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MORPHOLOGICAL AND BIOCHEMICAL RESPONSES TO BORON AND ZINC FERTILIZERS IN *STEVIA REBAUDIANA*

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Abstract

An experimental study was conducted in the experimental plot at the M.J.P. Rohilkhand University to investigate the impact of boron and zinc fertilization on growth, biomass and biochemical indexes in Stevia. Experimental plants were treated with three different concentrations of boron (as B_1, B_2 and B_3 with 2, 4 and 6 kg ha^{-1} respectively) and zinc (as Zn_1, Zn_2 and Zn_{3}- with 5, 10 and 15 kg ha^{-1} respectively) each. The data documented from the experiments shows that all studied parameters were enhanced by the nutrients application. However, Zn_3 was noticed with the highest values (per plant) for plant height (61.52 cm), number of branches (31.8), number of leaves (371.8), leaf area (2522.6 cm^2), leaf area index (4.41), fresh weight (72.61 g), dry weight (19.47 g), whereas biochemical indexes (in mg g^{-1}) were documented as chlorophyll a (3.939), chlorophyll b (1.410), total chlorophyll (5.347), carbohydrate (513.25), protein (85.02) and nitrogen (57.60).

Key words : boron, zinc, growth, biomass, nutrients.

Introduction

Stevia rebaudiana, a natural sweetener plant was originated in the South American countries (Paraguay and Brazil) and representing the family Asteraceae (Bertoni, 1905). Stevia is a new crop and its cultivation is gaining popularity in many countries of the globe because of the sweetness of leaves, which contain steviol glycosides. Stevia dry leaves are 20 to 30 times sweeter than the cane sugar without any processing and most importantly without any calorie. As it is non caloric sweetener it is a safe and beneficial source of sweetness and a good alternative of not only cane and beet sugar but also some artificially synthesized sweeteners like saccharine, acesulpham-K, aspartame and cyclamate which have some adverse effect on human health. Therefore, to avoid problems leading by the artificial sweeteners, the demand of Stevia sweeteners has been increased in many food supplements such as chocolates, biscuits, juices, marmalades, ice-creams, sweets, beverages and candy (Ojha *et al.*, 2010). Besides these Stevia is also have many medicinal and therapeutic uses such as hypoglycemic, obesity, diabetes, dental health, cardiovascular, digestion, oral contraceptive, hypertension, antimicrobial activity and skin problems (Dryskog *et al.*, 2005).

Commercially Stevia was first time cultivated in Paraguay in 1964 and since then its cultivation has been taken place in many countries like Japan, China, UK, USA, Canada, South Korea, Belgium, Thailand, Spain, Austrailia, Taiwan, Malaysia etc. (Chatsudthipong and Muanprasat, 2009; Pal *et al.*, 2015). Stevia has also been introduced in India and cultivated successfully in various parts of countries. Stevia mainly propagated via means of vegetative propagation because seeds are viable only for a short time period and show very poor germination rate. Additionally seeds raised plants also show variation from their mother plant so their steviol glycosides concentration may also be vary (Rank and Midmore, 2006). Stevia is propagated vegetatively via the means of stem cuttings. Loam or sandy loam soils with slightly acidic pH range are better for the growth of Stevia plant. It requires temperature ranging from 20 to 35ºC and long days for higher leaf yield and glycosides production as short days initiating flowering which reduced the glycosides concentration (Singh *et al.*, 2014).

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Stevia growth and biomass production is affected by several factors like genetic, environmental and nutrient availability. Nutrients required for plants growth may be categorized in macronutrients (such as nitrogen, potassium, phosphorus, calcium etc.) and micronutrients (such as boron, copper, manganese, zinc, molybdenum etc.). Some studies observe the significance of macronutrients (nitrogen, potassium and phosphorus) on Stevia growth but role of micronutrients are not much explored on Stevia growth and biomass yield. Therefore, in this research the role of boron (B) and zinc (Zn) is investigated on the Stevia.

B is crucial element required for the important functions like cell division, sugar transport, development of cell wall, seeds, fruits and hormone. B deficiency has numerous effects on various processes in higher plants like root elongation, carbohydrate metabolism, sugar translocation, IAA oxidase activity, growth of pollen tube and synthesis of nucleic acids (Blevins and Lukaszewski, 1998; Goldbach and Wimmer, 2007). B toxicity is also well-known and altered metabolism, reduces in chlorophyll quantity and photosynthetic rates, decreases root cell division and reduces suberin and lignin level in higher plants (Nable *et al.*, 1997; Reid, 2007).

