

DOI: 10.5586/aa.1793

**Publication history**

Received: 2019-04-19

Accepted: 2019-09-03

Published: 2019-11-26

**Handling editor**

Barbara Hawrylak-Nowak,  
Faculty of Environmental  
Biology, University of Life  
Sciences in Lublin, Poland

**Authors' contributions**

ZMS carried out all the experiments, analyzed the data, and prepared the manuscript; BT designed the experiment, supervised the task, and gave the inputs to finalize the manuscript

**Funding**

This research was financed from the fellowship obtained from the Department of Science and Technology – Innovation in Science Pursuit for Inspired Research (DST-INSPIRE), Government of India, No. DST/INSPIRE Fellowship/2016/160744 dated 2018-02-12.

**Competing interests**

No competing interests have been declared.

**Copyright notice**

© The Author(s) 2019. This is an Open Access article distributed under the terms of the [Creative Commons Attribution License](#), which permits redistribution, commercial and noncommercial, provided that the article is properly cited.

**Citation**

Shandilya ZM, Tanti B.  
Hydroponic screening of traditional rice varieties in Assam, India to estimate their potential resistance to Al toxicity under P deficiency. *Acta Agrobot.* 2019;72(4):1793. <https://doi.org/10.5586/aa.1793>

## ORIGINAL RESEARCH PAPER

# Hydroponic screening of traditional rice varieties in Assam, India to estimate their potential resistance to Al toxicity under P deficiency

Zina Moni Shandilya, Bhaben Tanti\*

Plant Molecular Biology Laboratory, Department of Botany, Gauhati University, Guwahati 781014, Assam, India

\* Corresponding author. Email: [btanti@gauhati.ac.in](mailto:btanti@gauhati.ac.in)

**Abstract**

Acid soils encompass nearly one-third of the available terrestrial land surface worldwide. Acidic soil is one of the major abiotic constraints for agricultural practices by potentially creating aluminum (Al) toxicity and/or phosphorous (P) deficiency. Assam, being an agricultural state of India, has the majority of its area covered by acidic soils due to the varied terrain in the region. Soil acidification increases the solubility of Al present in the soil from its nontoxic silicate or oxide forms into highly phytotoxic ionic Al (mainly the trivalent cation  $Al^{3+}$ ). Ionic Al can form complexes with the available phosphorous leading to plant nutrient deficiency. In the present investigation, screening of traditional rice varieties from Assam was conducted for tolerance to combined Al toxicity and P deficiency. Seedlings of 41 rice landraces from various agro-climatic locations were subjected to three different concentrations of Al (0, 50, 100  $\mu M$ ) for 24, 48, and 72 h under P deficiency in static nutrient culture to identify the extent of their resistance to these stressed conditions. Different morpho-physiological parameters (root and shoot lengths, fresh and dry weight yields, chlorophyll and relative water content) were evaluated after stress treatment. All the experiments were conducted in a randomized block design with three replicates. Based on the overall morphological characters, total stress response index (TSRI) was calculated which showed a variation ranging from 18 to 23. Accordingly, the varieties were classified into different groups of resistance. Varieties 'Moti' and 'Baismuthi' were found to be the least resistant, whereas 'Holpuna', 'Beto', and 'Soria Sali' were identified as most tolerant varieties to Al toxicity under P deficiency. The findings of the present investigation could be exploited for developing promising varieties in future rice breeding programs.

**Keywords**

aluminium toxicity; phosphorus deficiency; morpho-physiological parameters; *Oryza sativa* L.; total stress tolerance index

**Introduction**

Aluminum (Al) is one of the most abundant metals comprising approximately 7% in the Earth's crust. The chemical stability of Al depends upon the pH of the soil. At a neutral pH, Al minerals exist in an almost insoluble state. However, in acidic conditions Al is oxidized to  $Al^{3+}$ , which is toxic to most of the conventional crops including rice [1–3]. As such,  $Al^{3+}$  binds to soluble phosphorous (P) limiting the plant-available P. Therefore, the adverse effect of an acidic soil on plant growth is strongly related to both the toxicity of  $Al^{3+}$  and P deficiency.

Very little free  $\text{Al}^{3+}$  in the soil solution causes damage to plant systems. Soluble forms of Al inhibit root growth as well as the development of root hairs. This results in poor nutrient uptake and assimilation and thereby leads to shoot nutrient deficiencies and a reduction of productivity [1,4]. This inhibition of root growth is one of the significant symptoms of Al toxicity, which occurs due to the interaction between  $\text{Al}^{3+}$  ions and root cells and their components [5–7].

Plants have a wide range of adaptability to tolerate the soluble form of  $\text{Al}^{3+}$ ; some species can tolerate  $>1 \text{ mg L}^{-1}$ , whereas most will show an adverse effect even at concentrations  $<0.5 \text{ mg L}^{-1}$ . A large proportion of soil Al is locked up in soil minerals such as aluminosilicates, with much smaller fractions appearing in soluble forms that are capable of influencing biological systems [8].

Deficiency of P is one of the major limiting factors for rice growth and yield [9]. In upland conditions, P deficiency commonly occurs due to the strong binding affinity of P, which predominantly reacts with Al and iron (Fe) in acidic soils, whereas it reacts with calcium (Ca) in neutral to alkaline conditions and consequently forms partially soluble complexes [10]. As compared to other macronutrients, P is found to be one of the least mobile and available elements in the soil. Phosphorus in the soils occurring in both organic and inorganic forms and P availability is a prime limiting criterion for plant growth and development.

The alarming increase in human population demands the necessity to accelerate agricultural productivity. Hence, it is a challenge for the agricultural community to fulfil the increasing demands for future food supply across the globe. In this context, it is equally important to think about soil health status, which is being impacted due to various abiotic factors caused by climatic changes. Efforts are being made to understand the complex processes of agro-ecosystems and the various interactions governing the sustainability of agricultural lands [11]. However, it is becoming clear that conventional agricultural practices cannot sustain the production base to maintain a healthy plant/soil system indefinitely and, as such, augment crop productivity. Currently, agronomists depend heavily on chemical fertilizers. In this context, P is the next most limiting essential nutrient after nitrogen (N), restricting crop growth and productivity. In the global context of soil status, most tropical and subtropical soils are acidic in nature and are thus often P deficient [12].

Acid soils are a serious problem in Assam and most of the areas of Northeast India and Southeast Asia, including Myanmar, Bangladesh, and parts of China. The low pH of Assam soils adversely affects crop productivity, notably of rice [13]. As rice is widely cultivated in Assam, it is therefore imperative for plant biologists to identify promising rice varieties that can thrive under Al toxicity and P-deficient soil conditions. With this in mind, the present study was conducted to explore the potential of some traditional rice landraces from Assam and to identify tolerant varieties to be used in future rice breeding programs.

## Material and methods

### Plant material and growth conditions

Seeds of 41 traditional rice (*Oryza sativa* L.) varieties were collected from different agro-climatic zones of Assam, India. The collected rice samples with their vernacular names were: 'Baismuthi', 'Rupohi', 'Anjana', 'Kartik', 'Hung bora', 'Moti', 'Maguribao', 'Sokbonglong', 'Jahingia', 'Basful', 'Bejilahi', 'Adoliya', 'Sornosab', 'Kekuabao', 'Lucky', 'Batisali', 'Moinagiri', 'Boradhan', 'Bokulbora', 'Kolabora', 'Moinaxali', 'Guntibora', 'Chokuwa', 'Biyoisali', 'Soriasali', 'Kolajoha', 'Bhugpuri joha', 'Bor joha', 'Betguti', 'Lotasali', 'Malsora', 'Halda', 'Nalabora', 'Lauguti', 'Suwagmoni', 'Tilbora', 'Beto', 'Fulpakhri', 'Holpuna', 'Nania', and 'Prasad bhog'.

Seeds were surface-sterilized with 0.1%  $\text{HgCl}_2$  for 15 minutes and washed three–four times with sterile distilled water to remove the residues of previous treatments. Germination was carried out on paper film and sterile moistened cotton in a growth chamber at 25–28°C for 72 h. Twenty seedlings of almost equal size were transplanted into transparent plastic vessels and allowed to grow in Hoagland's nutrient solution

[14] in the growth chamber under white light with a photon flux density of  $220 \mu\text{mol m}^{-2} \text{s}^{-1}$  (PAR) over a 14-h photoperiod. The nutrient solution was replaced after 5 days. Nine-day old seedlings were subjected to a  $500 \mu\text{M CaCl}_2$  (pH 4.5) pretreatment for 24 h in order to maintain homeostasis, followed by treatment with  $\text{AlCl}_3$  at different concentrations (0, 50, and  $100 \mu\text{M}$ ) for 24, 48, and 72 h in a modified Hoagland's solution where  $\text{NH}_4\text{H}_2\text{PO}_4$  was replaced by  $\text{NH}_4\text{Cl}$  to maintain the P-deficient conditions. Plants grown in regular Hoagland's medium without P deficiency and Al stress were considered as the controls. Three biological replicates were maintained and cultures were placed in a randomized block design in the growth chamber.

