

IMPACT OF ALUMINIUM (Al³⁺) STRESS ON GERMINATION AND SEEDLING GROWTH OF FIVE WHEAT GENOTYPES

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ABSTRACT

A Petridish and hydroponic culture experiments were conducted at Crop Physiology Laboratory, Department of Crop Botany and Agriculture Chemistry Laboratory, Bangladesh Agricultural University, Mymensingh during the period from August to October 2011 to investigate the effect of aluminium on morphological characters and growth of wheat seedlings. The experiment comprised of two levels of aluminium concentrations viz., 0 μ M (control) and 100 μ M and five varieties viz; Kanchan, Shatabdi, Sourav, Bijoy (BARI-23) and Sufi (BARI-22). The experiment was laid out in two factors completely randomized design with three replications. Applications of 100 μ M aluminium had a profound influence on hypocotyls and epicotyls length, germination percentages, and root-shoot length, fresh and dry mass production in wheat. Results indicated that germination percentage, hypocotyls and epicotyls length, root and shoot length, leaf length, leaf sheath length, plant height, fresh and dry mass plant were greater in control than aluminium stress conditions. It revealed that wheat seedlings are susceptible to aluminium stress. However, among the varieties, the reduction of dry mass under aluminium stress was minimum in Shatabdi followed by Kanchan showed that Shatabdi was more tolerant to aluminium stress than the other varieties namely Sourav, Bijoy (BARI-23) and Sufi (BARI-22). Sufi and Sourav were more susceptible to aluminium stress.

Keywords: Hydroponic Culture, Hypocotyl, Epicotyl, Aluminium Ions, Germination, Growth

INTRODUCTION

In tropical climates, aluminium toxicity is a main factor for crop performance under acid soils (Barcelo and Porchenrieder, 2002). The presence of aluminium (Al) ions is the major constraints on crop production in acid soils which cover more than 30% of

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agricultural land around the world. Aluminium toxicity can be easily recognized by the inhibition of root growth by interfering with the regulation of root growth and development (Foy, 1988). Ionic Al rapidly suppresses root elongation and inhibits the uptake of nutrient and water, resulting in poor growth in plants. Although aluminium is not a heavy metal (specific gravity of 2.55-2.80), it makes up about 8% of the surface of the earth and is the third most abundant element (ATSDR ToxFAQs for Aluminium). It is readily available for human ingestion through the use of food additives, antacids, buffered aspirin, astringents, nasal sprays and antiperspirants from drinking water, automobile exhaust, tobacco smoke, aluminium foil, aluminium cookware, cans, ceramics and fireworks. Even though wheat is the second most important cereal crop in Bangladesh next to rice, the country still imports significant quantities of wheat to meet the domestic demand. Environmental stresses have adverse effects on wheat in growing stages, Al toxicity in acidic soils is one of them. Scientists reported that failure in grain set caused by Al toxicity causes yield reduction in wheat. One of the major factors leads to decrease wheat yield is aluminium toxic soil due to unavailability of aluminium tolerant cultivars. Some wheat cultivars have significant tolerance level and studies showed that wheat roots of aluminium resistant cultivars gather more Al^{3+} than those of aluminium sensitive ones (Aniol, 1983), whereas other works suggest that accumulation of Al^{3+} is alike to both resistant and sensitive wheat cultivars (Pettersson and Strid, 1989; Zhang and Taylor, 1989). Therefore, the identification of wheat cultivars which are tolerant to Al toxicity is of particular significance for their immediate use in regions where Al toxicity causes limited yield. So, the present research work was undertaken to screen out the resistant variety(s) among the selected varieties with the proper investigation of aluminium tolerance variability among the different wheat varieties and to know the mineral uptake variability of cultivars under aluminium stress.

