EVALUATION OF ALUMINUM TOXICITY TOLERANCE IN RICE *(ORYZA SATIVA L.)*

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ABSTRACT: *Aluminum toxicity is one of the major environmental constraints limiting rice growth and productivity. To overcome that constraint the study of rice crop-tolerance against Al toxicity is one of the best approaches to save the time and efficiency. The core objectives of the study were to evaluate the aluminum toxicity tolerance in introgression lines of rice. The experiment was conducted in hydroponic environment with two sets of reciprocal introgression lines derived from the cross of 02428/Minghui63 in japonica 02428 background (02428-ILs) and indica Minghui63 background (MH63-ILs) to evaluate aluminum toxicity tolerance (ATT) at the concentration of 1.5 mmol L^{-1} at the seedling stage. The relative root elongation (RRE) was recorded as tolerance-criterion. The parent 02428 has greater ATT than that of MH63. This study will be helpful in the development of aluminum toxicity tolerant (ATT) rice variety in future.*

Key words: Rice, Aluminum toxicity, Reciprocal Introgression lines, root, shoot.

INTRODUCTION

Aluminum (Al) is one of the primary limiting factors in the soil which affects plant growth and crop production in acidic soils (pH 5.0). The arable lands of the world contain ~ 40% of aluminum [1; 2; 3] and therefore, it constitutes about 7% of the total amount of soils in the world [4], particularly in the tropics and subtropics [1; 5]. It has been estimated that over 50% of world's potentially arable lands are acidic [6; 7]. Out of that, up to 60% of the acid soils are present in developing countries [6].

Crop productivity on acid soils is restricted by multiple abiotic stress factors. Since the forms of aluminum (Al) in soil and their solubility is high at acidic pH 5 or less. So for this, aluminum toxicity becomes one of the major growth limiting factors affecting plants in acid soils [8]. Under highly acidic soil conditions (pH 5.0), Al is soluble into the soil solution as Al^{3+}, which is highly phyto-toxic, causing a rapid inhibition of root growth that leads to a reduced and stunted root system, thus having a direct effect on the ability of a plant to acquire both water and nutrients [9].

Many researchers have reported the identification of Al-tolerant genotypes in rice and other cereals [10; 11; 12; 13 and 14]. The cereal crops have been a primary focus of Al tolerance research. This research has demonstrated that levels of Al tolerance vary widely both within and between species [8; 12]. In wheat, sorghum, and barley, Al tolerance is inherited as a simple trait, controlled by one or a few genes [13; 15]. However, in maize, rice, and Arabidopsis, tolerance is quantitatively inherited [1; 5]. Among the major cereal species that have been extensively studied, such as rice, maize, wheat, barley and sorghum, rice demonstrated superior Al tolerance under both field and hydroponic conditions [9; 12].

Based on high level of Al tolerance in rice and numerous genetic and genomic resources, rice provides a good model for studying the genetics and physiology of Al tolerance. Recently four mutant genes that lead to Al sensitivity in rice have been cloned, STAR1 (Sensitive to Al rhizotoxicity1), STAR2 (Sensitive to Al rhizotoxicity2), ART1 (Aluminum rhizotoxicity1), and Nrat1 (Nramp aluminum transporter 1) [16; 17]. Al tolerance trait in rice is mainly controlled by quantitative genes [9]. A recent study identified two genes STAR1 and STAR2 which function as bacterial-type ATP binding cassette (ABC) transporter to control Al tolerance toxicity in rice [18]

The core objectives of this study were to evaluate the effects of Aluminum toxicity in rice to assess the inherent aluminum toxicity tolerance at seedling stage in two sets of Reciprocal Introgression lines of rice and to find out the correlation among different environmental conditions for root length under aluminum toxicity tolerance.

i. MATERIALS AND METHODS

Two sets of introgression lines (ILs) were developed from a cross between MH63 an *indica* variety and 02428 a japonica variety. The F1 hybrids simultaneously back crossed two times with both parents to produce two BC_2F_1 populations. The two BC_2F_1 were allowed to self-produce for $BC_2F_{2:8}$ generation. Finally 2 sets of reciprocal introgression lines (RILs) were developed, consisting of 200 lines in the MH63 indica background and 200 lines in 02428 japonica backgrounds. These lines were brought under study.

