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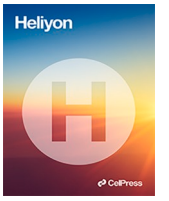

Research article

Evaluation of salt-tolerant germplasm of mulberry (*Morus* L.) through *in vitro* and field experiments under different salinity stresses

Ritwik Acharya [a], Debnirmalya Gangopadhyay [a,*], Phalguni Bhattacharyya [b], Amitava Ghosh [c]

[a] *Department of Sericulture, Raiganj University, Raiganj, 733134, West Bengal, India*
[b] *Department of Botany, Shibpur Dinobundhoo Institution (College), Howrah, 711102, West Bengal, India*
[c] *Department of Botany, Asutosh College, Kolkata, 700026, West Bengal, India*

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ABSTRACT

Twenty-five promising salt-tolerant mulberry germplasms from different *Morus* species were evaluated for growth under varying salinity levels (10.20–25.60 dS m^{-1}) typical of the coastal regions in South 24 Parganas, West Bengal. Evaluations were conducted using *in vitro* axillary bud culture and field experiments under natural conditions to identify superior salt-tolerant germplasms. Soil sample analysis revealed significant variation in salinity levels (34.37–17.09 dS m^{-1}) across different areas, with the highest in Kultali and the lowest in Canning I and II. Among the 25 germplasms, 6 were identified as highly salt-tolerant, 6 as moderately high salt-tolerant, 11 as salt-tolerant, and 2 as salt-sensitive. Survivability rate and root length were found to have the highest correlation with salt tolerance during early development. The six highly salt-tolerant germplasms, including English Black, Kolitha-3, C776, Rotundiloba, $BC_2$59, and S1 were further tested in field trials. English Black showed the highest survivability rate of 69.2 % in soil salinity of 18–20 dS m^{-1}. Results from *in vitro* and field trials were consistent, with a strong positive correlation between survivability rate and root length. This study establishes an effective method for evaluating salt tolerance in mulberry, providing a foundation for more efficient assessments.

1. Introduction

Salinity, or the presence of high levels of salts in the soil, can have several negative impacts on the physiological and developmental processes in plants [1–6]. Flowers et al. [7] observed that the survivability of most beneficial plant species gets greatly reduced when soil NaCl concentrations exceed 200 mM. Plants' responses to salinity are observed in the changes in morphological, physiological, biochemical, and molecular parameters [8–11]. For example, increased soil salinity creates an osmotic imbalance, affecting plants' ability to take up water from the soil [12]. This reduced water uptake can lead to water stress and dehydration, affecting various physiological processes in plants. Broadly, the growth of root and shoot cells gets instantly arrested due to a reduction in turgor pressure [13]. Prolonged exposure to elevated soil salinity causes ionic toxicity, disrupts nutrient balance, and leads to changes in

* Corresponding author. Department of Sericulture, Raiganj University, Raiganj, 733134, West Bengal, India.
E-mail address: deb_ganguly2003@yahoo.com (D. Gangopadhyay).

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cellular metabolite levels, ultimately resulting in high mortality, reduced and delayed growth, as well as symptoms like yellowing, necrosis, and wilting in plant leaves [14,15].

Mulberry is an economically important crop plant of the sericulture industry and contributes to over 2 lakh MT of global raw silk production annually [16]. There are more than 60 species of the genus *Morus* found in the subtropical, tropical, and temperate regions of Asia, Africa, and North America [17,18]. The major ones include *Morus alba*, *M. indica*, *M. nigra*, *M. rubra*, *M. australis*, *M. atropurpurea*, *M. cathayana, M. notabilis*, and *M. mesozygia*, etc. India enjoys a large biodiversity of sericultural flora and fauna. There are 1317 varieties of mulberry germplasm in the country's repository [19]. The leaf of the mulberry is extensively used for feeding *Bombyx mori* larvae, and the silk cocoon produced by the larvae is commercially exploited for the production of silk yarn. Though there remains a vast potential to explore sericulture for economic development, India has not achieved its true potential to meet the growing domestic demand for the silk industry during the last few decades. Degradation of land due to soil salinity becomes one of the major threats to popularizing sericulture in India. It is recorded that 6.73 million hectares of area are affected by salinity and sodicity stress, covering various states of the country [20]. The state, of West Bengal, for example, has the largest share of salinity-prone regions (0.82 million hectares) under coastal saline lands where more than 50 % of the coastal stretch is mainly confined to the districts of South 24 Parganas [21]. In this context, exploring mulberry genotypes under different salinity stresses is very important for several reasons. For example, identifying suitable salt-tolerant mulberry genotypes can lead to the development of new cultivars that are better adapted to saline conditions. This can ultimately increase mulberry yields in regions where salinity is a problem. Besides, the cultivation of salt-tolerant mulberry genotypes can have positive environmental implications by reducing the necessity for conventional soil salinity mitigation practices such as leaching excess salts from the soil. It also aids in the preservation of essential soil resources by reducing water consumption and plays a role in enhancing economic stability and food security, thereby supporting the sustainability of sericulture [22,23].

Plants' ability to tolerate salinity varies largely between and within the species, thus, facilitating the identification of superior plant genotypes that are critical for salinity stress tolerance [24–28]. The genotypic screening *in vitro* and field trials are most important in plant breeding research, especially when developing salt-tolerant germplasm for commercial exploitation. These complementary approaches help identify, characterize, and adapt genotypes to address the challenges posed by soil salinity, ultimately contributing to the sustainability and success of mulberry cultivation. A preliminary screening of a large number of mulberry germplasm *in vitro* under controlled environmental conditions of different salinity stresses proved to be more economical and efficient for rapid identification of superior germplasm before conducting rigorous field trials at diverse salinity-prone regions [29,30]. It has been shown that an increase in electrical conductivity (EC) of experimental soil substrate led to detrimental effects on the productivity of mulberry, including reduced chlorophyll fluorescence, plant height and shoot growth [31,32]. The effect of salinity stress also depends on the pH of the water and interacts significantly under mixed saline–alkali stress [11]. Mulberry seedlings when grown under different saline-alkali mixed stress conditions, the increased salt concentration with high pH treatments exhibited the highest reduction in leaf water content due to osmotic stress causing difficulty in water uptake by plant root [33].

Mulberry is a moderately salt-tolerant, important economic crop with huge natural genetic variations. Thus, in the screening of superior mulberry genotypes for salt tolerance breeding, reliable indices and traits must be determined. Various screening methods for salinity tolerance have been developed that include plant growth [34], germination rate, root growth [35], K^+/Na^+ discrimination [36], Cl^- exclusion [37], etc. In rice and wheat, root and shoot length and plant biomass were identified as good indicators of salt tolerance [38,39]. However, there are few studies focused on the salt tolerance of mulberry. Earlier, Tewary et al. [40], Vijayan et al. [31], Checker et al. [41], and Zhi et al. [42] testified the salt tolerance of mulberry genotypes *in vitro*, but no reliable salt tolerance index was presented. The present study has been undertaken with a broader aim to preliminary screen the superior salt-tolerant mulberry germplasm suitable for introduction along the salinity-prone coastal regions. In doing so, firstly, soil samples collected from salinity-prone zones were analyzed for salinity stress. Then various salinity stress environments were simulated by mixing different concentrations of salt in different proportions and plants' responses were measured to screen superior mulberry germplasm following *in vitro* axillary bud culture method. Finally, some of the highly salt-tolerant (HST) mulberry germplasms shortlisted based on *in vitro* screening were analyzed to evaluate their performance under different soil salinity stresses under natural field conditions. The study provides a basis for rapid, large-scale screening of salt-tolerant mulberry germplasm.

Table 1
A brief profile of the study area.

Blocks	Total area (sq. km)	Net area under cultivation (ha)	Saline Area (%)	Rural Population (no.)	Agricultural workers (%)	Cultivable land/agricultural worker (ha)
Gosaba	296.73	17000	32	222822	73.53	0.27
Canning I and II	402.80	31610	18	440594	55.53	0.45
Sagar	282.11	17436	23	185644	73.95	0.31
Namkhana	370.61	16910	21	160627	63.81	0.39
Kakdwip	252.74	15973	20	239326	53.26	0.36
Basanti	404.21	26151	19	278592	74.02	0.40
Kultali	306.18	19923	32	187989	71.61	0.47
Patharpratima	484.47	36429	25	288394	65.84	0.45

Data collected from Department of Planning and Statistics, Govt. of West Bengal. http://www.wbpspm.gov.in/SiteFiles/Publications/13_21062017112440.pdf/(Accessed August 11, 2022).

