Journal of Nature Studies 18(1): 11-21
Online ISSN: 2244-5226

EARLY VEGETATIVE GROWTH RESPONSES OF *Vigna radiata* L. (MUNGBEAN) cv NSIC Mg 17 TO BORON TOXICITY

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ABSTRACT – After the Philippines experienced a severe El Niño last 2015 to 2016, there is likelihood that this will recur given the continuous threat posed by climate change. This natural phenomenon could lead to extreme drought causing a dependence on groundwater as an alternative source of irrigation which is a contributory factor to boron toxicity in plants. With the likelihood that this could adversely affect the growth and yield of a local agricultural crop, this study was conducted. This aimed to investigate the vegetative growth responses to boron toxicity of *Vigna radiata* L. cv NSIC Mg 17, an economically important Philippine legume. Hydroponics culture method using Simple Nutrient Addition Program (SNAP) solution with increasing boron concentrations was utilized in the experiment. Results showed that concentrations as high as 25 mg L^{-1} significantly influenced plant survival percentage, root length and number of chlorotic and necrotic leaves. These findings indicated that an increase in boron concentration in the environment could adversely affect the vegetative growth of mungbean. Similar investigations on the effects of boron toxicity using other local crops are needed for additional information and as basis in making policies concerning the use of groundwater as irrigation water for crops.

Keywords: boron toxicity, growth response, hydroponics, Vigna radiata

INTRODUCTION

In 2015 to 2016, the Philippines experienced the strongest El Niño causing the worst dry spells recorded to date (Macas, 2015; The Manila Times, 2015). This similar phenomenon may again hit the country after several international monitoring and prediction models indicate warming in the central Pacific Ocean (Flores, 2018; Nelz, 2018). This could lead to extreme drought and may entail the use of an alternative source of irrigation water such as groundwater (Chatzissavvidis et al., 2004; Dobermann & Fairhurst, 2000). Irrigation water is a contributory factor to boron toxicity in addition to overuse and misuse of fertilizers, animal manures, fly ash, surface mining, and release to the environment of industrial boric acid and borate minerals (Pech et al., 2013; Kord et al., 2010; Dobermann & Fairhurst, 2000; Nable et al., 1997). This is actually not new to the Philippines after it has been recorded back in the 1980's in rice farms of the International Rice Research Institute, Los Baños, Laguna (Cayton, 1985), and

To cite this paper: Balinado, L.O., Manzano, R.M.E., Ate, C.N., Fernandez, M. & Chan, M.A. 2019. Early Vegetative Growth Responses of *Vigna radiata* L. (Mungbean) cv NSIC Mg 17 to Boron Toxicity. *Journal of Nature Studies*. 18(1), 11-21.

in the province of Albay where soils are formed on volcanic plant material (Dobermann & Fairhurst, 2000).

Locally, the maximum permissible concentration of boron in water used for irrigation purposes as stated in Department of Environment and Natural Resources (DENR) Administrative Order No. 34, series of 1990 (DENR, 1990) and Department of Agriculture (DA) Administrative Order No. 26, series of 2007 (DA, 2007), is 0.75 mg L^{-1}. Excessive boron can result to damages and visible toxicity symptoms in plants which in turn can have subsequent negative effects on country's agricultural crop yields (Herrera-Rodriguez et al., 2010; Ahmed et al., 2008). This could inhibit starch synthesis from sugars and negatively affect the formation of β-carbohydrate complexes (Dugger et al., 1957). In rice, high boron concentration could result to chlorotic tips and edges of older leaves during early exposure until it turns brown and becomes necrotic (Dobermann & Fairhurst, 2000). Similarly, boron toxicity may affect other local crops including *Vigna radiata* L. (mungbean) that is being used in crop rotation with rice (Lagasca, 2008).

V. radiata, locally known as "*mongo*" or "*balatong*", is one of the cheapest protein sources in the Filipino diet. It can easily be cultivated and it actually contributed 790 million pesos (or $39.2M) to the Philippine economy in 2006 (PCARRD-DOST, 2003). Growing this crop after rice also offers farmers a good opportunity to earn additional income since it only requires minimum inputs for production (Lagasca, 2008), while offering a variety of applications including *sotanghon* and *hopia* manufacturing, cereal flour and noodle production, bread and snack making, and the like (Sicat & Buño, 2014; Bureau of Agricultural Research, 2012).

