Foliar feeding of boron influencing biochemical attributes and enzyme activity in dragon fruit (*Selenicereus monacanthus*)

Ankita SAHU¹, Kundan KISHORE²*, Satya N. DASH³, Sarat C. SAHOO³, Rabi K. NAYAK³, Bijay K. BAIDYA³, Kuldeep K. SHUKLA³, Subhashree BARIK²

¹ICAR-Central Institute for Women in Agriculture, Baramunda, Bhubaneswar, 751003, India; sahankita29@gmail.com
²Central Horticultural Experiment Station (ICAR-IIHR), Aginia, Bhubaneswar, 751019, India; kkhort12@gmail.com (*corresponding author); sbaik.1997@gmail.com
³Odisha University of Agriculture and Technology, Siripur, Bhubaneswar, 751003, India; satya1992@yahoo.co.in; chsarat.sahoo@gmail.com; rabiosolorissa@gmail.com; bijay.baidya8@gmail.com; shuklahort7854@gmail.com

**Abstract**

Boron plays crucial role in metabolic processes during fruit ripening and in turn ensures better fruit quality. However limited studies have been conducted to assess the influence of boron on fruit quality of dragon fruit. In the present study, the efficacy of boron was investigated on red-fleshed dragon fruit (*Selenicereus monacanthus*). Four levels of boron (100 mg L⁻¹, 200 mg L⁻¹, 300 mg L⁻¹ and 400 mg L⁻¹) were applied on 7- and 14-day-old flower buds. The highest pollen germinability, seed weight, fruit weight (274.32 ± 36.72 g), pulp content (70.80 ± 1.79%) and pulp firmness (2.74 ± 0.18 N) were recorded when B was applied at 300 mg L⁻¹ on 7-day old flower bud. The same treatment also manifested higher soluble solid contents (17.42 ± 0.62 °Brix), sugar content, total carbohydrate (15.92 ± 1.12%), protein (1.33 ± 0.11%), ascorbic acid (112.66 ± 4.98 µg/g), betacyanin (32.86 ± 2.52 µg/g), total phenol (95.26 ± 3.72 µg GAE/100g), total flavonoid (37.65 ± 2.14 mg QE/100g) and anti-oxidative activity (27.71 ± 2.14 mM Fe II/100g). Correlation studies elucidated significant positive influence of pollen germinability on fruit weight, pulp content and pulp firmness. The activities of α-amylase, invertase and sucrose synthase enzymes were significantly upregulated with the application of B 300 mg L⁻¹ on 7-day old flower bud. On the other hand, the activities of cell wall degrading enzymes such as cellulase, polygalacturonase and pectin methyl esterase were reduced with increasing levels of boron. The principal component analysis (PCA) illustrated the maximal proximity of most of the quality attributes with B 300 mg L⁻¹, applied at 7-day old flower bud stage, thus exemplifying it as the best treatment.

**Keywords:** antioxidant activity; boron; dragon fruit; enzyme activity; pollen germination

**Introduction**

Nutrients play vital roles in influencing physiological and metabolic processes that regulate vegetative and reproductive development of plants. Boron, an essential micronutrient, has a well-established role in...
reproductive development of plants through pollen germination and other physiological events. Additionally, it also influences fruit development and improves fruit quality by regulating biochemical processes (Storey, 2007). Pollen with high germinability exhibit profound impact on fruit growth and quality (Kamiloglu, 2011; Marschner, 2011). Boron is involved in several metabolic pathways including sugar transport during fruit development and maturation. It also stimulates the synthesis of sucrose by upregulating the activity of sucrose synthase. Boron is also involved in maintenance of fruit firmness by reducing the activity of cell wall degrading enzymes (Muengkaew et al., 2018). It has also been reported that for obtaining better fruit quality, boron should be available in optimum range in plant tissues (Mousavi et al., 2020).

