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Aluminium toxicity tolerance of five wheat cultivars at germination and early vegetative stage

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ABSTRACT

A significant limitation of wheat production is Al^{3+} toxicity in acidic soils, which interferes with development of plants. This study was carried out to screen wheat varieties for Al tolerance by evaluating their morphological responses to aluminum (Al) stress during the germination and seedling stages. This research into the impact of Al stress on wheat growth was conducted between August and October 2018 in the Physiology Laboratory of the Department of Crop Botany at Bangladesh Agricultural University in Mymensingh, Bangladesh. There were two parts to the experiment i.e., germination and hydroponics phase. The two-factorial experiment was designed to be completely randomized (CRD) with three replicates. Stress treatment was applied at 0 µM Al (control) and 100 µM Al (stress). The evaluated wheat varieties were BARI Gom-21, BARI Gom-27, BARI Gom-28, BARI Gom-29 and BARI Gom-30. The results indicated significant variation in root length, shoot length and percent germination at germination stage and in root length, shoot length, plant height and dry weight at the seedling stage. BARI Gom-27 showed improved performance among all the varieties at germination stage under Al stress (100 µM Al) in all the studied parameters. In case of seedling stage, maximum shoot length, no. of roots plant^{-1}, fresh weight and dry weight were exhibited by BARI Gom-27 and the lowest were by BARI Gom-30 under stress treatment. Taken together, BARI Gom-27 was pointed out as Al stress-tolerant variety as it showed boosted stress tolerance indices in most of the studied variables. This variety could be used in future breeding programs for improved morpho-physiological and biochemical aluminium stress tolerant characters.

***Key Words**: Aluminium toxicity, Morphological characters, Hydroponics, Stress tolerance indices.*

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I. Introduction

Rising temperatures, rainfall unpredictability, seasonal shifts, increased salinity, soil acidity and extreme weather events are all signs of global climate change (Ma and Furukawa, 2003). The soil

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acidity problem worsens, and thousands of hectares of land fallow in Acid Basin Clays, Acid Sulphate Soils and Brown Hill Soils. Aluminium (Al), the most prevalent metal in the earth's crust, accounts for around 8% of its total mass (Tammam et al., 2018). One of the most significant limits on crop production in acid soils, representing more than 30% of agricultural area worldwide, is the presence of Al^{3+} (Agegnehu et al., 2021). Al^{3+} toxicity affects over 2.6 billion hectares of highly acidic soils worldwide (Gupta et al., 2013). Al stress primarily affects soil with a low pH (Gupta et al., 2013). In acidic soil conditions, Al concentration rises through increasing Al^{3+} solubility in the plant's rhizosphere (Iqbal et al., 2012). As a result, Al intrusion primarily affects the plant root by inhibiting root growth (Kochian et al., 2004) and inhibition of root elongation is the main symptom of aluminium toxicity (Rahman and Upadhyaya, 2021), which becomes apparent quickly. Even a small concentration of aluminium may induce root growth inhibition (Kochian et al., 2004). In general, root elongation is characterized by cell division and elongation; nevertheless, interruption of cell elongation is recognized as the primary cause of root growth inhibition. Aluminium poisoning also reduces water and nutrient intake and alters cellular activity in susceptible species (Ma and Furukawa, 2003).

Wheat (*Triticum aestivum* L.) is a member of the Poaceae family (Sagar et al., 2018). Wheat is the most important cereal crop in the world in terms of cultivated area (232 million ha) and grain production (595 million tons). It is Bangladesh's second most important cereal crop after rice; nevertheless, it imports substantial wheat to meet domestic demand (Barma et al., 2019). Soil acidity associated with aluminum toxicity and a scarcity of aluminum-tolerant cultivars is two main factors contributing to a drop in wheat yield in Bangladesh. However, developing tolerant cultivars is time-consuming and labor-intensive. However, identifying wheat cultivars resistant to aluminum toxicity is critical for their immediate adoption in locations where aluminium toxicity limits output. There has been little investigation into finding wheat cultivars resistant to aluminium toxicity among the wheat cultivars typically produced in Bangladesh. As a result, the current study project was launched to assess the ability of wheat cultivars to germinate under aluminium stress and used morphological parameters to screen the tolerant variety.