With the continuous environmental threat posed by climate change and the more frequent El Niño phenomenon it entails, and with the assumption of the possible negative effects of excessive boron to this economically important Philippine legume, the researchers investigated the growth influence to mungbean of high boron concentrations at an early vegetative stage. Studying this crop during this period was found important as the effects of boron to early growth characteristics would determine whether or not the plant would shift to seed-producing reproductive stage. This study involved the use of a locally developed mungbean cultivar, 'NSIC Mg 17' or 'Mabunga 4'. Being recommended as a national variety for both wet and dry seasons (NSIC, 2015) and with no existing studies involving this cultivar, investigation of its response to excessive boron could significantly provide a scientific basis for the establishment of governing policies on the proper use of irrigation water and nutrient supplementation to prevent unwanted crop loss.

METHODOLOGY

Acquisition of Mungbean Seeds

The local mungbean cultivar used in the study was 'NSIC Mg 17' (Figure 1) bought from the Bureau of Plant Industry–Los Baños National Crop Research and Development Center (BPI – LBNCRDC), Los Baños, Laguna. It is an approved local mungbean cultivar by the National Seed Industry Council (NSIC) for commercial release in the Philippines with local name "Mabunga 4". This variety is known to produce glossy green seeds with 24.12% crude protein content and gives a 1.28-1.54-ton/ha yield (Sicat & Buño, 2014).

Figure 1. Pods and seeds of mungbean cv NSIC Mg 17 or Mabunga 4. (Source: NSIC Seed Catalogue, 2011)

Seed Sowing

The mungbean seeds were allowed to germinate in a tray of sandy loam soil. Mungbean plants which exhibit root formation at seven days after sowing (7 DAS) were then transferred to hydroponics culture setup.

Hydroponics Culture Method

Mungbean seedlings at 7 DAS were uprooted and transferred to a non-circulating hydroponics culture setup and were grown in regularly replenished Simple Nutrient Addition Program (SNAP) hydroponics solution that is developed by and bought from the Institute of Plant Breeding, College of Agriculture, University of the Philippines-Los Baños, Laguna, Philippines. It was prepared in distilled water with calculated amounts of H_3BO_3 (Sigma-Aldrich ®) to obtain the following boron concentrations in solution: 5.0, 10.0, 15.0, 20.0, and 25.0 mg L^{-1} (Hasnain et al., 2011). These were higher than the 0.75 mg L^{-1} allowable boron concentration in water for plants (DA, 2007; DENR, 1990) and at the same time, fell within with the range of boron concentration in groundwater that could be as high as 300 mg L^{-1} (ATSDR, 2010). SNAP solution with 10 mg L^{-1} iron (*i.e.*, prepared as Fe-EDTA) concentration, a micronutrient known to cause visible toxicity symptoms to plants, was used as positive control; while a non-modified SNAP solution was utilized as normal control.

Experimental Design

A total of 30 mungbean plants selected in a completely randomized manner were used per treatment and this was performed in triplicate. Each replicate used one solidly-colored, black, plastic pail (to exclude light, thus reducing algal growth in the solution) where each of the 30 mungbean plants, supported by newspaper plugs in the lid, was equidistantly transferred (Figure 2a and Figure 3). Two additional openings were also made in the lid and were allotted for passive aeration to support nutrient uptake of the roots and for passage of SNAP hydroponics solution during replenishment (Figure 2b and Figure 3). This experimental setup stood for 14 days (21 DAS) (temperature: 25ºC ± 5, light: 12 hours natural daylength) to determine the effect of increasing boron concentration to mungbean.