MATERIALS AND METHODS

The experiment was conducted in the green house (growth chamber), Bangladesh Agricultural University, Department of Crop Botany, Mymensingh from August 2011 to June 2012. Seeds of wheat cultivars: Shatabdi, Kanchan, Sourav, Bijoy (BARI-23) and Sufi (BARI-22). Seeds were collected from the Bangladesh Agricultural Research Institute (BARI), Gazipur, Bangladesh. The hydroponic system was constructed according to the IRRI slanted protocol (Gregorio et al., 1997). Five seeds were soaked in distilled water on filter papers (Advance Technology, Tokyo, Japan) in each Petridish for 24 hours. Plants were transferred to 4×30×35 cm rectangular plastic trays after two days of sowing. The experiments were conducted in a green house at 25°C with 0.6 Cd m⁻² light intensity. The volume of each tray was 8 L. On the opening of each tray, nylon net and rectangular-shaped Styrofoam having 10 × 10 holes were placed. Hogland solution (N:P:K= 21:31:23) was added to supply nutrient to plants. The solution was renewed with fresh nutrient solutions in 5 days interval. When seedlings were about 4 cm, seedlings were

pretreated with 100 μM CaCl_2 for six hours. The pH was maintained 5.10 by pH meter using HCl and NaOH if necessary. Then the seedlings were subjected to (Al treatment) or non-subjected (control) in 8L of solution containing 100 μM AlCl_3 (pH 5.5) or 0 μM AlCl_3 (pH 5.0) for 24 hours. Parameters such as: hypocotyls length, epicotyls length, germination percentage were recorded at 4, 6 and 8 days after sowing of germinated seeds. Physical characteristics *viz.* root length, shoot length, number of roots plant⁻¹, leaf length, leaf sheath length, fresh weight, dry weight, total root and shoot weight, were also recorded at 10, 15 and 20 days after sowing in both Al (treatment) and control conditions.

Statistical analysis: Data were statistically analyzed for analysis of variance (ANOVA) using the MSTAT Statistical Computer package programme in accordance with the principles of Completely Randomized Design (Gomez, 1984). Least Significant Difference (LSD) was used to compare variations among the treatments.

RESULTS

1. Effect of aluminum levels on Seed related parameters of wheat grown in Petridish

1.1. Length of hypocotyl

The interactive effect of variety and aluminium level on hypocotyl length at 4, 6 and 8 DAS was significant (Table 1). The longest hypocotyl length was recorded in the treatment combination of variety Kanchan with 0 μM aluminium at all growth stages (5.6, 8.6 and 10.7 cm for 4, 6 and 8 DAS respectively) followed by variety Sufi with 0 μM aluminium (5.5 and 8.3 cm for 4 and 6 DAS respectively). On the other hand, the shortest hypocotyls length was more or less observed in variety Sourav with 100 μM aluminium at all growth stages (2.7, 7.2 and 8.8 cm for 4, 6 and 8 DAS respectively).

1.2. Length of epicotyl

The interactive effect of variety and aluminium level on epicotyls length at 4, 6 and 8 DAS was significant (Table 1). At 8 DAS, the longest epicotyl length was recorded in the treatment combination of Shatabdi (12 cm) with 0 μM aluminium followed by Kanchan and Sufi while the shortest was recorded in Bijoy (2cm at 4 DAS) with 100 μM aluminium.

Table 1. Combined effect of treatments and varieties on hypocotyls length and epicotyls length of wheat grown in petridish

Treatment	Variety	Hypocotyl length (cm)			Epicotyl length (cm)		
		4DAS	6DAS	8DAS	4DAS	6DAS	8DAS
T ₁ (0μM)	Kanchan	5.6a	8.6a	10.7a	3.3ab	9.3b	11.5ab
	Shatabdi	5.1bc	7.9bcd	9.8bc	3.4a	9.9a	12a
	Bijoy	4.5de	8.3ab	10.1b	3a-e	9b	11a-e
	Sufi	5.5ab	8.3ab	10.2b	2.8bcd	9.2b	11.2abc
	Sourav	5.2abc	8bc	10.0b	3.2abc	8.2c	10.9a-e
T ₂ (100μM)	Kanchan	4.4e	8bc	9.9b	2.7cd	8.4c	10.1cd
	Shatabdi	3.2g	7.5de	8f	2.1e	7.1de	9.8d
	Bijoy	4f	7.8cd	9.3d	2e	6.9e	9.8d
	Sufi	3.9cd	7.9bcd	9.4cd	2.7cd	8.1c	10.6bcd
	Sourav	2.7h	7.2e	8.8e	2.5de	7.5d	9.8d
LSD at 5%		0.19**	0.18**	0.19**	0.21**	0.18**	0.50**

LSD= Least Significant Difference; ** = Highly Significant

2. Effect of aluminum levels on germination percentage of wheat grown in Petridish

The interactive effect of variety and aluminium level on germination percentage at 4, 6 and 8 DAS was significant (Fig 1.). The highest germination percentage was recorded in the treatment combination of variety Shatabdi and variety Kanchan each with 0μM aluminium at all growth stages (98% and 100% at 8 DAS respectively). The lowest germination percentage was observed in Bijoy (84% at 8 DAS) with 100 μM aluminium.

