Variation in Boron Availability Alters Root Architecture Attributes at the Onset of Storage Root Formation in Three Sweetpotato Cultivars

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Abstract. The primary objective of this work was to generate species-specific information about root architectural adaptation to variation in boron (B) availability at the onset of storage root formation among three sweetpotato [*Ipomoea batatas* (L.) Lam] cultivars (Beauregard = BX; Murasaki = MU; Okinawa = OK). Three B levels were used: 0B (B was omitted in the nutrient solution, substrate B = 0.1 mg·kg⁻¹), 1XB (sufficient B; 0.5 mg·kg⁻¹), and 2XB (high B; 1 mg·kg⁻¹). The check cultivar BX showed evidence of storage root formation at 15 days in 0B and 1XB, whereas cultivars MU and OK failed to show evidence of root swelling. The 1XB and 2XB levels were associated with 736% and 2269% increase in leaf tissue B in BX, respectively, relative to plants grown in 0B. Similar magnitudes of increase were observed in MU and OK cultivars. There were no differences in adventitious root (AR) count within cultivars but OK showed 25% fewer AR numbers relative to BX across all B levels. 0B was associated with 20% and 48% reduction in main root length in BX and OK, respectively, relative to plants grown in 1XB and 2XB. 2XB was associated with a 10% increase in main root length in MU relative to plants grown in 0B and 1XB. 0B was associated with reduced lateral root length in all cultivars but the magnitude of responses varied with cultivars. These data corroborate findings in model systems and well-studied crop species that B deficiency is associated with reduced root growth. These data can be used to further understand the role of cultivar-specific responses to variation in B availability in sweetpotato.

The B requirement of plants is species-dependent, yet the range between B deficiency and toxicity in plants is narrow (Goldberg, 1997). Globally, B micronutrient

deficiency is considered widespread (Shorrocks, 1997; Tariq and Mott, 2007). Investigations of B deficiency in plants show that B is necessary for proper plant development, as it leads to root, leaf, flower, and meristem defects (reviewed in Matthes et al., 2020). Cumulative evidence appears to support the hypothesis that root growth is more sensitive to B deficiency than shoot growth (reviewed in Brdar-Jokanović, 2020). Soil B deficiency is typically found in humid regions with well-drained soils (Tanaka and Fujiwara, 2008). In contrast, under low rainfall conditions, B cannot be sufficiently leached and may accumulate to levels that may become toxic to plant growth (Reid, 2007). A primary phenotypic effect of B toxicity in plants is root growth inhibition (reviewed in Princi et al., 2016). Evidence also exists that supports the hypothesis that B interacts with the uptake of other mineral nutrients (reviewed in Bariya et al., 2014; Fageria, 2001). In sweetpotato (*Ipomoea batatas*), B has been associated with storage root disorders such as splitting (Byju et al., 2007) and blister (Miller and Nielsen, 1970). There are published leaf sufficiency ranges (Mills and Jones, 1996), but very little information is available about newer cultivars. Despite the documented role of B in sweetpotato storage root quality, considerable knowledge gaps still exist in terms of understanding how the sweetpotato adapts

to B deficiency and toxicity, especially during the critical stages of plant establishment, initial root growth, and storage root formation. Thus, expanding knowledge about B deficiency and toxicity in sweetpotato is of fundamental and practical importance in this globally important crop.

One of the means that plants adapt to variation in soil nutrient availability is by altering root system architecture (RSA) (reviewed in Giehl et al., 2014). In sweetpotato, the successful emergence and development of lateral roots, the main determinant of RSA, determines the competency of ARs to undergo storage root formation (Villordon et al., 2012). Hence, knowledge about intrinsic and environmental variables that control RSA development contribute to the understanding of storage root formation and productivity. The primary objective of this work was to generate species-dependent information about root architectural responses to variation in B availability at the onset of storage root formation in three sweetpotato commercial cultivars with contrasting storage root characteristics and storage root yield potential. A secondary objective was to generate preliminary information on the possible interaction of B with the uptake of other mineral nutrients.

Materials and Methods

Plant materials and experimental conditions. The greenhouse experiments were carried out from 24 Nov. to 8 Dec. and 15 to 30 Dec. 2020 in Chase, LA (lat. 32°6'N, long. 91°42'W). Supplementary plantings were conducted on 29 Mar. and 13 Apr. 2021. These supplementary plantings were harvested at 50 d to confirm the presence of storage roots in experimental treatments that showed delayed storage root formation (appearance of anomalous cambium) at 15 d after planting and to monitor the progression of deficiency and toxic symptoms in leaves (Supplemental Figs. 1A, 2B and C). In addition, supplemental 5-day plantings of cuttings grown in liquid medium (Supplemental Fig. 1B) were conducted to verify that B was not an absolute requirement of AR emergence from cuttings as documented in other species (Jarvis et al., 1983; Josten and Kutschera, 1999). The following cultivars were used: Beauregard (BX), Murasaki (MU), and Okinawa (OK). Cultivar descriptions and other pertinent information are presented in Table 1. The cultivar BX was considered as a “check” or “control” cultivar for storage root formation, defined as the appearance of anomalous cambium (Togari, 1950) at 15 d in the growing conditions used for the current study as well as prior work (Villordon et al., 2020). BX is a copper-skinned, orange-fleshed cultivar that is grown globally and is the highest storage root yielder with a potential for early harvest relative to the other cultivars used in the study (Table 1). MU and OK are white- and purple-fleshed cultivars, respectively, with contrasting yield potential (Table 1). Virus-tested generation 1 storage roots were bedded in plastic containers containing soilless media (Sungro; Sun Gro Horticulture, Bellevue, WA) and served as a source

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Table 1. Description of sweetpotato cultivars used in the study.