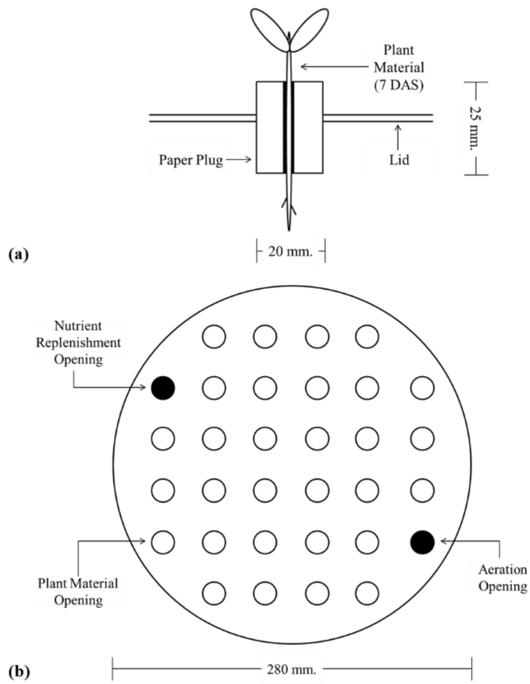

Figure 2. An illustration of the experimental setup showing (a) transferred mungbean material (7 DAS) supported by a paper plug in the lid; and (b) the topview of the container where openings for mungbean material, aeration and nutrient replenishment were placed.

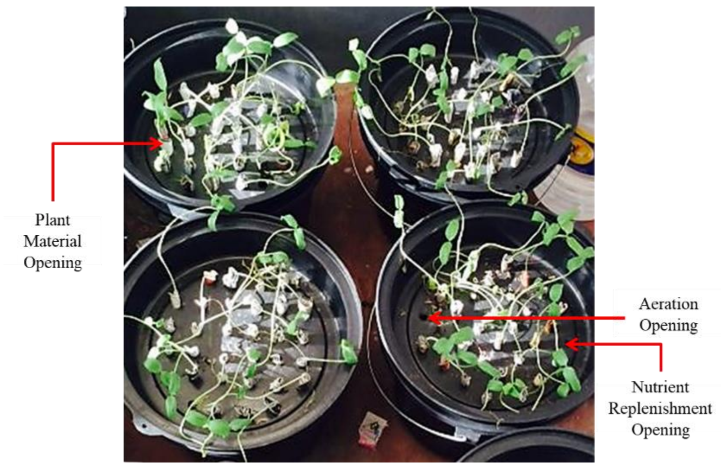

Figure 3. The actual experimental setup.

Growth Response Assessment to Boron Toxicity

The effect of excessive boron to the early vegetative growth of mungbean was determined using different growth parameters including plant survival percentage, root elongation, shoot elongation, percentage of plants with chlorotic and necrotic leaves, and dry weight.

Plant survival percentage was determined by counting the number of survived plants per 30 plants in each replicate. For root and shoot elongation, only replicates with at least 10 survived plants were subjected to measurement, in which each of the randomly selected 10 survived mungbean plants was measured (in mm) by subtracting the length of primary root (or shoot) at 21 DAS with its corresponding recorded root (or shoot) length at 7 DAS. Moreover, percentage of plants with chlorosis was measured by counting the number of plants with chlorotic leaves (*i.e.,* yellowing of leaf tissue) per total number of plants. The same procedure was done in determining percentage of plants with necrotic leaves (*i.e.*, browning or death of leaf tissue).

After measuring the aforementioned growth parameters, the 10 previously selected survived plants of 30 mungbean plants per replicate were used to determine the dry weight (in mg). This was done by drying the plant materials in an oven at 70°C for at least 48 hours and until a constant weight is obtained (Hasnain et al., 2011).

Statistical Analysis

The gathered data from the growth responses of mungbean to excessive boron were sorted, tallied and tabulated, and were then subjected to one-way analysis of variance (ANOVA) available in SPSS 16.0. The level of significance was interpreted as follows: p<0.05 was significant and p>0.05 was not significant. The ANOVA was followed by specific comparison of mean differences using Duncan's Multiple Range Test (DMRT) (Hasnain et al., 2011) available in the same statistical software.

RESULTS AND DISCUSSION

Mungbean is a boron-sensitive plant which can tolerate 0.75-1.00 mg L^{-1} concentration of the microelement for optimal growth and development (Ayers & Westcot, 1994). To determine how elevated amounts of boron could affect this plant, this investigation was performed. Mungbean plants at 7 DAS were transferred to hydroponics setup containing elevated concentrations of boron: 5, 10, 15, 20 and 25 mg L^{-1}. Growth responses were then recorded at 21 DAS based on plant survival percentage, root elongation, shoot elongation, percentage of plants with chlorotic and necrotic leaves, and dry weight.