#### Measurement of plant growth and biomass

Plant growth was measured in terms of shoot and root length, fresh weights, dry weights, relative water content (RWC), and chlorophyll content. For each treatment, three replicates consisting of 20 seedlings were considered, and from each replicate five plants were chosen randomly for the measurement of shoot and root lengths. Again, three plants from each replicate were randomly selected for measurement of fresh and dry biomass. Then shoot and root tissues were dried at  $80^\circ\text{C}$  for 48 h and weighed to obtain dry biomass.

To determine the relative water content (RWC), the fresh weight was initially measured, then the seedlings were placed in distilled  $\text{H}_2\text{O}$  for 4 h in diffuse light. When a shoot became fully turgid, it was reweighed. The dry weight was taken after drying for 72 h and the shoot RWC was then calculated [15].

#### Determination of chlorophyll concentration

For chlorophyll *a*, *b*, and total chlorophyll concentrations, fresh leaf samples of 300 mg were crushed in mortar and pestle with 5 mL of 80% acetone. The absorbance was measured at 663 nm and 645 nm using an UV-Vis spectrophotometer (Eppendorf Bio-Spectrophotometer Basic-603873) against 80% acetone as reference, and total chlorophyll was calculated by using a standard formula [15].

#### Determination of total stress response index

Total stress response index (TSRI) was calculated for the maximum stress duration (72 h) by using the established formula with modifications [16]. Calculation was carried out by dividing the average stress from the average control value for each variety at both Al concentrations ( $50 \mu\text{M}$  and  $100 \mu\text{M}$ ) for all the parameters determined individually. Summation of all the parameters was made to obtain the datasets for both Al concentrations. Finally, the mean tolerance values of both data sets were calculated to determine the TSRI. A dendrogram was generated by Ward's linkage method based on similarity in total tolerance indices.

#### Statistical analysis

As the experiments were conducted with a completely randomized design, summarized data were calculated as treatment means  $\pm SE$  for three replicates. Pearson's correlation coefficient analysis was carried out using the MS Excel data sets and statistical significance was considered at *p* values  $< 0.05$ . Data were analyzed using the XLSTAT 2017 statistical package [17]. A cluster analysis of the 41 rice varieties was carried out based on the TSRI data.

## Results

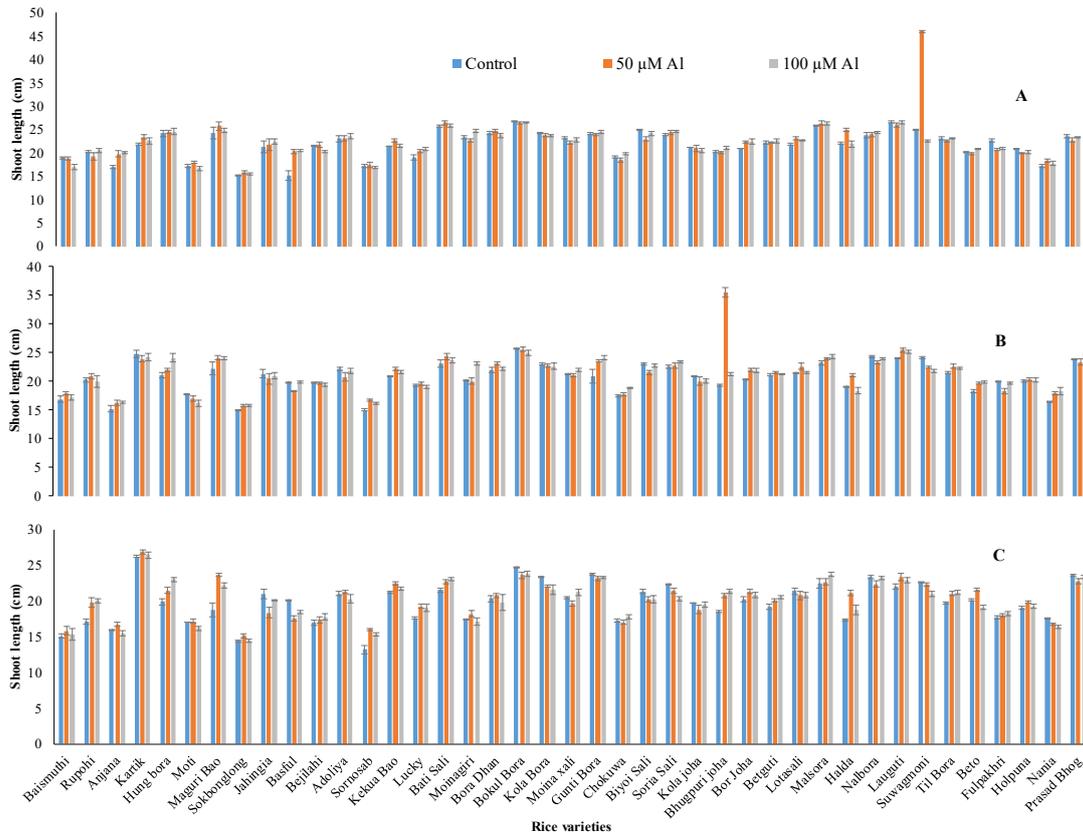
### Morpho-physiological parameters under Al treatment and P deficiency

**Shoot and root length.** The growth of the rice seedlings in Hoagland's solution under Al treatment and P deficiency was measured in terms of shoot and root lengths and revealed that the growth of most of the varieties was impacted. The shoot length of all the 41 traditional rice varieties ranged from 15.9 to 46.1 cm at 50  $\mu\text{M}$  Al and from 15.6 to 26.6 cm at 100  $\mu\text{M}$  Al after 24 h of the stress treatment. Forty-eight h of stress resulted in a range of shoot lengths between 15.0 and 40.8 cm at 50  $\mu\text{M}$  Al and between 15.8 and 25.1 cm in the 100  $\mu\text{M}$  Al treatment. After 72 h at this Al concentration, a range of 15.1–23.6 cm at 50  $\mu\text{M}$  Al and 15.2–26.4 cm was noted. Variety 'Suwagmoni' produced the maximum shoot length (46.1 cm), whereas 'Sokbonglong' showed the lowest (15.9 cm) at 50  $\mu\text{M}$  Al. Variety 'Lauguti' emerged as most tolerant at 100  $\mu\text{M}$  Al concentration after 24 h stress duration. However, after 48 h, 'Sokbonglong' had the lowest (15.0 cm) and 'Bhugpuri joha' the greatest (40.8 cm) shoot lengths. With 72 h Al exposure, 'Sokbonglong' showed the lowest (15.1 cm) and 'Maguri bao' the highest (23.6 cm) shoot lengths in the 50  $\mu\text{M}$  Al treatment, whereas 'Sornosab' showed the lowest (15.2 cm) and 'Kartik' the highest (26.4 cm) shoot lengths at 100  $\mu\text{M}$  Al (Fig. 1).