2. Materials and methods

2.1. Study area, soil sampling, and preparation of sample

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. A brief profile of the study area is given in Table 1. A total 8 coastal community development blocks, namely Gosaba, Canning-I and II, Sagar, Namkhana, Kakdwip, Basanti, Kultali, and Patharpratima of the South 24 Parganas district of West Bengal, were covered for the study. Soil samples were collected at random from multiple locations (at least five for each block), mostly from vast open fields or barren lands in March 2019, when the temperature was nearly above 40 °C and there were no interruptions from seasonal thunderstorms. The soils were brown to light grey, sandy to sandy-loam in texture and poor in structure characterized by limited aggregation. Surface (0–15 cm) and subsurface (15–30 cm) soil samples with a variety of textures were collected, air dried at room temperature away from direct sunlight, ground with a porcelain mortar and pestle, and sieved through a 2 mm sieve to separate the fine earth fraction (<2 mm) from the coarse earth fraction. Each soil sample was kept separate and labelled in a sealed polythene bag till further analysis.

2.2. Analysis of soil samples

The soil samples prepared were analyzed for electrical conductivity (EC) to measure salinity levels in the soil. The soil pH, nitrogen, phosphorus, and potassium contents were also analyzed to study the quality of soil samples following standard methods. The details of the methodology are described below.

2.2.1. Measurement of electrical conductivity (EC)

The EC of the soil samples was measured in a 1:5 soil-to-water suspension ratio using a digital electrical conductivity meter, type 308 (Systronics) following the method of Khan and Amin [43]. In a beaker, 10 g of dried soil sample was mixed with 50 mL of deionized water and shaken thoroughly for 1 h to dissolve soluble salts. A standard solution of 0.01 M KCl was used to calibrate the conductivity meter, and then the conductivity electrodes were dipped in the mixture and recorded values expressed in dS m^{-1}. All the reagents used were of analytical grade. All the measurements were carried out at room temperature (25 ± 2 °C).

2.2.2. Measurement of soil pH

The soil pH of the collected samples was measured in distilled, deionized water in soil-to-solution ratios of 1:1, following the standard procedures of Khan and Amin [43], using a type HI5222 pH meter (HANNA) bearing a glass electrode and a reference electrode, which had previously been calibrated with buffer solutions of pH 4 and 7. A scoop of 5 g soil sample was placed in a beaker, 5 mL of deionized water was added, it was vigorously stirred for 5–10 s, and then it was left to stand and settle for 10 min. Then, the electrodes were placed in the solution, swirled carefully, and a pH reading was taken immediately. To minimize the influence of contact time between solution and soil on soil pH readings, all measurements were done after 30 min of agitation, following the recommendations made by Qiu and Zhu [44].

2.2.3. Measurement of nitrogen (N) content

The total N content of the soil sample was determined using the Kjeldahl [45] digestion and distillation method. One gram of soil was mixed with 10 mL of concentrated H_2SO_4 in digestion tubes, stirred, and cooled. A mixture of 2.5 g $CuSO_4.7H_2O$–K_2SO_4 (1:24) was added, and the solution was heated at boiling for 3 h, then at 370 °C for 5 h with glass funnels to condense the H_2SO_4. After cooling, the solution was diluted with 20 mL distilled water and transferred to a distillation apparatus. Five milliliters of H_3BO_3 was placed in a 200 mL conical flask under the condenser, and 20 mL NaOH was added to the apparatus. About 100 mL of condensate was collected, rinsed, and titrated with H_2SO_4 using Tashiro's indicator until a violet-pink endpoint was reached. $C_6H_7NO_3S$ with known N content was used as a control following the method of Adam et al. [46].

2.2.4. Measurement of phosphorus (P) content

The P content in the soil sample was measured using the Olsen method [47]. Two grams of soil were mixed with 40 mL of 0.5 M $NaHCO_3$ (pH 8.5) and centrifuged at 200 rpm for 30 min. The clear extract was filtered through Whatman no. 2 filter paper. Five milliliters of this extract was diluted with 15 mL of distilled water, and 5 mL of a reaction reagent was added. After shaking vigorously and waiting 10 min for colour development, the optical density was measured at 882 nm using a UV–vis spectrophotometer (Shimadzu UV-1280). A standard curve was then used to determine the P content in the soil extract.

2.2.5. Measurement of potassium (K) content

The K was extracted from a 2 g soil sample by mixing it with 20 mL of 1 M neutral ammonium acetate and shaking for 5 min at 200 rpm, as per Adam et al. [46] with slight modification. The clear suspension was filtered through Whatman no. 2 filter paper. The filtered extract was analyzed using an atomic absorption spectrophotometer (Shimadzu AA-7000) in emission mode at 776 nm. A standard curve was prepared to measure the concentration of exchangeable K in the soil extract, using a standard K solution made by dissolving 0.9533 g of KCl in 500 mL of distilled, deionized water.

2.3. In vitro screening of salt-tolerant mulberry germplasm

Twenty-five different promising salt-tolerant mulberry germplasm samples (collected from the Central Sericultural Research and Training Institute, Berhampore, West Bengal, India) representing 4 different species of *Morus* were screened at different levels of salinity stress as prevalent in the coastal regions of the South 24 Parganas district of West Bengal following the *in vitro* axillary bud culture method [31]. The detailed list of mulberry germplasm used in the study is given in Supplementary Table S1. Axillary buds from the healthy shoots of 3-4-month-old plants were collected, washed under running tap water, and then immediately treated with 1% cetavlon prepared from 2% cetrimide (w/v) for 5 min followed by repeated washing (2–4 times) in sterile distilled water. Surface sterilization was carried out with 0.1% mercuric chloride solution for 5 min followed by washings in distilled water as described by Vijayan et al. [31] to avoid fungal and bacterial contamination during the axillary bud culture. After surface sterilization, explants were cultured on Murashige and Skoog (MS) medium (Hi-Media, TS-1076) supplemented with 8.8 μM N6-Benzyladenine and 30 g l^{-1} sucrose. For developing an *in vitro* saline condition, various amounts of NaCl were added, the pH of the medium was adjusted to 5.7±1 and solidified agent Difco-Bacto agar (7%) was added to it. In this way, different NaCl treatment sets (0.25, 0.50, 0.75, 1.00, and 1.25%) with varied EC (10.20, 12.90, 17.60, 20.80, 25.60 dS m^{-1} respectively) were prepared. Test tubes without NaCl were kept as control (EC- 7.10 dS m^{-1}). Both treatment and control sets were autoclaved at 103421.4 Pa/sq inch pressure for 15 min for sterilization before putting the axillary buds inside them. To study the rooting response, shoot-apices (30 days old) from shootlets (1–2 cm in length) were transferred separately to the MS medium containing 2.6 μM NAA and 30 g l^{-1} sucrose. The test tubes with explants were arranged in randomized block design on the rack. The culture was kept at 25±1 °C for a 16 h light cycle and an 8 h dark cycle. A daylight white fluorescent lamp was used in the experiment and the average light intensity was 3000 lx. Four parameters, such as survival rate (SR), shoot length (SL), shoot weight (SW), and root length (RL) of 250 experimental and 50 control batches were recorded on the 30th day to screen the salt-tolerant mulberry germplasms based on their performance under different levels of salinity stress. The whole experiment was repeated twice. The STI for each morphological trait in every mulberry germplasm was determined as a ratio of the value under salt treatment relative to the value of the control.

2.4. Short listing of salt-tolerant mulberry germplasm

The salt tolerance capacity of the mulberry germplasm on multiple parameters was evaluated and verified with the fuzzy comprehensive evaluation method using MFV [48]. The MFV of salt tolerance was calculated using the following equation:

$$Xi = \frac{X - Xmin}{(Xmax - Xmin)} \times 100$$

where Xi stands for the MFV of STI in a particular germplasm, X is the measured value of STI in a specific germplasm, and X_{max} and X_{min} denote the maximum and minimum values observed in each mulberry germplasm, respectively [49]. Based on the average value of the MFVs of each trait, the salt tolerance of the mulberry germplasm was evaluated. The MFVs of all mulberry germplasm ranged from 0 to 1. In each germplasm, the mean MFV is the average of the MFVs for SR, SL, SW, and RL. Each of the mulberry germplasm has its own mean MFV. A higher mean MFV indicates a higher salt tolerance of the respective germplasm.