Plant survival

A significantly decreasing percentage of survived mungbean plants (p<0.0001) was observed in response to increasing boron concentrations (Table 1). According to Nable et al. (1997), boron toxicity causes a decrease in survival of sensitive crops. As observed in sunflower, excessive boron leads to reduction in seed germination, root and shoot length and vigor index (Prathima, 2015) and ultimately leads to death, suggesting the use of optimal concentration between 0.5-1.0 mg L^{-1} (Eaton, 1940). Tomato (*Lycopersicum esculentum*), in addition, died at an elevated concentration of the micronutrient (*i.e.,* 165-195 mg/kg) in wastewater-contaminated soil (Onthong et al., 2011).

Table 1. Growth responses of *V. radiata* L. cv NSIC Mg 17 to increasing boron concentrations based on plant survival percentage.

BORON CONCENTRATION (mg L^{-1})	PLANT SURVIVAL PERCENTAGE (%)
Normal control	100.00 a
5	91.00 a
10	71.00 b
15	52.00 c
20	13.00 de
25	1.00 e
Positive control	31.00 d
$F_{\text{boron concentrations}}$	41.37*

Means followed by the same letter do not differ significantly from one another at 0.05 level of significance (DMRT);
* indicates the interpretation of *p* value as significant at 0.05 level of significance

Chlorosis and necrosis

In addition, a significantly increasing number of plants with chlorotic ($p<0.05$) and necrotic ($p<0.0001$) leaves was recorded (Table 2), as an early and a more severe symptom of boron toxicity, respectively. It was observed that increasing the boron concentration from 0 to 15 mg L^{-1} had caused an increase in the percentage of plants with chlorosis, while higher levels (*i.e.*, 20 and 25 mg L^{-1}) had resulted to a subsequent decline. This could then be attributed to an increase in number of plants with necrosis under these concentrations.

Table 2. Growth responses of *V. radiata* L. cv NSIC Mg 17 to increasing boron concentrations based on percentage of plants with chlorotic and necrotic leaves.

BORON CONCENTRATION (mg L^{-1})	PLANTS WITH CHLOROTIC LEAVES (%)	NECROTIC PLANTS (%)
Normal control	0.00 a	0.00 a
5	9.00 ab	9.00 a
10	11.00 ab	29.00 b
15	16.00 b	48.00 b
20	13.00 b	87.00 c
25	1.00 a	99.00 c
Positive control	31.00 c	69.00 b
$F_{\text{boron concentrations}}$	5.561*	41.37*

Means followed by the same letter do not differ significantly from one another at 0.05 level of significance (DMRT);
* indicates the interpretation of *p* value as significant at 0.05 level of significance

As shown in Figure 4, chlorosis started to become evident at the leaf tip and it progressed to leaf margins and even in the entire leaf blade as boron concentrations increased. Similar findings were also reported by Farag & Fang (2014) and Hasnain et al. (2011). This observation could be explained by the accumulation of boron at the end of the transpiration stream, *i.e.* leaf tips and margins, where osmotic imbalances could occur (Ozturk et al., 2010; Reid et al., 2004). Hasnain et al. (2011) even reported that necrosis progresses from the leaf tips and margins of the plants to the midrib of the leaves and at the base of the leaflets of the plants.

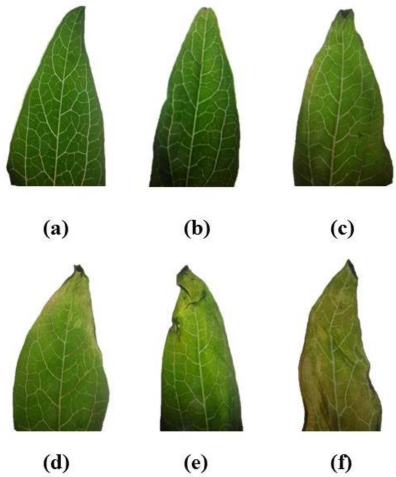

Figure 4. Leaves of *V. radiata* L. 'NSIC Mg 17' at 21 DAS after exposure to (a) normal control, (b) 5 mg L^{-1}, (c) 10 mg L^{-1}, (d) 15 mg L^{-1}, (e) 20 mg L^{-1}, and (f) 25 mg L^{-1} boron, as viewed under a dissecting microscope.