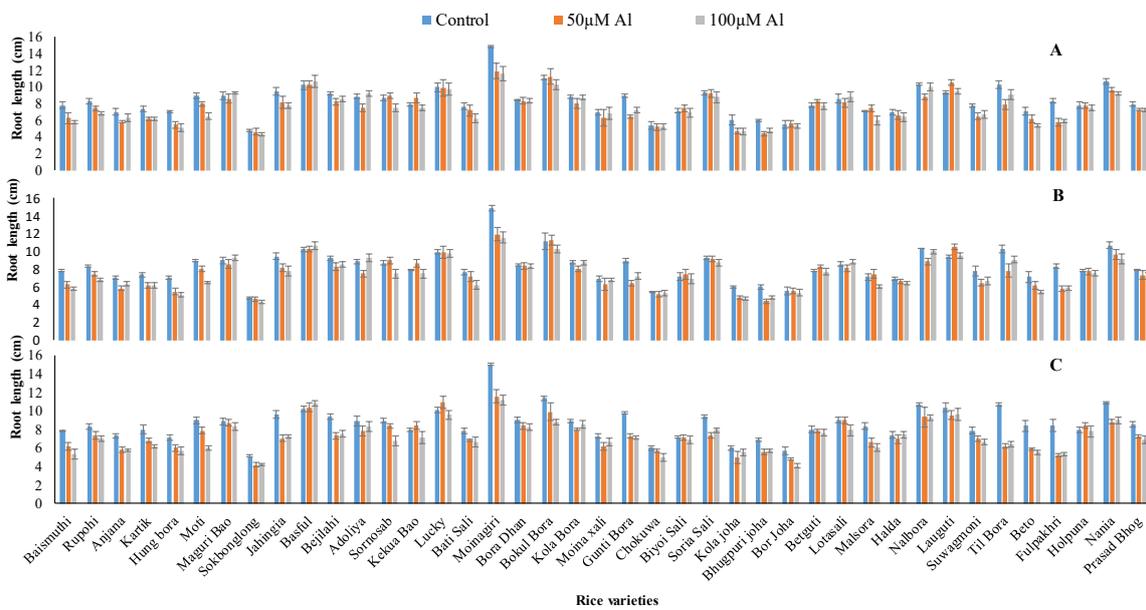
In the case of the roots, lengths ranged from 4.4–11.9 cm at 50  $\mu\text{M}$  Al to 4.7–11.5 cm at 100  $\mu\text{M}$  Al after 24 h. After 48 h, root lengths did not show marked differences at both the Al concentrations used. After 72 h, the root lengths ranged from 4.2 to 11.5 cm at 50  $\mu\text{M}$  Al and from 4.3 to 11.1 cm at 100  $\mu\text{M}$  Al. Variety 'Bhugpuri joha' showed the lowest (4.4 cm) and 'Moinagiri' was characterized by the highest (11.9 cm) root length at 50  $\mu\text{M}$  Al. Varieties 'Kola joha' and 'Moinagiri' were the lowest (4.7 cm) and the highest (11.5 cm), respectively, at 100  $\mu\text{M}$  Al after 24-h exposure. After 48 h, the lowest and highest root lengths at 50  $\mu\text{M}$  Al were found in 'Bhugpuri joha' (4.4 cm) and 'Moinagiri' (11.9 cm), respectively, and at 100  $\mu\text{M}$  Al in 'Sokbonglong' (4.3 cm) and 'Moinagiri' (11.5 cm), respectively. With an increased stress exposure time to 72 h, 'Sokbonglong' showed the lowest (4.3 cm) and 'Moinagiri' the highest (11.5 cm) root lengths at 50  $\mu\text{M}$  Al, whilst at 100  $\mu\text{M}$  Al, 'Sokbonglong' produced the lowest (4.3 cm) and 'Kartik' the highest (11.1 cm) root lengths (Fig. 2).

**Root and shoot fresh weights.** The root fresh weights ranged from 0.04 to 0.13 g at 50  $\mu\text{M}$  Al and from 0.04 to 0.17 g at 100  $\mu\text{M}$  Al after 24 h of exposure. After 48 h, they ranged from 0.05 to 0.13 g at 50  $\mu\text{M}$  Al and from 0.05 to 0.14 g under 100  $\mu\text{M}$  Al treatment, respectively. After 72 h, they ranged from 0.04 to 0.15 g at both Al concentrations used. After 24 h of treatment, 'Bhugpuri joha' showed the lowest root fresh weights (0.04 g) at both Al concentrations, whereas 'Bora dhan' showed the highest (0.12 g) root fresh weights at 50  $\mu\text{M}$  Al and 'Baismuthi' emerged as tolerant at 100  $\mu\text{M}$  Al (0.17 g). However, after 48 h of stress, 'Bhugpuri joha' had the lowest (0.05 g) and 'Bora dhan' the highest (0.13 g) root fresh weights at 50  $\mu\text{M}$  Al and Fulpakhri the lowest (0.04 g) and 'Bora dhan' the highest (0.13 g) root fresh weights in 100  $\mu\text{M}$  Al. After 72 h exposure, 'Bhugpuri joha' produced the lowest (0.04 g) and 'Rupohi' the highest (0.15 g) root biomass at 50  $\mu\text{M}$  Al. Varieties 'Nalbora' and 'Basful' had the lowest (0.05 g) and the highest (0.15 g) root fresh weights, respectively, at 100  $\mu\text{M}$  Al (Fig. 3).

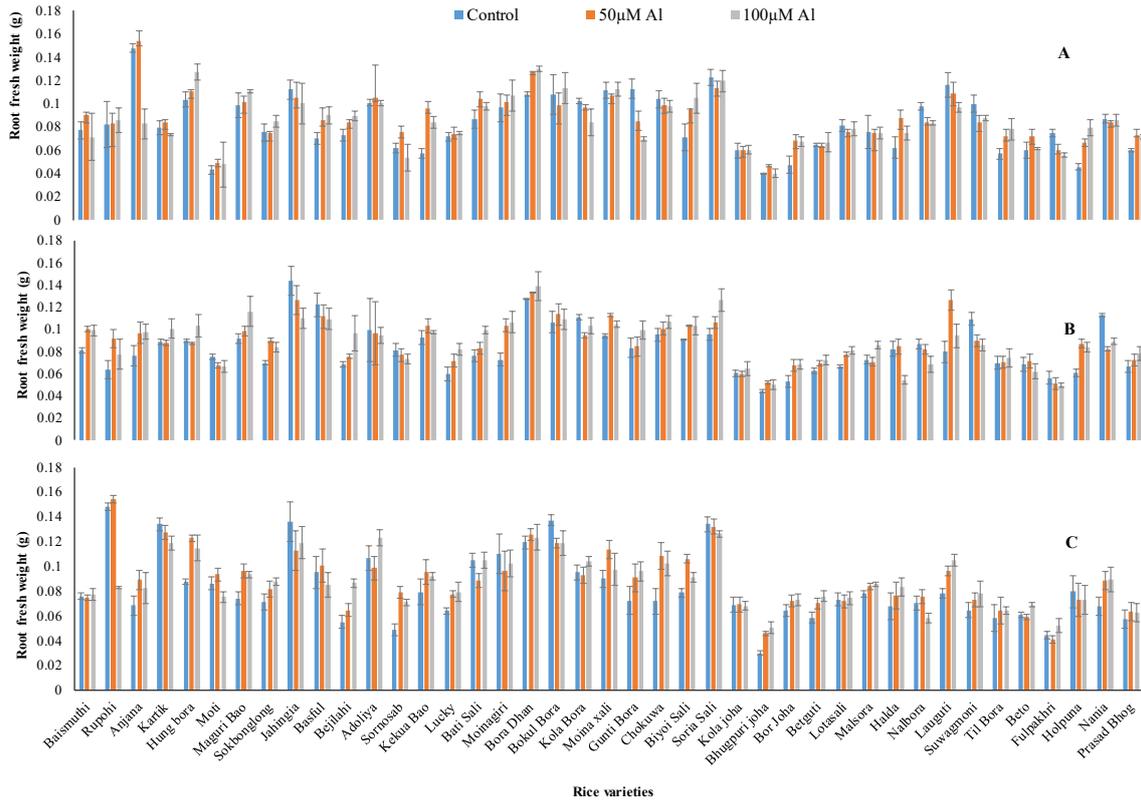
The fresh weight of shoots ranged from 0.09–0.31 g at 50  $\mu\text{M}$  Al to 0.18–0.31 g at 100  $\mu\text{M}$  Al after 24 h. However, after 48 h they ranged between 0.16 and 0.30 g at 50  $\mu\text{M}$  Al and between 0.18 and 0.38 g in the 100  $\mu\text{M}$  Al treatment. After 72 h, a range of 0.15–0.29 g in 50  $\mu\text{M}$  Al and 0.15–0.34 g in the 100  $\mu\text{M}$  Al treatment was noted. Variety 'Kola bora' produced the lowest shoot fresh weights (0.09 g) at 50  $\mu\text{M}$  Al and 'Kola joha' the lowest (0.18 g) at 100  $\mu\text{M}$  Al. Variety 'Lauguti' showed the highest shoot fresh weights (0.31 g) in both the Al treatments after 24 h. However, after 48 h, 'Bhugpuri joha' showed the lowest (0.16 g) and 'Lauguti' the highest shoot fresh weights (0.30 g) in 50  $\mu\text{M}$  Al. Simultaneously, 'Moti' produced the lowest (0.18 g) and 'Kekua bao' the highest (0.30 g) shoot fresh weights at 100  $\mu\text{M}$  Al. After 72 h of Al exposure, 'Bhugpuri joha' demonstrated the lowest shoot fresh weights (0.15 g) in both the Al treatments used and 'Adoliya' showed the highest (0.29 g) at 50  $\mu\text{M}$  Al. 'Kartik' had the greatest shoot fresh weights (0.34 g) at 100  $\mu\text{M}$  Al (Fig. 4).



