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SHORT COMMUNICATION

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Physio-biochemical attributes in two cultivars of mulberry (*Morus alba* L.) under $NaHCO_3$ stress

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Abstract

The purpose of this study was to evaluate the physiological and biochemical changes that occur in mulberry (cv. local and Sujanpuri) plants under alkaline conditions in order to understand the response of these cultivars to alkalinity. Mulberry plants were subjected to different treatments using $NaHCO_3$ and after 20, 40 and 60 days, different physiological and biochemical parameters were studied. Local cultivar was found to tolerate salt stress more than sujanpuri.

Keywords: Alkaline; Biochemical changes; Mulberry.

Introduction

Mulberry (*Morus alba* L.) belongs to family Moraceae, is a multipurpose woody perennial, deciduous tree and is mainly cultivated for sericulture. Salt problem is considered as one of the most important environmental factors, limiting plant growth and productivity of arid and semiarid regions.

During stress conditions plants need to maintain internal water potential below that of soil and maintain turgor and water uptake for growth. This requires an increase in osmotica, either by uptake of soil solutes or by synthesis of metabolic (compitable) solutes. One of the most common stress responses in plants is overproduction of different types of compitable organic solutes e.g. proline, protein, sugar etc. (Ahmad et al., 2006; Ahmad et al., 2008; Ahmad and Sharma, 2008).

The accumulation of proline is thought to function as osmoprotectant and is a unique plant response to environmental stress. Soluble proteins and sugars have also been shown to accumulate under salt stress (Ahmad and Jhon, 2005). Several biochemical processes are affected by salinity, particularly nitrate assimilation and nitrate reductases activity (NRA) (Ahmad and John, 2005). The present study was performed to evaluate the effects of salt stress on growth, water relations and gas exchange of two mulberry cultivars.

Materials and Methods

Plant material and $NaHCO_3$ treatment

Nodal cuttings of *Morus alba* (cv. local and Sujanpuri) were planted in pots containing soil and sand (3:1 proportion). Before planting, the required pH was attained in the soil by applying 0 (T1), 200 mM (T2), 300 mM (T3) and 400 mM (T4) of $NaHCO_3$. The pots were watered daily and kept in Micro model (botanical garden) IIT-Delhi, under natural photoperiod of 12 to 13 h and temperature of 28±4 °C. Data were collected on 20, 40 and 60 days after stress.

Measurements of net CO_2 assimilation rate (A) and transpiration (E) rate were made on a fully expanded youngest leaf of each plant using an open system LCA-4 ADC portable infrared gas analyzer (Analytical Development Company, Hoddesdon, England). Leaf relative water content (RWC) was measured in fully expanded leaves. The relative water content (RWC) was calculated by the following formula:

RWC (%) = (FW-DW/TW-DW) X 100

Total free amino acids were determined following Hamilton and Van Slyke (1943). Proline concentration was determined by the method of Bates et al., (1973). Soluble protein content in the leaves was estimated by the method of Bradford (1976) after precipitation with trichloric acetic acid, using bovine serum albumin as standard. Sugar was estimated by the method of Dey (1990). NRA was determined according to Heuer and Plaut (1978).

Results and Discussion

The addition of $NaHCO_3$ to the soil causes increase in pH which caused a progressive reduction in the survival percentage, fresh and dry weight of mulberry cultivars in the present study (Figure 1). NaCl reduced the cell division and cell elongation which in turn inhibits growth (Yasseen et al., 1987).

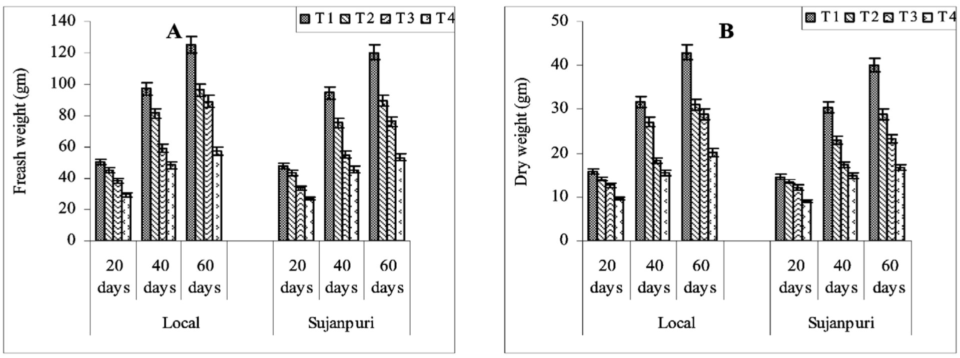

Figure 1. Effect of different concentration of $NaHCO_3$ on (A) fresh weight (gm) and (B) dry weight (gm) Values are significantly different p<0.05 from control (DMRT). Values are means ± S.E (n = 5).