3. Effect of aluminum levels on seedlings characters of wheat grown in hydroponic culture

3.1. Root length

The interactive effect of variety and aluminium on root length was significant (Fig. 2). The highest root length was observed in the treatment combination of variety Shatabdi with 0 μM aluminium (16.9 cm at 20 DAS) and the lowest was recorded in the treatment combination of Sourav with 100 μM aluminium (11.6 cm at 15 DAS).

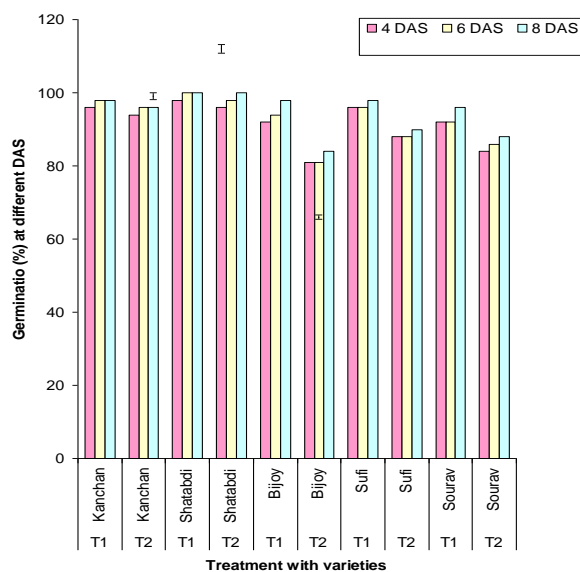


Figure 1: Combined effect of treatments and varieties on germination percentages in wheat grown in petridish. Bar represents at LSD_(0.05)

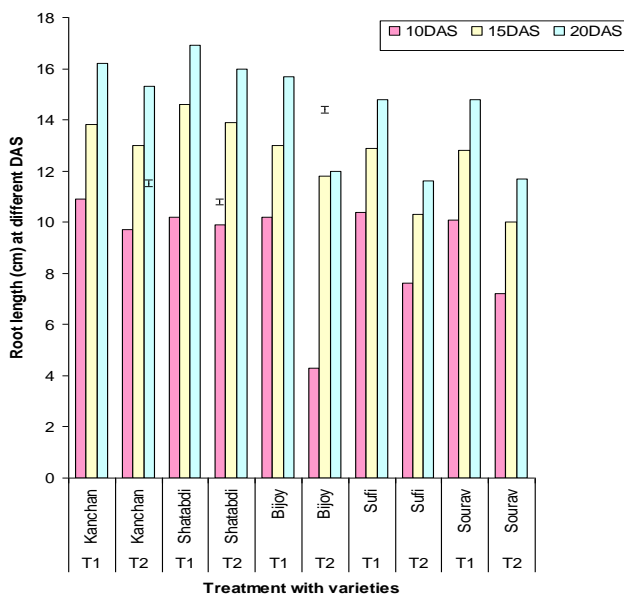


Figure 2: Combined effect of treatments and varieties on root length in wheat grown in hydroponic culture. Bar represents at LSD_(0.05)

3.2. Shoot length

The interaction effect of variety and aluminium level on shoot length at 10, 15 and 20 DAS was significant (Table 2). At 10 DAS, variety kanchan produced significantly lengthy shoots with 0 μM aluminium (15.5 cm) as compared to all other combinations. At 15 DAS and 20 DAS, Kanchan and Shatabdi produced the lengthy shoots at both aluminium treatments which were at par with each other but significantly lengthy from all other treatment combinations.