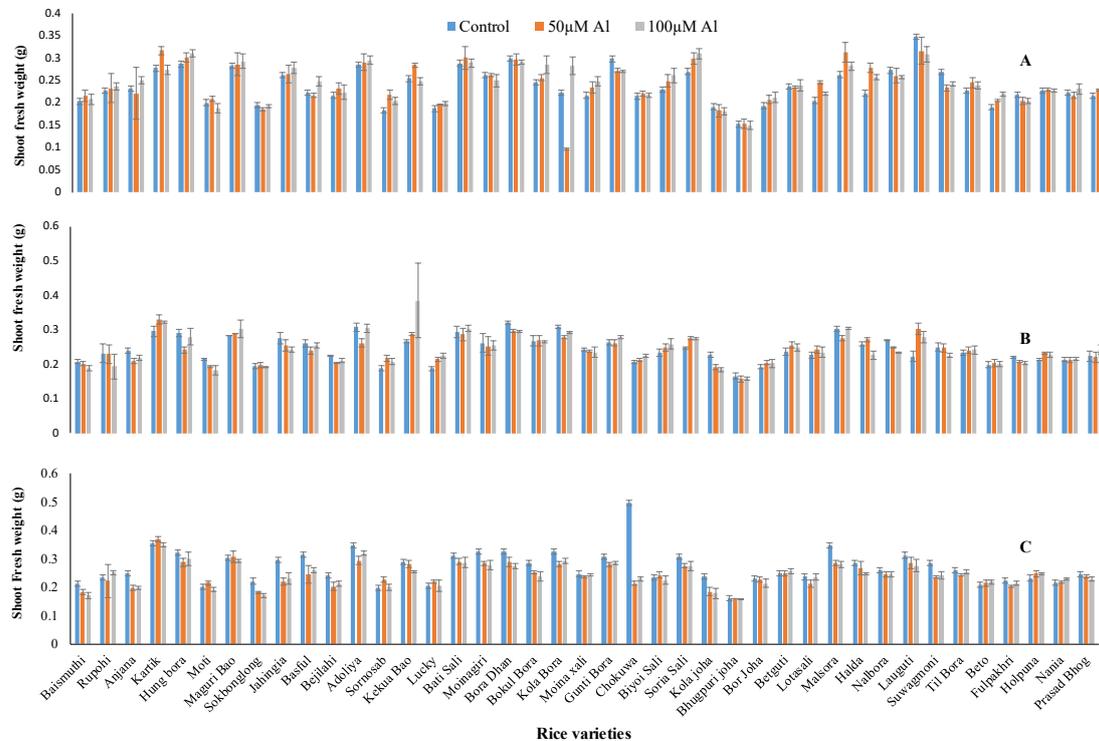
**Fig. 1** The length of rice shoots under Al toxicity and P deficiency after 24 h (A), 48 h (B), and 72 h (C) of Al stress exposure (data presented as means  $\pm$ SE,  $n = 3$ , where  $n$  indicates the number of replicates).



**Fig. 2** The root lengths under Al toxicity and P deficiency after 24 h (A), 48 h (B), and 72 h (C) of Al stress exposure (data presented as means  $\pm$ SE,  $n = 3$ , where  $n$  indicates the number of replicates).



**Fig. 3** The root fresh weights under Al toxicity and P deficiency after 24 h (A), 48 h (B), and 72 h (C) of Al stress exposure (data presented as means  $\pm$ SE,  $n = 3$ , where  $n$  indicates the number of replicates).



**Fig. 4** The shoot fresh weights under Al toxicity and P deficiency after 24 h (A), 48 h (B), and 72 h (C) of Al stress exposure (data presented as means  $\pm$ SE,  $n = 3$ , where  $n$  indicates the number of replicates).

**Root and shoot dry weights.** Root dry weights ranged from 4 to 10 mg at 50  $\mu\text{M}$  Al and from 3 to 17 mg at 100  $\mu\text{M}$  Al after 24 h in the stress treatments. After 48 hours, the dry weights ranged from between 3 and 58 mg at 50  $\mu\text{M}$  Al and between 3 and 50 mg at 100  $\mu\text{M}$  Al and after 72 h, a range of 3–10 mg in 50  $\mu\text{M}$  Al and 3–9 mg in the 100  $\mu\text{M}$  Al concentrations were found. With all the times of stress duration, 'Bhugpuri joha' showed the lowest root dry weights (3 mg) at both the Al concentrations used. 'Bora dhan' had the highest root dry weights (10 mg) at 50  $\mu\text{M}$  Al, whilst 'Rupohi' emerged as tolerant (17 mg) in 100  $\mu\text{M}$  Al after 24 h. However, after 48 h, 'Baismuthi' revealed the highest root dry weights (50 mg) in both Al treatments. After 72 h, 'Bhugpuri joha' demonstrated the lowest (4 mg) and 'Rupohi' and 'Kartik' the highest (10 mg) root dry weights at 50  $\mu\text{M}$  and 100  $\mu\text{M}$  Al, respectively (Fig. 5).

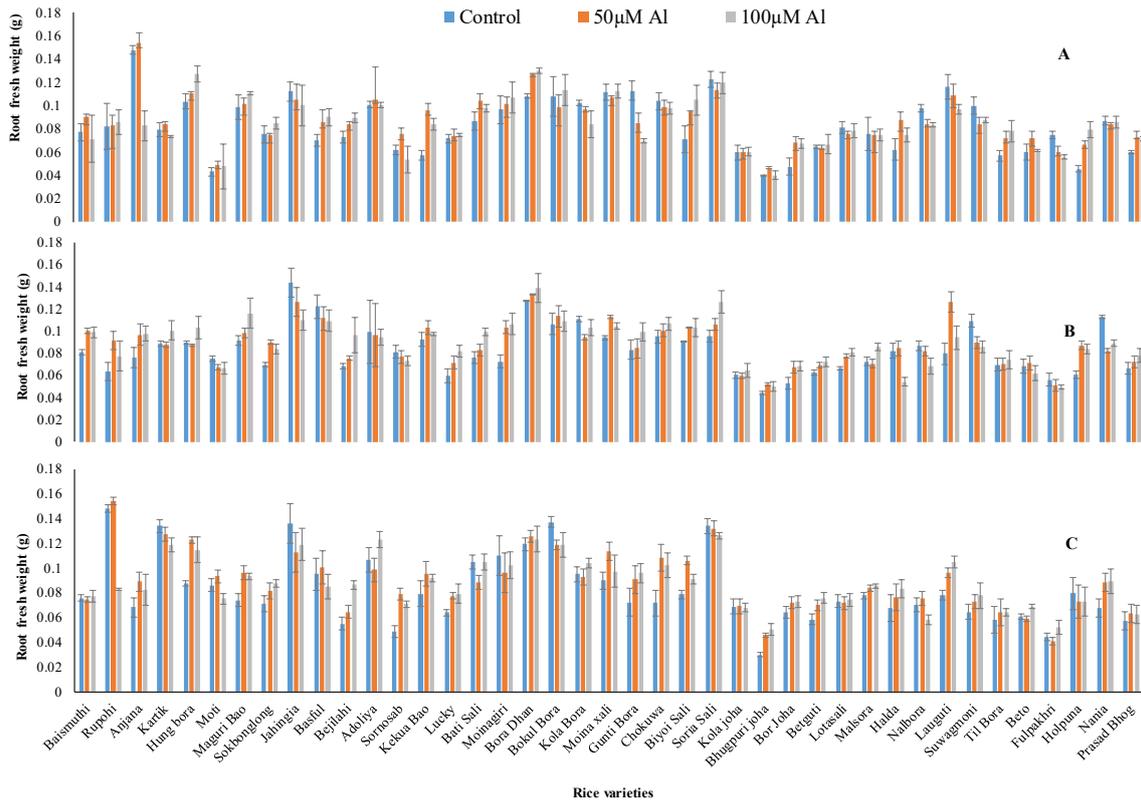
After 24 h, the shoot dry weights ranged from 18 to 80 mg in the 50 and 100  $\mu\text{M}$  Al treatments, respectively. After 48 h, they ranged from between 17 and 90 mg at 50  $\mu\text{M}$  Al and between 10 and 70 mg at 100  $\mu\text{M}$  Al. After 72 h, a range of 18–50 mg for 50  $\mu\text{M}$  Al and 19–60 mg for 100  $\mu\text{M}$  Al was noted. With 24-h treatment, varieties 'Lucky' and 'Bhugpuri joha' produced by the lowest shoot dry weights at 50  $\mu\text{M}$  Al and 100  $\mu\text{M}$  Al, respectively. 'Rupohi' was seen to have highest (80 mg) shoot dry weights. However, after 48 h, 'Bhugpuri joha' showed the lowest shoot dry weights (17 mg) at both Al concentrations and 'Anjana' the highest shoot fresh weights (90 mg) at 50  $\mu\text{M}$  Al and 'Kartik' showed the highest shoot dry weights (70 mg) at 100  $\mu\text{M}$  Al. Again, after 72-h stress treatment, 'Bhugpuri joha' showed the lowest shoot dry weights (18 mg) in both the Al concentrations and 'Rupohi' the highest shoot fresh weights (56 mg) at 50  $\mu\text{M}$  Al. Variety 'Anjana' produced the highest shoot dry weights (60 mg) in 100  $\mu\text{M}$  Al (Fig. 6).