II. Materials and Methods

These studies were conducted to screen the aluminium toxicity-tolerant wheat cultivars based on morphological characteristics. For that, we conducted two experiments at wheat stages, including the germination stage in petridish and seedling stage in hydroponics at Plant Physiology Laboratory, Department of Crop Botany, Bangladesh Agricultural University, Mymensingh during the period from August to October 2018. The seeds of five wheat varieties (BARI Gom-21, BARI Gom-27, BARI Gom-28, BARI Gom-29, BARI Gom-30) were collected from the Bangladesh Agricultural Research Institute (BARI), Gazipur, Bangladesh.

Germination experiment

Collected wheat seeds were sterilized with 5% sodium hypochlorite for 30 minutes before being washed with distilled water (DW). The two-factorial experiment was carried out in three repetitions using a Completely Randomized Design (CRD). However, thirty petri dishes with filter papers (5 kinds, 2 treatments and 3 replications) were set up. Ten healthy seeds of each kind were planted on petri plates for the germination experiment. The Al treatments of 0 M Al and 100 M Al were applied to the petri dish twice a day with $AlCl_3$ to confirm the wet environment for seed germination and to generate Al stress. At 8 days after seeding, the germination percentage, vigor index, root length and shoot length were measured (DAS). Furthermore, the stress tolerance indices of the investigated parameters were evaluated at 8 DAS using the formulas described in Sagar et al. (2019), Sagar et al. (2020), Shovon et al. (2021), Sultana et al. (2021) and Huqe et al. (2021).

$$Vigor\ Index\ (VI) = Seedling\ length \times \%\ germination$$

$$Stress\ Tolerance\ Index\ (STI) = \frac{Data\ at\ stress}{Data\ at\ control} \times 100$$

Hydroponics experiment

The experiment was conducted by a completely randomized design (CRD) with three repetitions. Seedlings were grown in a water pot with nutrients for 7 days. The hydroponic system was maintained in a plastic pot (3.5L). One week old seedlings were transferred to continuously aerated

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nutrient solution in 3.5L water pots on Styrofoam blocks with four holes and three plants per hole, supported with sponge to fix the young plants. After 7 days, seedlings were allowed to grow on modified Hoagland´s nutrient solution (pH 5.5–6) with following composition: $Ca(NO_3)_2.4H_2O$ (2 mM), KH_2PO_4 (0.2 mM), K_2SO_4 (1 mM), $CaCl_2.2H_2O$ (2 mM), $MgSO_4.7H_2O$ (0.5 mM), Fe-EDTA (200 µM), H_3BO_3 (1 µM), $CuSO_4.5H_2O$ (0.3 µM), $MnSO_4.6H_2O$ (2 µM), $(NH_4)_6Mo_7O_{24}$ (0.01 µM), $ZnSO_4.7H_2O$ (0.5 µM). Al stress was induced after seven days of nutrient addition in the pots and exposed for 8 days to observe the Al tolerance. The seedlings of five studied cultivars were evaluated under two levels of Al stress viz., 0 µM Al and 100 µM Al. A photoperiod of 16h was regulated, providing artificial lighting using high-pressure sodium lamps (HPS, 400 Watt) and approximately 350 µmol m^{-2} s^{-1} of Photosynthetic Photon Flux Density (PPFD) was provided for the seedling growth. An optimum growing temperature (30±2°Cdaytime and 25±1°C night) was maintained during the experiment. The nutrient solution was replaced once a week and aerated continuously using an individual air pump for each tank. Different morphological traits were observed to evaluate the Al tolerance, such as root length, shoot length, total fresh weight and total dry weight at 21 days of growing in hydroponics. Besides these, stress tolerance index of these parameters was also measured using the formula mentioned above.

All the data were analyzed by analyses of variance (ANOVA) using the statistical software package Statistix-10 by the principles of Completely Randomized Design (Gomez, 1984). Tukey's test was used to compare variations among the treatments.

III. Results

Germination experiment

Root length: The combined effect of Al stress on wheat varieties showed significant variation for root length at a 5% level of probability (Table 01). The results showed that root length decreased considerably when exposed to stress (100 µM AlC_3) in comparison with control condition (0 µM $AlCl_3$) at 8 DAS. In the control condition, maximum root length was found in BARI Gom-28 (11.79cm) and the minimum was in BARI Gom-30 (10.54cm). Under a stress level of 100 µM $AlCl_3$, BARI Gom-27 showed highest root length (10.25cm) and the lowest was for BARI Gom-30 (8.97cm).

