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Analysis of the Phytochemical Composition of Leaves of Six Superior Salt-Tolerant Mulberry Germplasm Grown Under Coastal Saline Soils of South 24 Parganas District of West Bengal, India

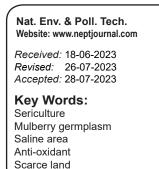
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ABSTRACT

The nutritive value of mulberry leaves makes it the only food of silkworms (Bombyx mori L.). It is recorded that 6.73 million hectares of area are affected by salinity and sodicity stresses covering various states of the country, which is becoming one of the major threats to popularizing sericulture in India. In the present study, chlorophyll, protein, catalase, peroxidase, and superoxide dismutase content of leaves of six mulberry germplasm viz., English Black, Kolitha-3, C776, Rotundiloba, BC₂59, and S1 grown under coastal saline soils of South 24 Parganas district of West Bengal, India was investigated. Results demonstrated a sharp decrease in the chlorophyll (2.35 to1.19 mg.g FW⁻¹) and protein (30.10 to 15.20 mg g FW⁻¹) contents of leaves of all the mulberry germplasm with increasing soil salinity (1.60 to 22.70 dS.m⁻¹). On the contrary, the number of stress-related antioxidant enzymes like catalase, peroxidases, and superoxide dismutase increased from 1.15 to 5.43, 1.43 to 4.76, and 8.65 to 25.15 g⁻¹ FW.min⁻¹, respectively. Overall, the field study indicated the superiority of Kolitha-3 and C776 grown in Canning (Canning I and II), Basanti, Namkhana, Kakdwip, and Sagar blocks of coastal regions of South 24 Parganas. The study deals with issues of the utilization of scarce land promoting income-generating avenues like sericulture in saline areas.

INTRODUCTION

The current state of competition for rapid urbanization and industrialization adversely brings an alarming rate of natural resource degradation along with biodiversity loss (Singh & Singh 2017), which has necessitated a paradigm of conversion of the conventional ways and means of agricultural production. The continued land degradation due to increased incidences of soil salinity becomes one of the major threats to agricultural sustainability (Sharma & Singh 2015). For instance, it is estimated that there is around 1128 m ha area in the world which is affected by salinity and sodicity stresses (Wicke et al. 2011). The rise in the sea level, the consequent increase in salt intrusion, and the increased frequency of cyclonic storms negatively impacted the productivity of coastal agroecosystems (Yeo 1998). In the coastal or deltaic regions, the salinity of soils occurs mainly by salts that contaminate the freshwater supply through the intrusion of seawater. Coastal agriculture may also be affected by saline aerosols, which are produced by violent wave activity during storms or heavy winds on the sea surface. These salts can also move inland up to a significant distance, but the most harmful effects occur on vegetation or crops grown near shore (Grieve et al. 2012). The state of West Bengal has the highest proportion of areas susceptible to salinity (0.82 million hectares) under coastal saline lands, with more than 50% of the coastal stretch of the state West Bengal confined primarily to the districts of South 24 Parganas (Bhowmick et al. 2020). Therefore, it is crucial to consider a wider perspective to address the concerns of optimal use of limited land resources while encouraging more avenues of income for those who live in salinity-prone areas.

The resilient, deeply rooted, and perennial mulberry tree is used to produce raw silk. Its leaves are fed to the monophagous silkworm *Bombyx mori* L. The sericultural flora and fauna of India are very diverse. The national repository has 1317 different types of mulberry germplasm (CSGRC Annual Report). Despite the immense potential for sericulture to be explored for economic growth, India has not

used its full potential to meet the rising local demand for the silk industry during the past few decades. Alongside that, the exploitation of mulberry in the food and pharmaceutical industries is also mere in our country, though there are a plethora of opportunities. The main challenge to the spread of moriculture in India is the degradation of land caused by soil salinity.

