Leaf gas exchange, physiological growth, yield and biochemical properties of groundnut as influenced by boron in soilless culture

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ABSTRACT
Crops specific proper concentration of micronutrient application is necessary to improve the yield and quality of crops. Therefore, an experiment was conducted to identify the optimum dose of boron for groundnut plant. Six level of boron (B) application, B0 (0 ppm), B1 (0.5 ppm), B2 (1 ppm), B3 (2 ppm), B4 (4 ppm) and B5 (8 ppm) were evaluated. Photosynthetic rate, transpiration and stomatal conductance were increased for boron application but leaf vapor pressure deficit decreased. Physiological growth parameters, yield and yield contributing character, and shelling percentage was highest for B3. The values of biochemical traits including protein, oil and vitamin E content were higher for B3. Thus, leaf gas exchange showed that boron can be used to culture groundnut as it provides high yield and biochemical properties.

Introduction
Groundnut is an important oil seed crops throughout the world (Onemli 2012) and boron is an essential nutrient element needed by the plant for normal growth and development as well as to improve the yield (Quamruzzaman et al. 2017). Boron regulated the carbohydrate metabolism, involved in protein synthesis and keeps a role in seed formation (BARC 2005). Boron had an important role in stigma receptive, sticky and making pollen grain fertile for enhancing the pollination (Kaisher et al. 2010). It also influenced in retaining flower and fruit setting in legume crops (Zhang 2001). As a result, boron would increase the number of pegs, pods as well as yield in groundnut (Naiknaware et al. 2015). Kabir et al. (2013) stated that boron was responsible for producing healthier seeds in groundnut. Successfully used this micronutrient improved the quality of groundnut seed including protein, oil and vitamin E content (Quamruzzaman et al. 2016b). The proper supply of adequate boron nutrition could help to improve the crop yield and quality. Thus, an understanding of proper supply of boron is important to achieve sustainable agriculture. Therefore, mistakes in application this micronutrient could result in serious yield reduction. Furthermore, excess boron application could cause a toxic effect in the root zone (Çikili et al. 2015).

Photosynthetic rate depends on the different factors such as thickness of leaves (Kalariya et al. 2015), age of leaves (Nautiyal et al. 1999) and availability of nutrient and water (Rahman et al. 2012). Boron nutrition also helped to increase the photosynthesis of plant (Pinho et al. 2010).

Stomatal conductance also increased due to boron application (Pinho et al. 2010). It has been shown that E and gS were decreased with increasing of LVPD (Choi et al. 2016). However, proper supply of boron could have an effect on the leaf gas exchange, growth, yield and quality of groundnut. Therefore, it is important to determine the optimal doses of boron application to groundnut plant.

Materials and methods

Experimental site and plant materials
A pot experiment was conducted at the Sher-e-Bangla Agricultural University, Dhaka from November 2015 to February 2016 and November 2016 to February 2017. BARI Chinabadam 8 cultivar was used in this experiment.

Experimental design and treatments
A completely randomized design with three replications was used in this study. The treatment considered as six level of boron (B) application, viz., B0 (0 ppm), B1 (0.5 ppm), B2 (1 ppm), B3 (2 ppm), B4 (4 ppm) and B5 (8 ppm) were evaluated. Photosynthetic rate, transpiration and stomatal conductance were increased for boron application but leaf vapor pressure deficit decreased. Physiological growth parameters, yield and yield contributing character, and shelling percentage was highest for B3. The values of biochemical traits including protein, oil and vitamin E content were higher for B3. Thus, leaf gas exchange showed that boron can be used to culture groundnut as it provides high yield and biochemical properties.

Growth environment
Eighteen plastic crates of 24 L pots were used for culturing the plants. Each crate was filled with a mixture of coco peat and broken bricks at the ratio of 60:40 (v/v), respectively. Rahman and Inden (2012) solution was used in this experiment as a standard where boron [Source: boric acid (16.5% boron)] was applied as per the treatments. The nutrient solution was applied by the drip irrigation system. The pH and electrical conductivity (EC) were maintained at approximately 6.0 and 3.0 dS/cm, respectively in the nutrient solutions. The growth condition, i.e. average maximum and minimum temperature were 27.38 and 15.38°C, relative humidity 63.75% and rainfall 20.95 mm were recorded.

Data collection
Three plants were considered as an experimental unit. Data were collected on physiological parameters, yield contributing characters and biochemical parameters including leaf area (LA), leaf area ratio (LAR), leaf mass ratio (LMR),
relative growth rate (RGR), net assimilation rate (NAR), number of pegs plant$^{-1}$, number of pods plant$^{-1}$, pod dry weight plant$^{-1}$, harvest index (HI), and protein and vitamin E content, respectively. Gas exchange parameters such as net photosynthetic rate ($P_n$), transpiration ($E$), stomatal conductance ($g_s$) and leaf vapor pressure deficit (LVPD) were also measured.