Cultivar	Storage root characteristics			Relative U.S. No. 1 yield potential ²	Cultivar release information
	Skin color	Flesh color	Shape		
Beauregard (BX) “B-14” ³	Light rose	Orange	Elliptic	—	Rolston et al. (1987)
Murasaki (MU)	Dark purple	White with yellow cast	Elliptic to round	≤25%	LaBonte et al. (2008)
Okinawan (OK) ⁴	Tan	Purple	Tapered, lobed	<75%	

²Relative U.S. No. 1 storage root yield grade vs. cultivar Beauregard harvested at 110–120 d as published in release notices and multiyear data from research plots at the Sweet Potato Research Station.

³Clone “B14” (LaBonte et al., 2004).

⁴“Okinawan” clone maintained by the LSU AgCenter sweetpotato foundation seed program. Originally sourced from a commercial nursery and subjected to virus testing procedures. This clone is presumed to be similar to the “Okinawan” cultivar grown in Hawai’i.

of transplants or vegetative cuttings for subsequent experiments. Baseline mineral leaf tissue of the source plants are shown in Supplemental Table 1. In each experiment, cuttings were set in 10-cm-diameter polyvinyl chloride (PVC) pots (height = 30 cm) with detachable plastic bottoms. Each plastic bottom had five drain holes (2 mm in diameter). In addition, each PVC pot had four rows of side drain holes (2 mm in diameter; 3 cm apart within row) that were located diametrically opposite each other. These side drain holes were added to help reduce the incidence of a perched water table (Bilderback and Fonteno, 1987). Washed river sand was used as a growth substrate for all experiments. The diameter of the sand particles varied from 0.05 to 0.9 mm with most (83%) in the 0.2- to 0.9-mm range. Nutrient analysis data for the base substrate used in the study (extractable B = 0.5 mg·kg⁻¹) as well as substrate samples from experimental treatments are shown in Supplemental Table 2. In all experiments, vegetative terminal cuttings with the following characteristics were used: 25 to 30 cm in length, five to six fully opened leaves, ≈5 mm diameter at the basal cut, and with uniform distribution of nodes.

All pots were provided with 1X Hoagland’s No. 1 solution modified to alter the B levels in the micronutrient stock solution (Hoagland and Arnon, 1950). Three B levels were used: 0.1 mg·kg⁻¹ (0B; B was omitted in the nutrient medium, substrate B = 0.1 mg·kg⁻¹ as shown in Supplemental Table 1), 0.5 mg·kg⁻¹ (1XB; sufficient B, control) and 1 mg·kg⁻¹ (2XB, high B). The 1 mg·kg⁻¹ B level was selected as the high B experimental treatment based on prior work (O’Sullivan et al., 1997). The published 1X Hoagland’s No. 1 solution was considered the control treatment (1XB) and contained 0.5 mg·kg⁻¹ of B. For the 0B and 2XB experimental treatments, the micronutrient stock solution was modified so that the final working solution contained 0 and 1 mg·kg⁻¹ B, respectively. During the critical establishment stage, defined as the appearance of ARs in the first 3 to 5 d (Villordon et al., 2009), 150 mL of the nutrient solution was provided daily. After establishment, 200 mL was provided every other day until the completion of the study. This is the equivalent to providing 25 mm·ha⁻¹ of water with each application. Cuttings were planted to a uniform depth of 6 cm with two to three nodes under the growth substrate surface, depending on cultivar. All experiments were arranged as a randomized complete block repeated across planting dates. Unless otherwise

indicated, there were four replicates in each experiment (one plant per pot = one replicate). Plants were grown for 15 d, after which near-intact root systems were collected. Leaf tissues (fourth, fifth, and sixth fully opened leaves) were sampled for leaf tissue analysis. Plant height measurements were taken using a ruler.

Growth substrate moisture was measured with ECH2O soil moisture sensors inserted vertically at the 2- to 7-cm depth (Model EC-5; Decagon Devices Inc., Pullman, WA). The moisture of the growth substrate ranged from 5% to 9% volumetric water content (VWC), where ≈50% of field capacity (FC) = 7% VWC. At saturation, the growth substrate typically ranges from 12% to 14% VWC (Villordon et al., 2012). The greenhouse temperature regimen for the first planting date (PD1) was 28 °C (SD = 2.3) for 14 h (day) and 24 °C (SD = 3.3) for 10 h (night). The relative humidity (RH), measured hourly, averaged 59% (SD = 13.4). The temperature regimen for the second planting date (PD2) was 31 °C (SD = 2.2) for 14 h (day) and 27 °C (SD = 2.7) for 10 h (night). The RH averaged 53% (SD = 15.7). The temperature and RH were monitored at the canopy level using an integrated temperature and RH sensor (Model RHT; Decagon Devices Inc.). Supplementary lighting was provided using light-emitting diode grow lights (Lumigrow Inc., Emeryville, CA) for 14 h per day. Photosynthetic photon flux (PPF) for PD1 ranged from 422 to 2731 mmol·m⁻²·s⁻¹; PPF for PD2 ranged from 582 to 3770 mmol·m⁻²·s⁻¹. PPF was measured at the canopy level with a quantum sensor (Model QSO-S; Decagon Devices Inc.). These experimental approaches have been used to validate storage root initiation timing in BX (Villordon et al., 2009, 2012) and measuring root architecture responses to biotic and abiotic variables (Villordon and Clark, 2014, 2018; Villordon et al., 2012, 2013).

