Research Article

Development of Novel Protocol to Al\(^{3+}\) Stress Tolerance at Germination Stage in \textit{Indica} Rice through Statistical Approaches

Nusrat Jahan \(^1\), Fazilah Abd Manan \(^2\), Arsala Mansoor \(^3\), Mudassir Asrar Zaidi \(^4\), Muhammad Naeem Shahwani \(^1\), and Muhammad Arshad Javed \(^2\)

\(^1\)Department of Biotechnology, Faculty of Life Sciences & Informatics, Balochistan University of Information Technology, Engineering & Management Sciences, Quetta, Pakistan
\(^2\)Faculty of Biosciences and Medical Engineering, Universiti Teknologi Malaysia, 81310 Johor Bahru, Malaysia
\(^3\)Faculty of Natural Sciences, University of Mississippi, MS 38677, USA
\(^4\)Department of Botany, University of Balochistan, Pakistan

Correspondence should be addressed to Nusrat Jahan; nusrat.jahanbuitems@gmail.com and Muhammad Arshad Javed; majaved@fbb.utm.my

Received 7 March 2018; Revised 21 May 2018; Accepted 19 June 2018; Published 26 September 2018

Academic Editor: Adriano Sfriso

Copyright © 2018 Nusrat Jahan et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Rice production is decreasing by abiotic stresses like heavy metals. In such circumstances, producing food for growing human population is a challenge for plant breeders. Excess of Al\(^{3+}\) in soil has become threat for high yield of rice. Improvement of crop is one of potential solution for high production. The aim of this study was to develop the new method for optimization of Al\(^{3+}\) toxicity tolerance in \textit{indica} rice at germination stage using two-way ANOVA and Duncan's multiple-range test (DMRT). Seeds of two \textit{indica} rice cultivars (Pokkali and Pak Basmati) were exposed in different concentrations (control, 5 mM, 15 mM, and 20 mM) of Al\(^{3+}\) toxicity at pH 4 ± 0.2 for two weeks. Germination traits such as final germination percentage (FG%), germination energy (GE), germination speed (GS), germination index (GI), mean time of germination (MGT), germination value (GV), germination velocity (GVe), peak value of germination (GPV), and germination capacity (GC) and growth traits such as root length (RL), shoot length (SL), total dry biomass (TDB), and germination vigour index (GVI) were measured. To obtain the maximum number of significance (≤ 0.01%) parameters in each concentration of Al\(^{3+}\) toxicity with control, two-way ANOVA was established and comparison of mean was done using DMRT. The results showed that 5 mM, 10 mM, and 15 mM have less significant effects on the above-mentioned parameters. However, 20 mM concentration of Al\(^{3+}\) produced significant effects (≤ 0.01%). Therefore, 20 mM of Al\(^{3+}\) is considered optimized limit for \textit{indica} cultivars (Pokkali and Pak Basmati).

1. Background

Acidic soils are one of the main constraints for crop production. Almost 30-40% of world soils have a pH below 5.5 [1]. The lower the pH, the more acidic the soil. Acidic soils are low in fertility due to the presence of combined mineral toxicities (Al\(^{3+}\), Mn\(^{2+}\), and Fe\(^{2+}\)) and deficiency of macronutrients (phosphorous (P), calcium (Ca), and magnesium (Mg)) [2]. At low pH of the soil, aluminum and other various species like Fe\(^{2+}\) and Mn\(^{2+}\) are solubilized into the soil, which are severely toxic to rice crop production. Heavy rainfall and high-temperature cause the rapid weathering of soil and the essential elements like Ca, P, and K leach from the soil; more stable compounds rich in Al\(^{3+}\) and Fe\(^{2+}\) oxides are left behind [3]. Al\(^{3+}\) is primarily found as a significant component of soil clays. Under highly acidic soil conditions (pH<5.0) it is solubilized to Al\(^{3+}\), which is highly phytotoxic. Al\(^{3+}\) affects the root growth rapidly that causes the reduced and stunted root system and has a direct effect on the ability of a crop to acquire both water and nutrients.