Significant reduction was found in Net CO_2 assimilation rate (A), transpiration rate (E) and stomatal conductance (g_s) and the results are depicted in Figure 2 (A-C). The lower net CO_2 assimilation rate was also demonstrated by Ashraf and Iram (2005) in drought sensitive species *Phaseolus vulgaris* than *Sesbania aculeata*. Stomatal conductance has long been considered an important selection criterion for salt resistance (Quarrie and Jones, 1979). Higher stomatal conductance in plants increases CO_2 diffusion into leaf thereby favoring higher photosynthetic rates. The accumulation of salts triggers a transient water deficit which induces an increase in ABA accumulation and causes stomatal closure (Aldesuquy and Ibrahim, 2001).

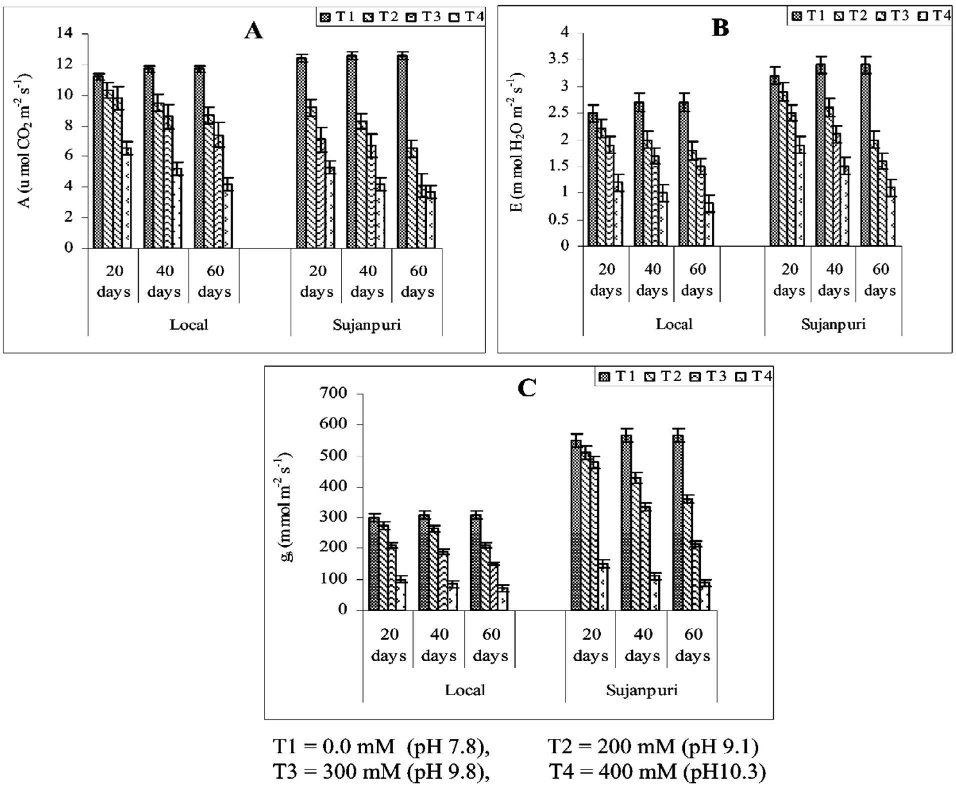

Figure 2. Effect of different concentration of $NaHCO_3$ on (A) Net CO_2 assimilation rate (A), (B) Transpiration rate (E) and (C) Stomatal conductance (g_s). Values are significantly different $P<0.05$ from control (DMRT). Values are means ± S.E (n = 5).

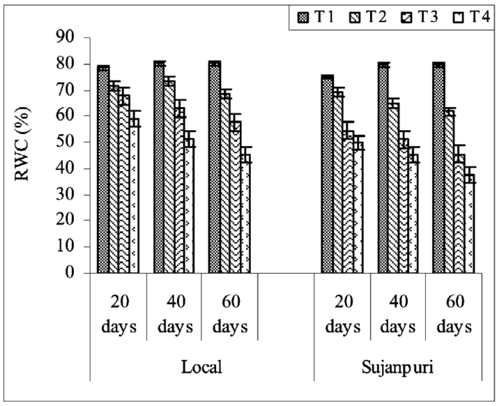

Figure 3. Effect of different concentration of $NaHCO_3$ on Relative water content (%).Values are significantly different *P*<0.05 from control (DMRT). Values are means ± S.E (n=5).