Dragon fruit is considered as an important exotic fruit crop on account of its nutritional significance and market potential. It is commercially grown in Southeast Asia, China, Central and tropical American countries. However, the crop is also being popularly cultivated in tropical regions of India (Wichienchot et al., 2010; Perween et al., 2018). Dragon fruit belongs to the family Cactaceae and genus *Selenicereus* (syn. *Hylocereus*). Among different species in *Selenicereus*, *S. monacanthus* is one of the most important species due to the presence of betalain in pulp. The fruit quality in dragon fruit is attributed to the presence of sugar, vitamins, minerals, dietary fibres, phenolic compounds and antioxidants (Korotkova et al., 2017; Priatni and Pradita, 2015). Additionally, the enzymatic activities in fruits during ripening contribute towards overall fruit quality (Lima et al., 2001). Dragon fruit is characterized with the occurrence of multiple flushes during May-October. Reproductive buds of dragon fruit appear singly (common) or in pair on areoles of a mature shoot. Bud grows rapidly and transform into fully developed flower within three weeks. On the other hand, fruit attains harvestable maturity after a month of anthesis (Kishore, 2016). Hence fruiting behaviour of dragon fruit (long spell) necessitates effective management of nutrients including micronutrients. In acidic soil, boron becomes one of the major limiting micronutrients which in turn affects fruit set, fruit development and fruit quality.

Unlike other micronutrients, boron has restricted mobility in plant system, hence foliar application is commonly followed in fruit crops to improve fruit quality attributes (Ali et al., 2017). Optimum quantification of boron application is vital for obtaining maximal fruit quality attributes. Additionally, plant species, as well as the genotypes within the species, dramatically differ in terms of boron requirements (Brdar-Jokanović, 2020). Since boron is considered as the most important micronutrient during fruit set and development, its deficiency significantly affects yield and quality. In India, dragon fruit is mainly cultivated in red and yellow soils which are acidic and low in boron content, and under such condition the quality of fruit is affected. Limited studies have been conducted on influence of boron on quality of dragon fruit. Hence, the present investigation is aimed to assess the influence of boron on pollen germination and fruit quality characteristics including enzyme activities of red-fleshed dragon fruit (*Selenicereus monacanthus*) under eastern tropical region. The study is based upon the hypothesis that optimization of boron concentration and stage of application will facilitate quality improvement in dragon fruit.

**Materials and Methods**

**Experimental site and plant materials**

The experiment was carried out during 2020 and 2021 at the Central Horticultural Experiment Station (ICAR-IIHR), Bhubaneswar, India located at latitude 20°27′ N, longitude 85°40′ E. The site is characterized by annual average air temperature of 26.9 °C, with relative humidity of 77.6% and annual rainfall nearly 1500 mm. The soil of the site was sandy loam and strongly acidic with low organic carbon. Soil was deficient in nitrogen, calcium, magnesium, sulphur and boron, and moderate in potassium content. In contrast, the content of phosphorous, iron, zinc, copper and manganese were high in the soil (Table 1). Red-fleshed dragon fruit (CHDF-1) were trained on single post system spaced at 2m x 3m spacing. Four plants were accommodated in
each hill. The plantation was installed with drip irrigation system, with the provision of mulching. The crops in the experiment field received a uniform package of practices for nutrient and disease management. For nutrient management a uniform dose of N, P and K was applied @ 500g, 200g and 300g hill⁻¹ year⁻¹, respectively in three splits (March, July and November). Diseases such as soft rot and anthracnose were managed with the removal of affected shoot and application of systemic fungicides such as tebuconazole and trifloxystrobin.