To categorize the level of salt tolerance of the mulberry germplasm, hierarchical cluster analysis was performed [29]. Based on hierarchical cluster analysis; the salt tolerance was divided into four clusters: highly salt-tolerant (HST), moderately high salt-tolerant (MHST), salt-tolerant (ST), and low salt-tolerant (LST). A multiple regression analysis was performed on the mean MFV (dependent variable Y) and STI value (independent variable Xi) for each germplasm. A mathematical evaluation model for salt tolerance was testified: $Y = \beta1X1 + \beta2X2 + \beta3X3 + \beta4X4 + \beta5X5 + \mu$, where Y is the mean MFV, X1 is the STI of SR, X2 is the STI of SL, X3 is the STI of SW, X4 is the STI of RL, β is the unstandardized coefficient, and μ is a constant. The random error term is denoted by the constant (μ).

2.5. Field evaluation of shortlisted salt-tolerant mulberry germplasm

The highly salt-tolerant (HST) mulberry germplasms namely, English Black, Kolitha-3, C776, Rotundiloba, $BC_2$59, and S1 were shortlisted for field evaluation. The field trials were conducted separately at 5 experimental farms of Canning I & II, Basanti, Namkhana, Kakdwip, and Sagar under the salinity stress ranging from 18 to 23 dS m^{-1}. The trials were conducted in a randomized complete block design (RCBD) with three replications and compared with the plants grown under non-saline conditions (EC within the range of 1.60 dS m^{-1}). The size of each plot was 4 m × 4 m while the distance between plants and rows was maintained at 0.9 m. 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 [17]. The plots were kept free of weeds throughout the experimental period by hoeing. No fertilizers or supplementary water through irrigation was applied during the trials. Major pests and diseases (leaf spot, leaf rust and powdery mildew) were controlled by 1–2 sprays (depending on pest pressure) using Bavistin (Carbendazim 50 % WP) and Dichlorvos (DDVP) 76% EC at recommended rates. Six parameters of the crop including survivability, plant height, leaf yield and N, P, and K contents of leaf tissue were analyzed to evaluate the performance of the salt-tolerant mulberry germplasm.

Total N content was analyzed after dry matter digestion in concentrated sulfuric acid, distillation with NaOH (10 mol L^{-1}) and boric acid 2% and titration with HCl (0.1 mol L^{-1}), according to Bremner [50] while P and K content was measured using UV–vis spectrophotometer (Shimadzu UV-1280) and an atomic absorption spectrophotometer (Shimadzu AA-7000) respectively, using the

phosphorus and potassium extracted from the samples after treatment with the di-acid (H_2SO_4–$HClO_4$) wet digestion method [51].

2.6. Statistical analysis

The data were subjected to analysis of variance (ANOVA) following Tukey's honestly significant difference (HSD) and Dunnett's post hoc analyses ($p \leq 0.05$) using GraphPad Prism version 9.4.0. Tukey's HSD test was used to compare pairwise significant differences between the groups while Dunnett's test was employed to compare significant differences between an experimental data set and the control values of that data set. Principal Component Analysis (PCA) was conducted on the 4 parameters (survivability rate, shoot length, shoot weight, and root length) of 25 salt-tolerant mulberry germplasms examined in this study. PCA components with eigenvalues greater than 1.0 (Kaiser's rule) were kept only. All analyses were carried out using XLSTAT statistics software version 2014.5.03 (Addinsoft, Paris, France). Hierarchical cluster analysis and multiple regression analysis on the mean MFV (the dependent Y variable), and STI value (the independent STI variable) were performed using IBM-SPSS Statistics version 28.0.1.1 (15).

3. Results and discussion

3.1. Analysis of soil samples

The soil EC, pH, N, P, and K content analyzed separately in surface (0–15 cm) and subsurface (15–30 cm) soil collected from Gosaba, Canning I and II, Sagar, Namkhana, Kakdwip, Basanti, Kultali, and Patharpratima blocks of South 24 Parganas district of West Bengal are given in Table 2. The results showed extremely high salinity in terms of measured EC values in Kultali (34.37 dS m^{-1}) while, it was lowest in Canning I and II (17.09 dS m^{-1}). EC measures the amount of salts in soils and is expressed in terms of the total concentration and composition of soluble salts present in the soil extracts as the electrical conductance of the saturation extract in units of dS m^{-1} [52]. It is an important indicator of soil health since the activity of soil microorganisms, availability of nutrients to plants and agricultural productivity are largely dependent on soil salinity [53]. Rhoades et al. [54] proposed that soil EC is sensitive to temperature and mostly soil salinity level tends to increase at higher temperatures. Soil salinity when measured during the mid-summer with an average temperature almost above 40 °C, the highest salinity was observed in Kultali (34.37 dS m^{-1}) while it was lowest in Canning I and II (17.09 dS m^{-1}). Sabareshwari and Ramya [55] reported the salinity (EC) range of the eastern Indian coastal area from 0.5 dS m^{-1} (monsoon) to 50 dS m^{-1} (summer). The high salt concentration in the Kultali block can be linked to its proximity to the coast, its position in downstream areas leading to salt-wedge estuaries, and the slow movement of water. On the contrary, the lower EC levels in Canning I and II may be attributed to their elevated topography. The results conform with the earlier findings of Hossain et al. [56].

Results on measured soil pH revealed that the soils in the coastal regions of South 24 Parganas district of West Bengal are slightly to moderately alkaline, ranging between 7.1 (Canning-I and II) and 8.2 (Gosaba). Earlier studies on eastern coastal regions of India showed soil pH within the range of 3–8.5 [57]. The availability of nutrients to plants is greatly affected by soil pH. It is reported that soil pH within the range of 6.0–7.5 is favourable to most plants for their optimum growth [56]. Several factors affect soil pH such as soil texture, soil salinity, climate, nitrogen content from fertilizer, organic matter, manure, and mineral content present in the soil etc. Among these, the salinity of soil (a salt molecule, when present in the soil-water mixture, separates into positively and negatively charged components or ions) plays a vital role in regulating soil pH measurement [58]. Even the soil pH values tend to vary randomly

Table 2
Analysis of soil samples collected from coastal regions of the South 24 Parganas district of West Bengal, India.

Block	Electrical conductivity (dS m^{-1})		pH		N (mg/kg)		P (meq/100 mg)		K (mg/kg)	
	0–15 cm	15–30 cm	0–15 cm	15–30 cm	0–15 cm	15–30 cm	0–15 cm	15–30 cm	0–15 cm	15–30 cm
Gosaba	34.20 ± 0.48^{a}	29.86 ± 0.58^{a}	8.20 ± 0.14	8.00 ± 0.16	95.12 ± 0.38^{a}	93.06 ± 0.30^{a}	19.20 ± 0.29	18.10 ± 0.23	26.20 ± 0.40^{a}	24.98 ± 0.43^{a}
Canning I and II	18.99 ± 0.23^{b}	17.09 ± 0.34^{b}	7.20 ± 0.29	7.10 ± 0.23	87.15 ± 0.17^{b}	83.25 ± 0.21^{b}	18.90 ± 0.10	17.54 ± 0.06	15.12 ± 0.08^{b}	13.20 ± 0.04^{b}
Sagar	24.26 ± 2.40^{c}	21.14 ± 1.54^{a}	7.70 ± 0.16	7.50 ± 0.22	76.16 ± 0.08^{c}	79.18 ± 0.14^{c}	17.47 ± 0.23	18.89 ± 0.18	17.20 ± 0.24^{b}	18.64 ± 0.18^{a}
Namkhana	23.12 ± 0.58^{b}	18.26 ± 0.46^{c}	7.30 ± 0.61	7.20 ± 0.75	76.10 ± 0.24^{c}	75.80 ± 0.29^{c}	14.65 ± 0.16	13.99 ± 0.15	17.96 ± 0.29^{b}	18.26 ± 0.22^{b}
Kakdwip	24.26 ± 1.74^{c}	17.89 ± 1.36^{c}	7.80 ± 0.63	7.20 ± 0.72	76.20 ± 0.88^{c}	77.38 ± 1.08^{c}	17.28 ± 0.12	18.72 ± 0.09	24.52 ± 0.14^{a}	25.14 ± 0.21^{a}
Basanti	21.51 ± 0.15^{b}	17.57 ± 0.06^{c}	7.20 ± 0.60	7.20 ± 0.44	89.18 ± 0.26^{b}	88.06 ± 0.18^{b}	14.79 ± 0.26	13.49 ± 0.19	21.97 ± 0.08^{a}	20.33 ± 0.13^{a}
Kultali	34.37 ± 0.26^{a}	29.84 ± 0.16^{a}	8.10 ± 0.17	8.10 ± 0.08	96.09 ± 0.16^{a}	92.07 ± 0.13^{a}	17.29 ± 0.27	16.87 ± 0.23	26.16 ± 0.29^{a}	24.40 ± 0.23^{a}
Patharpratima	27.01 ± 6.85^{c}	22.29 ± 6.29^{a}	7.60 ± 0.81	7.40 ± 0.60	83.16 ± 1.75^{b}	81.04 ± 1.25^{c}	16.80 ± 1.03	15.70 ± 0.84	21.19 ± 3.63^{a}	20.01 ± 5.02^{a}