**Relative water content (RWC).** To understand the effect of Al stress, relative water content (RWC) of the leaves was monitored in both control and treated plants. RWC showed significant differences in both the Al stress regimes. Varieties 'Jahingia' and 'Moti' showed higher RWCs at 24 h, 'Bati Sali' and 'Hung bora' at 48 h, and 'Betguti' and 'Bor joha' showed higher RWCs after 72 h. Varieties 'Nal bora' and 'Prasad bhog' showed the lowest RWCs at 24 h and 48 h, whereas at 72 h, a significant reduction in RWC was found in 'Anjana' at 50  $\mu\text{M}$  Al and in 'Baismuthi' at 100  $\mu\text{M}$  Al (Fig. 7).

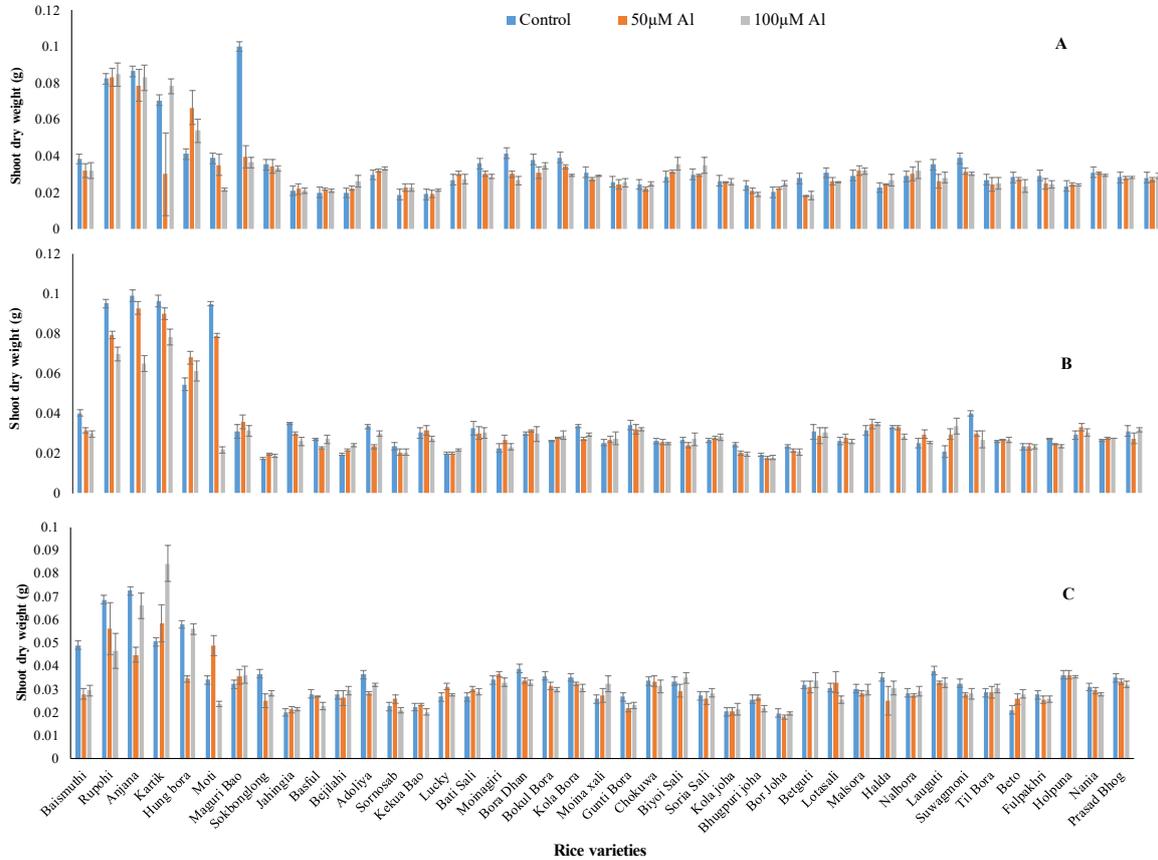
**Chlorophyll concentration.** All the rice varieties tested revealed variations in total chlorophyll content in the Al-treated plants compared to the control. The total chlorophyll concentrations ranged from 5.2 to 23.3  $\text{mg mL}^{-1}$  at 50  $\mu\text{M}$  Al and from 3.9 to 21  $\text{mg mL}^{-1}$  at 100  $\mu\text{M}$  Al after 24 h. After 48 h of treatment, ranges of 3.9–11.8  $\text{mg mL}^{-1}$  at 50  $\mu\text{M}$  Al and 3.8–11.56  $\text{mg mL}^{-1}$  at 100  $\mu\text{M}$  Al were determined. After 72 h, the ranges were 5.8–12.1  $\text{mg mL}^{-1}$  for 50  $\mu\text{M}$  Al and 5.3–10.6  $\text{mg mL}^{-1}$  for 100  $\mu\text{M}$  Al. Among all the rice varieties, 'Anjana' showed a significantly highest total chlorophyll concentration, whereas 'Moti', 'Maguri bao', and 'Basful' showed lower chlorophyll concentrations over the respective time frames (Fig. 8).

#### Correlation between the different morpho-physiological parameters of rice grown under Al toxicity and P deficiency

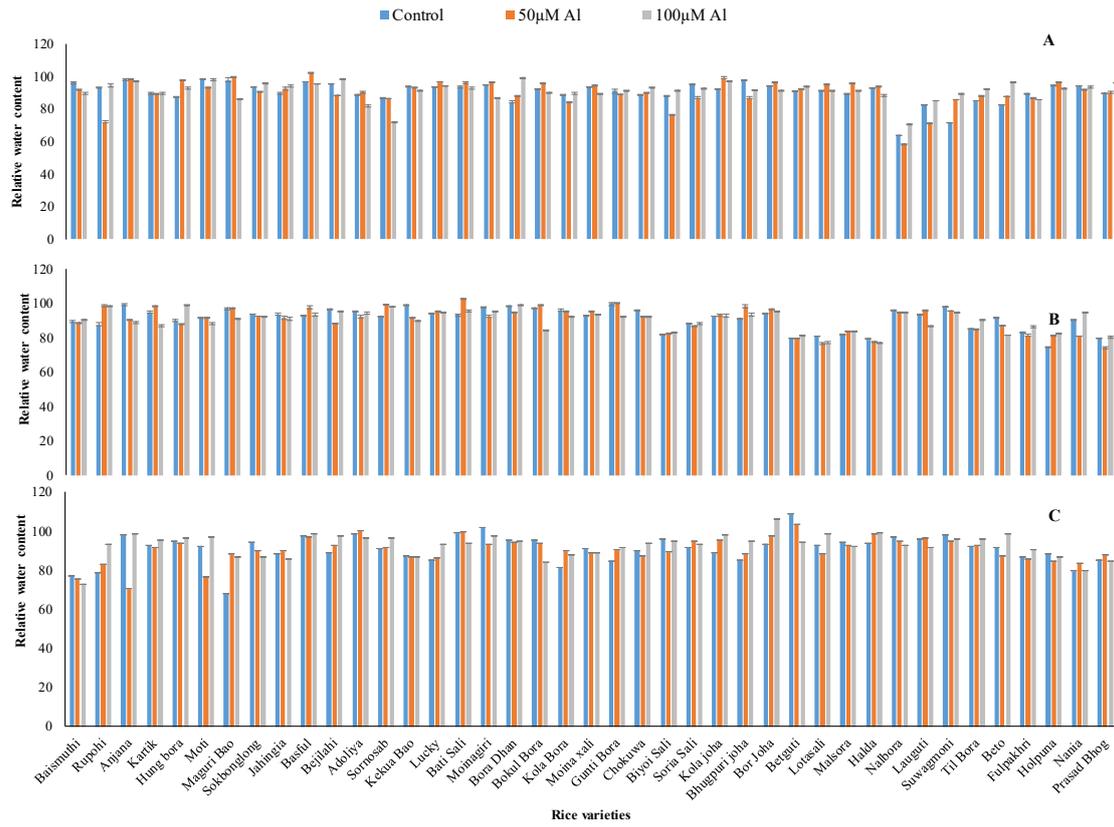
Pearson's correlation coefficients for comparisons between different morphological and physiological parameters are tabulated in Tab. 1. These showed significant positive relationships between shoot fresh weight (SFW) and shoot length (SL) ( $r = 0.64$ ) at 24 h of Al treatment, 0.55 at 48-h exposure, and 0.59 after 72 h. Shoot fresh weight showed significantly positive correlations with shoot length ( $p < 0.05$ ) at all durations of treatment. Similarly, a significant positive was revealed between root fresh weight (RFW) and SFW, and shoot dry weight (SDW) and root dry weight (RDW) for all Al treatment durations. After 72-h treatment, other significant positive correlations were observed: RDW–SFW, RDW–RFW, and RDW–SDW.