Al\(^{3+}\) toxicity is reducing production on acidic soils due to inhibition of root growth, reduction in cell division, and cell
elongation [4]. To reduce the cell elongation, Al$^{3+}$ may bind to free carboxyl groups of pectin, resulting in cross-linking of pectin molecules and a decrease in cell wall elasticity [5].

Acidic soils are becoming an issue with the changing environment; reduction of available arable lands due to weathering of soils, unsustainable farming and toxic soils, rigorous agricultural practices, acid rain, and climate change are the contributors to soil acidification [6, 7]. Cultural strategies, like application of lime (CaCO$_3$), could amend the few constraints of acidic soils and lead to increase in production [6]. However, liming is only effective at increasing the pH in the upper soil profile and is mostly unproductive when the subsoil is acidic [8]. It has reported that approximately 75% of the acidic soils in the world are influenced by subsoil acidity. In many regions of the world, liming is also not effective due to high cost and lacking of infrastructure.

Therefore, developing Al$^{3+}$ tolerant crops tolerating the acidic soils has great importance for breeding programs worldwide. Identification of QTL linked to tolerance traits is one of the important techniques. The aim of present study was to find the statistical approach that could ease for optimization of Al$^{3+}$ toxicity tolerance level for two commonly used indica rice Pokkali and Pak Basmati against high concentrations of Al$^{3+}$ toxicity at germination stage.

2. Materials and Methods

The parental genotypes, Pak Basmati and Pokkali, were exposed to different levels of Al$^{3+}$ toxicity to optimize the optimum stress limits. Seeds were kept in 50°C for five days to break dormancy and surface sterilized by dipping in 70% (v/v) ethanol for 1 min and in 2% (w/v) solution of NaOCl for 10 min followed by washing 4-5 times with 70% (v/v) ethanol for 1 min and in 2% (w/v) solution in deionized water [9]. Surface sterilized and imbedded seeds were then placed in wet Petri dishes for two weeks by the addition of Al$^{3+}$ for 15 mM, and 20 mM) at pH 4.0-4.2; each treatment had three replications where it has been determined to be a good standardization to natural soil condition where Al$^{3+}$ toxicity is the problem [10]. Experiments were conducted in control condition, where the light and dark periods were 14 hours and 10 hours, respectively, with humidity level of approx. 60%. Seeds were considered germinated when both the plumule (root) and radical (shoot) were extended to approximately more than 2mm [11]. Germination parameters such as final germination percentage (FG %), germination velocity (GVe), germination energy (GE), germination peak value (GPV), germination capacity (GC), germination index (GI), germination value (GV) and growth parameters like root length (RL), shoot length (SL), total dry biomass (TDB), and germination vigour index (GVI) were recorded by the following formulas.

\[
\begin{align*}
(i) \, FG\% &= \frac{\text{Number of germinated seeds}}{\text{Total number of seeds tested}} \\
(ii) \, GVe &= \sum \frac{G}{t}
\end{align*}
\]

\[(1)\]

where G is germination percentage and t is total germination time;

\[(iii) \, MGT = \frac{\Sigma Dn}{\Sigma n} \text{ (days)} \]

\[(iv) \, GE \% = \frac{\text{Number of germinated seeds at 4 DAS}}{\text{Total number of seeds tested}} \times 100\]

\[(v) \, GV = \text{(final) MDG} \times PV\]

\[(vi) \, SG\]

\[(\text{Number of germinated seeds}) + \ldots + \frac{\text{Number of germinated seeds}}{\text{days of final count}} (9 \text{ days})\]

\[(vii) \, GPV\]

\[= \text{Cumulative Percent Germination on each day} \times \frac{\text{number of days after germination elapsed}}{20}\]

\[(viii) \, GI = \frac{(N_{10} + N_{15})}{20 \times 100}\]

where N$_{10}$ is number of germinated seeds with 10 days of stress and N$_{15}$ is number of germinated seeds with 15 days of the stress.

\[(x) \, GVI\]

\[= \frac{\text{(Avg shoot length + Avg root length)}}{\text{Germination percentage}} \times (160)\]

\[(12-14)\].