Values are means (±SE) of three replications. Different superscript letters in the same column are significantly different ($p \leq 0.05$, ANOVA, Tukey-HSD). N, P, and K stand for Nitrogen, Phosphorus, and Potassium content respectively.

when the soil samples are collected from various location points or at different depths of soil [59]. However, analysis of pH values indicated no significant differences ($p \leq 0.05$) in the soils collected from surface to subsurface soil in the coastal areas of South 24 Parganas district.

The analysis of N, P, and K contents in the soil of coastal regions in the South 24 Parganas district showed significant variability, with concentrations ranging from 75.80 to 96.09 mg/kg for N, 13.49–19.20 meq/100 mg for P, and 13.20–26.20 mg/kg for K. In alluvial soil, nutrients like N, P, and K always tend to be on the higher side [60]. Hossain et al. [56] reported a great deal of variation in the physical and chemical properties of paddy field soils affected by salinity in Bangladesh. The soil of coastal areas in the South 24 Parganas district is mostly alluvial [61]. The high N content (96.09 mg/kg) found in the soils of the Kultali block in South 24 Parganas district might be due to either the normal retention capacity of the alluvial soil or the slow mineralization process of the N content under the extreme saline condition [62]. In saline soils, it has been observed that P content tends to be more strongly bound compared to N content [63]. The moderately higher rate of organic matter deposition in coastal soils, particularly in the Gosaba block, likely contributes to sufficient P availability in the soil. Additionally, the presence of a relatively higher amount of exchangeable potassium (K) in the Gosaba block can be attributed to both the abundance of K in the soil exchange complex and the tendency for coastal soils to exhibit an increase in exchangeable K [64].

Further, the correlation coefficient was studied among the soil pH, EC, N, P, and K contents to assess their relationship (Table 3). EC was significantly correlated with soil pH ($r = 0.92$, $p<0.01$), similar to the study of Ranjbar and Jalai [65]. However, no significant correlation was noticed in the relationship among the N, P, and K content recorded.

3.2. *In vitro* screening of salt-tolerant mulberry germplasm

Twenty-five mulberry germplasms screened to assess their responses on survivability, shoot length, shoot weight, and root length at different levels of salinity stress as prevalent in coastal areas of the South 24 Parganas district of West Bengal are given in Fig. 1(A–D). The results revealed significant variability, with English Black showing the highest survival rate of 54.35%, followed by Kolitha-3 at 44.20%, under a salinity stress level of 1.25% NaCl (25.60 dS m^{-1}). Overall, English Black, Kolitha-3, C776, and Rotundiloba exhibited survival rates exceeding 25% under the same salinity stress conditions (Fig. 1 A). In terms of shoot weight measured on a fresh weight basis, C776 recorded the highest weight at 95.15 g, followed by Rotundiloba at 75.17 g under the highest salinity treatment (1.25% NaCl) (Fig. 1 B). Furthermore, C776 displayed the most substantial shoot length growth (1.15 cm) at the highest saline treatment, with English Black following at 0.82 cm (Fig. 1C). Similarly, in case of root length, C776 (2.64 cm) and English Black (2.43 cm) demonstrated superior growth under 0.25% NaCl (10.20 dS m^{-1}) conditions (Fig. 1 D). Root formation was completely halted after 0.25% NaCl salinity treatment. Plants have certain inherent mechanisms for tolerating salt stress; however, the ability to resist salt stress varies widely among different plant species and cultivars [66]. The different responses of plant species under different stress conditions have been reported previously in *Triticum aestivum* [67], *Brassica napus* [29], and *Helianthus annuus* [30].

Morus sp. is a moderately salt-tolerant, important economic plant species of the sericulture industry. Continuous natural genetic variations in *Morus* show different responses to the same stress under different environmental conditions, even within the same species. This huge genetic diversity of *Morus* sp. has attracted the attention of many sericultural scientists. Vijayan et al. [31] made the first exhaustive *in vitro* screening attempt of the mulberry genotypes under salinity stress, but the selection method was mainly based on the survival percentage. Salt stress is one of the major abiotic stresses faced by plants, and soil salinity causes a significant reduction in plant productivity. The damage caused by soil salinity resulted in a variety of morphological, physiological, and biochemical changes in plants [68,69]. However, one of the most important steps in conventional salt tolerance breeding is the selection of suitable salt tolerance parameters. Several morpho-physiological parameters like survival percentage, seed germination rate, and root-shoot growth have been studied in different plant species to study their salt stress responses under different salinity levels [29,30]. In *Morus* sp., the effects of survival, germination, and root-shoot growth under various salt stresses have been reported by some researchers [31,40].

In the present study, 25 *Morus* germplasms have been screened to study their responses on survival rate, shoot length, shoot weight, and root length under different concentrations of salinity stress level following the *in vitro* axillary bud culture method. The differences in salt tolerance among mulberry germplasms were measured using the STI of each morphological trait. Each trait of each germplasm has its own STI. A correlation coefficient was studied to determine the relationship between the parameters of STI_{SR}, STI_{SL}, STI_{SW}, and STI_{RL} and the results are given in Table 4. Very highly significant correlation coefficients (r=0.7601, $p<0.001$) noticed between salt tolerant indices of survivability rate and root length as well as shoot length and shoot weight (r=0.6997, 0.8897 $p<0.001$) indicate that they have very strong positive correlation among each other. However, there was no such stronger relationship found between shoot weight and root length (r=0.5242, $p<0.01$) as well as shoot length and root length (r=0.5300, $p<0.01$). The process of evaluation

Table 3
Pearson's correlation coefficients of Nitrogen, Phosphorus, Potassium, Electrical conductivity, and soil pH.

Parameters	Phosphorus	Potassium	Electrical conductivity	pH
Nitrogen	0.16[a]	0.46[a]	0.69[a]	0.65[a]
Phosphorus		0.15[a]	0.35[a]	0.60[a]
Potassium			0.68[a]	0.72[b]
Electrical conductivity				0.92[c]

[a] non significant; [b], [c] significant at $p \leq 0.05$ or 0.01 respectively.