Shoot length: A significant effect was observed between Al stress and varieties on shoot length (Table 01). Shoot length of wheat genotypes was reduced by Al stress significantly (0.05). As found in case of root length, shoot length also decreased when subjected to Al stress, compared to control. Results exhibited that longest shoot (10.3cm) was recorded from BARI Gom-27 followed by BARI Gom-28 (10.2cm), while the shortest one was for BARI Gom-30 (9.7cm). The highest shoot length in stress condition (100 µM $AlCl_3$) was observed in BARI Gom-27 (8.53cm). On the contrary, it was lowest (7.60cm) in BARI Gom-30.

Table 01. Combined effects of aluminum level and varieties on seedling growth parameters of wheat grown in Petri dish at 8 DAS

Treatment	Variety	Shoot length (cm)	Root length (cm)	Seedling length (cm)	Percent germination (%)
T_0 (0 µM AlC_3)	BARI Gom-21	9.9c	11.30c	21.2	96.87b
	BARI Gom-27	10.3a	11.55b	21.85	99.23a
	BARI Gom-28	10.2a	11.79a	21.99	99.10a
	BARI Gom-29	9.77b	11.17d	20.94	95.87c
	BARI Gom-30	9.7b	10.54e	20.24	93.87d
T_1 (100 µM $AlCl_3$)	BARI Gom-21	7.92g	10.20f	18.12	85.87g
	BARI Gom-27	8.53d	10.25f	18.78	91.87e
	BARI Gom-28	8.23e	9.57h	17.8	86.87f
	BARI Gom-29	8.10f	9.67g	17.77	85.37g
	BARI Gom-30	7.60h	8.97i	16.57	83.87h
Level of Significance		**	**	NS	**

Similar letters in a column do not differ significantly at 5% level of significance.** = Significant at 1% level of probability. NS= non-significant.

Seedling length: Al stress and varieties' combined effect on seedling length were insignificant (Table 01). Results revealed that highest seedling length in control condition was observed in BARI Gom-28 (21.99cm), while it was lowest in BARI Gom-30 (20.24cm). When subjected to Al stress (100 µM AlC_3), BARI Gom-27 showed the best performance (18.78cm).

Percent germination: The percent germination of discussible wheat genotypes got decremented significantly (0.05) when exposed to Al stress (Table 01). BARI Gom-27 performed best both at control conditions and under Al stress. In control condition, the highest germination percentage was noted for BARI Gom-27 (99.23%), followed by BARI Gom-28 (99.10%) and the lowest value was observed in BARI Gom-30 (93.83%) at 8 DAS. As for Al stress, maximum germination percentage was found in BARI Gom-27 (91.87%) and BARI Gom-30 exhibited minimum value (83.87%).

Vigor index: Significant variation was noticed among the varieties under Al stress with a drastic alteration in vigor index. (Figure 01). All the wheat varieties tended to decline in vigor index under a stress level of 100 µM $AlCl_3$, contrasted to the control condition. Figure 01 depicted that the highest vigor index (2179.21) was observed in BARI Gom-28 followed by BARI Gom-27 (2168.25) and the lowest value was noted for BARI Gom-30 (1899.86) in control condition. At 100 µM AlC_3, BARI Gom-27 had the highest vigor index (1725.26), while BARI Gom-30 had the lowest one (1389.95).

