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Seedling vigour as a potential indicator for rapid screening of rice (*Oryza sativa* L.) genotypes for aluminium (Al) toxicity tolerance

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Aluminium (Al) is solubilized into a phytotoxic form [$Al(H_2O)_6^{3+}$] in acid soil, which is known as Al^{3+}. Al toxicity is the primary growth-limiting factor for plants in acid soils. Breeding of rice for enhanced Al tolerance considered to be an important approach for increasing grain yield in acid soils. In the present endeavour, we studied the effects of toxic levels of Al on vigour of rice seedlings under hydroponics and finally grouped the genotypes with varied degree of tolerance to Al toxicity based on vigour index. Good seed germination (%), high speed of germination and high vigour index indicate uniform crop stand. Germination ranged from 72-99.50%. It was significantly high for almost all genotypes (≥80%) except three. The speed of germination ranged from 9.75 to 29.99 in control. Most of the genotypes showed high speed of germination (>15.00) except a few. Remarkable reduction in seedling vigour has been reported earlier under stressed environments. Low seedling vigour has been identified as the key factor responsible for poor germination and uneven seedling establishment. Based on the vigour index, nine genotypes were classified as tolerant, 19 as moderately tolerant and 12 as susceptible. Among the tolerant genotypes, UBKVR-3 showed maximum value for vigour index followed by UBKVR 19, UBKVR 9, UBKVR 8, Parijat, MTU 1075 and Radhunipagal. Most susceptible genotypes based on seedling vigour were found to be Pusa Basmati, Aiswarya and Pratikha. It is to be mentioned that till now there is no clear and distinct divisions of vigour index for classification into different levels of tolerance towards Al toxicity. In our study, we have identified distinct classes for grouping of genotypes endowed with different levels of tolerance. The values of the vigour index depend upon root length, shoot length or some other characters used for calculation of vigour index. The magnitude of the vigour index may not be useful for classification uniformly across the crops as well as genotypes within the crop. Hence, the procedure used in this work may overcome the above mentioned constraints.

Keywords: Acid soil, Germination speed, Paddy, Soil acidity

The $Al(OH)^{2+}$ is of minor significance and exists over a narrow pH range. The Al^{3+} is predominant below pH 5.0, $Al(OH)^{2+}$ (between pH 4.7 and 6.5), $Al(OH)_3$ between 6.5 and 8.0 and $Al(OH)_4^-$ above pH 8.0. Acid soils are phytotoxic as a result of nutritional disorders, deficiencies, or unavailability of essential nutrients such as calcium, magnesium, molybdenum, and phosphorus, and toxicity towards aluminum, manganese, and hydrogen activity have been reported[1-4]. The solubility of soil compounds and, therefore, nutrient availability to plants is related to soil pH. It has been estimated that over 50% of the world's potential arable lands are acidic[5]. Soil acidity is important in tropical and subtropical zones. Aluminium (Al) toxicity is the most important factor, being a major constraint for crop production on 67% of the total acid soil areas[6]. In India, the acid soils are found in the Himalayan region, the eastern and North-eastern plains, peninsular India and the coastal plains under different agro-climatic situations. The soils occupy about 90 million hectares, constituting over one fourth of total geographical area of the country (NPSSLUP, Nagpur, India).

Al toxicity and tolerance mechanisms differ strikingly with its chemical form, and the study of Al related processes are complicated depending upon complex chemistry of Al. Therefore, the experimental results may differ with experimental conditions such as pH and prevailing ions, even if same concentration of Al is used[7]. The cellular components and processes, which have been proposed to be affected by Al are in wide range and some of the most important include; cell nuclei, mitosis and cell division[8-10], composition, physical properties and structure of the plasma membrane[11,12], uptake of Ca^{2+} and other ions[13,14], phosphoinositide-mediated signal transduction and

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cytoplasmic calcium homeostasis[15,16], oxidative stress[17], cytoskeletal dynamics[18] and the cell wall-plasma membrane-cytoskeleton continuum[19].