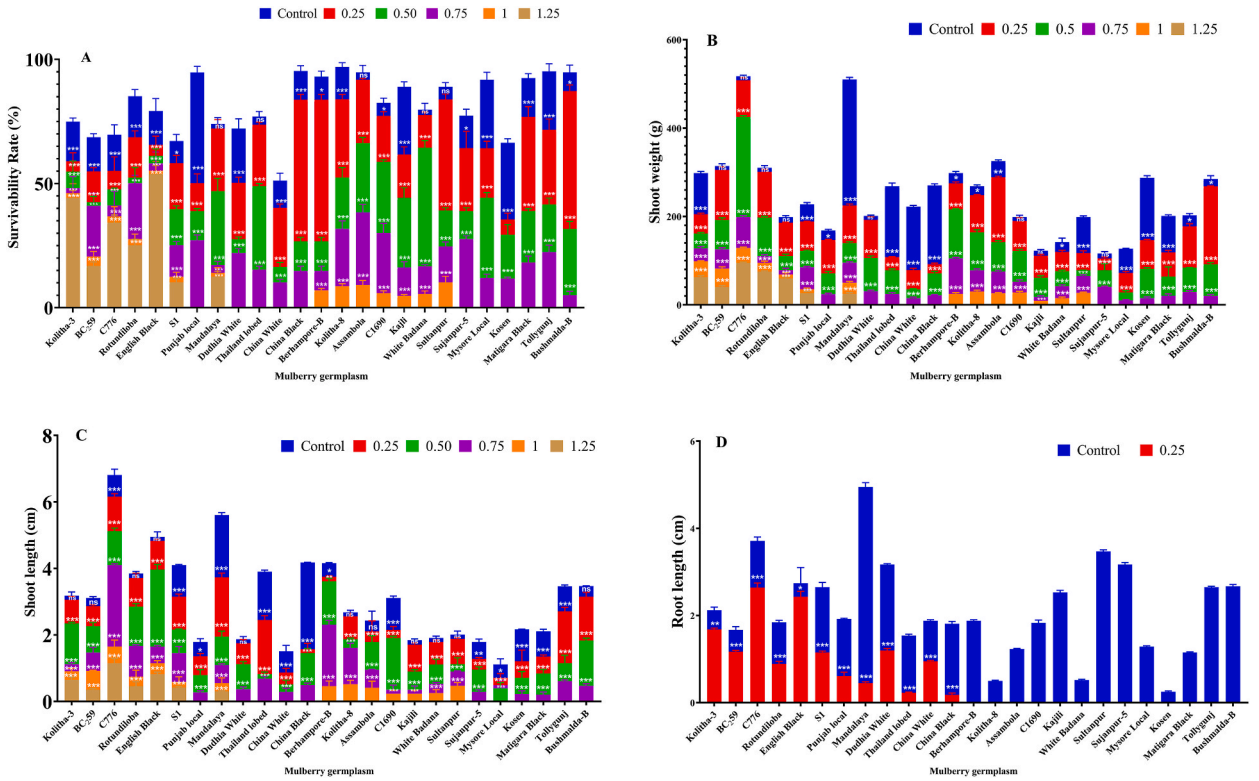

Fig. 1. (A–D). Survivability rate (A), shoot weight (B), shoot length (C), and root length (D) as affected by different concentrations of NaCl treatments (0.25, 0.50, 0.75, 1.0, and 1.25 %) along with control (without NaCl) in 25 salt-tolerant mulberry germplasm. Each value is expressed as mean ± standard error (n = 3). Dunnett's test was employed to compare significant differences between an experimental data set and the control values of that data set; ns: non significant and *, ** and ***: significant at $p < 0.05$, 0.01 and 0.001 respectively.

Table 4
Pearson's correlation coefficients of. salt tolerance indices of survival rate, shoot length, shoot weight and root length.

Parameters	STI_{SL}	STI_{SW}	STI_{RL}
STI_{SR}	0.6997***	0.7088***	0.7601***
STI_{SL}		0.8897***	0.5300**
STI_{SW}			0.5242**

STI_{SR}, STI_{SL}, STI_{SW} and STI_{RL} indicate salt tolerance indices of survival rate, shoot length, shoot weight and root length respectively; **, ***: significant at $p \leq 0.01$ and 0.001 respectively.

becomes more critical and cumbersome when more germplasm lines with multiple parameters are used for screening. Thus, the morphological and physiological indices related to salt tolerance are needed for the screening of salt-tolerant germplasm [30].

Principal Component Analysis (PCA) was conducted on the standardized data to explore the association between evaluated parameters and assessed germplasms. This approach often helps to uncover unforeseen connections among the parameters or variables, enabling better interpretations that would be difficult to achieve through other methods [70]. The PCA analysis resulted in two factors, each with eigenvalues greater than 1.0, according to Kaiser's rule. These two factors collectively accounted for 88.22% of the total variance (Table 5). The initial component, PC1 which represented 77.40% of the total variance was primarily characterized by shoot

Table 5
Eigenvalue, percentage variation and cumulative variance for the four factors derived from principal component analysis.

	PC1	PC2	PC3	PC4
Eigenvalue	3.096	0.433	0.404	0.068
Variability (%)	77.398	10.818	10.094	1.690
Cumulative %	77.398	88.216	98.310	100.000

PC1, the first principal component; PC2, the second principal component; PC3, the third principal component, and PC4, the fourth principal component.

length and shoot weight. Meanwhile, the second component, PC2, accounting for 10.80% of the variance, was predominantly influenced by the survivability rate and root length. The results of the PCA biplot illustrate the primary associations between the parameters and the principal components while also emphasizing connections among the parameters themselves (Fig. 2). The parameters such as survivability rate correlated positively with the exogenous germplasms such as English Black and Rotundiloba and indigenous germplasms of Kolitha-3 belonging to the species of *M. latifolia*, *M. rotundiloba* and *M. alba* respectively. However, these germplasms have a distant correlation with the parameters such as shoot weight and root length. Moreover, parameters like survivability rate and root length as well as shoot weight and shoot length exhibited positive relationships while the closeness between survivability rate and shoot length and shoot weight was found lesser than those of the other parameters studied. Vijayan et al. [71] observed a significant correlation between leaf yield and several component traits such as plant height, leaf size, shoot weight, root weight and root length of mulberry plants under conditions of severe salinity induced by a 1.00% NaCl solution. In another study, Ali et al. [38] observed a positive relationship between shoot length and root length on some rice landraces when examining the tolerance of these plants to different salinity stresses.

3.3. Short-listing of salt-tolerant mulberry germplasm

The salt tolerance of mulberry germplasm was evaluated across multiple parameters using the fuzzy comprehensive evaluation method, employing the mean MFV (Table 6). Additionally, a hierarchical cluster analysis, utilizing the furthest neighbour approach, was employed to construct a hierarchical arrangement of clusters (Fig. 3). Among the 25-mulberry germplasms evaluated, 6 showed high salt tolerance, 6 showed moderately high salt tolerance, 11 showed salt tolerance, and 2 showed salt sensitivity. Interestingly, some of the mulberry germplasms, like English Black, Kolitha-3, and C776, showed higher survivability (34.34–54.35%), shoot length (0.65–1.15 cm), and shoot weight (62.50–95.15 g) under 12.5 mg/mL NaCl as compared to the controls. A moderate salinity promotes significant vegetative and reproductive growth, which might be due to well-organized ion compartmentalization and succulence [72, 73]. Vijayan et al. [31] reported more adaptability of English Black, Kolitha-3, C776, and $BC_2$59 to salinity as compared to the other 58