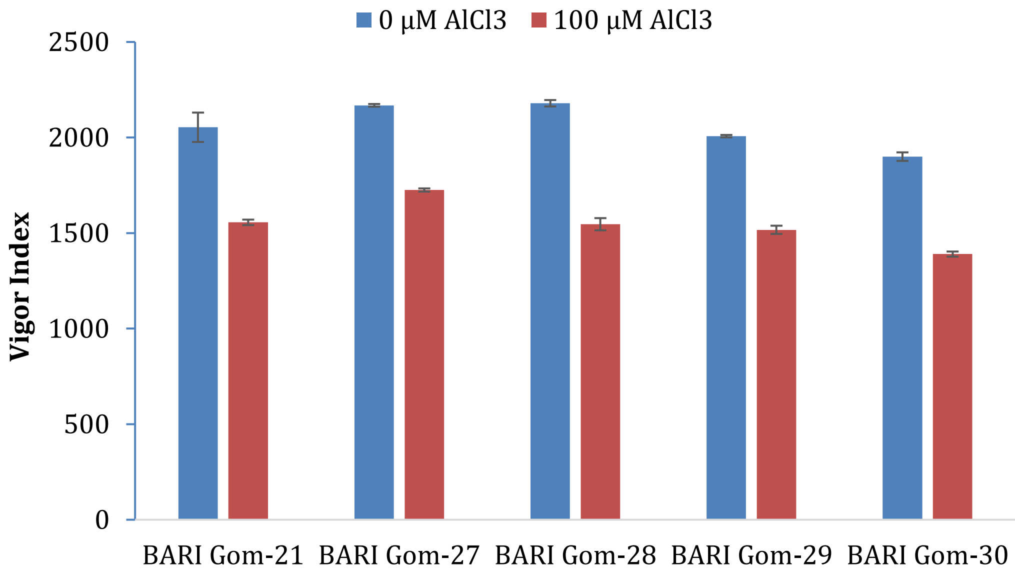

Figure 01. Combined effect of Al treatments and varieties on vigor index of wheat grown in petri dish as 8 DAS. The vertical bars represent the standard error of the means (*n*=3).

Stress tolerance indices: Aluminum stress substantially lowered the values for germination percentage, seedling vigor, root length and shoot length (Figure 02). Interaction between Aluminum stress and varieties at the germination stage exhibited a significant out-turn on the discussible parameters for stress tolerance index. BARI Gom-27 showed highest (92.58) germination stress tolerance index (GSTI) with minimum reduction, while the reduction in germination percentage under stress was found to be maximum in BARI Gom-28 (87.66) as compared to the control condition. As for vigor stress tolerance index (VSTI), the lowest reduction occurred in BARI Gom-27 (79.56), while the highest reduction (70.96) was found in BARI Gom-28 when Al stress was imposed. The highest value in the root length stress tolerance index (RSTI) was obtained from BARI Gom-21 (90.23) with minimum reduction. On the contrary, BARI Gom-28 exhibited the lowest value (81.11) with maximum reduction. BARI Gom-27 showed the highest (82.86) shoot length stress tolerance index (SSTI) with minimum reduction, while the reduction in shoot length under stress was found to be maximum in BARI Gom-30 (78.30) as compared to the control.

Hydroponics experiment

Root length: Combined effect of aluminum level and varieties on root length exhibited significant variation (Table 02). Maximum and minimum root length was found in BARI Gom-21 (17.38 cm) and BARI Gom-29 (16.73 cm) at 0 µM AlC_3, respectively (Table 02). On the other hand, BARI Gom-21 (15.94

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cm) and BARI Gom-30 (11.19 cm) had the highest and lowest root length at 100 µM $AlCl_3$, respectively (Table 02).