Salinity-induced stress results in many physiological and biochemical impairments in plants, such as the reduced amount of photosynthate, ionic imbalance, and oxidative injury to proteins (enzymes) (Zhu 2001, Xiong & Zhu 2002, Muchate et al. 2016). Salinity also affects mulberry plants in various ways. The first visible symptom of salt injury in mulberry is the appearance of yellow patches (Vijayan et al. 2008), which occur due to chlorophyll depletion by increasing the activity of the chlorophyllase enzyme (Singh & Singh 1999). Protein concentrations in the leaves of mulberry grown under salinity decline significantly (Vijayan et al. 2008) due to the breakage of electrostatic bonds and an increase in hydrophobic interactions (Melander & Horvath 1977). The adverse effect of salinity on the rate of photosynthesis is also found in mulberry plants like other woody plants (Kumar et al. 2003, Vijayan et al. 2008). Simultaneously, to defend themselves against oxidants, mulberry has developed specialized defense mechanisms that involve enzymes and antioxidant molecules (Sudhakar et al. 2001).

In woody plant species such as Thai neem (Cha-um et al. 2004), olive (Marin et al. 1995), pine (Khasa et al. 2002), and mulberry (Hossain et al. 1991, Tewary et al. 2000, Vijayan et al. 2003, 2008, Jhansilakshmi et al. 2016), screening for salt tolerance has been studied. The biochemical traits have been investigated in different salt-tolerant and saltsusceptible mulberry germplasm to study the mechanism of salinity tolerance at an early stage of growth (Vijayan et al. 2008). It has been shown that an increase in the electrical conductivity (EC) of an experimental soil substrate led to a detrimental effect on the productivity of mulberry, including reduced leaf protein content, chlorophyll fluorescence, and an extremely elevated level of antioxidants (Vijayan et al. 2008, Sudhakar et al. 2001).

In the recent past, the effectiveness of mulberry germplasm against salinity has been realized, and a few salinity-resistant and high-yielding mulberry germplasm have been identified (Tewary et al. 2000, Vijayan et al. 2003, 2008, Jhansilakshmi et al. 2016). The purpose of this study is to comprehend how the physicochemical characteristics of six different salt-tolerant mulberry germplasm in the soil of coastal blocks of the South 24 Parganas district respond. The study's findings are anticipated to assist the dual purposes of wasteland management and sericultural farm output while debilitating the impacts of increasing soil salinity, particularly in coastal areas.

MATERIALS AND METHODS

Study Area

The study was conducted along the coastal regions of South 24 Parganas district (located between latitudes 21°88' N and 22°16' N and longitudes 88°11' E and 88°82' E) in West Bengal, India. Separate field tests with salinity stresses ranging from 18 to 23 dS.m⁻¹ were carried out in the five experimental farms of Canning I and II, Basanti, Namkhana, Kakdwip, and Sagar. A brief profile of the study area is given in Table 1.

Plant Materials and Experimental Setup

For field testing, the salt-tolerant mulberry germplasms English Black, Kolitha-3, C776, Rotundiloba, BC₂59, and S1 were chosen (collected from the Central Sericultural Research and Training Institute, Berhampore, West Bengal, India). Details of the exploited germplasm are given in Table 2. Three replications of the trials were used and compared to plants grown in non-saline conditions (EC between 1.60 dS.m⁻¹) using a randomized complete block design (RCBD). The size of each plot was $4 \text{ m} \times 4 \text{ m}$, while the distance between plants was maintained at 0.9 m. The plantation was done through stem cuttings in March, whereas the performance of the crop was evaluated after the 60th day of pruning in the first week of May. Recommended agronomical practices were followed throughout the growth period (Datta 2000).

Table 1: Brief profile of the study area. Data collected from Department of Planning and Statistics, Govt. of West Bengal. http://www.wbpspm.gov.in/ SiteFiles/Publications/13_21062017112440.pdf/(accessed 16 June 2023).

Blocks	Total area [sq. km]	Net area under cultivation [ha]	Saline Area [%]	Salinity (EC) [dS.m ⁻¹]	Agricultural workers [%]
Canning I and II	402.80	31610	18	18.04	55.53
Basanti	404.21	26151	19	19.54	74.02
Namkhana	370.61	16910	21	20.69	63.81
Kakdwip	252.74	15973	20	21.08	53.26
Sagar	282.11	17436	23	22.70	73.95

The Physiological Study

For all biochemical studies, leaf samples were collected on the 60th day of the pruning. The 5th leaf from the top of each twig was used for the study (Vijayan et al. 2003).

Leaf Chlorophyll Content Assay

Leaf tissues of 100 mg were suspended in 10 mL of 80% acetone, mixed well, and kept at 4°C overnight in the dark. The supernatant was withdrawn after centrifugation at 5000 rpm (Remi PR-24), and absorbance was recorded at 663 and 645 nm in a spectrophotometer (Shimadzu, UV-1700). The amount of chlorophyll was calculated according to the equation given below, as mentioned by Arnon (1949).