Plant genetic resources are a rich source of valuable traits that could be used for crop improvement. Species have genotypic differences in growth response towards aluminum toxicity[20]. Yamamoto *et al.*[21], and Ishikawa & Wagatsuma[12] found that some ecotypes could be more tolerant to Al than others. Selection and breeding of Al-resistant genotypes are important to enhance increasing seed yield under acidic soil. The available screening methods for assessing Al tolerance in crops are based on the inhibition of root elongation in hydroponic culture and visual detection of Al tolerance levels by staining of seedlings root with hematoxylin[22]. Laboratory- and greenhouse-based techniques for screening for Al tolerance are widely used because those are quick, highly accurate, non-destructive, and can be applied at early developmental stages of plant growth. Hede *et al.*[23] used root vigour to classify the rye genotypes into different classes of tolerance to Al toxicity. In this endeavour, here, we made an attempt to use seedling vigour and its associated characters for classification of rice genotypes into three types *viz.* tolerant, moderately tolerant and susceptible.

Materials and Methods

Materials

Forty rice genotypes were used in this study. Those materials were consisted of 14 released varieties, one hybrid, eight aromatic Farmers' Varieties, one high yielding scented variety, 11 advanced lines developed at Uttar Banga Krishi Viswavidyalaya (Pundibari), four advanced lines of IVT-*Boro* 2011 received from Directorate of Rice Research (Hyderabad) and one maintainer line- IR58025B of KRH-2 hybrid rice.

Methods

The experiment was done at the Department of Genetics and Plant Breeding, Uttar Banga Krishi Viswavidyalaya, Pundibari, Cooch Behar, West Bengal. The experiment was designed to assess the rice genotypes under different concentrations of Al. To determine the concentration ranges of Al to impose toxicity to rice seedlings under different cultural conditions 30, 60 and 90 ppm concentrations of Al were used.

Seed vigour traits

To determine germination percent seeds were placed in 9.0 cm Petri dishes on two layers of filter paper (Whatman No. 1) moistened with 3.5 mL distilled water or test solution. Each treatment consisted of two replicates of 100 seeds. Germination counts were made daily for 16 days. Complete germination was considered when the radicle protruded 2 mm. Germination (%) was calculated following standard germination test according to the procedure of ISTA[24], speed of germination (SG) was calculated as described by AOSA[25] and the vigour index (VI) was calculated using the following equation[26,27].

$$\text{Germination percentage} = \frac{\text{No of seeds germinated}}{\text{No of seeds inoculated}} \times 100$$

$$\text{Speed of germination} = \frac{\text{No. of germinated seed}}{\text{Day of first count after inoculation}} + \frac{\text{No of germinated seeds}}{\text{Days of final count after inoculation}}$$

$$\text{Vigour Index} = (\text{Shoot length} + \text{root legth}) \times \text{germination }\%$$

Classification of genotypes based on vigour index

The optimal Al concentration for screening genotypes depends on the plant species. However, optimum Al concentration also depends on the purpose of screening. If it is part of an on-going breeding programme and the aim is simply to identify the most Al tolerant plants, higher Al concentrations can be applied. However, if the purpose is to quantitatively characterize the Al tolerance of the genotypes, a lower Al concentration has to be applied for better group of germplasm. In this present study, a good degree of separation in rice genotypic responses could be observed only at 30 ppm of Al concentrations. The higher concentrations, 60 and 90 ppm were found highly toxic to the rice genotypes. Separation of the effect of Al concentrations at higher concentrations is difficult to distinguish. Thus, 30 ppm of Al in nutrient solution was used for classification of the genotypes into three classes, viz. tolerant, moderately tolerant and susceptible.

Following Hede *et al.*[23] we used seedling vigour for classification of rice genotypes into three categorieses, such as tolerant, moderately tolerant and susceptible. Values for different levels of tolerance were decided as follows: the difference between the highest and lowest values of vigour index, i.e. 2421.94–1216.14=1205.78. Subsequently, the difference was divided by 3, i.e. 1205.78/3=401.92. One part of the difference, i.e. 401.92 was added to the minimum value of vigour index, i.e. 1216.14+401.92=1618.06 ≅1618.00. The genotypes having values of vigour index below 1618.00 were considered as susceptible. Furthermore, 401.92 was added to 1618.00 to find out the moderately tolerant class, i.e. 1618.06+401.92= 2019.98≅2020.00. The genotypes bearing the values of vigour index ≥1618.00 and <2020.00 were considered as moderately tolerant, and the genotypes bearing the values of vigour index ≥2020.00 were considered as tolerant towards Al toxicity at 30 ppm concentration.