Table 2. Combined effect of treatments and varieties on shoot length, leaf length, leaf sheath length of wheat grown in hydroponic culture

Treatment	Variety	Shoot length (cm)			Leaf length (cm)			Leaf sheath length (cm)		
		10DAS	15DAS	20DAS	10DAS	15DAS	20DAS	10DAS	15DAS	20DAS
T ₁ (0 μM)	Kanchan	12.5a	13.8a	15ab	14.8c	16.8a	18b	3.4a	5a	6a
	Shatabdi	11.9b	13.9a	15.1a	15.4a	17.0a	18.2a	3.5a	5a	6.2a
	Bijoy	11.2c	12.8b	14.8ab	13.9d	15.8b	17.8c	2.9bc	3.9b	6a
	Sufi	11.6bc	12.7bc	14.6b	13.5e	15.4bc	16.9e	2.6c	4b	5.7ab
	Sourav	11.4c	12.4c	14.5b	14d	15.3bcd	16.8e	2.8c	4.1b	5.3bc
T ₂ (100 μM)	Kanchan	9.9e	13.7a	14.8ab	14.7c	16.6a	17.8c	3.2ab	4.7a	5.7ab
	Shatabdi	9.8e	13.7a	14.9ab	15.2b	16.9a	18.1ab	3.5a	4.8a	6a
	Bijoy	9.7e	11.6d	14c	12.8f	14.9cd	17.2d	2.2d	3.2c	5.6ab
	Sufi	10.6d	11.4d	13.9c	11.7g	15.0cd	16.1f	2.0d	3.2c	5c
	Sourav	9.8e	11.0e	13.8c	13.5e	14.7d	16.2f	1.9d	3.0c	5c
LSD at 5%		0.17**	0.15**	0.47**	0.05**	0.27**	0.05**	0.14**	0.23**	0.24**

LSD= Least Significant Difference; ** = Highly Significant

3.3. Leaf length

The interactive effect of variety and aluminium levels on leaf length at all growth stages was significant (Table 2). The longest leaf length was recorded in the treatment combination of Shatabdi (15.4 cm at 10 DAS, 17.0 cm at 15 DAS and 18.2 cm at 20 DAS) with 0 μM aluminium followed by the same variety with 0 μM aluminium, which were statistically at par with each other but significantly lengthy from all other treatment combinations.

3.4. Leaf sheath length

The interactive effect of variety and aluminium concentration on leaf sheath length at 10, 15 and 20 DAS was significant (Table 2). All growth stages, Kanchan and Shatabdi with both aluminium treatments produced statistically similar leaf sheath length but significantly more than all other treatment combinations.

3.5. Root number

The interactive effect of variety and aluminium on root production was significant (Fig 3). The highest number of roots plant⁻¹ was observed in the treatment

combination of shatabdi (13 plant^{-1} at 20 DAS) with $0 \mu\text{M}$ aluminium and the lowest was recorded in the treatment combination of Sourav (8 plant^{-1} at 20 DAS) with $100 \mu\text{M}$ aluminium.

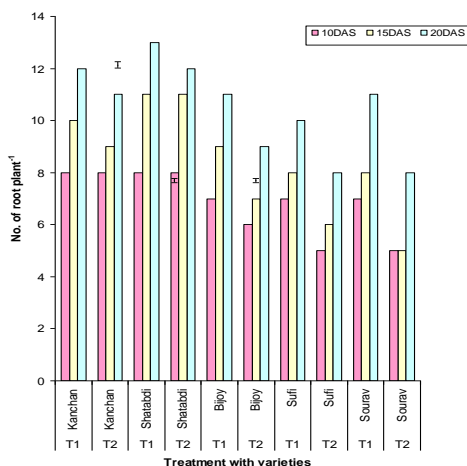


Figure 3: Combined effect of treatments and varieties on number of root plant⁻¹ of wheat grown in hydroponic culture. Bar represents at LSD (0.05)

3.6. Plant height

The interactive effect of variety and aluminium concentration on plant height at 10, 15 and 20 DAS was significant (Fig 4.). The longest plant height was recorded in the treatment combination of Shatabdi (26.3 cm at 20 DAS) with $0 \mu\text{M}$ aluminium. On the other hand, the shortest plant height was observed in Sourav (17.9 cm at 20 DAS) with $100 \mu\text{M}$ aluminium followed by Sufi (18.8 cm at 20 DAS).