Chlorophyll a $[\mu g.mL^{-1}] = 12.7 (A_{663}) - 2.69 (A_{645})$ Chlorophyll b $[\mu g.mL^{-1}] = 22.9 (A_{645}) - 4.68 (A_{663})$ Total chlorophyll $[\mu g.mL^{-1}] = 20.2 (A_{645}) + 8.02 (A_{663})$

Leaf Protein Assay

For estimating the total soluble proteins, the Lowry et al. (1951) method has been adopted. A 100-mg leaf sample was ground in 10% trichloroacetic acid. The TCA solution was removed by centrifugation (Remi PR-24), and 1N NaOH solution was added to the precipitated proteins. After thorough mixing, it was boiled at 65°C for 10 minutes in a water bath and then centrifuged. The supernatant liquid was taken for protein estimation. The assay mixture, containing 1 mL of protein solution and 3 mL of alkaline solution (50 mL of solution containing 2% sodium carbonate in 0.1 N NaOH and 2 mL of 1% CuSO₄.5H₂O in 1% sodium potassium

Table 2: A detailed list of mulberry germplasm utilized in the study.

tartrate), was allowed to stand for 10 min. Finally, 0.7 mL of folin-phenol reagent was added rapidly. The reaction was allowed to take place for a period of 30 min, and the blue color developed in the assay mixture was measured at 660 nm in a spectrophotometer (Shimadzu, UV-1700) against a blank containing sodium hydroxide instead of protein solution. The protein was estimated by using the standard curve, which was prepared by plotting the percentage transmittance against the standard protein concentration of bovine serum albumin.

Study of the Antioxidant Enzymes from Leaves

Catalase and Peroxidase: The leaf tissues, weighing about 200 mg, were homogenized with 10 mL of phosphate buffer pH 6.8 (0.1 M) and then centrifuged at 4°C for 15 min at 5000 rpm in a cold centrifuge (Remi CM-12 Plus). The clear supernatant was taken as the enzyme source. The activity of catalase, as well as peroxidase, has been assayed following the method of Chance and Maehly (1955). Three milliliters of the assay mixture for the catalase activity comprised 1 mL of 0.1% H₂O₂ and 2 mL of the enzyme extract. After incubation at 25°C for 15 min, the reaction was stopped by adding 3 mL of 5% (v/v) H_2SO_4 , and the residual H_2O_2 was titrated against 0.01 N KMnO₄ until a faint purple color persisted for at least 15 sec. A control was run at the same time in which the enzyme activity was stopped at "zero" time. One unit of catalase activity is defined as the amount of enzyme that breaks down 1 g of tissue per minute.

Five milliliters of the assay mixture for the peroxidase activity comprised 125 μ moles of phosphate buffer, pH 6.8, 50 mmoles of pyrogallol, 50 μ moles of H₂O₂, and 1 mL of the 20 times-diluted enzyme extract. This was incubated for

Sl. No.	Germplasm	Accession No.	Species	Origin	Salient feature
1.	English Black	ME-0004	<i>Morus latifolia</i> Poir.	Exogenous (France)	Branching semi-erect; homophyllous, deeply green, ovate-shaped leaves with a dentate edge.
2.	Kolitha-3	MI-0108	Morus alba L.	Indigenous	Semi-erect, straight-branched with heterophyllous, deep-ovate leaves and serrated margins.
3.	C776	MI-0158	Morus indica L.	English Black × M. multicaulis	Erect branching with straight branches, mature; homophyllous leaves are green in color, ovate in shape, and have a serrated margin.
4.	Rotundiloba	ME-0095	Morus rotundiloba Koidz.	Exogenous	Semi-erect branching, branches are slightly curved; leaves are green and homophyllous, ovate in shape with a serrated margin and slightly rough surface having free lateral, caducous stipule.
5.	BC ₂ 59	MI-0080	<i>Morus latifolia</i> Poir.	<i>M. indica var.</i> Matigara Local X Kosen	Branching erect, leaves are homophyllous green in color with dentate margin and smooth surface.
6.	S-1	ME-0065	Morus indica L.	Clonal selection from Mandalaya	Wide branching; branches are slightly curved; leaves are homophyllous, deep green, ovate with dentate margin and smooth surface.