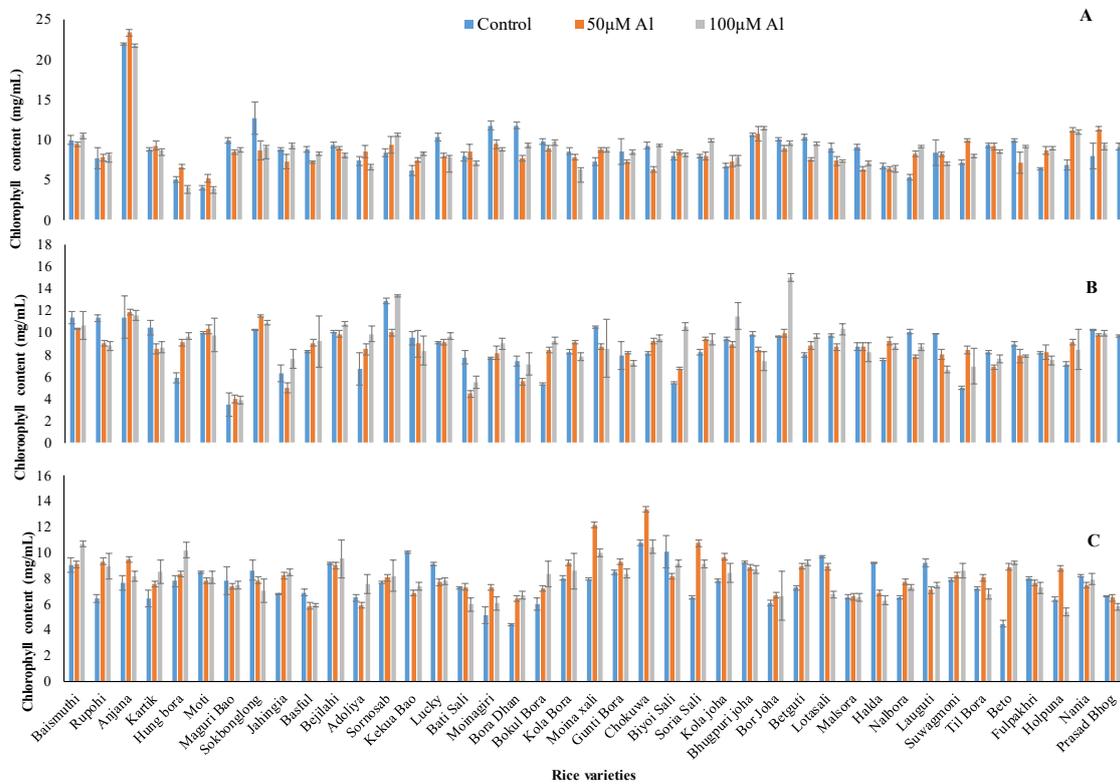
**Fig. 5** The root dry weights under Al toxicity and P deficiency after 24 h (A), 48 h (B), and 72 h (C) of Al stress exposure (data presented as means  $\pm$ SE,  $n = 3$ , where  $n$  indicates the number of replicates).



**Fig. 6** The shoot dry weights under Al toxicity and P deficiency after 24 h (A), 48 h (B), and 72 h (C) of Al stress exposure (data presented as means  $\pm$ SE,  $n = 3$ , where  $n$  indicates the number of replicates).



**Fig. 7** Relative water content under Al toxicity and P deficiency after 24 h (A), 48 h (B), and 72 h (C) of Al stress exposure (data presented as means  $\pm$ SE,  $n = 3$ , where  $n$  indicates the number of replicates).



**Fig. 8** Total chlorophyll concentration under Al toxicity and P deficiency after 24 h (A), 48 h (B), and 72 h (C) of treatment (data presented as means  $\pm$ SE,  $n = 3$ , where  $n$  indicates the number of replicates).

**Tab. 1** Pearson's correlation coefficients for different morpho-physiological rice parameters after 24-h, 48-h, and 72-h Al treatment.

Parameters	SL	RL	SFW	RFW	SDW	RDW	CHL	RWC
<b>24 h</b>								
SL	1							
RL	0.213	1						
SFW	0.644*	0.335	1					
RFW	0.389	0.288	0.668*	1				
SDW	0.023	-0.089	0.314	0.397	1			
RDW	-0.169	-0.037	0.060	0.177	0.576*	1		
CHL	-0.218	-0.068	-0.150	0.265	0.412	0.136	1	
RWC	-0.316	-0.157	-0.215	-0.062	0.084	-0.013	0.201	1
<b>48 h</b>								
SL	1							
RL	0.189	1						
SFW	0.550*	0.336	1					
RFW	0.140	0.388	0.603*	1				
SDW	-0.075	-0.161	0.182	0.061	1			
RDW	-0.228	-0.097	-0.163	0.068	0.321	1		
CHL	-0.583	-0.232	-0.554	-0.367	0.144	0.209	1	
RWC	0.022	0.103	0.130	0.349	0.087	0.056	-0.049	1
<b>72 h</b>								
SL	1							
RL	0.143	1						
SFW	0.594*	0.284	1					
RFW	0.293	0.264	0.624*	1				
SDW	0.221	-0.021	0.371	0.502*	1			
RDW	0.244	0.220	0.555*	0.717*	0.737*	1		
CHL	-0.248	-0.377	-0.199	-0.057	-0.056	-0.226	1	
RWC	0.234	0.067	0.300	0.052	-0.140	-0.179	-0.293	1

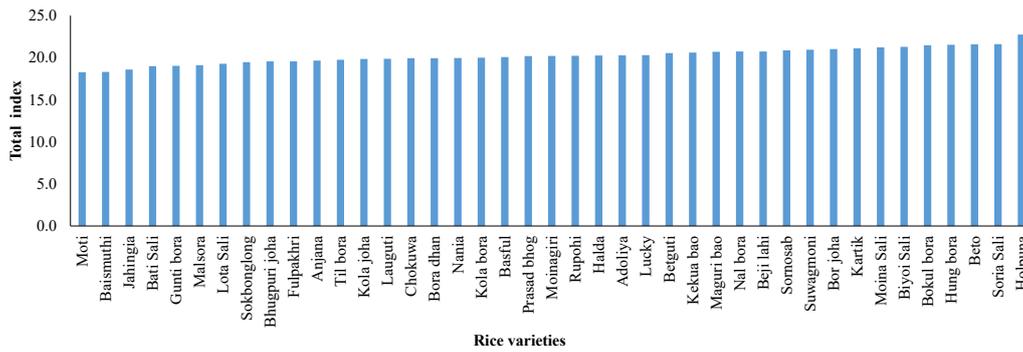
Level of significance: \*  $p < 0.05$  (two-tailed). RL – root length; SL – shoot length; RFW – root fresh weight; RDW – root dry weight; SFW – shoot fresh weight; SDW – shoot dry weight; RWC – relative water content; CHL – chlorophyll concentration.