Results

Analysis of variance

Germination (%), speed of germination and vigour index showed highly significant differences among the treatments at all levels of Al stresses (Table 1). Mean values of different seedling vigour traits also showed significant differences across Al concentrations in the nutrient solution (Table 2).

Seed germination

Germination (%) ranged from 72.00 (Heera-2) to 99.50 in the control. The germination (%) decreased with the increased concentrations of Al in the culture solution. It was found to be significantly high for almost all the genotypes and passed the Indian Minimum Seed Certification Standard for rice (≥80.0%) except IET 21255, Heera 2 and IVT4007-B in control (Fig. 1A). The genotypes — Satabdi, MTU 7029, IR 64, IR58025B, Kalobhog, Mohanbhog, UBKVR 3 and UBKVR 6 showed constantly high germination across different levels of Al stress in the culture solution.

Table 1 — ANOVA of vigour traits of 40 rice genotypes under Al stress tolerance in nutrient solution

Sources	d.f.	Mean sum of squares		
		Germination	Speed of germination	Vigour index
Total	319	84.671	20.969	314352.324
Replication	1	310.078	2.620	106546.422
Treatment	159	154.111**	41.298**	575072.533**
Error	159	13.813	0.755	54939.070
Conc. (C)	3	2668.594**	807.250**	20315450.843**
Variety (V)	39	228.741**	78.077**	385485.264**
C × V	117	64.761**	9.399**	132104.743**
Error	159	13.813	0.755	54939.070

[** denote significance at $P = 0.01$]

Table 2 — Mean values of four treatments of Al stress across the genotypes

Al concentration	Germination	Speed of germination	Vigour index
Control	91.337[a]	20.809[a]	2374.629[a]
30 ppm	84.687[b]	16.163[b]	1823.991[b]
60 ppm	82.825[c]	15.275[c]	1422.782[c]
90 ppm	77.337[d]	13.316[d]	1235.991[d]

[*values bearing same letter in the column are not significantly different at $P = 0.01$ of LSD]

Speed of germination

The speed of germination ranged from 9.75 (IVT4007-B) to 29.99 (MTU 7029) in the control (Fig. 1B). Most of the genotypes showed high speed of germination (>15.00) in control (Fig. 1B) except IVT4007-B (9.75), IET 22838 (11.77) and KMR 3 (12.97).

Speed of germination showed similar trend as that of control when nutrient solution was fortified with 30 µg/mL of Al. It ranges from 8.41 (UBKVR 8) to 24.42 (Satabdi). At 30 µg/mL of Al stress also most of the genotypes showed high speed of germination (>15.00) except MTU1010, Parijat, Pratikha, Heera 2, Pusa Basmati-1, UBKVR 4, UBKVR 8, UBKVR 11, UBKVR-15, IVT4007-B, IET 21255 and KMR-3 (Fig. 1B). Remarkable reduction was discernible in speed of germination at 60 and 90 µg/mL of Al stresses in the nutrient solution.

Seedling shoot length

Shoot length showed significant variation across the genotypes as well as Al concentrations and the interaction between genotypes and Al concentrations (Fig. 1C). The shoot length in control found in the range of 12.95 to 25.25 cm. Longest seedling was observed in UBKVR 3 followed by UBKVR 1, Heera 2, Khasha, UBKVR 8, UBKV 6 and UBKVR 4. Shortest seedling was observed in a traditional cultivar Chinakamani. The pattern of shoot growth at 30 ppm Al concentration was almost similar as that of control. It was in the rage of 13.38 to 24.42 cm. At 30 ppm of Al stress also the genotype UBKVR-3 showed longest shoot followed by UBKVR-4, UBKVR-9, UBKVR-19, UBKVR-8, UBKVR-6, Heera-2 and Kalojeera.