Root architecture measurements and anatomic sampling. At harvest, the detachable plastic bottoms were removed, and the pot was tilted and the growth substrate was gradually removed using a stream of water. The roots were then placed in water-filled trays and rinsed twice. The AR samples were stored in 50% alcohol solution before scanning. Storage root formation was confirmed by verifying the onset of anomalous cambium development in representative BX adventitious root samples ≥2 mm in diameter (Villordon et al., 2020).

Measurement of root architectural attributes followed the procedures described in

prior work (Villordon et al., 2020). Briefly, intact washed ARs were floated on waterproof trays and scanned using an Epson Perfection V850 Pro Photo Scanner (Epson Corporation, San Jose, CA). Image acquisition parameter was set to “high” accuracy (600 dpi; image size ≈18 MB), whereas analysis precision was set to “high.”

In prior work using the cultivar BX, preset intervals were used to classify root classes (Villordon et al., 2012, 2013); however, the variation in complexity of root samples among cultivars and overlapping root thickness led to classification errors among root classes in the current study. Hence, the following modifications of prior approaches were adopted for this work. For purposes of this work, AR specimens with labeled sections in Fig. 1 were used to standardize terminologies and clarify specific root class categories and attributes measured. First, main or primary root (MR) length (Fig. 1A and B) was manually measured using the segmented line feature in ImageJ (Schneider et al., 2012). Second, total root length (TRL) was measured using RhizoVision Explorer (Seethepalli and York, 2020). Debris such as sand particles and loose root tissue were excluded from the analysis by turning on the filter for non-root objects in RhizoVision Explorer (size = 25 pixels). Finally, lateral root (LR) length was calculated by subtracting the manual MR length measurements from TRL. A separate analysis was performed in RhizoVision Explorer to detect the length of MRL segments that were ≥3 mm to measure incidence of storage root (SR) swelling (Fig. 1A and B). An AR that failed to show evidence of anomalous cambium development is presented in Fig. 1C for comparison. Wilson and Lowe (1973) documented the onset of SR formation, defined as the appearance of anomalous cambium, in MR segments that were 1 mm in diameter or greater. In the present study, anomalous cambium was detected in BX segments with diameter ≥2 mm (data not shown).

Statistical analyses. Root length and counts were transformed using log 10 and square root transformation, respectively, to reduce heterogeneity of variance. The unbalanced data set was analyzed using SAS Proc Mixed (SAS 9.4; SAS Inc., Cary, NC). Fisher’s least significant difference test at the 0.05 *P* level was used to test for statistical significance. There were no significant planting date and cultivar effects as well as cultivar by B level effect for all root attributes; hence, data were combined. The R package

Fig. 1. Scanned images of 15-d-old 'Beauregard' adventitious roots labeled to clarify terminology as well as show evidence of storage root (SR) development. The main or primary root (MR) is labeled in A, along with a swollen section (SR) in the proximal 3- to 5-cm section associated with anomalous cambium activity. A representative lateral root (LR) is labeled in B along with another swollen section. An adventitious root with no evidence of swelling is shown in C. Scale bar = 1 cm (A). Adventitious roots were floated on waterproof trays and images were acquired using an Epson Perfection V850 Pro Photo Scanner (Epson Corporation).

ggplot2 (Wickham, 2009) was used to generate boxplots using R Studio (v.1.2.1335; R Studio Inc., Boston, MA) with R version 3.6 (R Development Core Team, 2019). The data presented were from nontransformed data. Pearson correlations among leaf tissue nutrient levels were calculated using 1000 bootstrapped correlations using the package boot in R.

Results and Discussion

Boron leaf tissue levels and their effect on other mineral nutrients. Sufficient (1XB) and high B (2XB) levels increased leaf tissue B in the check cultivar BX by 736% and 2269%, respectively, relative to plants grown in low B (0B) (Table 2). Similar magnitudes were observed in cultivars MU and OK (Table 2). At 15 d, leaf tissue toxicity symptoms were already visible in BX plants grown in 2XB, that is, pale green to whitish interveinal chlorosis (O'Sullivan et al., 1997) as well as the onset of necrotic tissue (Supplemental Fig. 2B). Supplemental plantings showed that at 50 d, necrosis progressed and premature leaf loss of older leaves was observed in BX (Supplemental Fig. 1C). These symptoms are consistent with B toxicity responses and distribution reported in other species, with B