A maximum decrease of 43.3% and 52.8% decrease in RWC was observed in local and Sujanpuri respectively after 60 days of stress at T4 concentration (Figure 4). Other reports also suggest that leaf RWC was decreased by salt stress in *Pisum sativum* (Ahmad and John, 2005) and *Brassica juncea* (Ahmad, 2009). Reduction in leaf RWC indicates loss of turgor that resulted in limited water availability for cell extension process (Katerji et al., 1997).

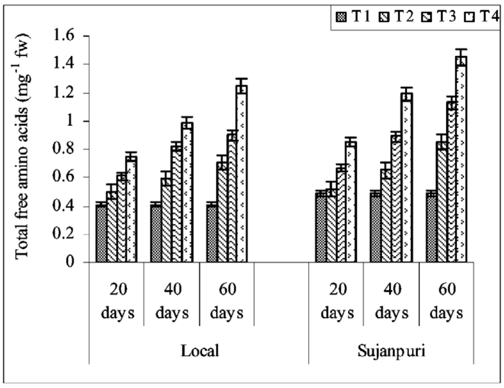

Figure 4. Effect of different concentration of $NaHCO_3$ on Total free amino acids. Values are significantly different *P*<0.05 from control (DMRT). Values are means ± S.E (n = 5).

Results of increased content of amino acids in salt sensitive cultivars of mulberry (sujanpuri) in present study (Figure 5) corroborates with the findings of Ashraf and Iram (2005) who also reported that more increase of free amino acids was observed in the leaves and nodules of drought sensitive *Phaseolus vulgaris* as compared to tolerant *Sesbania aculeata.*

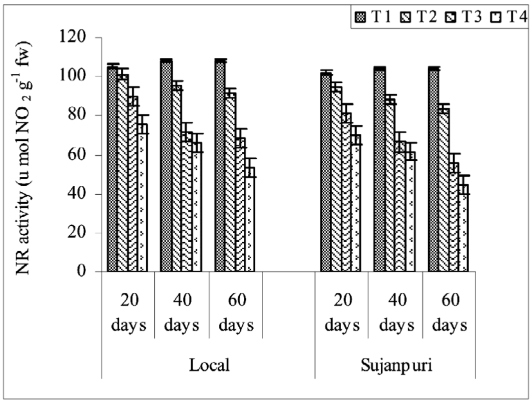

Figure 5. Effect of different concentration of $NaHCO_3$ on NR activity. Values are significantly different $p<0.05$ from control (DMRT). Values are means ± S.E (n = 5).

Proline increased with increasing concentration of NaHCO3 in present study (Table 1). Misra and Gupta (2005) reported an increase in proline content in two high yielding genotypes of green gram (*Phaseolus aureus*) under NaCl stress. A positive correlation between magnitude of free proline accumulation and salt tolerance has been suggested as an index for determining salt tolerance potentials between cultivars (Ramanjula and Sudhakar, 2001).

The protein content increased with the increase in $NaHCO_3$ in both the cultivars of mulberry plants as compared to control but the concentration was higher in local than Sujanpuri cultivar (Table 1). A higher content of soluble proteins has also been observed in salt tolerant cultivars of mulberry (Ahmad et al., 2006) and rice (Pareek et al., 1997). Proteins that accumulate in plants grown under saline conditions may provide a storage form of nitrogen that is re-utilized when stress is over and may play a role in osmotic adjustment.

$NaHCO_3$ stress caused a significant increase in levels of soluble sugar in both the cultivars at all stress level (Table 1). The accumulation of soluble carbohydrates in plants has been widely reported as a response to salt stress (Ahmad and Jhon, 2005).

The maximum decrease in NR activity of 50.6% and 57.2% in local and Sujanpuri respectively was observed after 60 days at the concentration of T4 (Figure 6). NR is sensitive to salt ions and is an indicator of the damaging effects of salt. Salt stress leads to nutritional imbalance which resulted in inactivation of enzymes such as NR (Ghosh et al., 2001).

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Table 1. Effect of different concentrations (T1, T2, T3, T4) of $NaHCO_3$ on proline (µg/g fw), protein (mg/g fw) and soluble sugar (mg/g fw) content in two varieties of *Morus alba* L.