Source: Compiled by the authors

5 min at 25°C, after which the reaction was stopped by adding 0.5 mL of 5% (v/v) H_2SO_4 . The amount of purpurogallin formed was determined by measuring the absorbency at 420 nm with a spectrophotometer (Shimadzu, UV-1700).

Superoxide dismutase: SOD was determined as described by Beauchamp and Fridovich (1971). Measured by the inhibition in the photochemical reduction of nitroblue tetrazolium. In the spectrophotometric assay, 1 mL reaction mixture contained 50 mM phosphate buffer (pH 7.8), 0.1 m M EDTA, 13 mM methionine, 75 µM nitroblue tetrazolium (NTB), 2 µM riboflavin, and 100 µL of the supernatant (prepared from leaf extract after centrifugation at 5000 rpm in Remi PR-24 centrifuge). Riboflavin was added at last, and the reaction was initiated by placing the tubes under two 15-W fluorescent lamps. The reaction was terminated after 10 min by removal from the light source. Non-illuminated and illuminated reactions without supernatant served as calibration standards. The reaction product was measured at 560 nm by spectrophotometer (Shimadzu, UV-1700). One unit of superoxide dismutase activity (expressed as unit.g⁻¹ FW.min⁻¹) is defined as the amount of enzyme required to cause 50% inhibition of nitroblue tetrazolium reduction, which was monitored at 560 nm.

Statistical Analysis

The data is presented as means \pm standard deviation (SD). The data were subjected to analysis of variance (ANOVA) following Tukey's honestly significant difference (HSD ($p \le 0.05$) using GraphPad Prism version 9.4.0. Tukey's HSD test was used to compare pairwise significant differences between the groups.

RESULTS AND DISCUSSION

The experimental trials of mulberry germplasm at relatively lower salinity levels showed superior performance than the trials at higher soil salinity stresses. All the mulberry germplasm propagated at soil salinity levels of 18 to

Table 3: Chlorophyll Content (mg.g FW ⁻¹)	Table 3:	Chlorophyll	Content (mg.g FW ⁻¹)
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 20 dS.m^{-1} (Canning I & II and Basanti), while no survivability was recorded in Rotundiloba, BC₂59, and S1 at salinity levels of 21 to 23 dS.m⁻¹ (Namkhana, Kakdwip, and Sagar).

Physiological Study

Leaf Chlorophyll Content Assay: Leaf chlorophyll concentration of the mulberry germplasm decreased in the soil of the all-salinity-prone blocks (Table 3). In Canning and Basanti, the percentage of decrease is on the lower side, whereas it is comparatively on the higher side in Namkhana, Kakdwip, and Sagar. In the most salinity-prone soils, superior performers like English Black, Kolitha-3, and C776 have a percentage decrease in chlorophyll content of 32.44%, 42.13%, and 21.54%, respectively. Salinity stress significantly lowered the amount of chlorophyll in the leaf, which may have been brought on by a high rate of chlorophyll breakdown or a reduction in pigment synthesis (Yeo & Flowers 1983). Vijayan et al. (2008) observed an initial increase in chlorophyll content when they performed an ex vitro experiment in NaCl-induced salinity-prone soil. Still, the amount of initial salinity in terms of EC observed there was comparatively on the lower side than the present experimental soils of the coastal blocks. In salt-tolerant germplasm, lesser depletion of the chlorophyll content may be considered as a preliminary selection criterion in mulberry for salinity stress (Jimenez et al. 1997).

Leaf Protein Assay: The protein content of almost all germplasm declined sharply at all the salinity-prone blocks (Table 4). The percentage of decrease in the protein content in Kolitha-3 is 41.10%, followed by English Black 27.39% at the most salinity-prone block, Sagar. However, in C776, protein concentration was greater in all salinity-prone blocks compared to other germplasm, which appeared similar to the observation of Vijayan et al. (2008). For many Lepidopteron larvae, leaf protein is a key nutrient quality indicator. It is well known that fibroin and sericin, which make up nearly 69% of the protein content of raw silk, are