accumulating at the end of the transpiration stream (reviewed in Nable et al., 1997). The cultivar OK grown in 2XB showed relatively lower B ($176 \pm 39 \text{ mg} \cdot \text{kg}^{-1}$) levels relative to BX ($363 \pm 17 \text{ mg} \cdot \text{kg}^{-1}$) and MU ($364 \pm 35 \text{ mg} \cdot \text{kg}^{-1}$) grown with similar B levels. B tolerant cultivars are characterized by a decreased B in leaf tissues under high B conditions in comparison with nontolerant cultivars (Nable et al., 1990). Follow-up work is needed to generate more data to support this hypothesis and its applicability to sweetpotato cultivars with similar responses to OK. Mills and Jones (1996) previously reported that leaf tissue was considered sufficient when B levels ranged from 25 to 75 $\text{mg} \cdot \text{kg}^{-1}$ at the midseason stage (≈ 60 d). Scott and Bouwkamp (1974) reported a range of 49 to 51 $\text{mg} \cdot \text{kg}^{-1}$ at 60 d in field-grown sweetpotato cultivars Centennial, Jewel, Nemagold, and Redmar grown with recommended practices. Schultheis and Campbell (1996) reported excessive B levels in leaf tissue ($>200 \text{ mg} \cdot \text{kg}^{-1}$) in field-grown sweetpotato cultivar 'Hernandez' when application levels exceeded 2.2 $\text{kg} \cdot \text{ha}^{-1}$. It is important to note that in the current study, a continuous supply of 1 $\text{mg} \cdot \text{kg}^{-1}$ B was provided during the study period for the 2XB experimental treatment. In field conditions, roots gradually

deplete nutrients in the root zone, leading to the formation of depletion zones (De Parseval et al., 2017). However, B is relatively mobile in the soil (Flis, 2019) such that rainfall or irrigation events may replenish the depletion zone.

Current available data support the hypothesis that B deficiency, sufficiency, and toxicity exert effects on other mineral nutrients in the plant, but the data are conflicting (reviewed in Bariya et al., 2014). The conflicting results may be due in part to different experimental systems and cultivar-specific B requirements such that specific B effects may vary under B sufficient and deficient conditions (reviewed in Bariya et al., 2014). These trends are consistent with the findings from the current study. When the correlation analysis was performed on the total data set, we failed to detect significant correlations between B and other mineral nutrients. However, when data from 0B-treated plants were removed from the data set, significant correlations were observed between B and the following: Ca (0.63, $P < 0.001$), Mg (0.70, $P < 0.001$), Zn (-0.4 , $P = 0.04$), and Cu (-0.45 , $P = 0.02$) (Fig. 2). Steinburg et al. (1955) reported similar conflicting results when analysis was performed separately on tobacco plants provisioned with B relative to plants without B. Francois (1986) reported that increasing B in the soil solution increased the concentration of B, P, K, and Mg in tomato leaves, whereas Ca and Na showed inconsistent trends. The negative correlation of B with Zn and Cu observed in the current work is consistent with findings reported by Leece (1978), although in the same report, Ca was negatively correlated. The negative correlation with Zn is consistent with available evidence that supports the hypothesis that Zn interferes with the absorption of Fe and B in plants and that the application of Zn is suggested as a measure to alleviate B toxicity in crops grown in B-rich soil (reviewed in Prasad et al., 2016). Hussein et al. (2007) and Aref (2011) suggested that B and Zn are antagonistic with respect to their concentration in plant tissue. However, Sinha et al. (2000) reported contrary results and observed a positive relationship between Zn and B.

Table 2. Leaf tissue nutrient concentrations in three sweetpotato cultivars as affected by boron level.²