Table 1. Physiochemical properties of the soil of experimental site

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sampling depth (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0-30</td>
</tr>
<tr>
<td>pH</td>
<td>5.08 ± 0.14</td>
</tr>
<tr>
<td>EC (dS m⁻¹)</td>
<td>0.60 ± 0.04</td>
</tr>
<tr>
<td>Texture</td>
<td>Sandy loam</td>
</tr>
<tr>
<td>Sand (%)</td>
<td>68.73 ± 2.12</td>
</tr>
<tr>
<td>Silt (%)</td>
<td>12.48 ± 1.13</td>
</tr>
<tr>
<td>Clay (%)</td>
<td>18.79 ± 1.43</td>
</tr>
<tr>
<td>Organic carbon (g kg⁻¹)</td>
<td>2.74 ± 0.32</td>
</tr>
<tr>
<td>Available N (mg kg⁻¹)</td>
<td>45.78 ± 3.22</td>
</tr>
<tr>
<td>Available P (mg kg⁻¹)</td>
<td>37.32 ± 4.11</td>
</tr>
<tr>
<td>Available K (mg kg⁻¹)</td>
<td>87.55 ± 7.22</td>
</tr>
<tr>
<td>Exchangeable Ca (mg kg⁻¹)</td>
<td>478.62 ± 35.98</td>
</tr>
<tr>
<td>Exchangeable Mg (mg kg⁻¹)</td>
<td>103.26 ± 5.98</td>
</tr>
<tr>
<td>Available S (mg kg⁻¹)</td>
<td>20.49 ± 3.21</td>
</tr>
<tr>
<td>Available Fe (mg kg⁻¹)</td>
<td>62.38 ± 8.32</td>
</tr>
<tr>
<td>Available Mn (mg kg⁻¹)</td>
<td>11.81 ± 1.23</td>
</tr>
<tr>
<td>Available Cu (mg kg⁻¹)</td>
<td>1.17 ± 0.11</td>
</tr>
<tr>
<td>Available Zn (mg kg⁻¹)</td>
<td>0.69 ± 0.03</td>
</tr>
<tr>
<td>Available B (mg kg⁻¹)</td>
<td>0.32 ± 0.03</td>
</tr>
</tbody>
</table>

Treatments and experimental design

Four-year-old healthy red-fleshed dragon fruit (*Selenicereus monacanthus*) plants with uniform growth and vigour were selected for experimentation. Two border rows were considered as guard rows. Prior to imposition of treatments, four mature shoots were tagged in each plant (16 mature shoots/hill) and only one flower bud was allowed to develop on the tagged shoots. The number of flower buds per hill was kept constant (16) under different treatments. Boron was applied @ 100 mgL⁻¹, 200 mgL⁻¹, 300 mgL⁻¹ and 400 mgL⁻¹, on 7- and 14-day-old flower buds in the month of July. Former stage of flower bud signified beginning of pollen development, whereas latter stage indicated pollen maturation (Kishore, 2016). Experiment was laid out in a randomized block design with 9 treatments (T1 to T9), each with three replications and each replication further comprised of 12 plants (3 hills). T1 indicates control (water spray), whereas T2, T3, T4 and T5 designated the application of B @ 100 mgL⁻¹, 200 mgL⁻¹, 300 mgL⁻¹ and 400 mgL⁻¹ on 7-day-old flower buds respectively. Application of B @ 100 mgL⁻¹, 200 mgL⁻¹, 300 mgL⁻¹ and 400 mgL⁻¹ on 14-day-old flower buds was represented by T6, T7, T8 and T9, respectively. Boron was applied in the form of H₃BO₃ (99.5% purity) in the afternoon (15.00 h – 16.00 h) and in order to ensure uniformity in B concentration, one litre of solution was sprayed on each hill containing four plants and 16 flower buds. Under all the treatments, flower buds were bagged a day before anthesis to ensure autogamy and to avoid cross pollination.

Pollen germination and physical traits

For *in-vitro* pollen germination test, agar plate method was followed with sucrose (20%) and boron (200 mgL⁻¹) as a medium and on the basis of number of germinated pollen and total number of pollen (n=400),
percentage germinability was worked out (Metz et al., 2000). For estimation of physical attributes forty ripe fruits (four weeks after anthesis) were randomly selected under each treatment and brought to laboratory. Fruits were washed and kept for about 30 minutes at ambient temperature 25 °C. Fruit weight (g) was measured with the aid of a physical balance and equatorial length was determined with vernier calliper. The pulp firmness was measured with a digital fruit firmness tester (Lutron, FR 5120) using a 3-mm tip and results expressed in Newton (N) /mm. Pulp percentage was worked out on the basis of pulp content and fruit weight. Peel thickness was measured with vernier callipers (5-6 measurements) and the expressed in mm. For estimation of number of seeds, 100g pulp preferably from the central region of the fruit was weighed and seeds were extracted and counted manually. The seed test weight was computed (g) by taking the weight of 1000 seeds.