2.1. Statistical Analysis. Statistical analysis was done with SPSS version 18 (Levesque, 2007). To establish the different significance of variables in each concentration of Al$^{3+}$ toxicity with control, analysis of variance (two-way ANOVA) was tested. Two significance levels, p (≤0.05 to ≤0.01), were used [15]. Differences between genotypes were compared using Duncan’s multiple-range test (DMRT). Al$^{3+}$ concentration was considered as optimized where most of germination and growth parameters exhibited high significant differences [16].

3. Results and Discussion

The inhibitory effects of Al$^{3+}$ toxicity were checked on rice genotypes Pak Basmati and Pokkali, germination and seedling growth parameters were examined over a wide range of AlCl$_3$ from 5 mM to 20 mM with three replications. ANOVA was applied to the germination and growth parameters of all treatments, i.e., 5 mM, 10 mM, 15 mM, and 20 mM.
Table 1: Analysis of variance of 5 mM and 10 mM of Al$^{3+}$ toxicity at pH 4 ± 0.2 in germination parameters on parental line Pak Basmati and Pokkali.

| Source of variations | df | FG% | GVe | GE  | SG  | GPV | GI  | Sum of Square |
|----------------------|----|-----|-----|-----|-----|-----|-----|---------------|
|                      |    |     |     |     |     |     |     | Final germination percentage, GVe = germination velocity, GE = germination energy, SG = speed of germination, GPV = germination peak value, GI = germination index, GC = germination capacity, GV = germination value, MGT = mean germination time, RL = root length, SL = shoot length, TDB = total dry biomass, and GVI = germination vigour index. |
| variety              | 1  | 0.00 | 0.00 | 208.33 | 17.45 | 3.46 | 0.00 | 15.64 | 0.00 | 154.15 | 0.03 | 4.66 | 23.46 | 23.46 |
| Stress               | 1  | 0.00 | 0.00 | 75.00 | 0.13 | 0.02 | 0.00 | 0.14 | 0.00 | 1.21 | 0.00 | 24.02 | 1.48 | 1.48 |
| variety × Stress     | 1  | 0.00 | 0.00 | 75.00 | 7.50 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 2.94 | 0.15 | 0.15 |
| Error                | 8  | 0.00 | 0.00 | 333.33 | 0.82 | 0.16 | 0.00 | 0.54 | 0.00 | 7.21 | 0.00 | 0.01 | 0.02 | 0.02 |
| Total                | 12 |     |     |       |     |     |     |       |     |       |     |       |     |     |

** Significant at 0.01, * significant at 0.05, ns = not significant, FG% = final germination percentage, GVe = germination velocity, GE = germination energy, SG = speed of germination, GPV = germination peak value, GI = germination index, GC = germination capacity, GV = germination value, MGT = mean germination time, RL = root length, SL = shoot length, TDB = total dry biomass, and GVI = germination vigour index.**
Table 2: Analysis of variance of 15 mM and 20 mM of Al$^{3+}$ toxicity at pH 4 ± 0.2 in germination parameters on parental line Pak Basmati and Pokkali.