### Total stress response index (TSRI)

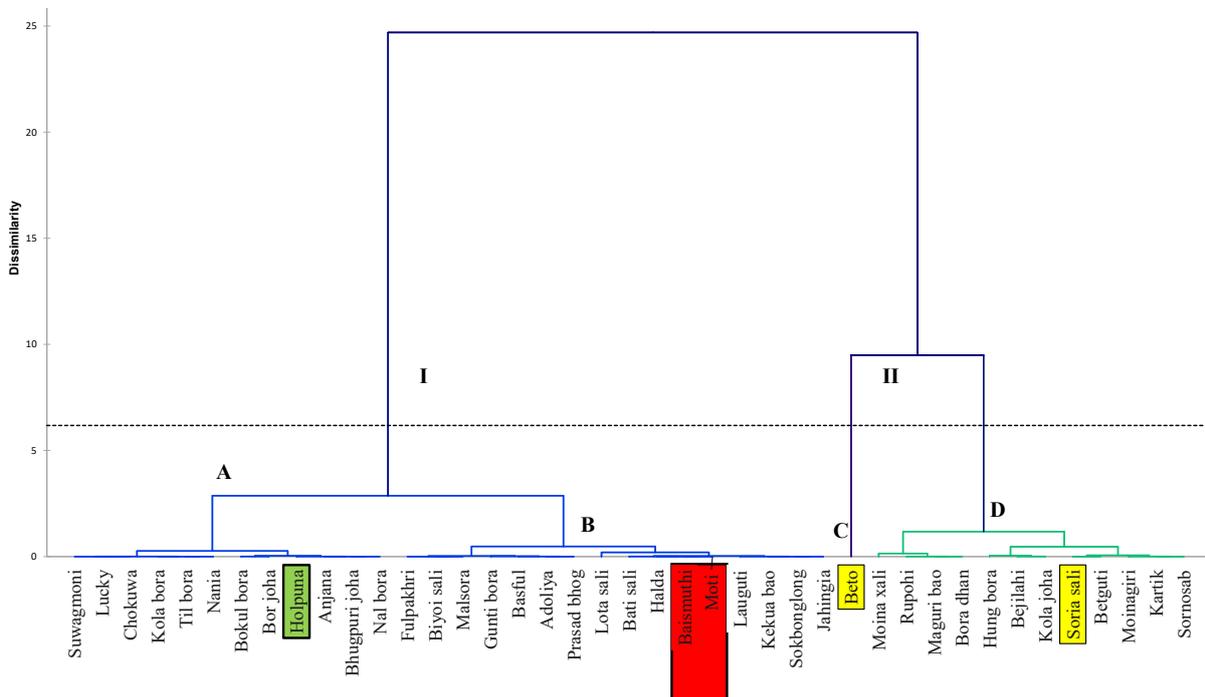
All the morpho-physiological parameters were statistically analyzed and based on these results, TSRI was calculated which clearly illustrated the differences in plant resistance to Al toxicity under P deficiency between the rice varieties. Based on its TSRI value, variety 'Holpuna' could be identified as the most resistant, 'Soria Sali' and 'Beto' as moderately resistant, whilst 'Baismuthi' and 'Moti' are the most sensitive (Fig. 9). On the basis of the differences of all the TSRI values, the varieties can be classified into four groups: highly resistant ( $\geq 4.0$ ), moderately resistant (3–4), susceptible (2–3), and highly susceptible ( $\leq 2.0$ ) to Al toxicity under P deficiency.

### Clustering based on morpho-physiological parameters of rice grown under Al toxicity and P deficiency

A dendrogram was constructed and a cluster analysis performed based on distance matrices which revealed two major clusters. Cluster I comprised 28 rice varieties and Cluster II 13 varieties. Additionally, Cluster I was further divided into two groups and



**Fig. 9** Total stress response indices based on morpho-physiological parameters of different rice varieties grown under Al toxicity and P deficiency.



**Fig. 10** Dendrogram showing a correlation cluster analysis of all the rice varieties based on the total stress response index.

Cluster II formed two major groups (C, D). Group D could be further divided into three subgroups. The selected susceptible varieties ‘Moti’ and ‘Baismuthi’ clustered together revealing their similarity in Al response. Varieties ‘Soria sali’ and ‘Beto’ clustered together again suggesting moderate similarity, whereas the highly tolerant ‘Holpuna’ emerged in a different cluster justifying our conclusions on the basis of morpho-physiological parameters under Al toxicity and P deficiency (Fig. 10).

### Discussion

It has been demonstrated in previous works that P supply can ameliorate Al toxicity possibly through precipitation of Al in the rhizosphere. There arises a relative ranking of Al tolerance in plants and the probable cause is due to P and Al interactions which exist in acidic soils. Under P-limiting conditions, it was observed that P-efficient genotypes acquire more P from soils and transport more P to the root tips resulting in an increase in Al tolerance [18]. Adaptation to low-P acidic soils might involve a superior ability to tolerate Al and utilize organic P efficiently. Keeping this in mind,

the present experimental model was designed with modification in composition from the basal Hoagland's nutrient solution. It is necessary to lower the P concentration in the growth medium to enable a conclusive comment on the effect of the applied Al concentration. In Hoagland's medium,  $\text{NH}_4\text{PO}_4$  is the P source and therefore, in our experimental growth medium,  $\text{NH}_4\text{PO}_4$  was substituted by  $\text{NH}_4\text{Cl}$  to create P-deficient conditions [19].

Here, the tolerance to different concentrations of Al (50  $\mu\text{M}$  or 100  $\mu\text{M}$ ) in the growth medium of the 41 rice cultivars collected from various agroclimatic zones of Assam, India was evaluated and the effect of  $\text{Al}^{3+}$  ions on different growth parameters was determined. Inhibition of root growth parameters such as root length and root fresh or dry weights was prominent in presence of the concentrations of Al employed in the growth medium. The roots showed the classical symptoms of Al toxicity, notably a reduction in root growth and the development of lateral roots. Stunted root growth was observed in almost all the experimental rice varieties under stress exposure and became more apparent with its duration. Root growth was reduced at both the Al concentrations and for all durations, and there was a distinct reduction at 100  $\mu\text{M}$  Al for 72 h. This observed reduced root growth behavior was similar to earlier reports by other authors [20–22].

Severe reduction of fresh and dry weight and relative water content of roots and shoots were observed in the Al treatments. A decrease in RWC and chlorophyll content was also observed to some extent. The effect was generally greater at the higher concentration of Al (100  $\mu\text{M}$ ). These observations also agree with previous results of other researchers [23–27].

Determination of correlations between various morpho-physiological parameters of rice would be useful to select the best combinations of attributes for experimental rice varieties when screening their resistance to Al stress under P deficiency. Correlations between all the eight parameters measured here were calculated which showed significant interaction ( $p < 0.05$ ) among the different traits. Shoot and root biomass were found to show a significant positive correlation in the presence of  $\text{Al}^{3+}$  ions in the growth medium.

Based upon our observations, we believe that the stress tolerance index can be considered as a suitable marker for screening of a large number of rice varieties to determine the tolerance to a particular environmental stress. In our experiment, this index was useful to differentiate all the screened rice varieties for their tolerance to Al stress and P-deficient conditions. This finding was also supported by the cluster analysis which was found to agree with earlier reports [16,28].

## Conclusion

In this experiment, only a few rice varieties were found to show higher resistance than others when exposed to Al stress under P deficiency. Root fresh weight, dry weight, and relative water content were all significantly decreased in the sensitive varieties. The effect of  $\text{Al}^{3+}$  ions was mostly shown by varieties 'Baismuthi' and 'Moti'. Based on the studies of several parameters, it was shown that variety 'Holpuna' is a comparatively resistant variety, whereas 'Moti' is the most susceptible of the 41 cultivars screened. 'Holpuna' can therefore be recommended for Assam farmers. The screening approach we used has also paved a way for further selection of rice varieties which can be used to study the mechanism of enhanced Al tolerance under P deficiency. It can also be beneficial for breeding programs to produce resistant genotypes or to determine gene regulation patterns, and then for producing a genetically-engineered Al tolerant variety.

## Acknowledgments

The authors are thankful to the DST-FIST and UGC-SAP (DRS-I), Government of India for using the infrastructures in the Botany Department, Gauhati University, Assam, India.