### Biochemical attributes

Total soluble solids (TSS) of fruit were evaluated with hand refractometer at 25 °C and expressed as °Brix. The reducing sugar of the sample was estimated by dinitro salicylic acid assay (DNS assay) following the protocol of Miller (1959), with minor modification. The dragon fruit juice was diluted 50 times with distilled water prior to estimation. The final absorbance of the reaction mixture was measured at 540 nm and the sugar content in the sample was expressed in percentage, quantified from the standard curve prepared with different concentration of D-glucose. The protocol laid down by Van Handel (1968) was used for sucrose determination. The methodology is based upon the principal of destruction of reducing sugar in the sample in hot conc. alkali (KOH), followed by determination of sucrose using anthrone reagent at low temperature. The absorbance was measured at 620nm and sucrose content in sample was quantified using the standard curve and expressed in percentage.

Total carbohydrate content of the fruit sample was estimated by phenol-sulphuric acid method following the protocol of Nielsen (2017). After incubation, absorbance of reaction mixture was measured at 490 nm. The standard curve was prepared with different concentration of D-glucose and content was expressed in percentage. For protein estimation, Bradford assay was followed. Clarified fruit juice 0.1 ml was mixed with Bradford reagent and incubated in dark for 20 minutes at room temperature. The absorbance was measured at a wavelength of 595 nm. A calibration curve was plotted by using Bovine Serum Albumin (BSA) as standard. Protein content was expressed in percentage.

Acidity in dragon fruit was measured in terms of citric acid following copper-ammonia complex method using dual wavelength (Farajzadeh and Nagizadeh, 2002). The reaction mixture comprised of sample, copper-ammonia complex and distilled water. Absorbance was measured at 600 nm and 750 nm. The citric acid content in fruit sample was expressed in percentage. The ascorbic acid was estimated following the method of Kapur et al. (2012) with slight modification. 2g of fruit sample was homogenized in 25 ml of 5% metaphosphoric acid and 10 % glacial acetic acid followed by centrifugation at 10000 rpm for 15 min. The supernatant was treated with bromine water, thiourea and 2,4-DNPH solution, followed by incubation at 37 °C for 3 hrs in water bath. The contents were cooled in ice, followed by addition of 85% sulphuric acid and absorbance was recorded at 521 nm. The ascorbic acid content in the fruit was quantified in terms of µg/g of fresh weight.

For estimation of betacyanin, total polyphenols, total flavonoids and FRAP activity, methanol (80%, v/v) was used as an extractant. The betacyanin content was quantified as per the protocol of Lim et al. (2011), where the absorbance of the extracted pigment was measured at 538 nm and betacyanin was quantified as BC (µg g⁻¹) = [(A × DF × MW × V x 1000) / (e × l x w)], where A is the absorbance, DF the dilution factor, MW the molecular weight of betacyanin (550g/mol), V the volume of extract (ml), e the molar extinction coefficient (65000 L/mol cm) and l the path length (1 cm) of the cuvette, w the fresh weight of extracted material (g). Total polyphenol content (TPC) was estimated with Folin-Ciocalteu (FC) reagent and expressed in gallic acid equivalent (GAE) 100 g⁻¹ fresh weight (fw) of edible portion of fruit (Ikram et al., 2009). Total flavonoid content (TFC) was estimated by aluminium chloride method and expressed in mg quercetin equivalent (QE)
Enzyme activity