| Source of variations | df | FG% | GVe | GE  | SG  | GPV | GI   | GC  | GV  | MGT | RL  | SL  | TDB  | VI  |
|----------------------|----|-----|-----|-----|-----|-----|------|-----|-----|-----|-----|-----|------|-----|
| variety              | 1  | 33.33$^{**}$ | 0.15$^{**}$ | 1200$^{**}$ | 27.36$^{**}$ | 6.44$^{**}$ | 133.33$^{**}$ | 71.74$^{**}$ | 133.33$^{**}$ | 371.85$^{**}$ | 0.028$^{**}$ | 3.842$^{**}$ | 24.74$^{**}$ | 514022.41$^{**}$ |
| Stress               | 1  | 33.33$^{**}$ | 0.15$^{**}$ | 833.33$^{**}$ | 2.90$^{**}$ | 0.94$^{**}$ | 133.33$^{**}$ | 25.64$^{**}$ | 133.33$^{**}$ | 77.72 | 0.01$^{**}$ | 27.09$^{**}$ | 2.314$^{**}$ | 48384768$^{**}$ |
| variety × Stress     | 1  | 33.33$^{**}$ | 0.15$^{**}$ | 833.33$^{**}$ | 1.07$^{**}$ | 0.45$^{**}$ | 133.33$^{**}$ | 21.17$^{**}$ | 133.33$^{**}$ | 46.65 | 0.001$^{**}$ | 3.663$^{**}$ | 0.27$^{**}$ | 13493.81$^{**}$ |
| Error                | 8  | 66.67 | 0.30 | 133.33 | 0.411 | 0.057 | 66.67 | 28.27 | 66.67 | 14.452 | 0 | 0.008 | 0.023 | 1615.74 |
| Total                | 12 |     |     |     |     |     |     |     |     |     |     |     |      |      |

** Significant at 0.01, * significant at 0.05, ns = not significant FG% = final germination percentage, GVe = germination velocity, GE = germination energy, SG = speed of germination, GPV = germination peak value, GI = germination index, GC = germination capacity, GV = germination value, MGT = mean germination time, RL = root length, SL = shoot length, TDB = total dry biomass, and GVI = germination vigour index.
## Table 3: Comparison of mean using DMRT for effect of Al<sup>3+</sup> on germination and growth traits of rice genotypes.

| Varieties | Treatments | FGP (%) | GVe | GEn | Sg | GPV | GI | GVI | TDW | RL | SL | GVI |
|-----------|------------|---------|-----|-----|----|-----|----|-----|-----|----|----|-----|
| **Pokkali** | control    | 100±0.0a | 6.67±0.0a | 100±0.0a | 18.34±0.2a | 8.13±0.16a | 100±0.0a | 200±0.0a | 17.06±0.1a | 54.36±0.8a | 0.28±0.0a | 4.43±0.0a | 7.49±0.0a | 1192.6±0.0a |
| 5 mM      | 100±0.0a   | 6.67±0.0a | 100±0.0a | 18.07±0.1a | 8.03±0.08a | 100±0.0a | 200±0.0a | 16.90±0.1ab | 53.54±0.5ab | 0.21±0.0ab | 3.60±0.0b | 6.94±0.0ab | 73.0±0.0b |
| 10 mM     | 100±0.0a   | 6.67±0.0a | 100±0.0a | 17.96±0.1ab | 7.98±0.08ab | 100±0.0a | 200±0.0a | 16.80±0.1ab | 53.21±0.5ab | 0.21±0.0ab | 3.20±0.0bc | 6.91±0.0bc | 724.0±0.0bc |
| 15 mM     | 93.3±0.5ab | 6.62±0.3ab | 66.66±0.5b | 14.45±0.1b | 6.42±0.07c | 90.00±0.0ab | 186.66±0.1b | 112.58±0.2b | 40.00±0.9b | 0.21±0.0ab | 1.11±0.0c | 5.79±0.0bc | 550.5±0.3c |
| 20 mM     | 86.6±0.5b  | 5.77±0.4b | 53.3±0.5c | 12.15±0.9c | 5.40±0.39d | 76.66±0.1b | 173.3±0.7b | 951.6±0.5c | 31.24±0.6c | 0.18±0.0b | 0.06±0.0cd | 2.74±0.0c | 243.0±0.1cd |
| **Pak Basmati** | control | 100±0.0a | 6.67±0.0a | 100±0.0a | 15.92±0.1a | 7.02±0.04a | 100±0.0a | 200±0.0a | 14.83±0.3a | 47.17±0.2a | 0.17±0.0a | 2.19±0.0a | 4.92±0.0a | 711.6±0.0a |
| 5 mM      | 100±0.0la  | 6.67±0.0a | 96.66±0.3ab | 15.73±0.0a | 6.99±0.04ab | 100±0.0a | 200±0.0a | 114.56±0.3ab | 46.63±0.2ab | 0.14±0.0ab | 0.35±0.0b | 3.96±0.0ab | 430.3±0.0b |
| 10 mM     | 93.3±0.0ab | 6.62±0.3ab | 86.66±0.5b | 14.34±0.2ab | 6.44±0.05ab | 90.0±0.5ab | 186.66±0.5ab | 112.90±0.3ab | 40.14±0.4bc | 0.14±0.0ab | 0.32±0.0bc | 3.76±0.0bc | 377.1±0.0bc |
| 15 mM     | 93.3±0.6ab | 6.62±0.3ab | 70.00±0.4bc | 14.15±0.2ab | 6.13±0.24bc | 86.6±0.0b | 186.66±0.5ab | 109.52±0.2bc | 38.14±0.6c | 0.13±0.0b | 0.08±0.0c | 3.24±0.0bc | 30.16±0.2c |
| 20 mM     | 70.66±0.5c | 5.70±0.1b | 63.3±0.4c | 13.76±0.1c | 5.90±0.3c | 70.00±0.0c | 164.43±0.7b | 346.86±1.0c | 30.07±0.7cd | 0.12±0.8bc | 0.06±0.0cd | 2.93±0.1c | 64.0±0.4d |

