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Interspecific Difference in Boron tolerance at Seed Germination Stage in three *Hibiscus* species

Research Article

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Abstract

Influence of boron toxicity on seed germination, seedling growth and activities of two antioxidant enzymes in three *Hibiscus* species namely, *Hibiscus cannabinus* L., *Hibiscus sabdariffa* L. and *Hibiscus tiliaceus* L. was investigated. Germination percentage of all the three *Hibiscus* species was decreased due to higher 'B' concentration (200 ppm). Higher dose of boron (200 ppm) caused marked reduction in germination percentage in *H. tiliaceus* (86%) and *H. cannabinus* (20%) while, in case of *H. sabdariffa* (2%) decline in germination percentage was evident. Root length of all the three *Hibiscus* species was declined due to higher concentration of boron (200 ppm). Root length of the three *Hibiscus* species was affected under 'B' stress in the order *H. cannabinus* > *H. sabdariffa* > *H. tiliaceus*. Shoot length was also similarly reduced due to 200 ppm 'B' concentration but the order of decrease was *H. tiliaceus* > *H. sabdariffa* > *H. cannabinus*. Higher dose (200 ppm) of boron concentration reduced the total seedling length in all three *Hibiscus* species. The percent reduction in seedling length at 200 ppm 'B' concentration was in the order *H. tiliaceus* > *H. sabdariffa* > *H. cannabinus*. Considerable reduction in vigour index of all the three *Hibiscus* species was noticed in response to 200 ppm 'B' treatment in following order *H. tiliaceus* > *H. cannabinus* > *H. sabdariffa*. Decrease in fresh weight per seedling of *H. cannabinus* and *H. sabdariffa* was noticed under 'B' toxicity while, in *H. tiliaceus* there was slight increase in fresh weight at 100 ppm 'B' concentration and thereafter it was decreased. Catalase and peroxidase activities in seedlings of *H. sabdariffa* were greatly enhanced due to 150 ppm and 200 ppm 'B' treatment respectively. Lower dose (100 ppm) caused slight increase in catalase activity of both *H. cannabinus* and *H. tiliaceus* seedlings. The activity of enzyme peroxidase was reduced in seedlings of *H. cannabinus* and *H. tiliaceus* over entire range of 'B' treatment. Thus an interspecific difference in boron tolerance at germination stage was evident in the three *Hibiscus* species.

Keywords: Germination; *Hibiscus*; Boron toxicity

Introduction

Boron toxicity is an important disorder which can limit plant growth on soils of arid and semi arid environments throughout world [1]. High concentrations of B may occur naturally in the soil or in groundwater and such sites have been noticed in Australia, North and South America, Asia, South Africa, Mediterranean and East Europe [1,2]. Boron deficiency can be corrected with the help of certain anthropogenic factors, such as irrigation water containing excess boron, the use of fly ash as an ameliorant in agriculture, boron fertilizers and excessive boron accumulation in soil may caused due to surface mining [1,2]. The occurrence of boron toxicity in Australian soils is recognized as factors limiting cereal (wheat and barley) production [3]. Furthermore, soil salinity is reported to aggravate the negative impact of boron on different agricultural plants [4]. On this back ground screening of various plant species and varieties for boron tolerance is essential to select boron tolerant species. In the present investigation an attempt has been made to study boron tolerance at seed germination stage in three *Hibiscus* species namely *H. cannabinus, H. sabdariffa* and *H. tiliaceus.* Out of these species the *H. cannabinus* is a fibre crop. *H. sabdariffa* and *H. cannabinus* are also leafy vegetables. *H. tiliaceus* is a halophytic tree species with ornamental value.

Material and Methods

The seeds of local cultivar of *H. cannabinus* were collected from local farmers while, seeds *H. sabdariffa* cultivar AS-73-CP-560 were obtained from University of Agricultural Sciences, Dharwad (Karnataka). The seeds of *H. tiliaceus* were collected from estuarine areas in Ratnagiri.