**Physiological parameters measured**
The physiological parameters were measured as described below:

\[
\text{LAR} = \frac{\text{LA}}{\text{PDW}},
\]
where, LAR = leaf area ratio, LA = Leaf area (cm$^2$), PDW = plant dry weight (g).

\[
\text{LMR} = \frac{\text{LDW}}{\text{PDW}},
\]
where, LMR = leaf mass ratio, LDW = leaf dry weight (g).

\[
\text{RGR} = \frac{\ln \text{PDW}_2 - \ln \text{PDW}_1}{(t_2 - t_1)},
\]
where $t$ = time. Subscripts 1 and 2 refer to the initial and sampling dates (days), respectively.

\[
\text{NAR} = \frac{\text{RGR}}{\text{LAR}},
\]

\[
\text{HI} = \frac{\text{Economic yield}}{\text{Biological yield}}.
\]

**Biochemical parameters measured**

**Protein analysis**: The protein content was evaluated by the multiplication of total nitrogen with 6.25, which was determined by following the Micro-Kjeldahl’s method (Kjeldahl 1883).

**Oil analysis**: In order to determine the oil content of groundnut seed, the Soxhlet extraction method was used (AOAC 1990).

**Vitamin E analysis**: The vitamin E content of groundnut seeds was analyzed using Ejoh and Ketiku (2013) procedure.

**Gas exchange parameter measured**
The $P_n$, $E$, $g_s$ and LVPD were measured using the LCpro portable photosynthetic system as described by Rajaona et al. (2013). The gas exchange parameters were recorded both in vegetative and reproductive growth stage during morning hours (between 10:00 and 12:00 h).

**Statistical analysis**
Data collected in two cropping seasons were mean together on account of the non-significant interaction. Then one-way ANOVA (analysis of variance) of means data of two trial experiments were analyzed by using IBM SPSS (Version 20.0) and mean separation was done at $P \leq 0.05$ by using Tukey’s test (Tukey 1977).

**Results and discussions**

**Transpiration, stomatal conductance and leaf vapor pressure deficit (LVPD)**
All of the parameters were studied both in vegetative growth stage and reproduce growth stages. Result revealed that application of boron enhanced the transpiration ($E$) and stomatal conductance ($g_s$), but it significantly decreased the leaf vapor pressure (LVPD) in groundnut (Table 1). The statistically significant increasing trend of $E$ and $g_s$ showed up to $B_3$ and then decreased but for LVPD, the decreased trend found up to $B_5$. Finding suggesting that the increased $E$ was associated with the increased $g_s$ whereas the negative response of $E$ and $g_s$ to LVPD was observed. The increased $E$ and $g_s$ are obtained in this study agreed with the finding of Lu et al. (2014). Choi et al. (2016) reported that transpiration, stomatal conductance was decreased in boron deficient area but increased in leaf vapor pressure.

**Net photosynthetic rate**
Boron application had a significant effect on net photosynthetic rate during vegetative and reproductive growth stage. Treatment $B_3$ facilitated the higher $P_n$ than the other treatments (Figure 1). Microscopic investigation indicated that the maximum stomatal pores in the epidermal layers of leaves were opened in boron treated plants. A recent study showed that, gas exchange (stomatal effects) as well as photosynthesis was induced by boron deficiency in coconut (Pinho et al. 2010). Reduced $g_s$ results in a decrease in uptake of CO$_2$ that can be used in carboxylation reactions (Brugnoli and Björkman 1992) and higher $g_s$ in plants increases the diffusion of CO$_2$ in the leaves and thus increases the rate of photosynthesis. The $P_n$ is progressively increased because of increasing stomatal conductance.

Result indicated that, the $E$, $g_s$, LVPD and $P_n$ were highest during reproductive growth stage compared to vegetative growth stages.