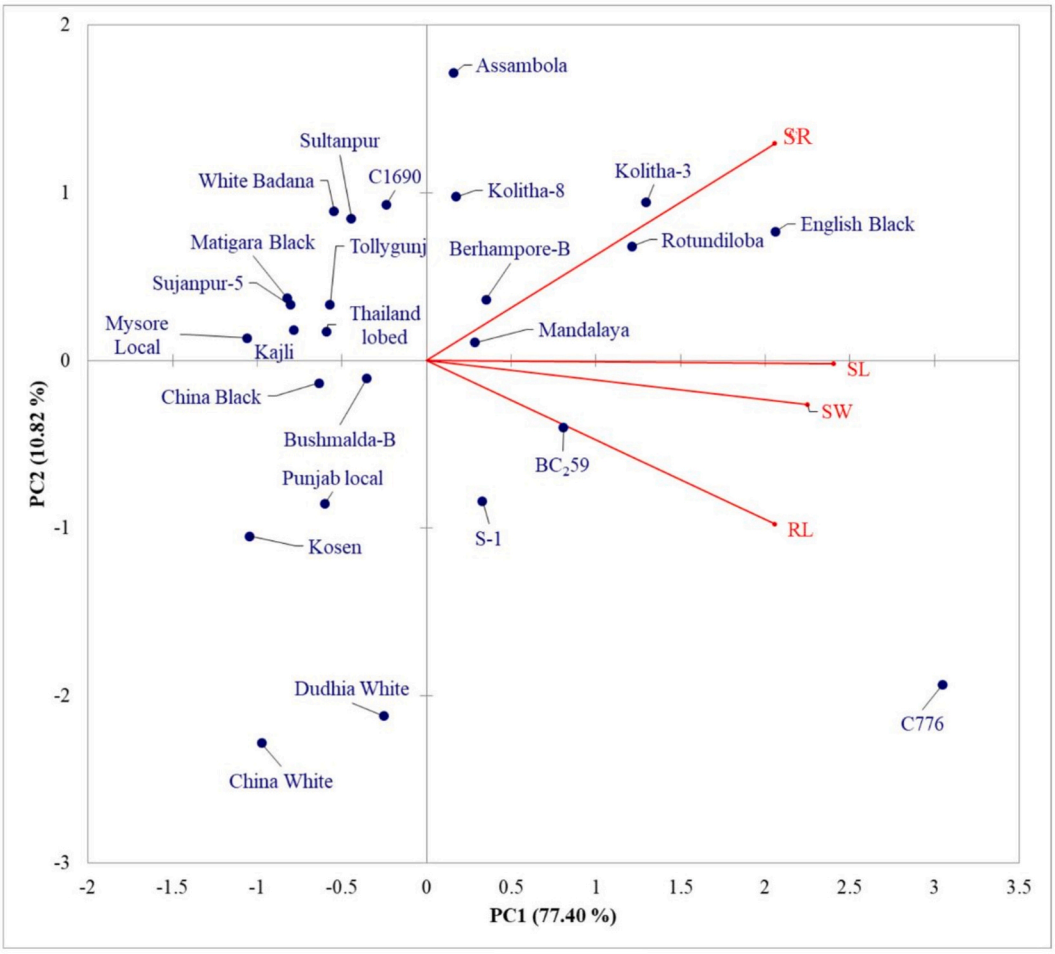

Fig. 2. Principal Components Analysis between evaluated parameters and assessed mulberry germplasm.

Table 6
Salt tolerance evaluation of multiple regression analysis with their mean MFVs.

Mulberry germplasm	STI^{a}_{SR}	STI^{b}_{SL}	STI^{c}_{SW}	STI^{d}_{RL}	Mean MFV[e]	Y[f]	Cluster[g]
Kolitha-3	0.68	0.51	0.44	0.79	0.60	0.62	HST
$BC_2$59	0.51	0.50	0.48	0.70	0.60	0.57	HST
C776	0.62	0.53	0.53	0.71	0.59	0.57	HST
Rotundiloba	0.53	0.49	0.50	0.48	0.58	0.48	HST
English Black	0.75	0.50	0.51	0.89	0.56	0.67	HST
S1	0.43	0.38	0.40	0.43	0.56	0.48	HST
Punjab local	0.25	0.27	0.29	0.32	0.54	0.46	MHST
Mandalaya	0.45	0.27	0.21	0.09	0.53	0.38	MHST
Dudhia White	0.28	0.35	0.35	0.39	0.53	0.47	MHST
Thailand lobed	0.36	0.20	0.16	0.15	0.52	0.42	MHST
China White	0.27	0.22	0.12	0.50	0.52	0.56	MHST
China Black	0.25	0.17	0.21	0.10	0.52	0.40	MHST
Berhampore-B	0.25	0.49	0.42	0.00	0.34	0.28	ST
Kolitha-8	0.35	0.49	0.38	0.00	0.34	0.28	ST
Assambola	0.41	0.43	0.38	0.00	0.33	0.30	ST
C1690	0.40	0.32	0.40	0.00	0.33	0.31	ST
Kajli	0.29	0.33	0.33	0.00	0.33	0.31	ST
White Badana	0.40	0.38	0.36	0.00	0.31	0.31	ST
Sultanpur	0.36	0.36	0.29	0.00	0.31	0.31	ST
Sujanpur-5	0.34	0.28	0.39	0.00	0.31	0.32	ST
Mysore Local	0.26	0.29	0.18	0.00	0.29	0.33	ST
Kosen	0.23	0.20	0.17	0.00	0.28	0.35	ST
Matigara Black	0.29	0.23	0.23	0.00	0.28	0.34	ST
Tollygunj	0.29	0.26	0.27	0.00	0.24	0.33	LST
Bushmalda-B	0.25	0.31	0.27	0.00	0.23	0.32	LST

[a, b, c,] and [d] denote salt tolerance indices of survivability rate (STI_{SR}), shoot length (STI_{SL}), shoot weight (STI_{SW}), and root length (STI_{RL}) in different concentrations of NaCl.

[e] Mean MFV indicates the calculated mean membership function value (MFV) of all the morphological traits viz., survivability rate, shoot length, shoot weight, and root length.

[f] represents the salt tolerance of mulberry germplasm. The larger Y value denotes higher salt tolerance ($Y = 0.39 + 0.07 \times STI_{SR} + (-0.20) \times STI_{SL} + (-0.10) \times STI_{SW} + 0.42 \times STI_{RL}$).

[g] Salt tolerance of mulberry germplasm is categorized into four clusters, where highly salt-tolerant (HST), moderately high salt-tolerant (MHST), salt-tolerant (ST), and low salt-tolerant (LST) clusters have Y values of 0.48–0.67, 0.38–0.56, 0.28–0.35, and 0.32–0.33 respectively.

genotypes in terms of higher survival.

The STI of each germplasm for each parameter was determined based on four parameters namely, SR, SL, SW, and RL and then MFV was determined based on these STIs to rank the germplasms. A comparable approach using MFV for the identification of salt-tolerant germplasm has previously been conducted in canola [29] and sunflower [30]. In this MFV-based ranking system, it was observed that among the four parameters, SR and RL had the most significant impact. The results of the PCA analysis also suggest that the salinity tolerance of mulberry germplasms, including varieties like English Black, Kolitha-3, and Rotundiloba, which are grouped within the HST cluster, is strongly associated with the SR. At the same time, it's worth noting that the performance of $BC_2$59 and S1, both belonging to the same cluster, is highly dependent upon RL. Overall, the present findings suggest that SR and RL are both reliable parameters for evaluating the salt tolerance of mulberry germplasm in *in vitro* studies.

Further, a mathematical model was applied by multiple regression analysis to establish the reliability of evaluating the salt tolerance of mulberry germplasm. The unstandardized coefficients of the STI of SR, SL, SW, and RL were 0.07, −0.20, −0.10, and 0.42, respectively. The random error term was 0.39 (Table 7). Therefore, $Y=0.39 + 0.07 \times STI_{SR} + (-0.20) \times STI_{SL} + (-0.10) \times STI_{SW} + 0.42 \times STI_{RL}$ ($p<0.05$), where Y represents the salt tolerance of mulberry germplasm. A higher Y value indicates greater salt tolerance. According to the classification criteria, the salt tolerance of mulberry germplasms has been divided into four clusters, where the highly salt-tolerant (HST) clusters have a Y value of 0.48–0.67, the moderately high salt-tolerant (MHST) and salt-tolerant (ST) classes have Y values of 0.38–0.56 and 0.28–0.35, respectively, and the low salt-tolerant (LST) class has a Y value of 0.32–0.33. To test whether the mathematical evaluation model can predict the salt tolerance of the mulberry germplasm, one germplasm from each of the different clusters (HST, MHST, ST, and LST) was randomly selected, and their Y values were calculated. The results indicated that the formula can be used to evaluate the salt tolerance of any mulberry germplasm. For example, the Y value of Kolitha-3 (HST) is $0.39 + (0.07 \times 0.68) + (-0.20 \times 0.51) + (-0.10 \times 0.44) + (0.42 \times 0.79)$; therefore, Y=0.62 and its mean MFV is 0.60; the Y of China White (MHST) is 0.56 and its mean MFV is 0.52; the Y of White Badana (ST) is 0.31 and its mean MFV is 0.31, and Y of Tollygunj (LST) is 0.33 and its mean MFV is 0.24. The values of the mean MFV and Y were very close. Thus, the model is reliable, and salt tolerance can be predicted by calculating the Y value of any of the 25-mulberry germplasm using the STIs of parameters such as SR, SL, SW, and RL by axillary bud culture. A high MFV indicates a higher salt tolerance level in the mulberry germplasm. The mean MFV is thus affected by the STI of SR, SL, SW, and RL, which also denotes a higher STI value for each indicator.