Genotype	$NaHCO_3$ conc.	No. of Days								
		20 days			40 days			60 days		
		Proline	Protein	Sugar	Proline	Protein	Sugar	Proline	Protein	Sugar
Local	T1	22.7 ± 0.8^{a}	18.2 ± 0.15^{a}	20.1 ± 0.6^{a}	22.7 ± 0.6^{a}	18.2 ± 0.15^{a}	20.1 ± 0.6^{a}	22.7 ± 0.8^{a}	18.2 ± 0.15^{a}	20.1 ± 0.6^{a}
	T2	27.2 ± 0.03^{b} (119)	19.0 ± 0.17^{a} (104)	20.7 ± 0.19^{a} (103)	37.2 ± 0.3^{b} (163)	21.3 ± 0.2^{ab} (116)	21.4 ± 0.21^{a} (106)	48.7 ± 1.4^{b} (214)	22.1 ± 0.24^{ab} (121)	22.1 ± 0.6^{a} (110)
	T3	54.1 ± 1.9^{c} (238)	22.4 ± 0.21^{b} (122)	23.4 ± 0.2^{ab} (116)	69.5 ± 0.41^{c} (305)	27.2 ± 0.22^{c} (149)	27.8 ± 0.25^{b} (138)	85.4 ± 1.5^{c} (376)	30.2 ± 0.31^{c} (165)	30.2 ± 0.41^{b} (150)
	T4	71.2 ± 1.5^{d} (313)	22.0 ± 0.2^{b} (120)	23.2 ± 0.2^{ab} (115)	90.5 ± 0.29^{d} (398)	26.8 ± 0.25^{c} (147)	26.1 ± 0.26^{b} (129)	130.2 ± 3.6^{d} (573)	30.1 ± 0.3^{c} (165)	30.0 ± 0.35^{b} (149)
Sujanpuri	T1	18.8 ± 0.24^{a}	16.9 ± 0.13^{a}	17.9 ± 0.21^{a}	18.8 ± 0.16^{a}	16.9 ± 0.17^{a}	17.9 ± 0.18^{a}	18.8 ± 0.5^{a}	16.9 ± 0.11^{a}	17.9 ± 0.14^{a}
	T2	27.4 ± 0.4^{b} (145)	17.7 ± 0.11^{a} (104)	$18.2 \pm 0.13_{a}$ (101)	31.3 ± 0.31^{b} (165)	18.5 ± 0.16^{a} (108)	18.9 ± 0.15^{a} (105)	38.7 ± 0.5^{b} (204)	19.1 ± 0.2^{ab} (112)	19.1 ± 0.36^{a} (107)
	T3	32.2 ± 0.43^{c} (170)	20.3 ± 0.3^{b} (119)	20.7 ± 0.14^{b} (115)	41.5 ± 0.26^{c} (219)	22.5 ± 0.21^{b} (132)	23.4 ± 0.14^{b} (130)	57.9 ± 1.2^{c} (306)	25.4 ± 0.3^{c} (149)	25.4 ± 0.35^{b} (142)
	T4	43.1 ± 0.5^{d} (228)	20.0 ± 0.25^{b} (109)	21.2 ± 0.16^{b} (118)	67.3 ± 0.31^{d} (356)	22.3 ± 0.15^{b} (131)	22.3 ± 0.21^{b} (124)	82.8 ± 2.2^{d} (438)	25.3 ± 0.4^{c} (149)	25.3 ± 0.31^{b} (141)

Different letters indicate significant difference between means at $P<0.05$ (DMRT). Values are means ± S.E (n = 5). Figures in parentheses indicate percent of control.

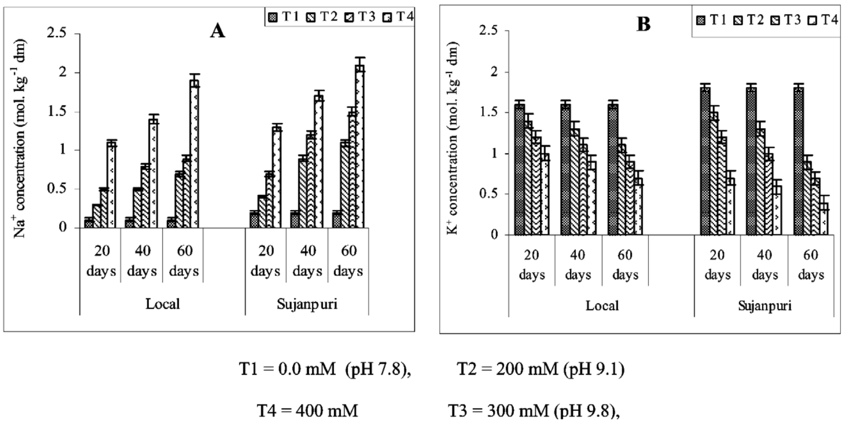

Figure 6. Effect of different concentration of $NaHCO_3$ on (A) Na^+ concentration (mol. Kg^{-1} DM) and (B) K^+ concentration (mol. Kg^{-1} DM). Values are significantly different $p<0.05$ from control (DMRT). Values are means ± S.E (n = 5).

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