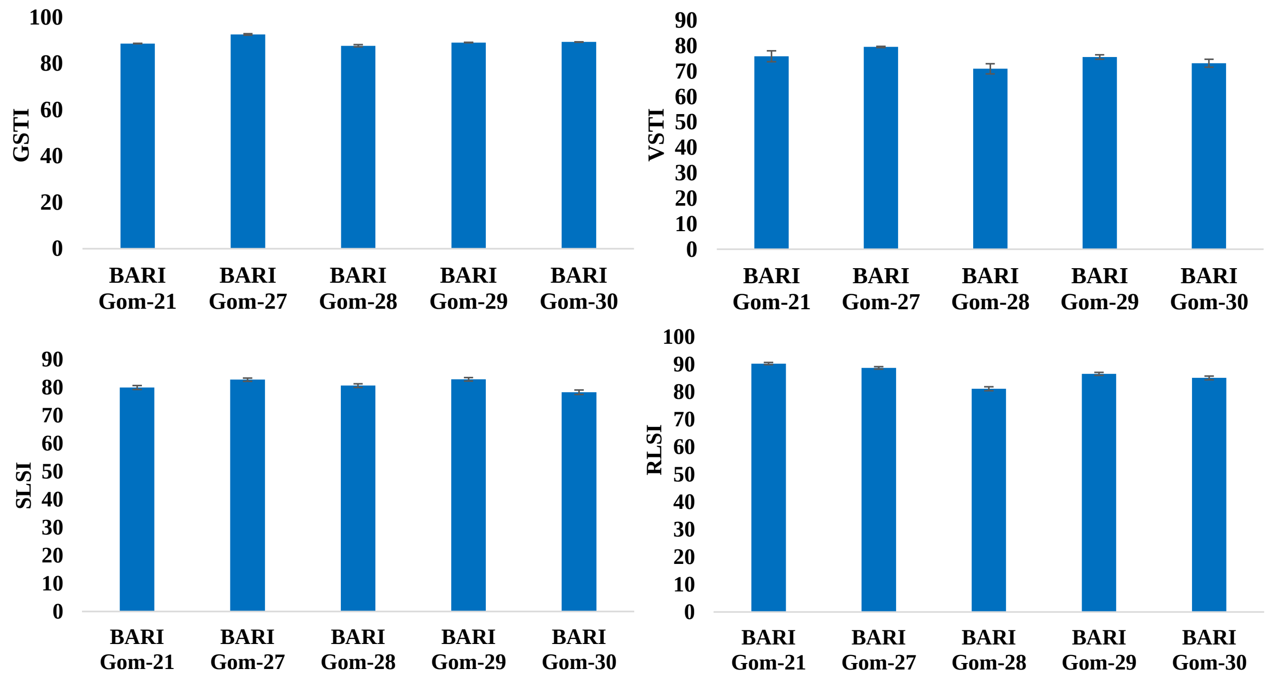

Figure 02. Stress tolerance indices of studied traits for percent germination (GSTI), vigor index (VSTI), shoot length (SLSI) and root length (RLSI) in five wheat varieties under aluminum stress (100 µM $AlCl_3$) conditions in petri dish at 8 DAS. The vertical bars represent the standard error of the means (*n*=3).

Hydroponics experiment

Root length: Combined effect of aluminum level and varieties on root length exhibited significant variation (Table 02). Maximum and minimum root length was found in BARI Gom-21 (17.38 cm) and BARI Gom-29 (16.73 cm) at 0 µM AlC_3, respectively (Table 02). On the other hand, BARI Gom-21 (15.94 cm) and BARI Gom-30 (11.19 cm) had the highest and lowest root length at 100 µM $AlCl_3$, respectively (Table 02).

Shoot length: The combined effect of aluminum level and varieties significantly varied on shoot length. The highest shoot length was found in BARI Gom-27 (15.20 cm) and the least was found in BARI Gom-30 (13.50 cm) under control conditions (Table 02). On the contrary, under Al stress condition BARI Gom-27 (12.82 cm) had the maximum and BARI Gom-30 (11.2 cm) had the minimum shoot length, respectively (Table 02).

Table 02. Combined effect of aluminum level and varieties on root length, shoot length and plant height of hydroponically grown wheat at 20 DAS

Treatment	Variety	Root length (cm)	Shoot length (cm)	Plant height (cm)
T_0 (0 µM AlC_3)	BARI Gom-21	17.38a	14.20bc	31.58c
	BARI Gom-27	17.23a	15.20a	32.43a
	BARI Gom-28	16.77a	14.94a	31.71b
	BARI Gom-29	16.73a	14.62ab	31.35d
	BARI Gom-30	16.95a	13.50cd	30.45e
T_1 (100 µM $AlCl_3$)	BARI Gom-21	15.94a	11.80f	27.74f
	BARI Gom-27	11.79b	12.82de	24.61h
	BARI Gom-28	12.16b	12.65e	24.81g
	BARI Gom-29	11.71b	12.28ef	23.99i
	BARI Gom-30	11.19b	11.2f	22.39j
Level of significance		**	**	**

Similar letters in a column do not differ significantly at a 5% level of significance. ** = Significant at 1% level of probability.

Plant height: Interaction between aluminum level varieties showed a significant variation in plant height. BARI Gom-27 (32.43 cm) and BARI Gom-30 (30.45 cm) indicated the highest and lowest plant height at 0 µM AlC_3, respectively (Table 02). At 100 µM $AlCl_3$, BARI Gom-21 (27.74 cm) and BARI Gom-30 (22.39 cm) had the maximum and minimum plant height sequentially (Table 02).

Number of roots per plant: BARI Gom-27 (26.57) and BARI Gom-30 (23.18) represented the highest and lowest number of roots per plant at control condition (Figure 03). On the other hand, number of roots per plant was maximum in BARI Gom-27 (23.18) and minimum in BARI Gom-30 (17.67), respectively, under Al stress conditions (Figure 03). Results delineate that the number of roots per plant varied significantly among the varieties at different treatments at 20 DAS.