The experiment was divided into two parts:

i.i. Phenotypic evaluation of Al^{3+} toxicity tolerant-related traits

1st: preliminary tests were carried out with applying different concentrations of aluminum toxicity to decide the suitable concentration of Al toxicity level for the RILs and two parents MH63 indica and 02428 japonica.

2nd: Regular experiments for evaluation of Al toxicity tolerance of mapping populations by using the suitable concentration of Al which was decided through 1st preliminary experiment.

i.ii. Detailed methodology

The seed of parents MH63 indica and 02428 japonica and RI lines from MH63 indica and 02428 japonica was surface sterilized with 1% hypochlorite solution for 10 minutes and rinsed well with distilled water. Then the seed was soaked in distilled water in the dark at 30 °C for 48 hours. The most uniform 10 emerged seeds for each RI line per replication were directly sown into perforated Styrofoam sheets covered with nylon net at the bottom. For each experimental condition (i.e. control and treated) most uniform 10 emerged seeds from parents MH63 indica and 02428 japonica were also sown in each container at random. All the material was twice replicated. The Styrofoam sheets were allowed to float on water up to 7 days and then transferred to Yoshida culture solution [19] without applying suitable concentrations of Al^{3+} for first 10 days and then root length for one replication was measured. On the 11th day of seeding, the Al^{3+} in the form of $AlCl_3$ at the rate of 1.5 mmol L1 was applied for 15 days. The pH of the solution on alternative day was adjusted to 4.5 with 1 *N* NaOH/HCl. The solution was renewed every fifth day. The experimental materials were laid out in two replications for all experiments (control and treated) at around temperature of 32/25 °C in day/night, 70-75% relative humidity and 12 hours photoperiod. The root length was recorded on 15th day of the Al application. The ratio of average root elongation under stress versus non stress conditions for each line in each replication was calculated as follows, as an indicator of the root resistance index:

RRE (%) = SRE/CRE x 100; (19).

Where, *RRE is the relative root elongation (%), SRE is the stress root elongation at 1.5 mmol L^1 Al and *CRE is the control root elongation in control (cm).

i.iii. DATA ANALYSIS

Analysis of variance (ANOVA) was used to compare the two parents for root length elongation. The means, SD, CV% and ranges for RILs under all conditions were also analyzed by using the SAS PROC GLM [20].

Correlation between different traits was determined by the SAS PROC CORR [20]. Phenotypic data of RILs obtained from Control, Al^{3+} stress condition as well as the ratio of Al^{3+} stress to Control condition was used as input data to identify the aluminum toxicity tolerance [21].

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ii. RESULTS

ii.i. Phenotypic performance of Aluminum toxicity tolerance-related trait of root elongation in Reciprocal Introgression Line Populations in MH63 *indica* background

ANOVA results (Table-1) showed that 02428 japonica parents had highly significant values for RL2 and RL3 than those of MH63 *indica* parents, except RL1 and RRE, for those traits, these had no any significant difference. The values of RILs for RL2 were significantly higher than those of RL1 and RL3. The trends of RL3 for RILs were also significantly higher than that of RL1, showed that root length for upcoming/next 15 days increased under control and Al^{3+} stress condition significantly. But RILs under Al^{3+} stress significantly lost 22.76% (in cm) while compared to RL2 under control with same days after seeding. While comparing the losses between MH63 parents under control before stress, the RILs under control before stress gained 3.90%. While the values of MH63 parents at RL2 and RILs at RL2 showed that MH63 parents lost 14. 49%. And at RL3, MH63 parents also lost 12.32% than that of RILs at RL3. So, these all losses of MH63 parents under all environmental conditions proved that MH63 parents had slower/decreased root growth than RILs under MH63 background. One hundred ninety three RILs means (96.5%) for RL1 have got significantly higher root length than that of MH63 *indica* parents under RL1 environment with a range of 3.64 to 7.01. One hundred one (51.5%) RILs under RL2 had achieved significantly greater root length than MH63 parents under RL2 environment with a range of 5.27 to 9.49. And one hundred one (51.5%) of RIls under RL3 showed significantly longer root length than those of MH63 parents under same environment with a range of 4.22 to 8.54. However, RRE for 02428 japonica background has indicated significantly higher trends than MH63 *indica* background, which has indicated that parents from 02428 japonica background has higher Al^{3+} toxicity tolerance than that of MH63 indica background