Invertase activity was estimated following the method of Topcu et al. (2022). The enzyme extract was prepared by using 50 mM sodium phosphate buffer, pH 7.4, containing 1 mM 3-mercaptoproethanol. The reaction mixture includes 120 ml of enzyme extract, 480 ml of 100 mM acetate buffer (pH 7.5) and 100 mM sucrose, followed by incubation at 37 °C for 1 hr. The amount of invert sugar produced was determined as per Dinitro salicylic acid assay and invertase activity was expressed as nmol glucose liberated/mg protein. The α-amylase activity was estimated by the protocol as described by Lima et al., (2001). The sugar liberated through enzyme activity represented the amylase activity of the fruit sample which was quantified in terms of µmole maltose/ml/min. Sucrose synthase assay was performed with enzyme extract and Hepes-NaOH buffer (pH 7.5) and the enzyme activity was expressed as mmol sucrose produced/ min/ mg protein (Panda et al., 2015).

Cellulase activity was assayed by the modified protocol of Mohan et al. (2014). Citrate buffer (0.1 M, pH 5) was used for enzyme extraction from fruit sample. The reaction mixture consisted of 0.9 ml carboxymethyl cellulose and 0.1 ml enzyme extract, followed by incubation at 37 °C for 15 mins. The glucose liberated was assayed by DNS protocol and cellulase activity was quantified in terms of µ mole of glucose liberated/mg protein/min. The polygalacturonase assay was carried out as per the protocol of Lohani et al. (2004) with minor modifications. The reaction mixture comprised of 0.4 ml sodium acetate buffer (0.2 M, pH 4.5), 0.1 ml of Sodium chloride (200mmol/ l), 0.4 ml of polygalacturonic acid (1 % aqueous soln. in sodium citrate buffer with pH adjusted to 4.5) and 0.1 ml of the enzyme extract. After 1 hr of incubation at 37 °C, the reaction was terminated by adding 1 ml DNS reagent. The enzyme activity was expressed as mole of D-galacturonic acid liberated/ min/g. Pectin methyl esterase activity was determined as per the protocol laid down by Hagerman and Austin (1986) with slight modification. The initial absorbance of the reaction mixture, comprising of 2ml 0.5 % pectin, 0.15ml 0.01% bromothymol blue indicator and 0.83ml water was measured at 620 nm. The reaction was initiated by adding 100µl of enzyme extract and the enzyme activity was expressed as ∆ A620/min/protein content (expressed in mg/ml extract).

Statistical analysis

Analysis of variance (ANOVA) was performed to test the significant differences among means of various attributes using XLSTAT statistical software, whereas means were compared using Tukeys’ HSD test (P<0.05). Correlation graphs were made using R-statistical software. Principal component analysis (PCA) was performed to maximize the variance and increase the interpretability of the relationship between the variables and the observations. This analysis was carried out using the statistical software package XLSTAT.

Results

Pollen germination and quality attributes

The perusal of data illustrated a significant impact of boron on pollen germination (Table 2). The germinability of pollen grains indicated that application of boron at varying concentrations on 7-day old bud resulted in significant difference in pollen germination of dragon fruit. On the other hand, application of B on 14-day old bud did not result in substantial improvement in pollen germination. When applied at 7-day old bud stage, the boron level 300 mgL⁻¹ (T4) resulted in maximal germination of pollen grains (~74% increase over control). However, reduction in pollen germinability was observed when boron was applied at higher concentration 400 mgL⁻¹ (T5). The number of seeds in fruit as represented in terms of seeds per 100g pulp
represented an inconsistent trend as there was no significant effect of boron application on no. of seeds. Nevertheless, the seed test weight manifested an increment with boron treatment with highest test weight (~36% increase over control) recorded under T4. Alternatively, the seed test weight reduced significantly when boron was applied at higher concentration (400 mgL$^{-1}$). It was clearly illustrated from data that foliar application of boron on 14-day old buds could not exhibit substantial effect on pollen germinability, seed test weight and seed number (Table 2).