Zn is also have a crucial role in many physiological and biochemical aspects and present as fundamental structural component in many proteins like transcription factors and metalloenzymes (Figueiredo *et al.*, 2012). In plants, it is necessary for oxidation-reduction reactions, metabolic reactions and various enzymatic reactions such as involve in nitrogen metabolism, protein synthesis and energy transfer (Hafeez *et al.*, 2013). Therefore, the deficiency of Zn has significant impact on these physiological and metabolic functions and affects growth, quality and productivity of plants. However, high quantity of Zn is also having toxic effects on plants and toxicity of Zn is depends on soil pH that maintains the Zn concentration in the soil solution (Daviscarter and Shuman, 1993).

Materials and Methods

An experimental study was carried out in the experimental plot of the Plant Science department of M. J. P. Rohilkhand University, Bareilly to observe the significance of B and Zn fertilizer applications on Stevia plants. Bareilly is situated at 79.5°E longitude and 28.5°N latitude with altitude of 172.21 meter above the sea level. The climate of Bareilly is humid subtropical, hot dry during summers and cold during winters. Generally, the average maximum temperature during summers is 42 to 43°C and the average minimum temperature during winters lies between 6.4 to 6.8°C. Bareilly has the average annual rainfall 787.16 mm and relative humidity between 87-100 percent from July to February which steadily decreasing upto 50 percent by first week of May.

The experimental field has the sandy clay loam soil. Stevia planting material was acquired from CIMAP Pantnagar, Uttarakhand, India. The size of experimental plots was 1×1 meter square and plants were planted at the spacing of 30×30 cm followed by light irrigation. The research experiment consisting three dosages of B (as B_1, B_2 and B_3 with 2, 4 and 6 kg ha^{-1} respectively) and zinc (as Zn_1, Zn_2 and Zn_{3-} with 5, 10 and 15 kg ha^{-1} respectively) each along with one control (C-without any treatment). The doses of B were applied by using boric acid (H_3BO_3) whereas Zn doses were applied by using zinc sulphate ($ZnSO_4.7H_2O$) in two equal splittings: first during the transplanting and second at the 30 days after transplanting. Various growth parameters like plant height, number of branches and leaves, leaf area (LA), leaf area index (LAI), fresh weight (FW) and dry weight (DW) were studied and recorded by selecting five plants randomly from each replicates after 70 days of transplanting. Chlorophyll content (Arnon, 1949) from the fresh leaves while carbohydrate (Morris *et al.*, 1949), protein (Lowry *et al.*, 1951) and nitrogen content (Lang *et al.*, 1958) from the dry leaves were also determined.

Results and Discussion

The mean data represented in table 1 show that the implementation of different doses of B and Zn has significantly enhances studied parameters in Stevia in comparison to untreated (control) plants. Zn_3 treatment shows the highest values for parameters like plant height (61.52 cm), branch number (31.8), leaf number (371.8), leaf area (2522.6 cm^2), LAI (4.41), fresh weight (72.61 g) and dry weight (19.47 g). Control has the lowest value for each parameter recorded as plant height (49.04 cm), branch number (23.4), leaf number (315.8), leaf area (2124.8 cm^2), LAI (4.07), fresh weight (58.65 g) and dry weight (15.85 g). However among the fertilizers applied treatments B_1 has recorded with the least plant height (51.88 cm), number of branches (25.2) while Zn_1 is recorded with least number of leaves (336.4), leaf area (2264.6 cm^2), LAI (4.14), fresh weight (62.24 g) and dry weight (16.78 g).

The different concentrations of B and Zn fertilization also affected chlorophyll, carbohydrate, protein and nitrogen content and observed data in dry leaves of Stevia was presented in table 2. These parameters were significantly influenced by the diverse concentrations of both nutrients (B and Zn). Zn_3 was noted with higher

Table 1: Influence of micronutrients (B and Zn) on growth and yield parameters.

Treatment	Parameters (per plant)						
	Plant Height (cm)	No. of Branches	No. of Leaves	Leaf Area (cm^2)	Leaf Area Index	Fresh Weight (g)	Dry Weight (g)
Control	49.04±1.880	23.4±1.342	315.8±12.518	2124.8±68.532	4.07±0.102	58.65±2.279	15.85±0.593
B_1	51.88±2.005	25.2±2.049	339.4±14.293	2285.2±57.159	4.18±0.007	62.69±3.132	16.90±0.788
B_2	57.26±2.327	28.2±1.924	358.8±13.846	2430.2±70.581	4.31±0.062	68.01±3.190	18.28±0.850
B_3	60.02±2.073	30.6±2.074	365.2±16.483	2484.0±61.968	4.34±0.054	70.25±3.376	18.84±0.741
Zn_1	52.38±1.663	26.8±1.304	336.4±13.885	2264.6±64.314	4.14±0.076	62.24±3.000	16.78±0.796
Zn_2	57.90±2.193	29.4±1.140	357.2±13.065	2419.8±74.905	4.29±0.079	67.48±2.821	18.13±0.695
Zn_3	61.52±2.363	31.8±2.387	371.8±14.446	2522.6±76.170	4.41±0.093	72.61±3.040	19.47±0.809

Table 2: Influence of micronutrients (B and Zn) on biochemical parameters.