The healthy viable seeds of the three species were sorted out and treated with 0.1% mercuric chloride for 5 min. for surface sterilization. The seeds were rinsed with distilled water for 4-5 times. Prior to the start of experiment, the seed coat of *H. tiliaceus* seeds was nicked opposite to embryo slightly with nail cutter [5]. The filter paper was moistened with 10 ml of boric acid solutions containing 100 ppm, 150 ppm and 200 ppm boron respectively and kept in petridish. The filter papers moistened with 10 ml of distilled water served as control. Experiments were carried out in the laboratory in the dark with an average temperature ranging from 28-30 ± 2 ˚C. Germination percentage was recorded after every 24 hours and seedling growth was studied after 120 hours. The seedlings were analyzed for Average shoot length, Average root length and Average fresh weight. Vigour index was calculated by using the formula proposed by Abdul-Baki and Anderson, [6]. VI = (Root length + Shoot length) × germination percentage. The activity of enzyme Catalase (EC 1.11.1.6) was assayed following the method of Luck [7] as described by Sadasivam and Manikam [8] and expressed as Δ OD min^{-1} mg^{-1} protein. Peroxidase (EC 1.11.1.7) activity was studied by the method of Horiguchi, [9]. The enzyme activity was expressed as Δ OD h^{-1} mg^{-1} protein. The soluble proteins in the enzyme were determined according to method of Lowry et al., [10]. All the values presented in the table are due the replicate experiments.

Results and Discussion

Effect of Boron toxicity on seed germination

Influence of boron treatment on germination percentage of the three *Hibiscus* species is depicted in Figure 1 and Table 1. It is clear from the Figure 1 that the germination percentage is decreased due to higher concentrations of boron in all the three species. About 80.00% (*H. cannabinus*), 97.82% (*H. sabdariffa*) and 13.33% (*H. tiliaceus*) of final germination of corresponding control is noticed under boron stress at 200 ppm level (Table 1). The glycophytic species *H. cannabinus* and *H. sabdariffa* showed relatively better tolerance to boron stress while, germination is markedly affected in halophytic species *H. tiliaceus*. Lower Boron levels (100 and 150 ppm) increased the germination percentage over control in *H. sabdariffa*.

Two wheat (Wyalkatchem and Mace) and four barley (Hamelin, Mundah, Gardiner and Buloke) varieties for their tolerance to different boron (0, 5, 10, 25, 50, 100 and 150 mg B kg^{-1}) concentrations and germination percentage was measured [3]. During early stages of germination, boron treatment showed significant differences between cultivars and concentrations. Boron concentration above 10 mg B kg^{-1} significantly reduced germination percentage during early stages of germination in wheat and barley cultivars. However, wheat cultivars

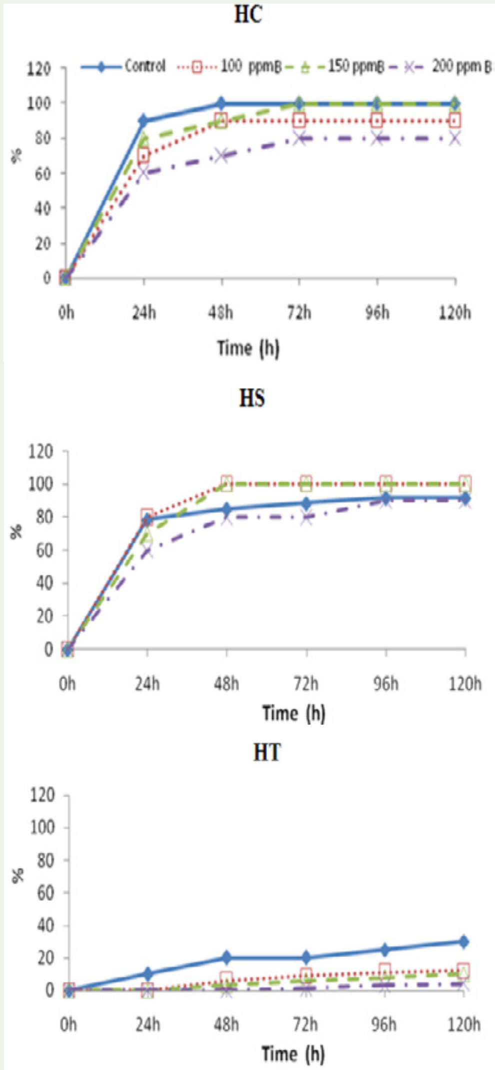

Figure 1: Influence of Boron toxicity on seed germination percentage in three *Hibiscus* species (HC=*Hibiscus cannabinus* L., HS=*Hibiscus sabdariffa* L. and HT=*Hibiscus tiliaceus* L.).

are more tolerant than the barley cultivars at germination stage. Higher (150 mg B kg^{-1}) boron concentration significantly reduced germination percentage in all the cultivars of wheat and barley on the first day performance of germination. He noticed inconsistent response of further germination in boron solution. He also stated that poorer germination may occur in cultivars sensitive to high boron treatments. In case of *H. cannabinus* and *H. sabdariffa* although the germination is delayed due to boron stress in initial phase the germination shows recovery latter and final germination percentage is only slightly lowered. But in case of *H. tiliaceus* inhibition of germination continues till the latter stages and it reflects sensitive nature of this species to boron stress.