Table 1. Performance of root-related traits in reciprocal introgression line populations in MH63 *indica* background for Al^{3+} stress toxicity

Trait	MH63 P1	02428 P2		RILs MH63 background		
	Mean	Mean	P1-P2	Mean ± SD	CV%	Range
RL1	4.61	5.18	-0.57	4.79±0.62	12.98	3.64-7.01
RL2	6.43	9	-2.57**	7.25±0.91	12.55	5.27-9.49
RL3	4.91	6.51	-1.6***	5.6±0.65	11.63	4.22-8.54
RRE	16.48	35	-18.52***	33.33±10.34	313.95	35.58-61.69

Trait= Trait of root elongation under different environmental conditions.

RL1= Root length of control after 10 days of seeding, before applying Al^{3+} stress.
RL2= Root length of control after 25 days of seeding (after 15 days of Al^{3+} stress).
RL3= Root length under Al^{3+} stress after 25 days of seeding and after 15 days of Al^{3+} stress.
RRE=Relative Root Elongation=SR/CR (Xue et al. 2006)
SR=RL3, **CR**=RL2.
Background= Reciprocal Introgression Line Populations in MH63 *indica* background.
P1= MH63 *indica* Parent 1.
P2= 02428japonica Parent 2.
P1-P2= Difference between the values of Parents 1 and Parents 2.
Mean= Average of values belonging to Reciprocal Introgression Lines (RILs) in MH63 *indica* background.
SD= Standard Deviation.
CV= Co-efficient co-variation=SD/Mean*100
Range= Minimum values and maximum values in RILs under MH63 *indica* background.
*****= Level of significance at $p< 0.05$, ****** = Level of significance at $p< 0.01$, ******* = Level of significance at the $p < 0.001$.

ii.ii. Phenotypic performance of aluminum toxicity tolerance-related trait of root elongation in two sets of reciprocal introgression line populations in 02428 japonica background

ANOVA (Analysis of variance) results (Table-2) showed that 02428 parents under control had significantly higher values for all traits of evaluation, i.e. RL1, RL2, and RL3 than those of MH63 parents, . *indica*ted that 02428 had got better root growth trait for growth period and under Al^{3+} stress tolerance than MH63 parents. Even the trends of RL3 for 02428 parents are also significantly higher than that of RL1, showed that root length for upcoming/next 15 days increased under control and Al^{3+} stress condition significantly higher than RL1. However, RILs under Al^{3+} stress lost 31.89% (in cm) while compared to RL2 under control with same days after seeding. While comparing the losses between 02428 parents under control before stress to the RILs under control before stress, the RILs lost 16.49%. While the values of 02428 parents at RL2 and RILs at RL2 *indica*ted that 02428 RILs lost 13.36%. And at RL3, 02428 RILs lost 13.43% than that of 02428 parents, *indica*ted that 02428 parents got more Al^{3+} stress tolerance than its RILs. One hundred twenty six (63.5%) of RILs under RL1 environment got significantly higher root length than that of 02428 parents with a range of 3.18 to 8.5. Seventy three (36.5%) of RILs under RL2 environment showed greater root length that of 02428 parents with a range of 6.6 to 14.2. Eighty three (41.5%) of RILs under RL3 environment got longer root length than that of 02428 parents under same environment with a range of 5.06 to 9.19. The root length elongation under all environments RL1, RL2 and RL3, and RRE clearly showed that root elongation increased under different environmental conditions differently, affected by total days of seeding under 0 and Al^{3+} stress toxicity as well

Table 2. Performance of root-related traits in reciprocal introgression line populations in 02428 japonica background for Al^{3+} stress