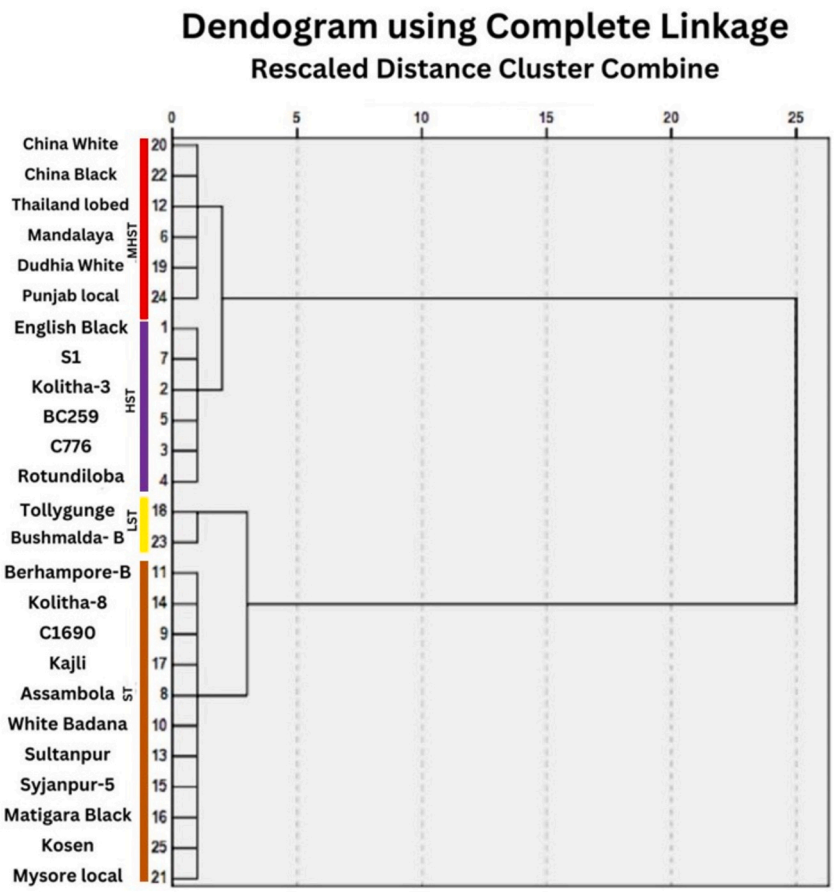

Fig. 3. Hierarchical cluster analysis based on the furthest neighbour to evaluate the salt tolerance of 25 salt-tolerant mulberry germplasm; HST (highly salt-tolerant), MHST (moderately high salt-tolerant), ST (salt-tolerant), and LST (low salt-tolerant).

Table 7
Multiple regression analysis for salt tolerance indices of survival rate (STI_{SR}), shoot length (STI_{SL}), shoot weight (STI_{SW}), and root length (STI_{RL}) in different concentrations of NaCl.

Model	Coefficient		t	Significance
	μ or β	SE		
Constant	0.39	0.06	6.63	0.00
STI_{SR}	0.07	0.22	0.32	0.00
STI_{SL}	−0.20	0.30	−0.66	0.00
STI_{SW}	−0.10	0.29	−0.35	0.00
STI_{RL}	0.42	0.08	5.02	0.00

Mean MFV is the dependent variable and β values are unstandardized coefficients. The constant μ represents the random error term.

3.4. Field evaluation of shortlisted salt-tolerant mulberry germplasm

Six highly salt-tolerant mulberry germplasms, *viz.,* English Black, Kolitha-3, C776, Rotundiloba, $BC_2$59, and S1 shortlisted based on *in vitro* assessment were further evaluated under field trial. The average performance of different mulberry germplasms cultivated at different soil salinity levels of 18–20 dS m^{-1} (Canning I & II and Basanti) and 21 to 23 dS m^{-1} (Namkhana, Kakdwip, and Sagar) is given in Table 8. Results revealed significant differences among the mulberry germplasms evaluated for almost all the characters. In general, the experimental trials of mulberry germplasm at relatively lower salinity levels showed superior performance than the trials at higher soil salinity stresses. Survivability of the mulberry germplasm ranged from 29.9 to 69.2% at soil salinity level of 18–20 dS m^{-1} while no survivability was recorded in Rotundiloba, $BC_2$59, and S1 at salinity level of 21–23 dS m^{-1}. Maximum survivability of 69.2 and 53.2% were recorded in English Black under soil salinity levels of 18–20 dS m^{-1} and 21 to 23 dS m^{-1} respectively and found even higher than that of 23.3% reported earlier [31]. Further, the association between the results of *in vitro* and field trials has been assessed through simple correlation analysis (Table 9). The results of *in vitro* and field trials of salt-tolerant mulberry germplasm

Table 8
Performance of salt tolerant mulberry germplasm under field trial during the month of March–May 2019.

Mulberry Germplasm	Survivability (%)		Plant height (cm)		Leaf yield (kg/ha)		Nitrogen (%)		Phosphorus (%)		Potassium (%)		Avg. STI
	EC 18-20	EC 21-23	EC 18-20	EC 21-23	EC 18-20	EC 21-23	EC 18-20	EC 21-23	EC 18-20	EC 21-23	EC 18-20	EC 21-23	
English Black	69.2 ± 2.8^{a} (−21.5 %)	53.2 ± 2.4^{a} (−39.7 %)	27.2 ± 0.6^{a} (−43.2 %)	10.5 ± 1.3^{a} (−77.2 %)	21.5 ± 2.7^{a} (−28.1 %)	11.5 ± 3.0^{a} (−61.5 %)	3.7 ± 0.1^{a} (−3.2 %)	3.4 ± 0.3^{a} (−11.6 %)	0.2 ± 0.0^{a} (−22.6 %)	0.2 ± 0.0^{a} (−33.3 %)	1.0 ± 0.0^{a} (−13.9 %)	0.8 ± 0.0^{a} (−34.7 %)	0.7
Kolitha-3	55.7 ± 0.4^{b} (−45.5 %)	38.2 ± 0.9^{b} (−61.8 %)	16.2 ± 0.9^{b} (−54.8 %)	8.9 ± 0.4^{a} (−77.2 %)	18.1 ± 1.6^{b} (−39.0 %)	12.6 ± 2.1^{a} (−57.7 %)	4.1 ± 0.2^{b} (−2.2 %)	3.9 ± 0.4^{a} (−5.2 %)	0.2 ± 0.0^{a} (−34.5 %)	0.1 ± 0.0^{b} (−50.6 %)	0.9 ± 0.0^{a} (−16.2 %)	0.7 ± 0.0^{a} (−34.9 %)	0.6
C776	65.1 ± 2.7^{a} (−34.8 %)	40.2 ± 2.6^{b} (−59.8 %)	18.6 ± 0.4^{b} (−55.9 %)	10.9 ± 1.0^{a} (−74.1 %)	17.1 ± 1.8^{b} (−43.5 %)	11.3 ± 2.0^{a} (−62.6 %)	4.3 ± 0.3^{b} (−1.2 %)	4.2 ± 0.0^{a} (−3.0 %)	0.2 ± 0.0^{a} (−23.3 %)	0.2 ± 0.0^{a} (−38.1 %)	1.1 ± 0.0^{a} (−12.0 %)	0.9 ± 0.0^{a} (−29.9 %)	0.6
Rotundiloba	26.1 ± 1.8^{c} (−69.8 %)	–	11.4 ± 1.0^{c} (−53.9 %)	–	16.2 ± 2.0^{b} (−53.4 %)	–	3.5 ± 0.2^{a} (−3.0 %)	–	0.1 ± 0.0^{b} (−59.6 %)	–	0.8 ± 0.0^{b} (−27.4 %)	–	0.3
$BC_2$59	29.9 ± 1.2^{d} (−60.8 %)	–	26.1 ± 0.4^{a} (−35.4 %)	–	19.7 ± 1.9^{a} (−35.6 %)	–	3.4 ± 0.2^{a} (−0.4 %)	–	0.1 ± 0.0^{b} (−54.0 %)	–	0.8 ± 0.0^{b} (−26.4 %)	–	0.3
S1	39.7 ± 2.6^{d} (−61.0 %)	–	21.1 ± 0.5^{d} (−33.9 %)	–	18.8 ± 1.7^{b} (−42.7 %)	–	3.0 ± 0.1^{a} (−4.2 %)	–	0.1 ± 0.0^{b} (−59.4 %)	–	0.8 ± 0.0^{b} (−35.1 %)	–	0.3

Values are means (±SE) of three replications; Different superscript letters in the same column are significantly different ($p \leq 0.05$, ANOVA, Tukey-HSD); Values in parentheses indicate percent change compared to the control. EC is the electrical conductivity of soil measured in dS m^{-1}.

