Ameliorative role of Boron on Morpho-physiological characters of Mandarin Orange Seedlings under Aluminium Stress Condition

ABSTRACT

Darjeeling mandarin orange is well known for its unique and delicious taste. It is also considered as one of the major cash crop of Darjeeling hills. But in recent past, the production has been declined drastically due to some of the biotic and abiotic factors. Acidic soils are mainly confined in this region which has a complex interaction of growth limiting factors that can restricts growth by inducing stress on plants through triggering aluminium stress and micronutrient Deficiency. This has resulted in declination in production and quality of mandarin orange grown in foothills of Darjeeling. Since boron is known to act as ameliorative role under aluminium toxicity, the present study was investigated to understand the effect of boron in mandarin orange seedlings grown under aluminium stress condition. Seedlings of Darjeeling mandarin orange were fertilized with Hoagland solution containing four levels of boron (*i.e*, 0µM, 5µM, 10µM and 25µM H_3BO_3) and two level of Aluminium (*i.e.*, 0µM and 1200µM $AlCl_{3.}6\ H_2O$) up to 22 weeks. Seedlings without boron and aluminium was treated as the control. Aluminium at higher concentration severely hinder physiological changes in terms of shoot length, leave numbers, leaf area, fresh shoot, root weight, chlorophyll content and carotenoid of mandarin seedlings. However, the application of boron improved the physiological attributes under higher concentration of aluminium. Furthermore, application of boron concentration at 10µM proved to be better treatment for mandarin seedlings grown under aluminium stress condition.

Keywords: Aluminium toxicity, Boron application, Mitigation, Darjeeling mandarin orange

1. INTRODUCTION

Darjeeling Mandarin orange (*Citrus reticulata* Blanco.) known as "Suntala" is one of the finest commercial fruit crops grown in foot hills located at northern region of West Bengal. Owing to its unique pleasant aroma and taste, it is considered as pride of Darjeeling. The prevalent soil type in this region is acidic in nature with pH (4.30- 5.44) and aluminium toxicity being one of the major constraints accompanied with deficiency of micronutrients caused by leaching due to heavy downpour as reported by Ray and Mukhopadhyay (24). Acidic soil is characterized by a deficiency in nutrients and toxicity by metals such as manganese (Mn), iron (Fe) and Aluminium (Al); with toxicity by aluminium being the main factor limiting plant growth in acidic soils. Kochian et al. (16); Gupa et al. (9). Some of the essential micronutrient such as boron, zinc and molybdenum are most deficient in acidic soils, with their deficiency reported in (45, 46 and 31 %) of the agricultural soils, respectively.

Singh (29). It is estimated over 50 percent of the world’s potentially arable soil is acidic in nature with aluminium toxicity being one of the limiting factors for crop productionYang et al. (35) as it is easily soluble under acidic conditions and might induce toxicity problems in plants. Aluminium toxicity hampers the plant root system by effecting the growth and development of the roots and leaves of the plant by causing cell death, imbalanced nutrient uptake and mobilization, and accumulation of reactive oxygen species. Corrales et al. (6); Poschenrieder et al. (22). Aluminium toxicity even at a negligible concentration decreased nitrogen, phosphorous, potassium, calcium, magnesium and sulphur uptake thereby inhibited the growth in different citrus crops. Guo et al. (8). The plant growth process implies a complex system consisting of cell integrity, cell division, and expansion which is degraded due to the presences of aluminium toxicity. Doncheva et al. (7). Boron having structural similarity with aluminium, deficiency of boron shares common effect on plant growth like aluminium toxicity which suggest a strong relationship between them. Stass et al. (30). It has also been reported that boron plays an essential role in improving aluminium toxicity tolerance in citrus, rice, pea and rape seedlings. Zhou et al. (38); Zhu et al. (36). Boron is considered as one of the most important micronutrients for citrus. It is essential for growth and development of higher plants. Voxeur and Fry (32) where boron plays an important role in supporting the formation of primary cell walls by cross-linking poly-pectic polysaccharides RG-II (rhamnogalacturonan II), suggesting,that the negative effect of aluminium toxicity by damaging cell wall integrity can be counteracted by cross-linked RG-II forming a stable and complex cell wall with reduced pore spaces, thus tightening the cell wall and restricting the entry of aluminium into the sensitive cell organelles, thus alleviating the negative effect of aluminium on plants O’Neill et al. (21); Corrales et al. (6). However, limited information is available on the effect of aluminium stress as well as interaction of boron under aluminium stress condition in Darjeeling mandarin. Considering the fact, the present investigation was carried out to investigate the ameliorative role of boron on aluminium stress condition in Darjeeling mandarin orange seedlings.