Seedling root length

The range of root length in control among the parents found in between 2.84 and 12.42 cm (Fig. 1D). Maximum root growth was observed in IR58025B followed by Heera-2, Pratikha, Gontra-Bidhan 1, UBKVR 4, Annada, Masuri, UBKVR 3, UBKVR 9 and UBKVR 8. Root length at 30 ppm varied from 2.62 to 8.34 cm (Fig. 1D). Longest root at 30 ppm was observed in Radhunipagal followed by UBKVR 19, Parijat, UBKVR 11, UBKVR 18, UBKVR 1, UBKVR-1, and MTU 1075. At 60 ppm of Al stress under hydroponic culture, the cultivar Kalobhog showed longest root followed by Krishna Hamsa, UBKVR 9, UBKVR 3, Gontra Bidhan 1, Chinakamani, UBKVR 1 and Mohanbhog. However, maximum root growth at 90 ppm of Al stress was observed for the advance

Fig. 1 — Graphical representation of (A) germination (%); (B) speed of germination; (C) shoot length; (D) root length; and (E) vigour index of 40 rice genotypes. [Genotypes were plotted in X-axis in all the figures. 1: Annada; 2: Satabdi; 3: MTU 1010; 4: MTU 1075; 5: Parijat; 6: Gontr-Bidhan-1; 7: MTU 7029; 8: IR 64; 9: IET5656; 10: Pratikha; 11: Aiswarya; 12: Masuri; 13: Krishna Hamsa; 14: IR58025B; 15: IET 21255; 16: Heera 2; 17: BRI-dhan 29; 18: IET 22838; 19: Gobindabhog; 20: Kalobhog; 21: Khasha; 22: Badshabhog; 23: Radhunipagal; 24: Kalojeera; 25: Mohanbhog; 26: Chinikamani; 27: Pusa Basmati 1; 28: UBKVR 11; 29: UBKVR 15; 30: UBKVR 18; 31: UBKVR 19; 32: UBKVR 16; 33: UBKVR 4; 34: UBKVR 8; 35: UBKVR 3; 36: UBKVR 9; 37: UBKVR 1; 38: UBKVR 6; 39: KMR 3; 40: IVT4007-B]

Table 3 — Classification of 40 rice genotypes based on vigour index

Vigour index	Classes	Genotypes	No. of genotypes
>2020	Tolerant	MTU 1075, Radhunipagal, Kalojeera, UBKVR-6, UBKVR 19, UBKVR 16, UBKVR 8, UBKVR 3, UBKVR 9	9
≥1618 ≤2020	Moderately tolerant	Annada, Satabdi, MTU 1010, Parijat, Gotra Bidhan 1, MTU 7029, IR 64, IET 5656, IET 21255, Kalobhog, Badshabhog, Mohanbhog, Chinakamani, UBKVR 1, UBKVR 4, UBKVR 11, UBKVR 15, UBKVR 18, KMR 3	19
<1618	Susceptible	Pratikha, Aiswariya, Masuri, Krishna Hamsa, IR58025B, Heera 2, BRI-dhan 29, IET 22838, Gobindobhog, Khasha, Pusa Basmati 1, IVT4007-B	12

line UBKVR-9 followed by Krishina Hamsa, UBKVR 1, Gontra Bidhan 1, Aiswarya and IR58025B.

Seedling vigour index

It varied from 1628.30 to 3410.35, 1216.14 to 2421.94, 921.00 to 2294.50 and 939.96 to 2037.77 in control, 30, 60 and 90 µg/mL of Al concentrations, respectively (Fig. 1E). Highest seedling vigour index was observed in UBKVR 3 which was at par with UBKVR 6, UBKVR 8, UBKVR 9, UBKVR 11, UBKV R18, UBKVR 19, Radhunipagal, Kalojeera, IR 64, Satabdi, MTU 1010, MTU 1075, KRM 3, Parijat nad Gontra Bidhan 1.

Classification of genotypes based on the vigour index

Differential response among rice genotypic responses was only observed at 30 µg/mL Al stress. Higher Al concentrations (60 and 90 µg/mL) were found to be highly toxic for the rice genotypes. The effects of Al at high concentrations are difficult to distinguish. Thus, the Al concentration of 30 µg/mL was used to classify the genotypes into three classes, namely, tolerant, moderately tolerant and susceptible.