Nutrient	Beauregard			Murasaki			Okinawa		
	0B	1XB	2XB	0B	1XB	2XB	0B	1XB	2XB
B, $\text{mg} \cdot \text{kg}^{-1}$	15 ± 1 e	128 ± 19 c	363 ± 17 a	15 ± 4 e	104 ± 12 cd	364 ± 35 a	14 ± 2 e	80 ± 10 e	176 ± 39 b
N, %	5.1 ± 0.1 ab	5.1 ± 0.3	5.1 ± 0.4	5.1 ± 0.2	5.2 ± 0.2	5.3 ± 0.1	4.8 ± 0.1	5.1 ± 0.3	4.7 ± 0.3
P, %	0.3 ± 0.01 a	0.3 ± 0.04 a	0.3 ± 0.01 a	0.3 ± 0.1 a	0.3 ± 0.03 a	0.3 ± 0.07 a	0.3 ± 0.04 a	0.3 ± 0.01 a	0.3 ± 0.05 a
K, %	5.3 ± 0.5 a	5.2 ± 0.8 a	5.1 ± 0.5 a	4.8 ± 0.3 a	5.1 ± 0.5 a	5.1 ± 0.2 a	5.2 ± 0.8 a	5.6 ± 0.5 a	4.9 ± 0.9 a
Ca, %	2.2 ± 0.1 a	2.2 ± 0.2 ab	2.1 ± 0.1 abc	1.6 ± 0.2 cd	1.7 ± 0.1 cd	1.7 ± 0.1 bcd	1.4 ± 0.2 d	1.5 ± 0.2 d	1.5 ± 0.3 d
Mg, %	0.8 ± 0.04 a	0.8 ± 0.07 a	0.8 ± 0.02 ab	0.6 ± 0.1 bc	0.7 ± 0.07 ab	0.8 ± 0.04 ab	0.5 ± 0.1 cd	0.5 ± 0.07 d	0.5 ± 0.1 d
S, %	0.4 ± 0.02 ab	0.5 ± 0.09 a	0.4 ± 0.05 ab	0.35 ± 0.1 b	0.3 ± 0.03 b	0.3 ± 0.02 b	0.4 ± 0.08 ab	0.4 ± 0.04 b	0.3 ± 0.03 b
Fe, $\text{mg} \cdot \text{kg}^{-1}$	140 ± 23 a	132 ± 30 a	136 ± 20 a	104 ± 13 a	119 ± 4 a	116 ± 3 a	123 ± 31 a	100 ± 16 a	118 ± 19 a
Mn, $\text{mg} \cdot \text{kg}^{-1}$	197 ± 16 ab	202 ± 4 a	171 ± 4 bc	167 ± 6 bc	166 ± 4 bc	161 ± 15 c	143 ± 28 cd	120 ± 25 de	111 ± 7 e
Al, $\text{mg} \cdot \text{kg}^{-1}$	21 ± 17 a	21 ± 18 a	12 ± 2 a	14 ± 4 a	15 ± 3 a	15 ± 2 a	24 ± 13 a	13 ± 3 a	12 ± 3 a
Zn, $\text{mg} \cdot \text{kg}^{-1}$	16.7 ± 2 d	17.7 ± 3 cd	16.7 ± 2 d	20.7 ± 1 cd	21 ± 2 bcd	22 ± 1 bc	25 ± 3 ab	21 ± 3 bcd	30 ± 2 a

²Values represent means (± se). Means within rows followed by a common letter are not significantly different based on Fisher's least significant different means separation test ($P \leq 0.05$). Values were $\log_{10}$ transformed. Corresponding nontransformed values are shown. Leaf tissue samples were collected 15 d after planting. 0B = B was omitted in the nutrient solution, substrate B = 0.1 $\text{mg} \cdot \text{kg}^{-1}$; 1XB = 0.5 $\text{mg} \cdot \text{kg}^{-1}$; 2XB = 1 $\text{mg} \cdot \text{kg}^{-1}$.

Fig. 2. Bootstrapped correlation values between boron and other macro- and micronutrients in sweetpotato leaf tissue. Solid black bars represent significant correlations at the $P < 0.05$ confidence level. Leaf tissue mineral nutrient data were combined from three cultivars and two planting dates. Leaf tissue samples were collected 15 d after planting.

There are other various conflicting findings about the influence of B on other mineral nutrients in plants. In sweetpotato, it is evident that follow-up studies are needed with standardized growth and experimental conditions.

SR formation timing. The check cultivar BX presented evidence of SR formation in the 0B and 1XB treatments but not in 2XB at 15 d (Figs. 1A and 3). Supplemental plantings that were harvested at 50 d showed evidence of SRs in 2XB treated plants (Supplemental Fig. 1B), supporting the hypothesis that the high B level used in this study did not suppress, but only delayed SR formation. The early onset of SR formation in BX relative to other cultivars is consistent with past findings in which SR formation was detected at 15 to 20 d in BX in similar growing conditions (Villordon and Clark, 2018; Villordon et al., 2009, 2020). Among the cultivars used in the current study, BX consistently produces relatively higher SR

yields in multiyear and multilocation field trials (Table 1; LaBonte et al., 2013). These data corroborate observations that early SR formation is an indicator of relative productivity in sweetpotato cultivars (Firon et al., 2013). Prior evidence shows that B leaf tissue concentrations exceeding $400 \text{ mg} \cdot \text{kg}^{-1}$ are associated with 30% to 50% growth reductions (Ayers and Westcot, 1989). Sweetpotato has been previously classified as semitolerant to high B levels ($2 \text{ mg} \cdot \text{kg}^{-1}$) in irrigation water (Ayers and Westcot, 1989); However, there is no current available data on the role of B on sweetpotato SR yield in field conditions.

AR number. We failed to detect differences in AR number within cultivars and across B levels but OK showed 25% less AR number relative to BX across all B levels (Fig. 4). AR number variation at the onset of SR formation among sweetpotato cultivars has also been reported previously (Lowe and Wilson, 1974;

Nakatani and Watanabe, 1986). The requirement for B in the growth of ARs formed on stem cuttings was first demonstrated by Hemberg (1951) and subsequently reported in cuttings of other plant species such as sunflower (*Helianthus annuus*) (Josten and Kutschera, 1999) and mung bean (*Phaseolus aureus*) (Jarvis et al., 1983). In sweetpotato, the presence of B in the substrate does not appear to be a requirement for AR emergence. Evidence from prior work (Lowe and Wilson, 1974; Ma et al., 2015) indicates that there is no direct correlation between total ARs per plant and final SR number at harvest. Lowe and Wilson (1974) cited limiting factors that influenced final SR yield, including assimilate production and transport.