Table 9
Correlation coefficients of parameters between *in vitro* and field trials of salt tolerant mulberry germplasm.

	PH^F	LY^F	N^F	P^F	K^F	RL^{IV}	SW^{IV}	SL^{IV}	SR^{IV}
SR^F	0.81*	0.97**	0.92**	0.97**	0.97**	0.93**	0.60*	0.63*	0.74*
PH^F		0.83*	0.61*	0.70*	0.72*	0.79*	0.62*	0.45^{ns}	0.48^{ns}
LY^F			0.90**	0.96**	0.92**	0.83*	0.69*	0.46^{ns}	0.76*
N^F				0.98**	0.97**	0.85*	0.46^{ns}	0.66*	0.69*
P^F					0.97**	0.87*	0.50^{ns}	0.59^{ns}	0.75*
K^F						0.94**	0.45^{ns}	0.77*	0.68*
RL^{IV}							0.71*	0.74*	0.65*
SW^{IV}								0.97**	0.66*
SL^{IV}									0.79*

SR, PH, LY, N, P, K, RL, SW, and SL stand for survivability rate, plant height, leaf yield, nitrogen, phosphorus, potassium, root length, shoot weight, and shoot length respectively; $^{IV, F}$ results of *in vitro* and field trial; ^{ns} non-significant; *, ** significant at $p \leq 0.05$ or 0.01 respectively.

showed a similar observation. The survivability rate of field trials was positively correlated with the root length (0.93, $p \leq 0.01$) and survivability rate and shoot length (0.74, 0.63, $p \leq 0.05$) of *in vitro* studies. Survivability is one of the important parameters to evaluate the performance of any salt-tolerant genotype since it greatly influences the yield potential of the crop. Mangal and Singh [74] and Rajkumar et al. [75] reported high survivability in the vegetable crop spinach (50%) and industrial crop date palm (40–50%) of commercial importance grown under moderate soil salinity levels of 10–15 dS m^{-1}. Plant height and leaf yield productivity in the mulberry germplasms ranged from 8.9 cm (Kolitha-3) to 27.2 cm (English Black) and 11.3 kg/ha (C776) to 21.5 kg/ha (English Black) respectively under soil salinity level of 18–20 dS m^{-1} and 21 to 23 dS m^{-1}. However, the results of plant height and leaf yield under field trial showed no significant relationships with the survivability rate and shoot length of *in vitro* studies respectively. Ahmad et al. [76] found that an increase in the EC of an experimental soil substrate led to a detrimental effect on the productivity of mulberry, including reduced chlorophyll fluorescence, plant height, and shoot growth. A total number of 22 tossa jute genotypes which were evaluated for various quantitative characteristics including survivability, plant height, and fibre yield exhibited superior performance at the soil salinity threshold level of 7.93 dS m^{-1} [77].

The macronutrients like nitrogen, phosphorus, and potassium are essential for plant's overall growth. The soil's nitrogen (75.80–96.09 mg/kg), phosphorus (13.49–19.20 meq/100 mg), and potassium (13.20–26.20 mg/kg) levels were positively correlated with the nitrogen (r^2 = 0.906), phosphorus (r^2=0.938), and potassium (r^2=0.951) levels in plants. However, increasing salinity significantly (P<0.001) impacted the nitrogen (3–4.3%), phosphorus (0.1–0.2%), and potassium (0.7–1.1 %) content in the leaf tissues of mulberry plants, as shown in Table 8 and Table S2. Under salinity stress, there was a notable decrease in the leaf tissue content of nitrogen (r^2=−0.950), phosphorus (r^2=−0.915), and potassium (r^2 =−0.842). Additionally, a significant positive correlation was observed between the nitrogen, phosphorus, and potassium content of mulberry plants grown under salinity stress and their survival rate (r^2 = 0.968, 0.807, and 0.959), plant height (r^2=0.965, 0.870, and 0.944), and leaf yield (r^2=0.947, 0.871, and 0.869). Maintaining optimal levels of nitrogen, phosphorus, and potassium in both soil and plants is essential for enhancing salt resistance, ensuring healthy growth, and improving crop yields under saline conditions [78]. The variation in the N, P, and K content of leaf tissues of mulberry germplasm under different salinity stresses might be attributed to the plant's ability to uptake mineral composition from the saline soils. A similar observation was noticed between the results of *in vitro* and field trials where the nitrogen and potassium contents in leaves of mulberry germplasm grown under the field condition showed a positive correlation with the root length, shoot length and survivability rate of *in vitro* studies (Table 9). Costa and Medeiros [79] reported significant variations in the N, P, and K content of the watermelon vegetative part influenced by the EC of the irrigation water. Interestingly, some of the morphological characteristics like the plant's height and leaf yield productivity were found to be influenced by a decrease of P and K contents in the leaf. A similar result was also observed for plant height and crop yield when the canola [80] and wheat [81] were evaluated in fields with extremely high soil salinity stress levels. In India, over 6.73 million hectares of land are affected by salinity stress [20]. To harness the potential of salt-tolerant mulberry germplasms like English Black, C776, and Kolitha-3 for poverty alleviation in rural areas and sustainable sericulture, there is a push to promote these cultivars extensively. The importance of biochemical indices such as malondialdehyde (MDA), proline, catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD) in salt tolerance has gained significant recognition. Our recent findings showed that stress-related antioxidant enzymes like CAT, POD, and SOD increased from 1.15 to 5.43, 1.67 to 4.76, and 8.65 to 25.15 g^{-1} FW.min^{-1}, respectively with increasing soil salinity (1.60–22.70 dS m^{-1}) in salt tolerant mulberry germplasm lines [82]. Furthermore, a thorough investigation into other morphological, physiological, biochemical traits, and antioxidant properties of salt-tolerant mulberry germplasms like English Black, C776, and Kolitha-3 has been undertaken. The results of the present study establish a foundation for a simpler and more efficient evaluation of salt tolerance in mulberry germplasm. The proposed evaluation method and regression formula can be used for rapid, large-scale screening of other mulberry germplasm lines, aiding further exploration in breeding salt-tolerant mulberry cultivars.

4. Conclusions

The study conducted in the coastal areas of South 24 Parganas district of West Bengal, India, revealed significant variation in soil salinity levels and nutrient content across different blocks. Screening of mulberry germplasm through *in vitro* axillary bud culture method showed diverse responses to salinity stress, with some germplasms exhibiting high salt tolerance. A mathematical model based

on multiple regression analysis proved reliable in evaluating salt tolerance. Field evaluation of highly salt-tolerant germplasm identified three superior varieties such as English Black, C776, and Kolitha-3. The correlation analysis emphasized the effectiveness of the *in vitro* method in rapidly identifying salt-tolerant germplasm. Root length and survivability rates emerged as important criteria, with a positive correlation observed across different salt concentrations in both *in vitro* and field trials. This underscores the importance of evaluating root growth and survivability when selecting mulberry germplasm. However, further research is needed to assess the food value of the salt-tolerant mulberry germplasm for silkworm performance and silk quality. The increasing soil salinity in coastal areas poses challenges for both agricultural productivity and livelihoods, highlighting the significance of the study in initiating widespread screening of salt-tolerant germplasm to address these concerns.

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Data availability statement

Data will be made available on request.

CRediT authorship contribution statement

Ritwik Acharya: Methodology, Investigation, Formal analysis. **Debnirmalya Gangopadhyay:** Writing – review & editing, Writing – original draft, Supervision, Conceptualization. **Phalguni Bhattacharyya:** Validation, Supervision. **Amitava Ghosh:** Validation, Formal analysis, Data curation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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