Based on the above criteria of classification, nine genotypes were classified as tolerant, 19 genotypes were classified as moderately tolerant and 12 genotypes as susceptible (Table 3). Among the tolerant genotypes, UBKVR 3 showed highest value for seedling vigour index followed by UBKVR 19, UBKVR 9, UBKVR 8, Parijat, MTU 1075 and Radhunipagal. Most susceptible genotypes as per the seedling vigour were the Pusa Basmati followed by Aiswarya and Pratikha.

Discussion

Ample variations were observed among the genotypes under Al toxicity[28]. The germination decreased with the increased concentrations of Al in the culture solution. Germination was very high for almost all the genotypes at control, 30 and 60 ppm of Al concentrations and passed the Indian Minimum Seed Certification Standard for rice (≥80.0%). The genotypes- Satabdi, MTU 7029, IR 64, IR 58025B, Kalobhog, Mohanbhog, UBKVR 3 and UBKVR 6 showed constantly high germination across the Al stresses in the culture solution. Retention of higher germination under stressed condition indicated better tolerance of those genotypes towards higher concentrations of Al in nutrient solution. It is important to note that IR58025B showed higher germination across the Al-concentration gradient, but it was classified as susceptible based on seedling vigour index. This may be due to shorter shoot and root length of this genotype at higher concentrations of Al in the nutrient solution. Whereas, the variety UBKVR 15 showed highest germination in control, but there remarkable reduction was observed under stressed environments. Higher concentrations of Al in culture medium also found to be toxic callus induction and plantlet regeneration of rice under *in vitro* condition[29,30].

Speed of germination is a good parameter to adjudge the uniformity of crop stand in the field. High speed of germination indicates highly uniform crop stand. Low speed of germination indicates that the seeds sown on same date, germinated in different days after sowing, the age of the individual plant of a population was different, which ultimately may lead to uneven maturity of the crop in the field condition.

In general, speed of germination of a genotype decreased with the increased concentrations of Al in the nutrient solution. Satabdi and IET 24171 showed almost constant speed of germination across different level of stresses in the medium (Fig. 1B). High germination indicates uniform crop stand. It showed their ability to germinate immediately after sowing in the field or most of the sown seeds germinated immediately after sowing. The variety MTU7029 showed maximum seed germination (%) as well as speed of germination in control, failed to maintain the same in higher doses of Al in the nutrient solution indicating its susceptibility towards the higher concentrations of Al.

Most of the genotypes showed high seedling vigour at control. Low seedling vigour was primarily due to poor germination. This shows that a satisfactory selection programme for improvement of these seed quality characters is possible in rice.

Hede *et al*.[23,31] used root vigour to classify the rye genotypes into different classes of tolerance to Al toxicity. In this endeavour, seedling vigour index was also used for classification of rice genotypes into three classes- tolerant, moderately tolerant and susceptible. Till now no clear and distinct division of vigour index for classification into different levels of tolerance is available. In our study we have elaborated the distinct classes for grouping of genotypes under different levels of tolerance (see the materials and method). The values of the seedling vigour index depend upon root length[32,33], shoot length[31,32] or some other parameters used for calculation of vigour index[31,32]. The magnitude of the vigour index may not be useful for classification uniformly across the crops as well as across the genotypes within the crop, because the parameters (shoot length, root length, seedling fresh weight, seedling dry weight and germination) used for the calculation of seedling vigour may differ genotype to genotype and/or crop to crop. Hence, the procedure used in this work will overcome the above mentioned difficulties.

Conclusion

Our above findings indicate that the seedling vigour index could be used as a potential index for rapid screening of rice genotypes for Al toxicity tolerance under hydroponic culture at green house or net house condition. The values of the vigour index depend on root length, shoot length or some other parameters used for calculation of vigour index. The magnitude of the vigour index may not be useful for classification uniformly across the crops as well as genotypes within the crop. This new method of classifying rice genotypes into different catagories of tolerance against abiotic stresses, Al in particular, overcomes the conventional challenges.

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