Effect of Boron toxicity on seedling growth

The root length is decreased due to higher concentrations of

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Table 1: Influence of Boron toxicity on germination and seedling growth in three *Hibiscus* species (*Hibiscus cannabinus* L. *Hibiscus sabdariffa* L. and *Hibiscus tiliaceus* L.).

Hibiscus species	Treatments Boron (ppm)	Germination percentage (%) after 120 h	Root length (cm)	Shoot length (cm)	Total length of seedling (cm)	Vigour Index	Fresh weight (mg seedling^{-1})
HC	Control	100	6.50±1.14	9.78±1.49	16.28±4.07	1600.32	528
	100	90	4.04±0.98	9.47±1.07	13.51±3.79	1080.80	355
	150	90	1.43±0.5	4.06±0.59	5.49±1.76	494.10	225
	200	80	1.48±0.42	3.00±0.72	4.48±0.93	313.6	148
HS	Control	92	2.55±0.82	7.20±2.28	9.75±3.82	897.00	560
	100	100	2.99±0.62	7.82±1.34	10.81±1.43	1081.00	355
	150	100	0.74±0.84	2.36±0.85	3.1±1.42	279.00	225
	200	90	0.76±0.27	1.86±0.56	2.62±0.96	235.00	148
HT	Control	30	0.74±0.05	2.70±0.90	3.44±0.90	103.2	25
	100	12	0.79±0.28	2.74±1.26	3.79±1.10	45.48	35
	150	10	0.41±0.07	3.00±0.36	1.13±0.40	11.30	20
	200	4	0.31±0.00	0.36±0.07	0.67±0.07	2.64	18

HC=*Hibiscus cannabinus* L., HS=*Hibiscus sabdariffa* L. and HT=*Hibiscus tiliaceus* L. ± SD.

boron in all the three *Hibiscus* species. About 22.77% (*H. cannabinus*), 29.80% (*H. sabdariffa*) and 41.89% (*H. tiliaceus*) reduction of root length is noticed over control under boron stress at 200 ppm level. The halophytic species *H. tiliaceus* showed higher tolerance to boron stress while, glycophytic species *H. cannabinus* and *H. sabdariffa* showed lower tolerance, with respect to seedling root growth. But at the same time slower growth of root in this species cannot be overlooked. Brdar-Jokanovic et al., [11] reported intercultivar differences regarding reduction in primary root length of wheat seedlings under 'B' stress. Such reduction was more prominent in cultivar Renan while, it was less in cultivar Trakija. Cultivar Stephens showed maximum primary root length among studied cultivars. According to Nable [12], tolerant wheat and barley cultivars maintain lower concentrations of boron in roots than sensitive genotypes. Liu et al., [13] observed that boron toxicity reduced the root cell division. This may be cause of reduction in root length due to 'B' toxicity. The shoot length is decreased due to 200 ppm concentrations of boron in all the three *Hibiscus* species. But at lower concentrations (100 ppm) in case of *H. sabdariffa* and (100 and 150 ppm) in case of *H. tiliaceus* there is increase in shoot length over control. In *H. cannabinus* there is continuous decrease in seedling shoot length along with increase in boron concentration. About 30.60 % (*H. cannabinus*), 25.83 % (*H. sabdariffa*) and 13.33 % (*H. tiliaceus*) control of shoot length is noticed under boron stress at 200 ppm level. The glycophytic species *H. cannabinus* showed higher tolerance to boron stress while, halophytic species *H. tiliaceus* showed lower tolerance, with respect to seedling shoot length at this boron concentration. According to Nable [12], tolerant wheat and barley cultivars maintain lower concentrations of boron in shoots than sensitive genotypes. Boron toxicity symptoms have been quoted to start occurring at 14 mg B kg^{-1}, for barley [14]. The shoot lengths of seedlings of the two chickpea cultivars (Go¨kce and Ku¨smen) were affected differently due to B treatment. Increasing concentrations of B (1.6 and 6.4 mM) significantly increased shoot length in cultivar Go¨kce, but decreased shoot length of cultivar Ku¨smen [15]. These workers found higher 'B' accumulation in cultivar Ku¨smen than in cultivar Go¨kce and it has been demonstrated that 'B' toxicity is correlated with intracellular 'B' concentration [16]. Boron at 10 mg B kg^{-1} caused greatest mean shoot length, suggesting need of boron during early growth [3]. Such stimulation of shoot growth is also seen in *H. sabdariffa* and *H. tiliaceus* at low 'B' concentration. The tolerance mechanisms amongst plants are very variable, however the tolerance mechanism to reduce uptake of boron in shoot seems to be common as that of roots. According to Farr [3], shoots show significant reduction in size and growth under boron toxic conditions. At 200 ppm 'B' levels similar picture is seen in all the three *Hibiscus* species. Boron treatment might be influencing root and shoot growth in seedlings of these three species in a different manner. In case of *H. cannabinus* there is greater decrease in root length as compared to the shoot length. In case of *H. sabdariffa* there is greater decrease in shoot length due to high 'B' concentration. In *H. tiliaceus* there is no consistent effect of 'B' on root to shoot growth.