<table>
<thead>
<tr>
<th>Stage of flower bud</th>
<th>Boron level (mgL$^{-1}$)</th>
<th>Treatment</th>
<th>Pollen germination (%)</th>
<th>No. of seeds/100g pulp</th>
<th>Seed test weight (g)</th>
<th>Fruit wt. (g)</th>
<th>Pulp content (%)</th>
<th>Pulp firmness (N)</th>
<th>Peel thickness (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7-day old</td>
<td>Control</td>
<td>T1</td>
<td>39.12 ± 3.21$^a$</td>
<td>3927.88 ± 313.76$^b$</td>
<td>198.82 ± 23.54$^c$</td>
<td>64.92 ± 1.23$^d$</td>
<td>2.38 ± 0.17$^e$</td>
<td>3.11 ± 0.22$^f$</td>
<td>6.32 ± 0.15$^g$</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>T2</td>
<td>48.73 ± 4.41$^a$</td>
<td>3778.36 ± 298.67$^b$</td>
<td>221.52 ± 29.32$^c$</td>
<td>66.86 ± 1.42$^d$</td>
<td>2.43 ± 0.15$^e$</td>
<td>3.11 ± 0.22$^f$</td>
<td>6.32 ± 0.15$^g$</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>T3</td>
<td>54.32 ± 4.56$^a$</td>
<td>3820.76 ± 198.77$^b$</td>
<td>257.86 ± 31.22$^c$</td>
<td>68.72 ± 1.49$^d$</td>
<td>2.48 ± 0.17$^e$</td>
<td>3.11 ± 0.22$^f$</td>
<td>6.32 ± 0.15$^g$</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>T4</td>
<td>68.34 ± 5.22$^a$</td>
<td>3978.62 ± 178.65$^b$</td>
<td>274.32 ± 36.72$^c$</td>
<td>70.80 ± 1.79$^d$</td>
<td>2.74 ± 0.18$^e$</td>
<td>3.11 ± 0.22$^f$</td>
<td>6.32 ± 0.15$^g$</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>T5</td>
<td>47.33 ± 3.88$^a$</td>
<td>3819.87 ± 273.82$^b$</td>
<td>246.17 ± 33.52$^c$</td>
<td>67.55 ± 1.54$^d$</td>
<td>2.65 ± 0.14$^e$</td>
<td>3.11 ± 0.22$^f$</td>
<td>6.32 ± 0.15$^g$</td>
</tr>
<tr>
<td>14-day old</td>
<td>100</td>
<td>T6</td>
<td>40.88 ± 3.44$^a$</td>
<td>3989.18 ± 376.32$^b$</td>
<td>219.07 ± 28.77$^c$</td>
<td>66.70 ± 1.34$^d$</td>
<td>2.40 ± 0.15$^e$</td>
<td>3.11 ± 0.22$^f$</td>
<td>6.32 ± 0.15$^g$</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>T7</td>
<td>43.42 ± 3.55$^a$</td>
<td>3877.29 ± 355.32$^b$</td>
<td>221.33 ± 31.87$^c$</td>
<td>67.55 ± 1.49$^d$</td>
<td>2.49 ± 0.14$^e$</td>
<td>3.11 ± 0.22$^f$</td>
<td>6.32 ± 0.15$^g$</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>T8</td>
<td>42.22 ± 3.27$^a$</td>
<td>3827.88 ± 345.22$^b$</td>
<td>226.47 ± 21.54$^c$</td>
<td>67.52 ± 1.52$^d$</td>
<td>2.61 ± 0.16$^e$</td>
<td>3.11 ± 0.22$^f$</td>
<td>6.32 ± 0.15$^g$</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>T9</td>
<td>43.11 ± 3.41$^a$</td>
<td>3865.22 ± 331.32$^b$</td>
<td>227.41 ± 25.32$^c$</td>
<td>66.32 ± 1.34$^d$</td>
<td>2.62 ± 0.13$^e$</td>
<td>3.11 ± 0.22$^f$</td>
<td>6.32 ± 0.15$^g$</td>
</tr>
</tbody>
</table>

Mean values followed by the same letter in each column show non-significant difference at $P < 0.05$.