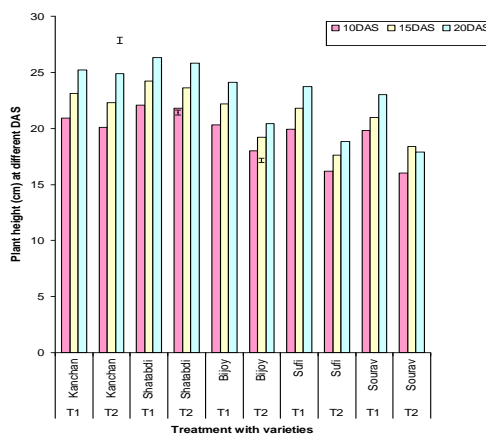


Figure 4: Combined effect of treatments and varieties on plant height of wheat grown in hydroponic culture. Bar represents at LSD (0.05)

3.7. Fresh weight plant⁻¹

The interactive effect of variety and aluminium level on fresh weight plant⁻¹ at 10, 15 and 20 DAS was significant (Table 3). The highest fresh weight plant⁻¹ was recorded in the treatment combination of Shatabdi (810, 1610 and 1830 mg plant⁻¹ for 10, 15 and 20 DAS respectively) with 0 μ M aluminium followed by Kanchan (800, 1590 and 1800 mg plant⁻¹ for 10, 15 and 20 DAS respectively) which were statistically at par with each other but significantly higher than all other treatment combinations at all three growth stages.

3.8. Dry weight plant⁻¹

The interactive effect of variety and aluminium level on dry weight plant⁻¹ at 10, 15 and 20 DAS was significant (Table 3). The highest dry weight plant⁻¹ was recorded in the treatment combination of Shatabdi (205, 480 and 550 mg plant⁻¹ for 10, 15 and 20 DAS respectively) with 0 μ M aluminium significantly higher than all other treatment combinations at all three growth combinations.

Table 3. Combined effect of treatments and varieties on fresh weight and dry weight plant⁻¹

Treatment	Variety	Fresh weight (mg) plant ⁻¹			Dry weight (mg) plant ⁻¹		
		10DAS	15DAS	20DAS	10DAS	15DAS	20DAS
T ₁ (0 μ M)	Kanchan	800a	1590b	1800ab	200b	470b	530b
	Shatabdi	810a	1610a	1830a	205a	480a	550a
	Bijoy	550e	1380f	1760bc	100g	370e	500c
	Sufi	640d	1450e	1750bc	105f	390d	480d
	Sourav	720b	1500d	1790ab	130e	400c	430f
T ₂ (100 μ M)	Kanchan	600b	1210c	1510ab	105d	160d	280c
	Shatabdi	650a	1285b	1560ab	115c	175c	290b
	Bijoy	300f	675g	910cd	50h	150f	200e
	Sufi	310f	705f	900d	45i	152f	180h
	Sourav	370c	710f	925cd	52h	145g	190g
LSD at 5%		11.89**	4.66**	23.32**	3.56**	2.33**	2.33**

LSD= Least Significant Difference; ** = Highly Significant

3.9. Total root fresh weight and dry weight

The interactive effect of variety and aluminium concentration on total root fresh weight was significant (Table 4). The highest total root fresh weight and dry weight were recorded in the treatment combination of Bijoy (5.98g and 5.95 g respectively) with 0 μ M aluminium where the result have shown 50%-60% loss compared to the fresh weight. Almost similar was the case with total shoot weight.

Table 4. Combined effect of treatments and varieties on total root fresh weight and dry weight and total shoot fresh weight and dry weight

Treatment	Variety	Total root fresh wt (g)	Total shoot fresh wt (g)	Total root dry wt (mg)	Total shoot dry wt (mg)
T ₁ (0 µM)	Kanchan	5.83c	2.98a	583ab	280ab
	Shatabdi	5.67d	2.87b	561b	275abc
	Bijoy	5.98a	3.02a	595a	292a
	Sufi	5.87b	2.99a	587a	281ab
	Sourav	4.93f	2.85b	490d	271abc
	Kanchan	4.80h	2.20cd	430d	250bc
T ₂ (100 µM)	Shatabdi	4.89g	2.45cd	450d	260bc
	Bijoy	3.36i	1.95e	290d	195c
	Sufi	3.03e	2.00bc	300c	200abc
	Sourav	3.20g	1.85de	275d	175c
LSD at 5%		0.01**	0.03**	0.07**	0.08**

LSD= Least Significant Difference; ** = Highly Significant

DISCUSSIONS

Effect of aluminium levels on seed, germination percentage and seedlings characters of wheat grown in Petridish and hydroponic culture