		Community Development Blocks of South 24 Parganas							
Germplasm	Control	Canning I and II	Basanti	Namkhana	Kakdwip	Sagar			
English Black	2.25±0.25 ^a	1.89±0.17 ^a	1.76±0.25 ^a	1.66±0.25 ^a	1.62 ± 0.14^{a}	1.52±0.21 ^a			
Kolitha-3	2.16±0.04 ^a	1.78±0.11 ^a	1.68 ± 0.19^{a}	1.46 ± 0.10^{a}	1.48±0.21 ^a	1.25 ± 0.11^{b}			
C776	1.81 ± 0.22^{b}	1.72±0.21 ^a	1.74 ± 0.18^{a}	1.54 ± 0.17^{a}	1.59 ± 0.15^{a}	1.42 ± 0.10^{a}			
Rotundiloba	2.35 ± 0.30^{a}	2.29 ± 0.08^{b}	2.18 ± 0.05^{b}	-	-	-			
BC ₂ 59	1.68 ± 0.27^{b}	1.36±0.20 ^c	1.19±0.13 ^c	-	-	-			
S1	1.52 ± 0.18^{b}	1.56±0.16 ^c	1.75±0.27 ^a	-		-			

Values are means (\pm SD) of three replications. Different superscript letters in the same column are significantly different ($p \le 0.05$, ANOVA, Tukey-HSD).

		Community Development Blocks of South 24 Parganas						
Germplasm	Control	Canning I and II	Basanti	Namkhana	Kakdwip	Sagar		
English Black	24.10±2.36 ^a	26.70±2.25 ^a	24.40±1.32 ^a	21.60±2.01 ^a	19.70±1.99 ^a	17.50±2.08 ^a		
Kolitha-3	25.80±0.49 ^a	24.20±1.66 ^a	24.10±1.77 ^a	20.50±1.93 ^a	18.20 ± 2.40^{a}	15.20 ± 2.02^{a}		
C776	23.60 ± 1.07^{a}	27.90±1.73 ^a	26.60±1.31 ^b	24.10±1.87 ^b	23.10±0.26 ^b	21.20 ± 0.80^{b}		
Rotundiloba	29.60 ± 2.16^{b}	26.40 ± 1.02^{a}	26.80±2.10 ^b	-	-	-		
BC ₂ 59	28.50 ± 2.63^{b}	27.30±2.23 ^a	26.30±0.99 ^b	-	-	-		
S1	30.1 ± 0.45^{b}	28.8±0.31 ^a	25.9±0.15 ^b	-	-	-		

Table 4: Protein content (mg.g FW⁻¹).

Values are means (\pm SD) of three replications. Different superscript letters in the same column are significantly different ($p \le 0.05$, ANOVA, Tukey's-HSD).

directly biosynthesized from mulberry leaf protein, with the remaining 31% coming from silkworm body tissue and hemolymph protein (Bose & Bindroo 2001). Different mulberry germplasm's protein contents had a direct impact on larval development, particularly on the development of silk glands and the features of silkworm cocoons.

Study of the Antioxidant Enzymes from Leaves

Catalase: The catalase activity was significantly elevated in the leaves of all the germplasm, along with the increasing salinity stress levels in the coastal blocks (Table 5). The rate of increase in the enzyme activities was dependent on the severity of the salinity stress level. In comparison to the control, the catalase activity in Kolitha-3 and C776 increased almost 3.6 and 4.1 times, respectively. Catalase is mostly present in peroxisomes and glyoxysomes in plants, where it works to eliminate H₂O₂ produced during photorespiration or the oxidation of fatty acids in glyoxisomes. By lowering the harmful amounts of hydrogen peroxide generated during cell metabolism, an increase in catalase activity is thought to be an adaptive feature that may aid in overcoming the damage to tissue metabolism (Rasheed & Mukherji 1991). In the present study, the greater increase in catalase activity of C776 than other germplasm may suggest its effective scavenging mechanism to remove H₂O₂.

Table 5: Catalase (g⁻¹ FW.min⁻¹).

Peroxidase: The increased rate of peroxidase activity in C776 is almost 3.9 times, followed by Kolitha-3 and English Black almost 2.6 and 2.3 times, respectively (Table 6). The ability of plants to tolerate salt has been linked to enhanced antioxidant levels (Gossett et al. 1994; Hernandez et al. 1995). POD activity was shown to be higher in tolerant plant species, allowing the plants to defend themselves against oxidative stress, while this high activity was not seen in sensitive plant species (Sudhakar et al. 2001). Similarly, in the current study, in all three germplasm, peroxidase activity increased significantly.