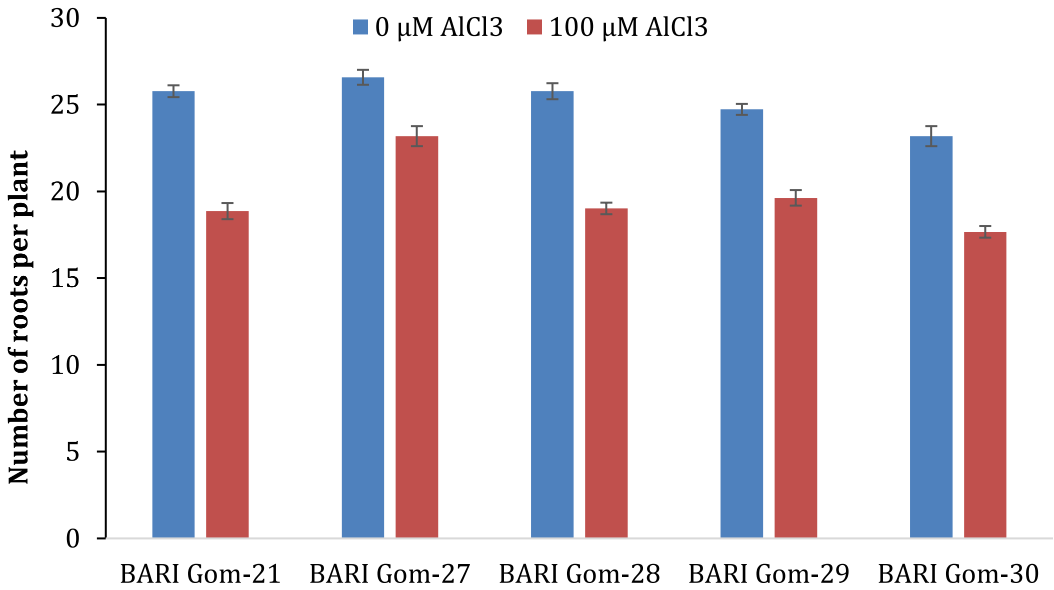

Figure 03. Combined effect of Al treatments and varieties on number of roots of hydroponically grown wheat at 20 DAS. The vertical bars represent the standard error of the means (*n*=3).

Fresh weight per plant: Combined effects of aluminum level and varieties on fresh weight hydroponically grown wheat at 20 DAS had significant variation (Table 03). At 0 µM AlC_3, maximum and minimum fresh weight/plant were found in BARI Gom-27 (1831.3 mg) and BARI Gom-21 (1752.7 mg), respectively (Table 03). At 100 µM AlC_3, BARI Gom-27 (1561.7 mg) had the highest and BARI Gom-21 (902.0 mg) had the lowest fresh weight/plant (Table 03).

Table 03. Combined effects of aluminum level and varieties on fresh and dry weight of hydroponically grown wheat at 20 DAS

Treatment	Variety	Fresh weight/plant (mg)	Dry weight/plant (mg)
T_0	BARI Gom-21	1752.7c	480.67c
(0 µM AlC_3)	BARI Gom-27	1831.3a	551.00a
	BARI Gom-28	1802.0b	531.00ab
	BARI Gom-29	1792.0b	502.00bc
	BARI Gom-30	1760.3c	431.00d
T_1	BARI Gom-21	902.0g	184.00f
(100 µM $AlCl_3$)	BARI Gom-27	1561.7d	257.67e
	BARI Gom-28	1528.7e	247.33e
	BARI Gom-29	925.7f	202.00f
	BARI Gom-30	910.0fg	181.00f
Level of significance		**	**

Similar letters in a column do not differ significantly at a 5% level of significance. ** = Significant at 1% level of probability.

Dry weight per plant: Results indicate that the combined effects of aluminum stress treatment and varieties on dry weight hydroponically grown wheat at 20 DAS had significant variation (Table 03).

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Maximum and minimum dry weight/pant were observed in BARI Gom-27 (551 mg) and BARI Gom-30 (431 mg) at 0 µM AlC_3. On the other hand, at 100 µM AlC_3 BARI Gom-27 (257.67 mg) and BARI Gom-30 (181 mg) exhibited the highest and lowest dry weight/plant, respectively (Table 03).

Stress tolerance indices: Graphs indicate that combined effects of aluminum level and varieties on shoot length stress tolerance index (RLSI), root length stress tolerance index (SLSI), Fresh weight/plant stress tolerance index (FWSI) and dry weight/plant stress tolerance index (DWSI) showed significant variation (Figure 04). In case of SLSI, BARI Gom-21 had the maximum value. BARI Gom-28 had the maximum RLSI. However, the highest FWSI and DWSI were recorded in BARI Gom-27 under control to 100 µM $AlCl_3$ induced aluminum stress (Figure 04).