Treatment	Parameters (mg per g)					
	Chlorophyll a	Chlorophyll b	Total Chlorophyll	Carbohydrate	Protein	Nitrogen
Control	2.956±0.009	1.044±0.006	4.000±0.014	433.50±1.446	67.51±1.829	46.08±1.731
B_1	3.029±0.016	1.022±0.010	4.050±0.026	450.50±1.639	70.04±1.197	48.48±1.543
B_2	3.437±0.005	1.170±0.003	4.606±0.007	486.25±0.901	78.34±1.004	54.24±0.831
B_3	3.806±0.012	1.335±0.007	5.141±0.019	510.75±0.661	84.79±0.610	56.64±1.731
Zn_1	3.042±0.005	1.013±0.003	4.054±0.007	446.50±0.433	70.73±0.922	48.96±1.686
Zn_2	3.389±0.002	1.227±0.004	4.615±0.006	483.75±0.750	79.72±1.613	54.72±1.466
Zn_3	3.939±0.016	1.410±0.011	5.347±0.025	513.25±1.250	85.02±1.161	57.60±0.831

values (in mg g^{-1}) chlorophyll a (3.939), chlorophyll b (1.410), total chlorophyll (5.347) in fresh leaf while carbohydrate (513.25), protein (85.02) and nitrogen (57.60) content in dry leaf powder of Stevia than the all nutrient treatments and control. Control was recorded for the lowest values for all parameters, while among nutrient applied treatments B1 recorded least values (in mg g^{-1}) for chlorophyll a (3.029), total chlorophyll (4.050), protein (70.04), nitrogen (48.48) and Zn1 recorded with least value (in mg g^{-1}) for chlorophyll b (1.013) and carbohydrate (446.50).

Data obtained from the present study on plant growth parameters showed an increase with the B and Zn fertilizers application. Quddus *et al.*, (2011) found that in mungbean application of B and Zn increase plant height, pod number and seeds number per plant. Quddus *et al.*, (2014) also found similar effect on lentil with B and Zn application. In radish, Deepika and Pitagi, (2015) with the Zn and B application, noticed a rise in plant height and leaf number. Alam and Islam, (2016) noticed increased branch number, plant height, pod length, pod number, seeds number in mungbean plants treated with B and Zn fertilizers whether alone or in combination. In rice, Kumar *et al.*, (2017) also reported an enhancement in plant height with boron and zinc. In Pakistan, Bhutto *et al.*, (2018) noticed the influence of B and Zn on productivity and protein content in five cultivars of rice and found stimulatory effects on both parameters. Bhat *et al.*, (2018) perform the investigation to observe the impact of B and Zn in onion and found enhanced growth parameters including plant height, leaf size and number. Karuppaiah (2019) investigated the influence of B and Zn on the tuberose and found effective to enhance the plant height, leaf area, leaf number, dry mass, chlorophyll content and yield attributes.

Uikey *et al.*, (2018) studied the role of foliarly applied B and Zn that positively affected to plant height, leaf number, branch number, leaf area and LAI in brinjal. Haleema *et al.*, (2018) also found similar effect with foliarly applied B and Zn in tomato. Hisamitsu *et al.*, (2001) and Samreen *et al.*, (2013) reported that Zn enhances the protein and chlorophyll content in mungbean plants grown in hydroponic system. Dalal *et al.*, (2011) suggested that foliarly applied B and Zn positively affected nitrogen content and yield in Ber. Singh *et al.*, (2015) noticed that Zn application increase the protein content in wheat. Ali *et al.*, (2015) studied the influence of B fertilization in tobacco leaves and concluded that nitrogen content initially increased but decreased at higher concentrations of B.

Conclusion

The results obtained from the current experimental study concluded that B and Zn nutrient supply improve the growth and biomass yield parameters compared to control. These nutrients also enhance the biochemical attributes like chlorophyll, carbohydrate, protein and nitrogen content with increasing concentrations of B from 2-6 kg ha^{-1} while Zn from 5 to 15 kg ha^{-1}. B is a key micronutrients and important for development of cell wall, cell division, activity of hormones and sugar transport. However, higher concentration of B may results to reduction in chlorophyll that leads to reduction in photosynthetic rates and also causes cell division inhibition. In plants, Zn is also an important micronutrient to regulate and maintain protein synthesis and nitrogen metabolism. Zn at above the optimum level may also have a negative impact on plant growth and plant biomass yield.

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