Aluminium in different concentrations has influence in different parameters of seedlings characters such as length of epicotyls, length of hypocotyls and germination percentage. Foy (1996) found that the increase of aluminium concentration brings about the lowest length of epicotyls and germination percentage range (70-82%). Aluminium toxicity brings about an allelopathic effect on seed especially aluminium stress susceptible variety such as Bijoy showed significant variation in length of epicotyls and germination percentage. Fleming (1983) found that epicotyls and germination had positive co-relation so that decrease of epicotyls length brought about lower possibility of germination. It is suggested that epicotyls play a vital role in vigorous seedlings. Aluminium toxicity brings about dead seedling at an early stage. Increase of aluminium concentration inhibits epicotyls growth and development that promotes scarcity of water and nutrients in plants. The inhibitory action of aluminium plays a significant role in root elongation in Durum wheat (Foy and Fleming, 1978). On the contrary, Aluminium stress tolerance is a complex trait in plants that influence plant to uptake mineral and water by avoiding Aluminium concentration at a great extent that is shown by variety Shatabdi in the conducted

experiment. Aniol (1984) found that Al phytotoxicity was responsible for low fresh weight, dry weight as well as dwarf plant, lower leaf length and leaf sheath length. Al toxicity hampers calcium-dependent metabolism, Plasma membrane's lipid peroxidation and also quick disruption of cell elongation and division. Al^{3+} can bind to different sites of plants such as cell wall, plasma membrane and interfere with their functions. Lipid peroxidation brings about lower leaf length and lower sheath length in plants caused by aluminium toxicity (Sivaguru and Paliwal, 1993). The main symptom of oxidative stress is peroxidation of unsaturated lipids in membrane that limits the protein degradation and lowers the capacity of ion transport which ultimately brings about cell death. Limiting ion transport brings about stunted growth of plants which brings about growth parameters disruption of plants. Al toxicity brings about significant variation in seedling growth parameters and found root length has a negative correlation with relative root induction. Hydrolysis of endosperm and starch and supply of oxygen is needed for the development of seminal root caused by aluminium toxicity. In this experiment, wheat variety 'Sourav' and 'Sufi' showed susceptibility to aluminium toxicity in most cases.

CONCLUSION

Hypocotyls and epicotyls length and germination percentage decreased under 100 μ M aluminium compared to control at 4,6 and 8 days after sowing indicating that 100 μ M concentration is toxic to wheat seedlings growth and development. The interactive effect of variety on hypocotyls and epicotyls length and germination percentages was significant. The highest hypocotyls length was observed in Kanchan (10.7 cm) while the highest epicotyls length was recorded in Shatabdi (12 cm). In contrast, the lowest hypocotyls length was observed in Sourav (2.7 cm) and the lowest epicotyls length was observed in Bijoy (2 cm). The interactive effect of hypocotyls and epicotyls length and germination percentages was significant. The highest hypocotyls length was observed in the treatment combination of Kanchan with control at all growth stages while the highest epicotyl length was observed in the treatment combination of Shatabdi with control. However, the lowest hypocotyl length was observed in the treatment combination of Bijoy and Sourav with 100 μ M aluminium while the lowest epicotyls length was observed in Sourav with 100 μ M aluminium. Again the highest germination percentages were recorded in the treatment combination of Shatabdi with control. The effect of concentrated aluminium on root and shoot length, root number, leaf length fresh and dry weight was significant. The root and shoot length, fresh and dry weight were greater in control compared to 100 μ M aluminium. In genotypes, the highest shoot length was observed in Shatabdi which resulted in highest dry mass $plant^{-1}$ at 15 and 20 DAS. In contrast, the lowest fresh and dry weight was recorded in Sourav and Sufi. The interaction effect of variety and aluminium on root and shoot length, fresh and dry

weight plant⁻¹ was significant. For dry mass reduction due to concentrated aluminium, result showed that the lowest dry mass reduction due to aluminum was observed in Shatabdi indicating this variety more tolerant to aluminium than others. In contrast, the higher dry mass reduction was observed in Sourav and Sufi indication these two varieties were susceptible to aluminium. By measuring the minerals uptake of roots and shoots it may be concluded that at 100 μM Al treatment, based on the experimental results, it may be concluded that the aluminum concentration of 100 μM had profound negative effect on germination percentages, growth and development of seedlings; and among the varieties, Shatabdi had highest tolerance to aluminium toxicity in respect with growth and development.

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