2. MATERIALS AND METHODS

The present study was carried out during the year 2021-2022 on Darjeeling mandarin orange seedlings grown in a polyhouse of Department of Pomology and Postharvest Technology at Uttar Banga Krishi Viswavidyalaya, Cooch Behar, West Bengal, India under the following condition: day temperature 22-31ºC; relative humidity (RH), (67.5-93%) and a photoperiod of 5- 8 hours. Anonymous (1). Geographically the district lies in the foothills of eastern Himalayas and is located at 28º58’86’’ N latitude, 81º66’’73’’ E longitude at an elevation of 42 m above mean sea level. The fruits of mandarin orange were obtained from the orchard of commercial growers of Darjeeling and immediately brought to the Pomology and Postharvest Technology laboratory, Uttar Bang Krishi Vishwavidyalaya, Cooch Behar. The seeds were extracted from the fruits collected and washed properly with tap water and then seeds were immersed in distilled water after surface sterilizing for 1 minutes in diluted sodium hypochlorite. The seeds were sown in portrays filled with sterilized sand having pH 6-7 following with the light irrigation and kept under polyhouse for a month. After the germination, uniform seedlings were selected and transplanted to 3 litre imperforated plastic pots containing sterilized sand having pH 6-7. Each pot was supplied with 500 ml of Hoagland nutrient solution containing H_3BO_3 and $AlCl_3.6H_2O$ combinations as per treatment details on every three days for 22 weeks after transplanting. The nutrient solution pH was adjusted to 4.1- 4.5 using hydrochloric acid. There were eight treatments in total including four levels of boron (*i.e*, 0µM, 5µM, 10µM and 25µM H_3BO_3) and two level of Aluminium (*i.e.*, 0µM and 1200µM $AlCl_{3.}6\ H_2O$). Seedlings without boron and aluminium was considered as control. Experiment was laid out in factorial CRD (complete randomized design) with four boron levels and two aluminium levels of treatments, and each treatment had three replications After 22 weeks of treatment, ten representative seedlings were selected from a treatment to measure different growth parameter of Darjeeling mandarin seedlings as per the standard protocol. The data were analysed by Fisher's Analysis of Variance (ANOVA) using R Software (R core team, 2018). Different letters behind the values indicates a significant difference between the treatments (P <0.05).

3. RESULTS AND DISCUSSION

3.1. **Effect of boron on plant growth parameters under aluminium stress condition**

The shoot length and leaf numbers increased significantly with the increase in the boron content in all the treatments. Among the treatments, application of boron concentration at B_2Al_0 (B_2-10µM, Al_0-0µM)recorded the highest value for shoot length, leaf number with an increment of 51.15 %, 22.27 %, whereas, the plant growth parameters significantly decreased under high aluminium stress (B_0Al_1) with a decline of 2.70 %, 2.31 %. However, the improvement of plant growth characters was noticed when plants supplied with boron under aluminium stress condition, as noticed in treatment B_2Al_l with an increment of plant height, number of leaves by 23.19 %, 18.26 % respectively followed by $B_3Al_{l.}$