Superoxide dismutase: Superoxide dismutase activity was significantly elevated in the stressed plants over control plants for all germplasm at all stress regimes (Table 7). In the salinity stress level of Sagar block, there was a significant increase in enzyme activity in English Black, Kolitha-3, and C776. Nevertheless, the increase in enzyme activity was higher in Kolitha-3 (2.6 times), followed by C776 (2.3 times) and English Black (2.2 times). Reportedly, SOD plays an important role in cellular defense against oxidative stress because its activity can directly modulate the amount of O_2^- and H_2O_2 , the two Haber-Weiss reaction substrates (Sudhakar et al. 2001). Salinity caused a significant increase in SOD activity in all the germplasm. The rate of increase was higher in Kolitha-3 than in other germplasm. The observed

		Community Development Blocks of South 24 Parganas						
Germplasm	Control	Canning I and II	Basanti	Namkhana	Kakdwip	Sagar		
English Black	1.43±0.26 ^a	2.83±0.23 ^a	2.90±0.29 ^a	3.40±0.16 ^a	3.46±0.17 ^a	4.80±0.29 ^a		
Kolitha-3	1.50±0.22 ^a	2.90±0.22 ^a	3.10±0.22 ^a	4.47 ± 0.46^{b}	4.57 ± 0.46^{b}	5.43 ± 0.62^{b}		
C776	1.27±0.19 ^a	2.67±0.33 ^a	3.10±0.14 ^a	3.20±0.14 ^c	3.27±0.05 ^c	5.17 ± 0.12^{b}		
Rotundiloba	1.15 ± 0.04^{b}	2.85 ± 0.37^{a}	3.20 ± 0.24^{a}	-	-	-		
BC ₂ 59	1.33±0.05 ^a	2.80 ± 0.16^{a}	3.10 ± 0.08^{a}	-	-	-		
S1	1.33±0.09 ^a	2.56±0.25 ^b	3.43 ± 0.20^{b}	-	-	-		

Values are means (\pm SD) of three replications. Different superscript letters in the same column are significantly different ($p \le 0.05$, ANOVA, Tukey's-HSD).

		Community Development Blocks of South 24 Parganas						
Germplasm	Control	Canning I and II	Basanti	Namkhana	Kakdwip	Sagar		
English Black	1.70±0.22 ^a	3.10±0.14 ^a	3.13±0.09 ^a	3.46±0.09 ^a	3.50±0.14 ^a	3.93±0.54 ^a		
Kolitha-3	$1.80{\pm}0.08^{a}$	3.16±0.09 ^a	3.23 ± 0.05^{b}	3.93 ± 0.05^{b}	4.13±0.12 ^b	4.60 ± 0.09^{b}		
C776	1.67 ± 0.05^{a}	3.20 ± 0.08^{a}	3.26 ± 0.12^{b}	4.23±0.16 ^c	4.20 ± 0.08^{b}	4.76±0.09 ^c		
Rotundiloba	1.73 ± 0.12^{a}	3.13±0.09 ^a	3.30 ± 0.16^{b}	-	-	-		
BC ₂ 59	$1.80{\pm}0.14^{a}$	2.93±0.05 ^b	3.13 ± 0.05^{a}	-	-	-		
S1	1.43 ± 0.18^{b}	3.00±0.16 ^a	3.33 ± 0.12^{b}	-	-	-		

Table 6: Peroxidase (g⁻¹ FW.min⁻¹).

Values are means (\pm SD) of three replications. Different superscript letters in the same column are significantly different ($p \le 0.05$, ANOVA, Tukey's-HSD).

Table 7: Superoxide dismutase (g⁻¹ FW.min⁻¹).