Trait	MH63 P1	02428 P2		RILs 02428 background		
	Mean	Mean	P1-P2	Mean ± SD	CV%	Range
RL1	6.38	7.76	-1.38*	6.48±0.8	12.28	3.81-8.5
RL2	11.04	12.56	1.52	10.88±1.42	13.02	6.6-14.2
RL3	7.19	8.56	1.37*	7.41±0.72	9.67	5.06-9.19
RRE	17.38	16.67	0.71	21.14±-12.90	-352.7	44.80-12.11

Abbreviations are same as above in Table 2.

iii. Correlations between control, Al^{3+} stress and relative root elongation trait under MH63 *indica* and 0242 *japonica* background

All the traits under MH63 *indica* background, RL1, RL2, RL3, and RRE under all conditions of control before Al^{3+} stress, control after Al^{3+} stress and Al^{3+} treated plants were significantly positive correlated with one another except RRE with RL1, which have only positive correlation with each other (Table-2), indicating that plants got high effect of Al^{3+} stress toxicity on all traits, so, Al^{3+} stress did not allow them to grow better under stress condition. However, all the traits under 02428japonica background have also positively significant correlation with one another except RL2 under both MH63 *indica* and 02428japonica backgrounds has a negative significant correlation with the ratios of RRE, which suggests that root length per plant have a similar relationship with their components under Al^{3+} stress condition

Table 3. Correlation coefficients for root related trait detected in the reciprocal introgression lines MH63 *indica* and 02428 japonica backgrounds:

	RL1	RL2	RL3	RRE
RL1		0.62***	0.74***	0.06
RL2	0.41***		0.67***	-0.5***
RL3	0.75***	0.60***		0.29**
RRE	0.17*	-0.69***	0.15*	

Table 3 Upper diagonal digits are for MH63 and lower diagonal digits are for 02428
=** Level of significance at $p<0.05$, ***=** Level of probability at $p< 0.01$, **=** Level of significance at $p>0.0001$

iv. DISCUSSION

The tolerance of rice crop against aluminum (Al^{3+}) toxicity is usually measured through the phenotypic performance of root growth and its development [22; 23; 24]. Therefore, root length of control (untreated) and treated plants as well as relative root elongation (RRE) were kept as a base for observing the ill-effects of Al^{3+} toxicity on RILs of both MH63 indica and 02482 japonica backgrounds. It was recorded that the root as well as root hair of Al^{3+} treated plants were bitterly affected in both rice populations MH-63 indica and 02428 japonica. The length of root under Al^{3+} toxicity was comparatively lower than untreated rice plants. Besides that the formation of root hairs in Al^{3+} treated rice plants was also lesser than untreated plants. On the other side, the root elongation of ten days old seedlings was also lesser than the seedlings of 25 days either under Al^{3+} treated and untreated conditions .These results clearly proved that root elongation increased under different environments differently as affected by the total days of seeding under 0 and Al^{3+} toxicity conditions. These results are in similarity with those of P. Wu *et al*.,[25] who had also observed that root length increased under Al treated and untreated environments as the time passed but the root length of treated rice plants was lesser than that of untreated plants.; 27] Similar results have been recorded by Chen-Wu and Ching Huei. [26] Pham and Nguyen [27] and observed that as the levels of aluminum toxicity increased so, the root growth decreased.

v. CONCLUSION

The results of phenotypic data showed that aluminum stress toxicity at the rate of 1.5 mmol. L^{1} had checked the root elongation at significant levels as compared to root elongation of plants under control (untreated) condition. But in spite of that, MH63 *indica* and 02428 japonica backgrounds had some important Al^{3+} stress toxicity tolerance lines which might lead us to further enhancement in the evolve and release of Al^{3+} toxicity tolerant varieties in future. Therefore, in the light of our careful practical study, it has been noted that aluminum treated plants with higher pH levels (non-acidic, 7.5 pH or above), the seedlings of rice could strive well. However, the aluminum treated plants with lower pH at acidic levels (4.5 pH or lower), showed negative effects on plant growth and development. Hence, as the concentration of aluminum increased with decreasing pH damaged the plants critically. Therefore, the rice crop can be grown in aluminum contaminated soils with higher pH (non-acidic) conditions.

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