As results indicated that shoot length of mandarin seedlings was significantly inhibited under the influence of boron and aluminium stress condition. The present findings in terms of shoot growth inhibition due to boron deficiency is in concord with the results of Ishii et al. (13) who reported decrease in growth is accompanied by cell wall thickening and a decrease in borate cross-linking of RG-II. The improvement in shoot length of mandarin seedlings can be attributed to the role of boron in stabilizing certain constituents of cell wall and plasma membrane, enhancement of cell division, tissue differentiation and metabolism of nucleic acids, carbohydrates, protein, auxins and phenols. Brown, (4). The probable cause of aluminium triggering the shoot length growth might be due to the bound aluminium content that can transform to soluble Al^{3+} under acidic condition and can be easily absorbed by plants, thereby inducing toxic problems to the plant growth as stated by George et al. (7); Poschenrieder et al. (22). The better performance of shoot length of seedlings with the application of boron under aluminium might be due to the role of boron in cross-linking of RG-II and the formation of a stable network of cell wall with reduced pore size and tightening the cell wall, thus restricting the entry of aluminium into the sensitive cell organelles reported by Jiang et al. (15). The increment in number of leaves of seedlings may be attributed to the higher level of photosynthetic activity and root growth as observed in our study whereas under boron deficiency leaf area decreased. The leaf is an important attribute of efficient photosynthetic system and efficient management of leaf nutrients, an increased number of leaves along with having increased leaf area indicate healthy growth and development of the seedlings. The application of boron tends to maintain the root elongation and better absorption of the nutrients and also increasing the photosynthetic activity. The present finding is also in concord with the reports of Ishii et al. (13) who reported that boron deficiency supresses growth in aerial plant parts, such as leaf number and leaf area. The leaf number were also found to be decrease under aluminium stress which could be possibly due to leaf senescence and ion toxicity Seguel et al. (27) and this results in induced impairment of membrane integrity and affect all physiological activities linked to membrane functioning as reported by Rouphael et al. (25). However, the application of boron under aluminium stress enhanced leaf numbers which may be due to the boron induced alleviation of aluminium toxicity by triggering relatively less amount of aluminium transportation form roots to leaves. Similar findings have also been reported by Jiang et al. (15) in *Citrus grandis*.

Table 1. Effect of boron on plant height, number of leaves and leaf area of mandarin orange seedlings under aluminium stress condition

Boron (B)	**Plant height (cm)/30 seedlings**			**Number of leaves/ 30 seedlings**		
	Aluminium (Al)		Mean	Aluminum (Al)		Mean
	Al_0	Al_I		Al_0	Al_I	
B_0	12.59 ^{f}	12.25 ^{f}		14.23 ^{cd}	13.90 ^{d}	14.06 ^{c}

		(-2.70)	12.42 ^{d}		(-2.31)	
B_1	16.27 ^{c} (29.22)	14.62 ^{e} (16.12)	15.44 ^{c} (-24.31)	16.70 ^{a} (17.35)	14.93 ^{bc} (4.91)	15.81 ^{b} (12.44)
B_2	19.03 ^{a} (51.15)	15.54 ^{d} (23.19)	17.29 ^{a} (-39.21)	17.40 ^{a} (22.27)	15.43 ^{b} (11.01)	16.41 ^{a} (16.71)
B_3	18.1 ^{b} (43.76)	14.89 ^{e} (18.26)	16.49 ^{b} (32.76)	16.90 ^{a} (18.76)	15.16 ^{b} (6.35)	16.03 ^{ab} (14.01)
Mean	16.50 ^{a}	14.33 ^{b} (-13.15)		16.30 ^{a}	14.85 ^{b} (-8.89)	
SOV	S.E(m)	CD at 5.0 %		S.E(m)	CD at 5.0 %	
B	0.123	0.373		0.176	0.532	
Al	0.087	0.264		0.124	0.376	
B×Al	0.174	0.528		0.249	0.752	