		Community Development Blocks of South 24 Parganas						
Germplasm	Control	Canning I and II	Basanti	Namkhana	Kakdwip	Sagar		
English Black	9.15 ± 0.04	12.65 ± 0.10	12.79 ± 0.17	14.65 ± 0.11	14.95 ± 0.02	20.30 ± 0.17		
Kolitha-3	9.83 ± 0.03	12.15 ± 0.04	12.45 ± 0.06	18.25 ± 0.05	19.05 ± 0.28	25.15 ± 0.07		
C776	8.65 ± 0.07	11.67 ± 0.05	12.12 ± 0.07	15.15 ± 0.07	15.28 ± 0.08	19.19 ± 0.09		
Rotundiloba	9.95 ± 0.04	12.45 ± 0.07	12.85 ± 0.10	-	-	-		
BC ₂ 59	10.14 ± 0.43	12.85 ± 0.09	12.95 ± 0.03	-	-	-		
S1	9.57 ± 0.29	12.56 ± 0.12	12.78 ± 0.09	-	-	-		

Values are means (\pm SD) of three replications. Different superscript letters in the same column are significantly different ($p \le 0.05$, ANOVA, Tukey's-HSD).

increase in SOD activity could increase the ability of the leaves to scavenge O_2^- radicals, which on the other hand, can also lead to severe membrane damage.

CONCLUSIONS

In conclusion, mulberry is generally moderately tolerant to salinity, but significant varietal differences exist in salt tolerance. Mulberry germplasm in the soil of the coastal South 24 Parganas district showed varied physiological and biochemical responses. Overall, the experiment revealed that mulberry germplasm C776, Kolitha-3, and English Black established its nutritional and salinity tolerance superiority with respect to total proteins and total chlorophyll contents alongside the antioxidant responses. From the observations, it is also clear that the mulberry germplasm, particularly C776, and Kolitha-3, turned out to be superior in leaf biochemical analysis compared to other germplasm studied under the same agro-climatic conditions. It may be recommended for silkworm rearing experimentation at the field level to test the cocoon parameters to confirm the possibilities of the establishment of sericultural farms in the socio-economically backward coastal regions of South 24 Parganas district of West Bengal. This establishment is important from both the socio-economic perspective and the effective utilization of the salinity-prone barren lands.

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REFERENCES

- Arnon, D.I. 1949. Copper enzymes in isolated chloroplasts: Polyphenoloxidase in Beta vulgaris. Plant Physiol., 1)24): 1.
- Beauchamp, C. and Fridovich, I. 1971. Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. Anal. Biochem., 1)44): 276-287.
- Bhowmick, M.K., Srivastava, A.K., Singh, S., Dhara, M.C., Aich, S.S., Patra, S.R. and Ismail, A.M. 2020. Realizing the potential of coastal flood-prone areas for rice production in West Bengal: prospects and challenges. New Front. Stress Manag. Agric., 16: 543-577.
- Bose, P.C. and Bindroo, B.B. 2001. A comparative biochemical study of seven promising mulberry (Morus alba L.) varieties under rainfed conditions of subtropical region. Indian J. Seric., 40: 171-173.
- Chance, B. and Maehly, A. C. 1955. Assay of catalases and peroxidases. Meth. Biochem. Anal., 1: 357-424
- Cha-Um, X., Suriyan, K. and Kirdmanee, C. 2008. Assessment of salt tolerance in eucalyptus, rain tree, and Thai neem under laboratory and field conditions. Pak. J. Bot., 40(5): 2041-2051.
- CSGRC Annual Report. 2020-21 Annual Report of CSGRC, Central Silk Board, Ministry of Textiles, Govt. of India. Retrieved from http://csgrc. res.in/downloads/csgrc_ar_2020_21.pdf/ (Accessed 28 July 2022).