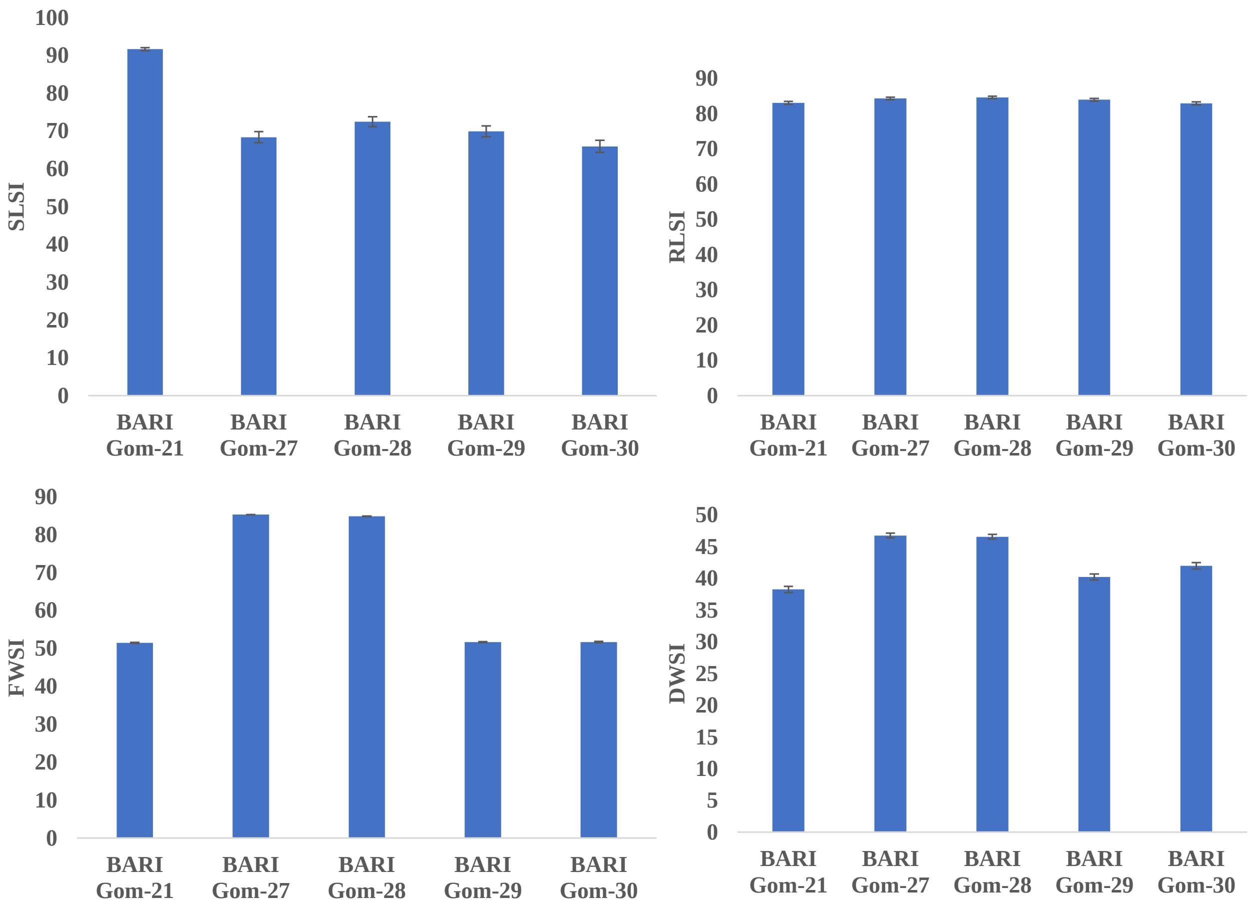

Figure 04. Stress tolerance indices of studied traits for shoot length (SLSI), root length (RLSI), fresh weight/plant (FWSI) and dry weight/plant (DWSI) in five wheat varieties under aluminum stress (100 µM $AlCl_3$) condition in hydroponic at 20 DAS. The vertical bars represent the standard error of the means (*n*=3).