**Physiological growth analysis**
The growth parameters of groundnut were significantly influenced by boron application (Table 2). The result showed that

| Treatments | Vegetative growth stage | Reproductive growth stage |
|------------|-------------------------|---------------------------|
| $E$ (mmol m$^{-2}$ s$^{-1}$) | $g_s$ (mol m$^{-2}$ s$^{-1}$) | LVPD (kPa) | $E$ (mmol m$^{-2}$ s$^{-1}$) | $g_s$ (mol m$^{-2}$ s$^{-1}$) | LVPD (kPa) |
| $B_0$ | 9.59 e | 0.207 e | 4.42 a | 10.22 e | 0.253 d | 4.58 a |
| $B_1$ | 10.69 de | 0.333 d | 3.99 b | 11.47 de | 0.363 c | 4.18 b |
| $B_2$ | 12.48 bc | 0.427 b | 3.55 c | 12.84 bc | 0.453 b | 3.65 c |
| $B_3$ | 15.32 a | 0.520 a | 3.10 d | 16.07 a | 0.543 a | 3.22 d |
| $B_4$ | 13.59 b | 0.417 bc | 2.97 d | 14.04 b | 0.453 b | 3.06 e |
| $B_5$ | 11.08 cd | 0.357 cd | 2.25 e | 11.80 cd | 0.377 c | 2.55 f |

Notes: $B_0$ = 0 ppm, $B_1$ = 0.5 ppm, $B_2$ = 1 ppm, $B_3$ = 2 ppm, $B_4$ = 4 ppm, $B_5$ = 8 ppm. $P$ = probability. Means were separated by Tukey’s test at $P \leq 0.05$. All of the parameters were studied both in vegetative growth stage and reproduce growth stages.
the values of LA, LAR, LMR, RGR and NAR were the highest for B3, while the values of the traits were the lowest for B0. Higher LA accelerates the production of metabolites. Prieto et al. (2007) stated that higher LA resulted in an increase in the plant’s ability to intercept light. The higher the growth parameter i.e. LA, LAR and LMR might be because of supply of 2 ppm boron containing the required nutrient which has the ability to produce higher metabolites in groundnut. The growth analysis data suggest that boron at 2 ppm provided better nutrition to the plant. This was most relevant in higher RGR and NAR because of application boron at 2 ppm. Moreover, the optimal supply of boron in treatment B3 could have the resulted in the maximum physiological growth.

Yield and yield contributing characters

Boron application affects the yield and yield contributing character of the groundnut. Significant differences were observed in a number of pegs plant$^{-1}$, number of pods plant$^{-1}$ and pods dry weight plant$^{-1}$ as a result of boron application (Table 3). Treatment B3 produced the maximum number of pods plant$^{-1}$ and pegs plant$^{-1}$. This might be because of an adequate supply of boron provides by treatment B3 resulting in the maximum reproductive growth. Kaisier et al. (2010) reported that boron had a pronounced effect on stigma receptivity, sticky and to make the pollen grain fertile for enhancing the pollination. As a result, boron resulted in the maximum number of pegs, pods (Naiknaware et al. 2015) and pods dry weight plant$^{-1}$ (Quamruzzaman et al. 2016a). The present finding consistent with their findings.

Shelling percentage

Shelling percentage obtained for the varying levels of boron were significantly different (Table 3). The highest value of shelling percentage was recorded for B3 than the other treatments. The reason behind the finding might be because of the boron was involved in cell development and grain formation (Naiknaware et al. 2015). Kabir et al. (2013) stated that boron was responsible for producing healthier seeds in groundnut. The result agreed with these findings.

Table 2. Effect of boron on physiological growth of groundnut.

| Treatments | LA (cm²) | LAR (cm² g⁻¹) | LMR (g g⁻¹) | RGR (g g⁻¹ d⁻¹) | NAR (g cm⁻² d⁻¹) |
|------------|----------|---------------|-------------|-----------------|------------------|
| B0         | 105.99 d | 74.40 e       | 0.38 d      | 0.036 e         | 0.000489 b       |
| B1         | 113.43 bc| 79.45 cd      | 0.42 c      | 0.039 cd        | 0.000495 ab      |
| B2         | 117.59 ab| 82.41 b       | 0.45 bc     | 0.042 b         | 0.000510 ab      |
| B3         | 122.11 a | 86.11 a       | 0.52 a      | 0.046 a         | 0.000530 a       |
| B4         | 115.127 bc| 80.53 bc     | 0.49 ab     | 0.041 bc        | 0.000513 ab      |
| B5         | 110.65 cd| 77.37 d       | 0.44 c      | 0.038 de        | 0.000491 b       |

Notes: B0 = 0 ppm, B1 = 0.5 ppm, B2 = 1 ppm, B3 = 2 ppm, B4 = 4 ppm, B5 = 8 ppm. $P$ = probability. Means were separated by Tukey’s test at $P \leq 0.05$. $\mu$mol m⁻² s⁻¹

Figure 1. Effect of boron on net photosynthetic rate under different photosynthetic photon flux (PPF) at vegetative (A) and reproductive (B) growth stage of groundnut. B0 = 0 ppm, B1 = 0.5 ppm, B2 = 1 ppm, B3 = 2 ppm, B4 = 4 ppm, B5 = 8 ppm. Vertical bars represent the SE of the treatment means.
et al. 2015). The similar results were also reported by Naiknaware and Pal 2014. This finding consistent with the finding of Quamruzzaman et al. (2013).