The seedling length is decreased due to higher concentrations of boron in all the three species. It is further evident from Table 1 that, the seedling length in all the three species decreased with increasing boron concentration. Except at lower concentrations (100 ppm) *H. sabdariffa* and *H. tiliaceus* exhibited higher seedling length. About 27.52% (*H. cannabinus*), 26.87% (*H. sabdariffa*) and 14.83% (*H. tiliaceus*) of seedling length of control seedlings is noticed under boron stress at 200 ppm level. The glycophytic species *H. cannabinus* and *H. sabdariffa* showed approximately equal tolerance to boron stress while, halophytic species *H. tiliaceus* showed less tolerance with respect to seedling length. The vigour index is decreased continuously along with increase in concentrations of boron in *H. cannabinus* and *H. tiliaceus*. In case of *H. sabdariffa* vigour index is slightly increased at 100 ppm 'B' but thereafter there is marked decline. It is further evident from Table 1 that, the vigour index in all the three species is considerably decreased at highest (200 ppm) boron concentration except at lower concentrations (100 ppm) *H. sabdariffa* showed

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higher value over control. About 19.59% (*H. cannabinus*), 26.19% (*H. sabdariffa*) and 2.55% (*H. tiliaceus*) of vigour index is noticed over control under boron stress at 200 ppm level. The glycophytic species *H. sabdariffa* showed higher tolerance to boron stress while, halophytic species *H. tiliaceus* showed lower tolerance, with respect to vigour index. Although there are no reports are available about influence of boron toxicity on vigour index of seedlings, the observations of Paull [14] indicated that wheat plants subjected to boron toxicity showed low vigour. Influence of boron toxicity on fresh weight of three *Hibiscus* species is depicted in Table 1. It is evident from the Table 1 that the fresh weight per seedling is decreased due to higher concentrations of boron in *H. cannabinus* and *H. sabdariffa*. In *H. tiliaceus* the fresh weight of seedlings treated with 100 ppm B is slightly higher but there after there is decline in fresh weight. The decrease in fresh weight (in comparison to control) at highest concentration (200 ppm) is more significant in *H. cannabinus* and *H. sabdariffa* as compared to *H. tiliaceus*. At seedling stage uptake of water mainly contributes to fresh weight besides increase in cell number and cell size. It is obvious that boron toxicity affects all these three parameters in the three *Hibiscus* species. About 28.03% (*H. cannabinus*), 26.43% (*H. sabdariffa*) and 72.00% (*H. tiliaceus*) of control fresh weight is noticed under boron stress at 200 ppm level.

Effect of Boron toxicity on antioxidative enzymes

Effect of boron (B) toxicity on activity of enzyme catalase (CAT) and peroxidase (POX) in seedlings (120 h) of *H. cannabinus, H. sabdariffa* and *H. tiliaceus* is shown in Table 2. It is clear from Table 2, that the maximum enhancement of CAT and POX activity in case of *H. sabdariffa* seedlings is noticed under high boron concentrations 150 ppm and 200 ppm respectively. A slight stimulation of CAT activity is also visible in case of *H. cannabinus* and *H. tiliaceus,* due to treatment of 100 ppm concentration of boron. The activity of enzyme POX is declined in seedlings of *H. cannabinus* and *H. tiliaceus* due to entire range of boron treatment. Boron toxicity is reported to lead to synthesis and accumulation of ROS in case of *Citrus* leaves [17], barley leaves [18] and apple rootstocks [19]. Antioxidant enzyme catalase plays important role in detoxification of H_2O_2 generated during dismutation of superoxide radical by SOD along with oxygen [18]. Dube et al., [20] reported that excess boron in sunflower leaves caused increase in catalase activity. There are several reports which indicate that the boron toxicity brings about increase in catalase activity in tobacco [21], barley [22] and in grapevine [23]. Similarly, apple rootstocks [24] and tomato [25] exhibited increased activity of catalase in response to B toxicity. In contrast to above reports, there are few reports of reduction in CAT activity in response to boron treatment in plants. Roots have lower catalase activity than leaves in wheat and barley under boron stress [3]. Keles et al., [17] also reported decreased activity of CAT in citrus leaves due to B toxicity.