There is evidence from other plant species that ethylene mediates variation in AR number response. We have previously documented that cultivar-specific AR emergence is mediated by differential ethylene sensitivity in cultivars (Villordon et al., 2012); however, there is very limited information about specific ethylene effects in sweetpotato RSA and storage root formation in general.

MR length. The 0B treatment was associated with 20% and 48% reduction in main root (MR) length in BX and OK, respectively, relative to plants grown in 1XB and 2XB (Fig. 5). 2XB was associated with a 10% increase in MR length in MU relative to plants grown in 0B and 1XB. These results in part corroborate current findings as regards the association of B deficiency with primary or MR length reduction in other species including mung bean (Middleton et al., 1978), squash (*Cucurbita pepo*) (Lukaszewski and Blevins, 1996), and wheat (*Triticum aestivum*) (Holloway and Alston, 1992). This growth arrest is due to a reduction in cell elongation and cell division at the growing tips, but the precise molecular mechanism is still under active investigation (reviewed in Matthes et al., 2020). Additional roles for B

Fig. 3. Box plots of main root segments with diameter $\geq 2 \text{ mm}$ in the check sweetpotato cultivar 'Beauregard' (BX) grown in three boron levels. Boxes represent the interquartile range (IQR, or middle 50%) of values for each feature. Shaded diamonds represent mean values. Bold horizontal lines indicate median values. Upper box plot whiskers represent the last data point within the range of 75% quantile + 1.5 IQR, lower box plot whiskers represent the last data point within the range of 25% quantile - 1.5 IQR. Mean comparisons were performed on transformed data; corresponding nontransformed values are shown. Boxes with different letters differ significantly at the 5% level by Fisher's least significant difference. Boron levels: 0B = B was omitted in the nutrient solution, substrate B = $0.1 \text{ mg} \cdot \text{kg}^{-1}$; 1XB = $0.5 \text{ mg} \cdot \text{kg}^{-1}$; 2XB = $1 \text{ mg} \cdot \text{kg}^{-1}$. Data were combined from two planting dates; adventitious roots were sampled at 15 d.

Fig. 4. Box plots of adventitious root (AR) number $\geq 2 \text{ cm}$ in length of sweetpotato cultivars grown in three boron levels. Boxes represent the interquartile range (IQR, or middle 50%) of values for each feature. Shaded diamonds represent mean values. Bold horizontal lines indicate median values. Upper box plot whiskers represent the last data point within the range of 75% quantile + 1.5 IQR, lower box plot whiskers represent the last data point within the range of 25% quantile - 1.5 IQR. Dots represent outliers (values smaller or larger than the median ± 1.5 times the interquartile range). Mean comparisons were performed on transformed data; corresponding nontransformed values are shown. Boxes with different letters differ significantly at the 5% level by Fisher's least significant difference. Boron levels: 0B = B was omitted in the nutrient solution, substrate B = $0.1 \text{ mg} \cdot \text{kg}^{-1}$; 1XB = $0.5 \text{ mg} \cdot \text{kg}^{-1}$; 2XB = $1 \text{ mg} \cdot \text{kg}^{-1}$. Cultivars: BX = Beauregard; MU = Murasaki; OK = Okinawa. Data were combined from two planting dates; ARs were sampled at 15 d.

Fig. 5. Box plots of main root length of sweetpotato cultivars grown in three boron levels. Boxes represent the interquartile range (IQR, or middle 50%) of values for each feature. Shaded diamonds represent mean values. Bold horizontal lines indicate median values. Upper box plot whiskers represent the last data point within the range of 75% quantile + 1.5 IQR, lower box plot whiskers represent the last data point within the range of 25% quantile - 1.5 IQR. Dots represent outliers (values smaller or larger than the median $\pm$ 1.5 times the IQR). Mean comparisons were performed on transformed data; corresponding nontransformed values are shown. Boxes with different letters differ significantly at the 5% level by Fisher's least significant difference. Boron levels: OB = B was omitted in the nutrient solution, substrate B = 0.1 mg·kg⁻¹; 1XB = 0.5 mg·kg⁻¹; 2XB = 1 mg·kg⁻¹. Cultivars: BX = Beauregard; MU = Murasaki; OK = Okinawa. Data were combined from two planting dates; adventitious roots were sampled at 15 d.

have been proposed and demonstrated related to metabolism, membrane processes, and phytohormone signaling, although it is difficult to separate primary and secondary effects of B deficiency (reviewed in Matthes et al., 2020). In sweetpotato, knowledge of variables that affect MRL is of practical importance because this contributes to the understanding of the determination of SR shape.