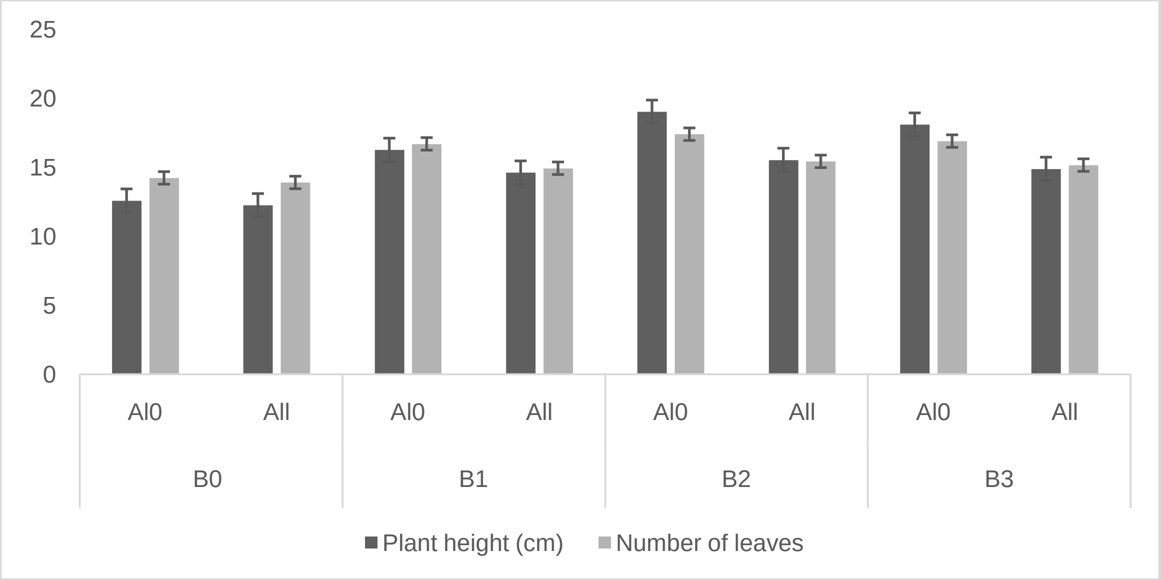

Fig 1: Effect of boron on plant height, number of leaves and leaf area of mandarin orange seedlings under aluminium stress condition

3.2. Shoot and Root fresh weight and Dry weight(g)

As shown in Table 2, the shoot and root weight of mandarin seedlings underwent a significant variation with the application of boron. The shoot and root fresh weight and shoot and root dry weight was recorded maximum with treatment B_2Al_0 by an increment of 66.92 %, 61.60 %, 42.85 %, 58.62 %. On the other hand, plants grown under aluminium stress condition as observed in treatment B_0Al_1 significantly decreased shoot fresh weight and root fresh weight (2.30 %, 2.38 %) and shoot dry weight and root dry weight (14.40 %, 10.34 %) respectively as compare with the control whereas, the plants grown with supplementation of boron under aluminium stress condition as observed in treatment B_2Al_1 showed significant improvement in biomass content with an increment of 48.06 %, 16.66 %, 20.80 %, 20.68 % . However, no significant variation was noticed between the treatments B_2Al_1 and B_3Al_1.

The seedlings fresh shoot, root weight and dry weight significantly increased with the boron application as shown in Table 2. Similar results were also recorded by Hussain et al. (12) in mustard leaves. The improvement in shoot and root weight can be attributed to the role of boron in stabilizing certain constituencies of cell wall and plasma membrane, enhancement of cell division, tissue differentiation and metabolism of nucleic acids, carbohydrates, proteins, auxins and phenols Marschner (18). Whereas, the toxic effect of aluminium on seedlings shoot and root dry weight was evident from the presented data as revealed in our study. Likewise, the similar findings are in conformity with the reports of Jiang et al. (14) who reported declination in the shoot and root dry weight in *citrus grandis* due to aluminium stress. The combined treatments containing both boron and aluminium treatment attributed significant increase in shoot and root weight. The results specify the role of boron which may be attributed to the alleviation of toxic effect of aluminium in seedlings as boron increases phenolic compounds which regulate polar auxin transport. The increased auxin activity triggers the oxidative effects of aluminium on seedlings, thereby results in increase of shoot and root dry weight of seedlings Gurjar et al. (10). The present findings are in accordance with the studies of Yalin et al. (34); Siddique et al. (28).

Table 2. **Effect of boron on shoot and root fresh weight and dry weight of mandarin orange seedlings under aluminium stress condition**