In the leaf tissue of C_3 species such as tobacco, sunflower, barley, tomato and chickpea due to photorespiratory process there is accumulation of H_2O_2 and catalase localized in peroxisomes plays important role in breakdown of H_2O_2. Hence, increase in catalase activity in the leaf tissue may takes place to regulate H_2O_2 level elevated as a result of increased photorespiration in response to boron treatment. An increase in SOD activity in roots and shoots in response to boron stress can also result in elevation of H_2O_2 level [15,26]. In seedling tissue probably due to disturbances in respiratory electron transport ROS production can take place under boron toxicity and the increase in catalase activity may help in scavenging the H_2O_2 level. Among the three *Hibiscus* species, seedlings of *H. sabdariffa* possess such adaptive feature as the activity of catalase is markedly increased due to boron treatment. In the other two species however, such stimulation in catalase activity is noticed only in response to lower dose (100 ppm) of boron.

Peroxidase is an oxidative enzyme which plays multiple roles in the cells. The enzyme play important role in secondary metabolism and lignin biosynthesis and possible involvement of this enzyme in auxin metabolism has been also discussed. Peroxidase plays important role in detoxification of H_2O_2 generated during dismutation of superoxide radical by SOD along with oxygen [18]. This is due to the fact that there are several isozymes of peroxidase which are localized in different regions and organelles in the cell. In view of Castillo, [27] an increase in POX activity may be an early response to several stress like B and may provide cells with tolerance to H_2O_2 formed when plants are exposed to stress. Palavan-Unsal et al., [28] reported promotion of activity of enzyme POX under 'B' stress and also detected presence of POX in lignified tissues.

Table 2: Effect of boron toxicity on the activities of enzymes catalase and peroxidase in seedlings of *Hibiscus cannabinus* L., *Hibiscus sabdariffa* L. and *Hibiscus tiliaceus* L.

Treatments	Catalase Δ OD min^{-1} mg^{-1} protein (% Control)			Peroxidase Δ OD h^{-1} mg^{-1} protein (% Control)		
	HC	HS	HT	HC	HS	HT
Control	100	100	100	100	100	100
100 ppm B	106.45	145.87	110.70	69.72	111.48	91.11
150 ppm B	87.50	155.09	80.70	79.40	118.58	71.66
200 ppm B	43.43	120.76	60.20	70.44	126.08	51.45

Control values:

Enzyme Catalase	**Enzyme Peroxidase**
HC=6.39 Δ OD min^{-1} mg^{-1} protein,	HC=21.07 Δ OD h^{-1} mg^{-1} protein,
HS= 0.62 Δ OD min^{-1} mg^{-1} protein and	HS= 7.34 Δ OD h^{-1} mg^{-1} protein and
HT= 0.39 Δ OD min^{-1} mg^{-1} protein.	HT= 0.39 Δ OD h^{-1} mg^{-1} protein

HC=*Hibiscus cannabinus* L., HS=*Hibiscus sabdariffa* L. and HT=*Hibiscus tiliaceus* L.

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POX activity was found to be increased in shoots of two chickpea cultivars due to 6.4 mM B treatment [15]. Above reports on the behavior of enzyme peroxidase are from full grown plants and germinating seeds are seldom investigated. Bhamburdekar [29] noticed a decline in peroxidase activity in pigeonpea seedlings due to boron toxicity. Such decline in enzyme activity is evident in seedlings of two *Hibiscus* species *H. cannabinus* and *H. tiliaceus* in response to boron toxicity but in case of *H. sabdariffa* opposite trend is noticed. This free radical scavenging enzyme system appears to be more efficient in *H. sabdariffa* than *H. cannabinus* and *H. tiliaceus* under the condition of boron excess.

Conclusion

From foregoing account of result it is concluded that *H. sabdariffa* is more tolerant to boron stress than *H. tiliaceus* and *H. cannabinus* with respect germination percentage, seedling growth, vigour index and activities of antioxidative enzymes. An interspecific difference in boron tolerance at germination stage was evident in the three *Hibiscus* species.

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Conflict of Interest

Authors declare that they have no conflict of interest.

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