Boron had a significant effect on the harvest index of groundnut plant (Table 3). Treatment B3 resulted in the lowest HI while the highest was obtained for B4. The reason for this could be that the economic yield was higher in B3 compared to other treatments. The result is in close conformity with the findings of Kabir et al. (2013).

The result indicated that along with the increase in boron concentration (>2 ppm), the physiological growth, yield contributing character, yield, shelling percentage and harvest index decreased.

Biochemical properties

Statistically significant differences were observed in the protein and oil content after different boron doses (Table 4). B4 produced the highest protein and oil content and lowest for B0. This might be because of the appropriate supply of boron was containing the required nutrient to produce the higher protein as well as oil. Because boron played an important role in the synthesis of essential amino acids, and protein that acts as an electron carrier in the photosynthetic process required for producing of oil (Naiknaware et al. 2015). The similar results were also reported by Quamruzzaman et al. (2016b).

There was a significant difference in the vitamin E content of different boron treatments. The highest vitamin E content was recorded for B4 (Table 4). The possible reason behind the finding was that boron helped to uptake the highest value of potassium (Nasef et al. 2006) and potassium involved to increase the Vitamin E content (Caretto et al. 2008). So, this result might be because of adequate boron supplementation. This finding consistent with the findings of Quamruzzaman et al. (2016b).

The result indicated that along with the increasing in boron concentration (>4 ppm), protein, oil and vitamin E content decreased.

Harvest index

Boron had a significant effect on the harvest index of groundnut plant (Table 3). Treatment B3 resulted in the lowest HI while the lowest was obtained for B0. The reason for this could be that the economic yield was higher in B3 compared to other treatments. The result is in close conformity with the findings of Kabir et al. (2013).

The result indicated that along with the increase in boron concentration (>2 ppm), the physiological growth, yield contributing character, yield, shelling percentage and harvest index decreased.

Table 3. Effect of boron on yield and yield contributing characters of groundnut.

| Treatments | No. of peg | No. of pod | 100 Seed weight (g) | Pod weight plant (gm) | Shelling % | HI (%) |
|------------|-----------|------------|---------------------|----------------------|------------|--------|
| B0         | 32.67 d   | 60.33 e    | 85.33 d             | 55.09 e              | 63.94 e    | 30.71 e |
| B1         | 36.33 c   | 64.00 d    | 88.33 c             | 57.50 d              | 67.68 d    | 35.62 d |
| B2         | 41.00 b   | 71.33 bc   | 93.67 b             | 62.58 bc             | 71.73 bc   | 39.34 bc |
| B3         | 46.66 a   | 78.00 a    | 99.33 a             | 67.49 a              | 75.89 a    | 43.32 a |
| B4         | 43.33 b   | 73.67 b    | 96.00 b             | 64.06 b              | 73.73 b    | 41.30 ab |
| B5         | 38.33 c   | 68.67 c    | 90.33 c             | 60.83 c              | 69.73 c    | 37.32 cd |

Boron (B)

Notes: B0= 0 ppm, B1= 0.5 ppm, B2= 1 ppm, B3= 2 ppm, B4= 4 ppm, B5= 8 ppm. P = probability. Means were separated by Tukey’s test at P ≤ .05.

Table 4. Effect of boron on biochemical properties of groundnut.

| Treatments | Protein (%) | Oil (%) | Vitamin E (mg/100 g) |
|------------|-------------|---------|----------------------|
| B0         | 32.27 e     | 44.39 e | 5.47 d               |
| B1         | 33.49 d     | 46.41 d | 6.57 c               |
| B2         | 35.73 c     | 48.31 c | 7.04 c               |
| B3         | 38.36 ab    | 51.71 b | 7.73 b               |
| B4         | 40.32 a     | 53.78 a | 9.27 a               |
| B5         | 37.10 bc    | 50.22 b | 8.67 a               |

Boron (B)

Level of significance (P) at 5%

Notes: B0= 0 ppm, B1= 0.5 ppm, B2= 1 ppm, B3= 2 ppm, B4= 4 ppm, B5= 8 ppm. P = probability. Means were separated by Tukey’s test at P ≤ .05.

Conclusion

In conclusion, the values obtained for all physiological growth parameters, well as gas exchange (except LVPD), yield and yield contributing character for treatment B3 were higher than those obtained for other treatments. Moreover, values obtained for biochemical traits were higher in B4 than other treatments. Thus, B3 can be used to culture groundnut as it provides higher gas exchange, yield and B4 can be used to get higher protein, oil and vitamin E content.

Disclosure statement

No potential conflict of interest was reported by the authors.

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