- Datta R.K. 2000. Mulberry Cultivation and Utilization in India. Proceedings of the FAO Electronic Conference on Mulberry for Animal Production (*Morus* L.), Rome, Italy, 2000, pp. 45-62.
- Gossett, D.R., Millhollon, E.P. and Lucas, M.C. 1994. Antioxidant response to NaCl stress in salt-tolerant and salt-sensitive cultivars of cotton. Crop Sci., 34(3): 706-714.
- Grieve, C.M., Grattan, S.R. and Maas, E.V. 2012. Plant salt tolerance. ASCE Manual Rep. Eng. Prac., 71: 405-459.
- Hernandez, J.A., Olmos, E., Corpas, F.J., Sevilla, F. and Del Rio, L.A. 1995. Salt-induced oxidative stress in chloroplasts of pea plants. Plant Science, 105(2): 151-167.
- Hossain, M., Rahaman, S.M. and Joarder O.I. 1991. Isolation of sodium chloride-resistant genotypes in some mulberry cultivars. Bull. Seric. Res., 2: 67-73.
- Jhansilakshmi, K., Borpuzari, M.M., Rao, A.A. and Mishra, P.K. 2016. Differential response of mulberry (Morus spp.) accessions for salinity stress. J. Appl. Biosci., 42(1): 30-35.
- Jimenez, M.S., Gonzalez-Rodriguez, A.M., Morales, D., Cid, M.C., Socorro, A.R. and Caballero, M. 1997. Evaluation of chlorophyll fluorescence as a tool for salt stress detection in roses. Photosynthetica, 33: 291-301.
- Khasa, P.D., Hambling, B., Kernaghan, G., Fung, M. and Ngimbi, E. 2002. Genetic variability in salt tolerance of selected boreal woody seedlings. Forest Ecol. Manag., 257-269: (3-1)165.
- Kumar, S.G., Reddy, A.M. and Sudhakar, C. 2003. NaCl effects on proline metabolism in two high-yielding genotypes of mulberry (*Morus alba* L.) with contrasting salt tolerance. Plant Sci., 6)165): 1245-1251.
- Lowry, H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. 1951.Protein measurement with the Folin phenol reagent. J. Biol. Chem., 193: 265-275.
- Marin, L., Benlloch, M. and Fernández-Escobar, R. 1995. Screening of olive cultivars for salt tolerance. Sci. Horti., 113-116 :(2-1)64.
- Melander, W. and Horváth, C. 1977. Salt effects on hydrophobic interactions in precipitation and chromatography of proteins: an interpretation of the lyotropic series. Arch. Biochem. Biophys., 1)183): 200-215.
- Muchate, N.S., Nikalje, G.C., Rajurkar, N.S., Suprasanna, P. and Nikam, T.D. 2016. Plant salt stress: Adaptive responses, tolerance mechanism and bioengineering for salt tolerance. Bot. Rev., 82: 371-406.

Rasheed, P. and Mukerji. S. 1991. Changes in catalase and ascorbic acid

oxidase activities in response to lead nitrate treatments in mung bean, Indian J. Plant Physiol., 34: 143-146.

- Sharma, D.K. and Singh, A. 2015. Salinity Research in India: Achievements, Challenges and Future Prospects. Springer, Singapore
- Singh, A.K. and Singh, R.A. 1999. Effect of salinity of photosynthetic pigments in chickpea (Cicer arietinum L.) leaves. Indian J. Plant Physiol., 4: 49-51.
- Singh, R.L. and Singh, P.K. 2017. Global environmental problems. Principles and applications of environmental biotechnology for a sustainable future. Indian J. Plant Physiol., 4: 13-41.
- Sudhakar, C., Lakshmi, A. and Giridarakumar, S. 2001. Changes in the antioxidant enzyme efficacy in two high-yielding genotypes of mulberry (Morus alba L.) under NaCl salinity. Plant Sci., 3)161): 613-619.
- Tewary, P.K., Sharma, A., Raghunath, M.K. and Sarkar, A. 2000. In vitro response of promising mulberry (Morus sp.) genotypes for tolerance to salt and osmotic stresses. Plant Grow. Reg., 1)30): 17-21.
- Vijayan, K., Chakraborti, S.P. and Ghosh, P.D. 2003. In vitro screening of mulberry (Morus spp.) for salinity tolerance. Plant Cell Rep., 22: 350-357.
- Vijayan, K., Chakraborti, S.P., Ercisli, S. and Ghosh, P.D. 2008. NaCl induced morpho-biochemical and anatomical changes in mulberry (Morus spp.). Plant Grow. Reg., 56: 61-69.
- Wicke, B., Smeets, E., Dornburg, V., Vashev, B., Gaiser, T., Turkenburg, W. and Faaij, A. 2011. The global technical and economic potential of bioenergy from salt-affected soils. Energy Environ. Sci., 8)4): 2669-2681.
- Xiong, L. and Zhu, J. K. 2002. Molecular and genetic aspects of plant responses to osmotic stress. Plant Cell Environ., 2)25): 131-139.
- Yeo, A. 1998. Molecular biology of salt tolerance in the context of wholeplant physiology. J. Exp. Bot., 323)49): 915-929.
- Yeo, A.R. and Flowers, T.J. 1983. Varietal differences in the toxicity of sodium ions in rice leaves. Physiol. Plant., 2)59): 189-195.
- Zhu, J.K. 2001. Plant salt tolerance. Trends Plant Sci., 2)6): 66-71.

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