*Same letters are not significantly different at probability (p<5%) error by Duncan’s multiple test. Values are means ± SD=standard deviation FG%=final germination percentage, GVe=germination velocity, GE=germination energy, SG=speed of germination, GPV=germination peak value, GI=germination index, GC=germination capacity, GV=germination value, MGT=mean germination time, RL=root length, SL=shoot length, TDB=total dry biomass, and GVI=germination vigour index.
The genotypes Pokkali and Pak Basmati showed significant difference (p<0.01) when exposed to optimized concentration, i.e., 20 mM (2000 mg/L). The genotype Pokkali showed stronger tolerance than the Pak Basmati in all parameters, especially in root length. Al^{3+} concentration is considered as optimized where most of germination and growth parameters exhibited high significant differences. In addition, promising statistical approaches for optimization of toxicity limits are being developed for phenotyping of population and identifying QTLs that could be used in crop improvement.

**Data Availability**

The data used to support the findings of this study are available from the corresponding author upon request.

**Conflicts of Interest**

The authors declare that they have no conflicts of interest.

**Acknowledgments**

Research Management Centre (RMC) of Universiti Teknologi Malaysia (UTM) is acknowledged for the financial assistance Cost Center no. Q.J130000.2545.05H93. Thanks are due to Dr. Rashid Ahmed (Department of Physics, Faculty of Science, Universiti Teknologi Malaysia) and my research fellows Ms. Farah and Mrs. Atiqah Saniullah Khan and Muhammad Waseem Chughtai.

**References**

[1] S. C. Hodges, *Soil Fertility Basics*, Soil Science Extension, North Carolina State University, Raleigh, NC, USA, 2010.

[2] A. R. Hede, B. Skovmand, and J. L. Cesati, *Acid Soils And Aluminium Toxicity*, International Maize and Wheat Improvement Center, 2001.

[3] L. V. Kochian, “Cellular mechanisms of aluminum toxicity and resistance in plants,” *Annual Review of Plant Biology*, vol. 46, pp. 237–260, 1995.

