57.5 (±3.1)
6.0	130.0 (±2.1)	10.5 (±0.9)	17.5 (±2.1)
11.0	91.2 (±1.8)	4.9 (±0.78)	7.2 (±1.4)

Values expressed as  $\mu\text{ mol h}^{-1} \text{ mg}^{-1}$  chlorophyll.

Similar observation has also been made in corn (Nieman and Clark, 1976). This accumulation may be due to the reason that the reducing power is not generated that fast in the bundle sheath chloroplasts. Downton and Pylotis (1971) have stated that bundle sheath chloroplasts of sorghum are agranal and that the agranal bundle sheath chloroplasts lack photosystem II. They cannot generate reducing potential photochemically. Further the accumulation of salt in photosynthetic tissue may possibly be responsible for slow utilization of PEP + PGA. This salt accumulation can inhibit reducing power (Joshi *et al.*, 1962; Gee *et al.*, 1965).

More label in alanine (9%) at 11.0 EC<sub>e</sub> indicates the disturbed metabolic process or it may be either not utilized or it is added via other routes. Thomas and Long (1978) while studying temperate salt water marsh plant *Spartina townsendii* have reported that the radioactive alanine is most probably derived from 3-PGA through glycolytic enzymes or it may also be derived from the C<sub>4</sub> acids after randomisation of <sup>14</sup>C through fumarate. It is also possible that the conversion of alanine into pyruvate for TCA cycle is not very much in the picture at higher concentration of salt. The results in Table II clearly depict that high salt stress results in inhibition of sugar synthesis and hence more amino acid formation. Reduction in sugar synthesis is an indication of the lowered photosynthetic ability of the plant. This lowered photosynthetic ability of the plant can be correlated with the decreased activity of the photosynthetic enzymes (Table III). Sankhala and Huber (1974) have studied the effect of NaCl salinity on the activities of PEP and RuBP carboxylases in *Pennisetum typhoides* and observed inhibition of the activity of these enzymes and thereby the decrease in <sup>14</sup>CO<sub>2</sub> fixation rate.

Strogonov (1964) has elucidated mechanism of salt stress and metabolic disturbances. He suggested that more amino acid synthesis leads to accumulation and synthesis of toxic metabolites which ultimately induce salt injury. From Table II it seems that 6.0 and 11.0 EC<sub>e</sub> are the toxic levels for sorghum. Appreciable label

in organic acid fraction, citrate, malate, succinate and fumarate is indicative of disturbed operation of Krebs cycle in the tissue. Increased labels in leucine, phenylalanine and valine at 6.0 and 11.0 EC<sub>e</sub> show that toxicity has set in. Incorporation of label in glycine + serine, glycolate, glyoxylate and glycerate could be accounted for the increased photorespiration induced by salt stress.

Salinity has caused considerable decline in the incorporation of radioactivity in sucrose which suggests that salt affects the carbohydrate metabolism. According to Downton (1977) under salt stress condition, impaired carbon flow through the glycolate pathway would effectively decrease the rate of sucrose formation.

Thus, it appears that sorghum hybrid CSH-5 is a salt sensitive cultivar and when grown in saline soil, the photosynthetic ability of the plant is inhibited and thereby the basic <sup>14</sup>C utilization is disturbed. This ultimately results in reduction in sugar synthesis and low yield.

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