Boron (B)	Shoot (fresh weight g^{-1})			Shoot (dry weight g^{-1})			Root (fresh weight g^{-1})			Root (dry weight g^{-1})		
	Aluminium (Al)		Mean	Aluminium (Al)		Mean	Aluminium (Al)		Mean	Aluminium		Mean
	Al_0	Al_l		Al_0	Al_l		Al_0	Al_l		Al_0	Al_l	
B_0	1.30^{de}	1.27^{e} (-2.30)	1.29^{c}	0.42^{cd}	0.41^{d} (-2.38)	0.42^{c}	1.25^{e}	1.07^{f} (-14.40)	1.16^{c}	0.29^{cd}	0.26^{d} (-10.34)	0.27^{c}
B_1	2.06^{a} (58.46)	1.43^{cd} (10.00)	1.74^{b} (34.88)	0.51^{b} (21.42)	0.44^{cd} (4.76)	0.48^{b} (14.28)	1.81^{b} (44.80)	1.31^{de} (4.8)	1.56^{b} (34.48)	0.37^{b} (27.58)	0.32^{bc} (10.34)	0.34^{b} (25.92)
B_2	2.17^{a} (66.92)	1.64^{b} (26.15)	1.91^{a} (48.06)	0.60^{a} (42.85)	0.49^{bc} (16.66)	0.54^{a} (28.57)	2.02^{a} (61.6)	1.51^{c} (20.80)	1.76^{a} (51.72)	0.46^{a} (58.62)	0.35^{b} (20.68)	0.40^{a} (48.14)
B_3	2.11^{a} (62.30)	1.50^{c} (15.38)	1.80^{b} (39.53)	0.48^{b} (14.28)	0.50^{b} (19.04)	0.46^{b} (9.52)	1.90^{ab} (52.00)	1.43^{cd} (14.40)	1.66^{ab} (43.10)	0.42^{a} (44.82)	0.33^{bc} (13.79)	0.38^{ab} (40.74)
Mean	1.91^{a}	1.46^{b} (-23.56)		0.51^{a}	0.45^{b} (-11.76)	0.48	1.74^{a}	1.33^{b} (-23.56)	1.53	0.38^{a}	0.32^{b} (-15.78)	0.35
SOV	S.E(m)		CD at 5.0 %	S.E(m)	CD at 5.0 %		S.E(m)	CD at 5.0 %		S.E(m)	CD at 5.0 %	
B	0.033		0.098	0.02	0.047		0.04	0.113		0.013	0.038	
Al	0.023		0.069	0.01	0.033		0.03	0.080		0.009	0.027	
B×Al	0.046		0.139	0.02	0.067		0.05	0.160		0.018	0.053	

*Boron level: $B_{0=}$ 0µM, $B_{1=}$ 5µM, $B_{2=}$ 10µM and $B_{3=}$ 25µ; Aluminium level: $A_{0=}$ 0µM and $Al_{1=}$ 1200µM
*Different letters behind the values indicates a significant difference between the treatments (*P* <0.05).
*Values in parentheses represent the percentage increase or decrease with respect to their control. Negative values represent percentage decrease and positive values represent percentage increase from control.

Boron (B)	**Chlorophyll a (mg g^{-1})**			**Chlorophyll b (mg g^{-1})**			**Total chlorophyll (mg g^{-1})**			**Carotenoids (mg g^{-1})**		
	Aluminium (Al)		Mean	Aluminium (Al)		Mean	Aluminium (Al)		Mean	Aluminium		Mean
	Al_0	Al_I		Al_0	$Al_{I200\ \mu M}$		Al_0	Al_I		Al_0	Al_I	
B_0	1.05^{ef}	0.90^{f} (-14.28)	0.97^{c}	0.73^{b}	0.72^{b} (-1.38)	0.73^{b}	1.75^{d}	1.63^{d} (-6.85)	1.69^{c}	0.52^{cd}	0.44^{d} (-15.38)	0.48^{c}
B_1	1.48^{bc} (40.95)	1.15^{de} (9.52)	1.31^{b} (35.05)	0.86^{ab} (19.4)	0.75^{b} (4.16)	0.80^{b} (9.58)	2.31^{bc} (56.00)	2.07^{c} (18.28)	2.19^{b} (29.58)	0.58^{bc} (11.53)	0.65^{ab} (25.00)	0.60^{b} (27.08)
B_2	1.81^{a} (72.38)	1.26^{d} (20.00)	1.54^{a} (58.76)	1.08^{a} (50.00)	0.93^{ab} (29.16)	1.01^{a} (36.98)	2.80^{a} (60.00)	2.43^{b} (38.85)	2.61^{a} (54.43)	0.69^{ab} (32.69)	0.73^{a} (40.38)	0.71^{a} (47.91)
B_3	1.66^{ab} (58.09)	1.29^{cd} (22.85)	1.48^{a} (52.57)	0.87^{ab} (20.83)	0.81^{b} (9.00)	0.84 ab (15.06)	2.50^{ab} (42.85)	2.30^{bc} (31.42)	2.42^{a} (43.19)	0.67^{ab} (28.84)	0.70^{a} (34.61)	0.69^{ab} (43.75)
Mean	1.50^{a}	1.15^{b} (-23.33)		0.88^{a}	0.079^{b} (-9.09)		2.34^{a}	2.12^{b} (-9.40)		0.63^{a}	0.61^{b} (-3.16)	
SOV	S.E(m)		CD at 5 %	S.E(m)	CD at 5 %		S.E(m)	CD at 5 %		S.E(m)	CD at 5 %	
B	0.044		0.133	0.063	0.188		0.072	0.213		0.026	0.079	
Al	0.031		0.094	0.045	0.133		0.051	0.151		0.019	0.056	
B×Al	0.062		0.188	0.126	0.267		0.103	0.302		0.037	0.113	