LR length and count. Low B reduced LR length (Fig. 6) and count (Fig. 7) in all cultivars, but the magnitude of responses varied with cultivars. For example, in the check cultivar BX, OB was associated with a 32% reduction in LR length relative to 1XB;

however, increasing B to 2XB did not lead to further LR length increase. In contrast, MU showed a 60% increase in LR growth when B was increased from OB to 2XB. The cultivar OK showed similar significant increases in LR length with 1XB and 2XB relative to plants grown in OB. Relative to BX and MU, the cultivar OK had the longest LR at 1XB and 2XB. Similar trends were observed with LR counts. These results are consistent with current evidence supporting the hypothesis that B deficiency is associated with reduced root growth but the response varies with genotype, for example in *Citrus* (Mei et al., 2011). In general, B deficiency is associated

Fig. 6. Box plots of lateral root length of sweetpotato cultivars grown in three boron levels. Boxes represent the interquartile range (IQR, or middle 50%) of values for each feature. Shaded diamonds represent mean values. Bold horizontal lines indicate median values. Upper box plot whiskers represent the last data point within the range of 75% quantile + 1.5 IQR, lower box plot whiskers represent the last data point within the range of 25% quantile - 1.5 IQR. Dots represent outliers (values smaller or larger than the median $\pm$ 1.5 times the interquartile range). Mean comparisons were performed on transformed data; corresponding nontransformed values are shown. Boxes with different letters differ significantly at the 5% level by Fisher's least significant difference. Boron levels: OB = B was omitted in the nutrient solution, substrate B = 0.1 mg·kg⁻¹; 1XB = 0.5 mg·kg⁻¹; 2XB = 1 mg·kg⁻¹. Cultivars: BX = Beauregard; MU = Murasaki; OK = Okinawa. Data were combined from two planting dates; adventitious roots were sampled at 15 d.

with reduction in root systems as documented in melons (*Cucumis melo*) (Edelstein et al., 2007). Kouchi and Kumazawa (1975) reported that the primary effect of B deficiency was the rapid cessation of root elongation followed by browning and some morphological changes in the root tips, such as abnormal enlargement and dense appearance of LRs. We observed browning and necrosis of OK LR tips sampled from plants grown with OB (Supplemental Fig. 2A). We failed to detect plant height differences within cultivars during the 15-d study period in our experimental conditions (Supplemental Fig. 3). This is consistent with observations in other species that the root system is more sensitive to B deficiency than shoots (reviewed in Brdar-Jokanović, 2020); however, it is conceivable that B deficiency may limit vine growth during the rapid bulking of SRs in field conditions, typically starting at 50 to 60 d with the remobilization of nutrients from the shoots to the developing SRs (Scott and Bouwkamp, 1974).

Knowledge about plant adaptation to B availability will lead to the optimization of B management approaches and selection of resilient cultivars that can tolerate the narrow range of B deficiency and toxicity. In general, the findings from the current study corroborate cumulative evidence from other species that B deficiency is associated with reduced root growth. Second, the results are consistent with current understanding of the relative narrow range and crop species specificity of B effectivity. Third, the results are consistent with the hypothesis that B response varies with cultivar. Currently, there are no specific published recommendations for B management for commercially grown sweetpotato cultivars and represents a significant gap in B management knowledge in sweetpotato. Our work represents an initial step toward an in-depth understanding of the role of B in sweetpotato productivity as well as provides some baseline information for follow-up studies on developing specific recommendations for managing B in sweetpotato production to address low soil B conditions and to minimize the risk of overapplication. This work as well as current available data indicate that for commercial sweetpotato production, a best practices approach for B management should include soil B measurements in soil testing to determine if any B applications are necessary as well as account for any B presence in other fertilizer materials that will be applied. Currently, B is not included in routine soil analysis (McAuley et al., 2016). In sweetpotato, B remobilization from shoots to roots, as documented by Scott and Bouwkamp (1974), is another variable that can modulate sweetpotato response to low B. Prior work has shown that B is remobilized and transported into plant tops, whereas a small but adequate amount was remobilized and transported to the roots (Oertli, 1993). Follow-up studies might include the role of B status of planting materials and how this interacts with soil B availability.

Fig. 7. Box plots of lateral root number of sweetpotato cultivars grown in three boron levels. Boxes represent the interquartile range (IQR, or middle 50%) of values for each feature. Shaded diamonds represent mean values. Bold horizontal lines indicate median values. Upper box plot whiskers represent the last data point within the range of 75% quantile + 1.5 IQR, lower box plot whiskers represent the last data point within the range of 25% quantile - 1.5 IQR. Dots represent outliers (values smaller or larger than the median $\pm$ 1.5 times the interquartile range). Mean comparisons were performed on transformed data; corresponding nontransformed values are shown. Boxes with different letters differ significantly at the 5% level by Fisher's least significant difference. Boron levels: OB = B was omitted in the nutrient solution, substrate B = 0.1 mg·kg⁻¹; 1XB = 0.5 mg·kg⁻¹; 2XB = 1 mg·kg⁻¹. Cultivars: BX = Beauregard; MU = Murasaki; OK = Okinawa. Data were combined from two planting dates; adventitious roots were sampled at 15 d.

Conclusion

Although some of the findings corroborate data from model plant species, significant gaps exist in understanding sweetpotato response to B deficient and toxic conditions, especially during the critical storage root formation phase. The experimental data support the hypothesis that sweetpotato cultivars vary in response to low and excessive B and that low B generally reduces overall root mass. Follow-up studies with more diverse materials are needed, including the measurement of transpiration stream to help explain the differential B uptake among cultivars. Even in model systems, significant gaps exist in knowledge about plant responses to deficient and toxic B levels.