IV. Discussion

One of the most significant ion stresses for plants is Al stress. Al has the most detrimental effects on crop productivity in tropical and subtropical areas in acidic soils (Zerrouk et al., 2016). The most delicate physiological stages in plants are during seed germination and seedling development because the defense mechanisms are impacted. These variables are frequently used as an essential index to measure plant tolerance to heavy metals (Talebi et al., 2014). Root growth had decreased as a result of the Al stress. One of the first signs of Al stress in plants is the inhibition of root growth, which can be seen within minutes of exposure to even very low concentrations of Al in solution (Singh et al., 2017). Root length is frequently used as a point of comparison to demonstrate Al stress tolerance. In studies on sorghum (Ohki, 1987), wheat (Kerridge et al., 1971), soybean (Hanson and Kamprath, 1979) and rice (Ohki, 1979), relative root length (RRL) has been used to gauge Al tolerance (Zhang et al., 2007). In the current study, it was found that BARI Gom-27 performed better in a stressful environment and that it may have Al stress tolerance potential.

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When Al stress was applied, shoot length decreased and BARI Gom-27 performed best under those circumstances. Zhao et al. (2013) also noted that the shoot growth rate was lower in the Al-stressed culture solution than in the Al-free culture solution. Al primarily slows down root growth and interferes with plant metabolism by reducing water uptake and mineral nutrition, which slows down shoot growth (Bidhan and Bhadra, 2014).

According to Delhaize and Ryan's 1995 study of its effects on a few plants, aluminium is toxic to growing plants. The results of the current study demonstrated that germination percentage decreased when imposed Al stress was present, with BARI Gom-27 exhibiting the highest rate of germination percentage in both the controlled and stressed conditions. Additionally, Cajanus cajan (Marin et al., 2004), rice (Peres et al., 2010), kidney beans (Roshani et al., 2014) and wheat all showed diminished germination when exposed to aluminium (Zhu et al., 2015). The responses to the parameters discussed impacted the plants' vigor index, which decreased in the stressed condition compared to the controlled condition. A similar decline in vigor index was also observed in *Lactuca sativa* (Silva and Matos, 2016). When vigor index was considered, BARI Gom-27's performance improved.

Al toxicity prevents root growth by increasing the production of reactive oxygen species and disrupting the homeostasis of antioxidant metabolism (Mora et al., 2006). The first signs of Al toxicity are changes in root morphology, such as atrophy of root hair, swelling of root tips and a loss of cell wall elasticity and plasticity (Ma et al., 2004; Horst et al., 2010; Huang et al., 2014). As a result, much effort has been made to address the problem in recent years. There is an effort to create new cultivars with greater Al tolerance because Al stress severely restricts plant growth in acidic soils. In order to determine the Al toxicity in hydroponically grown wheat plants that were 20 days old, a study was carried out.

The results of this study demonstrated that hydroponically grown wheat exposed to Al stress (100 M Al alone) experienced significant reductions in root length, shoot length, plant height, the number of roots per pant, fresh weight per plant and dry weight per plant (Table 02 and Table 03, Figure 03 and Figure 04). Another study observed a decrease in root elongation with increasing Al concentrations (Tammam et al., 2012). With time, among the varieties at Al treatment alone, the length of the roots, shoots, total fresh weight and total dry weight of the plant all significantly decreased (Table 02 and Table 03). Aluminum ions were found to affect plasma membrane permeability (Stass and Horst, 1995), fluidity (Gunes et al., 2007) and protein-lipid interactions, which may account for the observed lower values for fresh and dry weight upon Al treatments (Molassiotis et al., 2006). In light of these modifications brought on by Al, plasma membrane function, ion transport, water uptake and fresh mass of wheat were all significantly affected under the current experimental conditions. The growth, dry weight and fresh weight of the roots and shoots of *Cucumber sativus* were all reduced at concentrations of 100, 500, 1000 and 2000 mM $Al_2(SO4)_3$, according to earlier research by Pereira et al. (2006). Aluminum likely interfered with the growth of root cells, resulting in a reduction in the production of cell walls, as aluminium prevents the Golgi apparatus' secretory function (Pereira et al., 2006).

V. Conclusion

The study disclosed that BARI Gom-27 exhibited maximum root length (cm), shoot length (cm), seedling length (cm), germination percentage and vigor index at the germination stage. Besides, it revealed the highest shoot length (cm), plant height (cm), number of roots plant^{-1}, fresh weight (g) and dry weight (g) at seedling stage in hydroponics. The screened-out variety 'BARI Gom-27' might show boosted growth and high yield in aluminium stress prone areas. This study results indicate that BARI Gom-27 may be utilized in future breeding programs for obtaining aluminium stress tolerant wheat varieties.

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