## References

- Kochian LV, Piñeros MA, Liu J, Magalhaes JV. Plant adaptation to acid soils: the molecular basis for crop aluminum resistance. *Annu Rev Plant Biol.* 2015;66:571–598. <https://doi.org/10.1146/annurev-arplant-043014-114822>
- Amonette JE, Russell CK, Carosino KA, Robinson NL, Ho JT. Toxicity of Al to *Desulfovibrio desulfuricans*. *Appl Environ Microbiol.* 2003;69:4057–4066. <https://doi.org/10.1128/AEM.69.7.4057-4066.2003>
- Zhang J, Jia W, Yang J, Ismail AM. Role of ABA in integrating plant responses to drought and salt stresses. *Field Crops Res.* 2006;97(1):111–119. <https://doi.org/10.1016/j.fcr.2005.08.018>
- Taylor GJ, McDonald-Stephens JL, Hunter DB, Bertsch PM, Elmore D, Rengel Z, et al. Direct measurement of aluminum uptake and distribution in single cells of *Chara corallina*. *Plant Physiol.* 2000;123:987–996. <https://doi.org/10.1104/pp.123.3.987>
- Horst WJ, Wang Y, Eticha D. The role of the root apoplast in aluminium-induced inhibition of root elongation and in aluminium resistance of plants: a review. *Ann Bot.* 2010;106(1):185–197. <https://doi.org/10.1093/aob/mcq053>
- Ma JF. Role of organic acids in detoxification of aluminum in higher plants. *Plant Cell Physiol.* 2000;41(4):383–390. <https://doi.org/10.1093/pcp/41.4.383>
- Wang J, Raman H, Zhou M, Ryan PR, Delhaize E, Hebb DM, et al. High-resolution mapping of the *Alp* locus and identification of a candidate gene *HvMATE* controlling aluminium tolerance in barley (*Hordeum vulgare* L.). *Theor Appl Genet.* 2007;115(2):265–276. <https://doi.org/10.1007/s00122-007-0562-9>
- Guodao YJLL. Research on soil acidification and acidic soil's melioration. *Journal of South China University of Tropical Agriculture.* 2006;1:001–004.
- Cakmak I, Horst WJ. Effect of aluminium on net efflux of nitrate and potassium from root tips of soybean (*Glycine max* L.). *J Plant Physiol.* 1991;138(4):400–403. [https://doi.org/10.1016/S0176-1617\(11\)80513-4](https://doi.org/10.1016/S0176-1617(11)80513-4)
- Holford ICR. Soil phosphorus: its measurement, and its uptake by plants. *Soil Res.* 1997;35(2):227–240. <https://doi.org/10.1071/S96047>
- Pradhan AK, Shandilya ZM, Lahkar L, Hasnu S, Kalita J, Borgohain D, et al. Comparative metabolomics approach towards understanding chemical variation in rice under abiotic stress. In: Hasanuzzaman M, Fujita M, Nahar K, Biswas JK, editors. *Advances in rice research for abiotic stress*. Duxford: Woodhead Publishing; 2019. p. 537–550. <https://doi.org/10.1016/B978-0-12-814332-2.00026-5>
- Gaume A, Mächler F, Frossard E. Aluminum resistance in two cultivars of *Zea mays* L.: root exudation of organic acids and influence of phosphorus nutrition. *Plant Soil.* 2001;234(1):73–81. <https://doi.org/10.1023/A:1010535132296>
- Kalita J, Pradhan AK, Shandilya ZM, Tanti B. Arsenic stress responses and tolerance in rice: physiological, cellular and molecular approaches. *Rice Sci.* 2018;25(5):235–249. <https://doi.org/10.1016/j.rsci.2018.06.007>
- Kapoor N, Pande V. Effect of salt stress on growth parameters, moisture content, relative water content and photosynthetic pigments of fenugreek variety RMT-1. *Journal of Plant Sciences.* 2015;10(6):210–221. <https://doi.org/10.3923/jps.2015.210.221>
- Hoagland DR, Arnon DI. The water-culture method for growing plants without soil. Berkeley, CA: University of California; 1950. [Circular (California Agricultural Experiment Station); vol 347].
- Singh B, Reddy KR, Redona ED, Walker, T. Screening of rice cultivars for morpho-physiological responses to early-season soil moisture stress. *Rice Sci.* 2017;24(6):322–335. <https://doi.org/10.1016/j.rsci.2017.10.001>
- Lahkar L, Tanti B. Study of morphological diversity of traditional aromatic rice landraces (*Oryza sativa* L.) collected from Assam, India. *Annals of Plant Science.* 2017;6(12):1855–1861. <https://doi.org/10.21746/aps.2017.6.12.9>
- Zheng SJ, Yang JL, He YF, Yu XH, Zhang L, You JF, et al. Immobilization of aluminum with phosphorus in roots is associated with high aluminum resistance in buckwheat. *Plant Physiol.* 2005;138:297–303. <https://doi.org/10.1104/pp.105.059667>

19. Yu-Mei Du, Jiang Tian, Hong Liao, Chang-Jun Bai, Xiao-Long Yan, Guo-Dao Liu. Aluminium tolerance and high phosphorus efficiency helps *Stylosanthes* better adapt to low-P acid soils. *Ann Bot.* 2009;103:1239–1247. <https://doi.org/10.1093/aob/mcp074>
20. Hore P, Nahar S, Hasampura M, Tanti B. Phylogenetic analysis and homology modelling of betaine aldehyde dehydrogenase – an aroma producing protein in rice (*Oryza sativa* L.). *International Journal of Multidisciplinary Approach and Studies.* 2017;4(3):139–148.
21. Lahkar L, Tanti B. Morpho-physicochemical and cooking characteristics of traditional aromatic Joha rice (*Oryza sativa* L.) of Assam, India. *Biocatal Agric Biotechnol.* 2018;16:644–654. <https://doi.org/10.1016/j.bcab.2018.10.001>
22. Nahar S, Kalita J, Sahoo L, Tanti B. Morphophysiological and molecular effects of drought stress in rice. *Annals of Plant Science.* 2016;5(9):1409–1416. <https://doi.org/10.21746/aps.2016.09.001>
23. Nahar S, Vemireddy LR, Sahoo L, Tanti B. Antioxidant protection mechanisms reveal significant response in drought-induced oxidative stress in some traditional rice of Assam, India. *Rice Sci.* 2018;25(4):185–196. <https://doi.org/10.1016/j.rsci.2018.06.002>
24. Chutia J, Borah SP, Tanti B. Effect of drought stress on protein and proline metabolism in seven traditional rice (*Oryza sativa* L.) genotypes of Assam, India. *Journal of Research in Biology.* 2012;2(3):206–214.
25. Alvim MN, Ramos FT, Oliveira DC, Isaias RM, Franca MG. Aluminium localization and toxicity symptoms related to root growth inhibition in rice (*Oryza sativa* L.) seedlings. *J Biosci.* 2012;37(1):1079–1088. <https://doi.org/10.1007/s12038-012-9275-6>
26. Pandey P, Srivastava RK, Dubey RS. Salicylic acid alleviates aluminum toxicity in rice seedlings better than magnesium and calcium by reducing aluminum uptake, suppressing oxidative damage and increasing antioxidative defense. *Ecotoxicology.* 2013;22(4):656–670. <https://doi.org/10.1007/s10646-013-1058-9>
27. Awasthi, JP, Saha B, Regon P, Sahoo S, Chowra U, Pradhan A, et al. Morpho-physiological analysis of tolerance to aluminum toxicity in rice varieties of North East India. *PloS One.* 2017;12(4):e0176357. <https://doi.org/10.1371/journal.pone.0176357>
28. Nahar S, Sahoo L, Tanti B. Screening of drought tolerant rice through morpho-physiological and biochemical approaches. *Biocatal Agric Biotechnol.* 2018;15:150–159. <https://doi.org/10.1016/j.bcab.2018.06.002>

### **Selekcja tradycyjnych odmian ryżu w celu oszacowania ich potencjalnej odporności na toksyczność glinu (Al) w warunkach niedoboru fosforu**

#### **Streszczenie**

Gleby kwaśne zajmują prawie jedną trzecią dostępnych gleb uprawnych na świecie. Gleba kwaśna stanowi jedno z głównych ograniczeń abiotycznych dla praktyk rolniczych, które wywołuje toksyczność glinu (Al) i niedobór fosforu (P). Assam jest rolniczym regionem Indii, a na większości obszaru występują gleby kwaśne. Zakwaszenie gleby zwiększa rozpuszczalność obecnego w glebie Al z jego nietoksycznych form krzemianowych lub tlenków w wysoce fitotoksyczne formy jonowe (głównie trójwartościowy jon  $Al^{3+}$ ). Jonowa forma Al tworzy ponadto kompleksy z dostępnym fosforem, co prowadzi do niedoborów niezbędnych składników mineralnych dla roślin. W prezentowanych badaniach przeprowadzono selekcję tradycyjnych odmian ryżu z rejonu Assam pod kątem ich tolerancji na toksyczność Al i niedobór P. Czterdzieści jeden lokalnych odmian ryżu pochodzących z różnych lokalizacji agroklimatycznych poddano działaniu trzech różnych stężeń Al (0, 50, 100  $\mu M$ ) przez 24, 48 lub 72 godziny w warunkach niedoboru P, aby określić zakres ich odporności na tego typu warunki stresowe. Po oddziaływaniu stresu oceniono różne parametry morfofizjologiczne roślin, tj. długość korzenia i pędu, świeżą i suchą masę, zawartość chlorofilu i względną zawartość wody (RWC). Wszystkie eksperymenty przeprowadzono w układzie zrandomizowanym blokowym z trzema powtórzeniami. W oparciu o ogólne cechy morfologiczne roślin obliczono wskaźnik całkowitej reakcji na stres (TSRI), który wahał się w zakresie od 18,2 do 22,73. Stosownie do jego wartości odmiany zostały zaklasyfikowane do różnych grup odporności. Odmiany 'Moti' i 'Baismuthi' okazały się najmniej odporne, podczas gdy 'Holpuna', 'Beto' i 'Soria' zostały zidentyfikowane jako odmiany najbardziej odporne na toksyczność Al w warunkach niedoboru P. Wyniki przeprowadzonych badań można wykorzystać do opracowania obiecujących odmian w przyszłych programach hodowli ryżu.