[4] S. De Dorlodot, S. Lutts, and P. Bertin, “Effects of ferrous iron toxicity on the growth and mineral composition of an interspecific rice,” *Journal of Plant Nutrition*, vol. 28, no. 1, pp. 1–20, 2005.

[5] H.-B. Chen, B.-S. Chiou, Y.-Z. Wang, and D. A. Schiraldi, “Biodegradable pectin/clay aerogels,” *ACS Applied Materials & Interfaces*, vol. 5, no. 5, pp. 1715–1721, 2013.

[6] M. E. Sumner and A. D. Noble, “The world story,” in The *Handbook of Soil Acidity*, 2nd ed., Z. Rengel, Ed., pp. 1–28, Marcel Dekker, Inc, New York, NY, USA, 2000.

[7] S. Solomon, *Climate change 2007-the physical science basis: Working group I contribution to the fourth assessment report of the IPCC*, vol. 4, Cambridge University Press, 2007.

[8] H. Marschner, *Mineral Nutrition of Higher Plants*, Academic Press, London, UK, 1995.

[9] A. L. Ranawake, O. E. Manangkil, S. Yoshida, T. Ishii, N. Mori, and C. Nakamura, “Mapping QTLs for cold tolerance at...
germination and the early seedling stage in rice (Oryza sativa L.),” *Biotechnology & Biotechnological Equipment*, vol. 28, no. 6, pp. 989–998, 2014.

[10] B. D. Nguyen, D. S. Brar, B. C. Bui, T. V. Nguyen, L. N. Pham, and H. T. Nguyen, “Identification and mapping of the QTL for aluminum tolerance introgressed from the new source, Oryza rufipogon Griff., into indica rice (Oryza sativa L.),” *Theoretical and Applied Genetics*, vol. 106, no. 4, pp. 583–593, 2003.

[11] J. R. Peralta, J. L. Gardea-Torresdey, K. J. Tiemann et al., “Uptake and effects of five heavy metals on seed germination and plant growth in alfalfa (Medicago sativa L.),” *Bulletin of Environmental Contamination and Toxicology*, vol. 66, no. 6, pp. 727–734, 2001.

[12] S. Kendall and S. Penfield, “Maternal and zygotic temperature signalling in the control of seed dormancy and germination,” *Seed Science Research*, vol. 22, no. 1, pp. S23–S29, 2012.

[13] A. F. Ologundudu, A. A. Adelusi, and K. P. Adekoya, “Effect of Light Stress on Germination and Growth Parameters of Corchorus olitorius, Celosia argentea, Amaranthus cruentus, Abelmoschus esculentus and Delonix regia,” *Notulae Scientiae Biologicae*, vol. 5, no. 4, 2013.

[14] S. K. Pradhan, D. K. Nayak, S. Mohanty et al., “Pyramiding of three bacterial blight resistance genes for broad-spectrum resistance in deepwater rice variety, Jalmagna,” *Rice*, vol. 8, no. 1, pp. 1–14, 2015.

[15] F.-B. WU, J. DONG, G.-X. JIA, S.-J. ZHENG, and G.-P. ZHANG, “Genotypic Difference in the Responses of Seedling Growth and Cd Toxicity in Rice (Oryza sativa L.),” *Agricultural Sciences in China*, vol. 5, no. 1, pp. 68–76, 2006.

[16] M. A. Carson and A. N. Morris, “Germination of,” *BIOS*, vol. 83, no. 3, pp. 90–96, 2012.

[17] H. G. Nass and P. Crane, “Effect of Endosperm Mutants on Germination and Early Seedling Growth Rate in Maize (Zea mays L.),” *Crop Science*, vol. 10, no. 2, p. 139, 1970.

[18] N. Pandey and C. P. Sharma, “Effect of heavy metals Co²⁺, Ni²⁺ and Cd²⁺ on growth and metabolism of cabbage,” *Journal of Plant Sciences*, vol. 163, no. 4, pp. 753–758, 2002.