Table 3. **Effect of boron on chlorophyll and carotenoid content of mandarin orange seedlings under aluminium stress condition**

*Boron level: $B_{0=}$ 0µM, $B_{1=}$ 5µM, $B_{2=}$ 10µM and $B_{3=}$ 25µ; Aluminium level: $Al_{0=}$ 0µM and $Al_{1=}$ 1200µM

*Different letters behind the values indicates a significant difference between the treatments (*P* <0.05).

*Values in parentheses represent the percentage increase or decrease with respect to their control. Negative values represent percentage decrease and positive values represent percentage increase from control.

3.3. Chlorophyll content and Carotenoid content (mg)

The content of chlorophyll and carotenoid content of seedlings were significantly affected by boron treatments as shown in Table 3. The chl a, chl b, total chlorophyll and carotenoid content was recorded maximum with a treatment B_2Al_0 with an increment of (72.38 %, 50.00 %, 60.00 % and 32.69 %) respectively which was also found to be statistically being at par with $B_3Al_{0.}$ Leaf pigments was significantly affected by higher aluminium condition (stress) B_0Al_1 leading to decline chl a, (14.28 %), chl b (1.38 %), total chlorophyll (6.85 %) and carotenoid content (15.38 %)$_.$ as compared to control. However, the boron application under aluminium stress as shown in treatment B_2Al_1 found to recover chl a, chl b, total chlorophyll, carotenoids (20.00 %, 29.16 %, 38.85 %, 40.38 %) respectively as compared to control. However, no significant variation was observed between treatment B_2Al_1 and $B_3Al_{1.}$

Our findings are in parallel with Peixoto et al. (21) who reported decrease in chlorophyll content in sorghum. The declination in chlorophyll content under aluminium stress might be also due to peroxidation processes in the chloroplast membrane lipids by reactive oxygen species as reported by Sandalio et al. (26); Chen et al. (5) as our results indicated. Application of boron under aluminium stress enhanced the chlorophyll and carotenoid concentration of seedlings in our study. The findings showed similarity with Wojcik (33) who reported that boron prevents the inhibition of root and shoot growth and the decrease in chlorophyll concentration. The reduction of carotenoid under boron deficient condition of seedlings may be an integral consequence of disturbed biosynthesis or maximum degradation of thylakoids Vassilev et al. (31). Carotenoids pigment act as scavengers of reactive oxygen species accumulation and in decrease level, it enhances the likelihood of reactive oxygen species accumulation which probably happened in seedlings under the presence of aluminium toxicity and boron deficient condition. The result of the present work suggested that the application of boron in the presence or absence of aluminium improved photosynthetic pigment content. Boron behaves as an antioxidant which helps to scavenge harmful reactive oxygen species, therefore helps in reducing oxidative damage to the chloroplast caused by aluminium stress and hence, improves the chlorophyll and carotenoid content in seedlings. Our results are in line with Jiang et al. (14) who suggested that boron improved photosynthesis, may be due to less aluminium uptake and mobilization in upper part of the plant.

4. CONCLUSION

Boron had a significant interactive influence on seedlings growth as well as leaf pigments under aluminium stress condition. Plants under aluminium stress resulted in decreased seedlings growth and development leading to decline in shoot length, leaf numbers, leaf area, shoot and root weight and leaf pigments. However, with the application of boron under aluminium stress condition improved plant morpho-physiological parameters. Likewise, the application of boron at a concentration of 10µM proved to be the effective treatment for Darjeeling mandarin seedlings grown under aluminium stress condition.

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