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Supplemental Fig. 1. Storage root development at 50 d in sweetpotato cultivar Beauregard grown in three boron levels (A). Adventitious root development at 5 d in sweetpotato cultivar Beauregard cuttings grown in nutrient solutions with 0.5 mg·kg⁻¹ boron and without boron (B). Scale bar = 1 cm. Boron levels: 0B = B was omitted in the nutrient solution, substrate B = 0.1 mg·kg⁻¹; 1XB = 0.5 mg·kg⁻¹; 2XB = 1 mg·kg⁻¹.

Supplemental Fig. 2. Symptoms associated with boron deficiency and toxicity in sweetpotato cultivars: 'Okinawa' adventitious roots at 15 d from plants grown without boron (A); 'Beauregard' leaf tissue at 15 d (B) and 50 d (C) from plants grown with excessive boron. IVC = interveinal chlorosis; NLT = necrotic leaf tissue; NRT = necrotic root tip; LA = leaf abscission.

Supplemental Fig. 3. Box plots of plant height of three sweetpotato cultivars grown in three boron levels. Boxes represent the interquartile range (IQR, or middle 50%) of values for each feature. Shaded diamonds represent mean values. Bold horizontal lines indicate median values. Upper box plot whiskers represent the last data point within the range of 75% quantile + 1.5 IQR, lower box plot whiskers represent the last data point within the range of 25% quantile - 1.5 IQR. Mean comparisons were performed on transformed data; corresponding nontransformed values are shown. Boxes with different letters differ significantly at the 5% level by Fisher's least significant difference. Boron levels: 0B = B was omitted in the nutrient solution, substrate B = 0.1 mg·kg⁻¹; 1XB = 0.5 mg·kg⁻¹; 2XB = 1 mg·kg⁻¹. Data were combined from two planting dates; plant height was measured at 15 days.

Supplemental Table 1. Mineral nutrient analysis of source plants used in the study.^z

Nutrient	Cultivar		
	Beauregard	Murasaki	Okinawa
N, %	3.6 ± 0.19	3.5 ± 0.09	3.1 ± 0.06
P, %	0.26 ± 0.017	0.28 ± 0.0	0.25 ± 0.03
K, %	2.3 ± 0.04	2.1 ± 0.19	1.9 ± 0.25
Ca, %	1.42 ± 0.09	1.37 ± 0.11	1.27 ± 0.01
Mg, %	0.43 ± 0.03	0.46 ± 0.03	0.44 ± 0.02
S, %	0.23 ± 0.02	0.12 ± 0.05	0.20 ± 0.03
B, mg·kg ⁻¹	32.0 ± 2.1	40.5 ± 3.5	40.0 ± 2.0
Cu, mg·kg ⁻¹	8.0 ± 1.0	7.0 ± 0.0	6.5 ± 0.5
Fe, mg·kg ⁻¹	83 ± 16	105 ± 35	67.5 ± 2
Mn, mg·kg ⁻¹	95 ± 15	79 ± 4	77 ± 13
Al, mg·kg ⁻¹	33.7 ± 3.8	41.5 ± 21.5	26.0 ± 5.0
Zn, mg·kg ⁻¹	44 ± 6.6	41 ± 3.0	47 ± 1.0

^zSource plants were grown from virus-tested generation 1 storage roots were bedded in plastic containers containing soilless media (Sungro; Sun Gro Horticulture, Bellevue, WA).

Supplemental Table 2. Laboratory analysis results of the growth substrate used in the study as well as samples from each boron treatment.^z

Nutrient	(mg·kg ⁻¹)			
	Untreated	0B	1XB	2XB
Phosphorus	3.0 ± 0.6	8.7 ± 0.7	7.7 ± 0.3	12.3 ± 0.3
Potassium	6.3 ± 0.3	26 ± 1.0	35 ± 2.5	33 ± 0.9
Calcium	135 ± 37	107 ± 5	105 ± 3	172 ± 27
Magnesium	17 ± 0	21 ± 0.6	25 ± 1.2	21 ± 0.3
Sulfur	3.7 ± 0.7	13.3 ± 1.8	14.0 ± 1.6	10.0 ± 3.2
Boron	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.03
Copper	0.17 ± 0.03	0.2 ± 0.0	0.27 ± 0.03	0.17 ± 0.03
Iron	15.7 ± 1.2	16.7 ± 0.9	17.7 ± 0.8	15.7 ± 0.3
Manganese	2.3 ± 0.3	2.0 ± 0.0	4.0 ± 0.6	2.7 ± 0.3
Zinc	0.17 ± 0.03	0.10 ± 0.0	0.27 ± 0.03	0.23 ± 0.03
Sodium	7.3 ± 0.3	6.3 ± 0.3	7.0 ± 0.7	7.0 ± 0.6
pH	6.5 ± 0.03	7.0 ± 0.06	6.9 ± 0.03	6.7 ± 0.07
Organic matter	0.23 ± 0.07	0.30 ± 0.06	0.27 ± 0.03	0.2 ± 0.06

^zValues represent means (± SE). Treated substrate samples were collected at 15 d. 1X Hoagland's was used as the base nutrient solution with different boron (B) levels: 0B = B was omitted in the nutrient solution, substrate B = 0.1 mg·kg⁻¹; 1XB = 0.5 mg·kg⁻¹; 2XB = 1 mg·kg⁻¹.