Root and shoot elongation

Results also showed that there was significant variability of mean difference recorded for root elongation ($p<0.05$) while, insignificant for shoot elongation (Table 3). This could be attributed to the relatively low concentration of boron in the roots and shoots under toxicity conditions in comparison to leaves (Nable et al., 1997). Negative result for root elongation indicates a decrease in root length due to death of root tissue caused by toxicity to boron. This result was in agreement with other studies which showed an inhibitory effect of boron on root growth of broadbean, *Vicia faba* L. (Liu et al., 2000), corn, *Zea mays* L. (Esim et al., 2013), wheat, *Triticum aestivum* L. (Ashagre et al., 2014 [a]), safflower, *Carthamus tinctorius* L. (Ashagre et al., 2014 [b]) and chickpea, *Cicer arietinum* L. (Ardic et al., 2009); while insignificant difference in shoot elongation was in contrary with other studies which reported a decline on shoot elongation (Ashagre et al. (2014 [a]; Nable et al. (1997) that could be due to the negative effects of excessive boron on cell division and elongation (Brown et al., 2002) or photosynthetic rate (Chatzissavvidis et al., 2008).

Dry weight

Further, insignificant differences on dry weights were observed (Table 4). Increasing the length of exposure to boron treatments could be essential to provide more accurate and reliable inference on the effects of excessive boron content. In the study conducted by Hasnain et al. (2011), a lesser root and shoot biomass of Z*ea mays* was obtained in comparison to the control as the concentration increased to 6.60 ppm (Ogunwole et al., 2015). The same gradual reduction in dry matter yield of roots and shoots was also reported in some wheat cultivars (Metwally et al., 2012).

Table 3. Growth responses of *V. radiata* L. cv NSIC Mg 17 to increasing boron concentrations based on root and shoot elongation.

BORON CONCENTRATION (mg L^{-1})	ROOT ELONGATION (mm)	SHOOT ELONGATION (mm)
Normal control	3.17 a	136.53 a
5	-6.50 a	131.50 a
10	-58.65 ab	139.40 a
15	-88.67 b	75.20 b
20	-	-
25	-	-
Positive control	-	-
$F_{\text{boron concentrations}}$	6.016*	4.197^{ns}

Means followed by the same letter do not differ significantly from one another at 0.05 level of significance (DMRT);
* indicates the interpretation of *p* value as significant at 0.05 level of significance
^{ns} indicates the interpretation of *p* value as not significant at 0.05 level of significance

Table 4. Growth responses of *V. radiata* L. cv NSIC Mg 17 to increasing boron concentrations based on dry weight.

BORON CONCENTRATION (mg L^{-1})	DRY WEIGHT (mg)
Normal control	476.67 a
5	463.33 a
10	445.00 a
15	456.67 a
20	-
25	-
Positive control	-
$F_{\text{boron concentrations}}$	0.562^{ns}

Means followed by the same letter do not differ significantly from one another at 0.05 level of significance (DMRT);
^{ns} indicates the interpretation of *p* value as not significant at 0.05 level of significance

CONCLUSION

This study was able to determine the responses of *V. radiata* cv NSIC Mg 17 to increasing boron concentrations based on different growth parameters including plant survival percentage, root and shoot elongation, percentage of plants with chlorotic and necrotic leaves, and dry weight. Exposure of mungbean to boron up to 25 mg L^{-1}, that is way beyond the 0.75-1.00 mg L^{-1} tolerable concentration, can result to a marked decrease in plant survival percentage and to visible toxicity symptoms including a decrease in root length and an increase in the number of chlorotic and necrotic leaves. These findings suggest that elevated boron concentration is detrimental to the growth and development of *V. radiata* 'NSIC Mg 17' cultivar. This also implies the adverse effects of application of boron-rich groundwater, especially during severe drought, to irrigate mungbean plantation; hence, selection of an irrigation water with no potential of developing toxicity, or if not available, adopting other management options to reduce toxicity and improve production are, therefore, necessary.

STATEMENT OF AUTHORSHIP

The first four authors conducted the literature search, prepared the conceptual framework, performed the study, and formulated recommendations. The first author undertook the writing up and finalization of the manuscript. The last author identified some issues, formulated recommendations, and reviewed the paper.

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JOURNAL OF NATURE STUDIES
(formerly Nature's Bulletin)
Online ISSN: 2244-5226