The physical attributes of fruits such as fruit weight, pulp content, pulp firmness and peel thickness established substantial variation with boron treatment (Table 2). Foliar application of boron on 7-day old bud manifested an improvement in desirable fruit physical attributes, which magnified with increasing boron level. Maximal fruit weight, pulp content and pulp firmness were achieved when boron was applied @300 mgL$^{-1}$ on 7-day old bud (T4). The fruit weight under T4 manifested an increase of about 38% over control, while pulp content increased by about 9%. A significant surge in pulp firmness (~15% over control), however, a decline in peel thickness (~14% over control) were also recorded under T4. The application of boron clearly illustrated that the influence on fruit physical attributes was not remarkable when boron was applied on 14-day old bud. It was evident from the results that higher concentration of boron (400 mgL$^{-1}$) did not strike substantial enhancement in desirable fruit physical characteristics.

Correlation studies clearly indicated positive influence of pollen germinability on fruit weight ($r = 0.90^{**}$), pulp content ($r = 0.91^{**}$) and pulp firmness (Figure 1). Conversely, pollen germination established a negative correlation with peel thickness ($r = -0.69^{*}$). The fruit weight was positively correlated with seed test weight ($r = 0.95^{**}$). Additionally, the pulp content also established positive significant correlation with seed test weight ($r = 0.91^{**}$) while the peel thickness correlated negatively with seed test weight ($r = -0.95^{**}$).
**Biochemical attributes**

The efficacy of treatments on biochemical attributes, specified a substantial effect of boron when applied on 7-day old bud (Table 3).

**Table 3.** Influence of boron on biochemical attributes of dragon fruit

<table>
<thead>
<tr>
<th>Stage of flower</th>
<th>Boron level (mgL⁻¹)</th>
<th>Treatment</th>
<th>TSS (°Brix)</th>
<th>Reducing sugar (% glucose)</th>
<th>Sucrose (%)</th>
<th>Protein (%)</th>
<th>CHO (%)</th>
<th>Citric acid (%)</th>
<th>Ascorbic acid (µg g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7-day old</td>
<td>Control</td>
<td>T1</td>
<td>16.15 ± 0.71</td>
<td>13.17 ± 0.44</td>
<td>1.62 ± 0.05</td>
<td>0.91 ± 0.07</td>
<td>14.52 ± 1.07</td>
<td>0.46 ± 0.04</td>
<td>87.31 ± 3.72</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>T2</td>
<td>16.55 ± 0.43</td>
<td>13.52 ± 0.32</td>
<td>1.79 ± 0.04</td>
<td>0.97 ± 0.06</td>
<td>15.45 ± 1.18</td>
<td>0.42 ± 0.03</td>
<td>98.64 ± 3.75</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>T3</td>
<td>16.82 ± 0.46</td>
<td>13.77 ± 0.35</td>
<td>1.87 ± 0.06</td>
<td>1.24 ± 0.12</td>
<td>15.81 ± 1.23</td>
<td>0.43 ± 0.05</td>
<td>106.87 ± 4.14</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>T4</td>
<td>17.42 ± 0.62</td>
<td>14.71 ± 0.51</td>
<td>2.30 ± 0.05</td>
<td>1.33 ± 0.11</td>
<td>15.92 ± 1.12</td>
<td>0.43 ± 0.03</td>
<td>112.66 ± 4.98</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>T5</td>
<td>16.69 ± 0.69</td>
<td>13.51 ± 0.39</td>
<td>1.76 ± 0.07</td>
<td>1.21 ± 0.09</td>
<td>15.34 ± 1.09</td>
<td>0.41 ± 0.04</td>
<td>92.25 ± 3.25</td>
</tr>
<tr>
<td>14-day old</td>
<td>100</td>
<td>T6</td>
<td>16.22 ± 0.48</td>
<td>13.36 ± 0.42</td>
<td>1.75 ± 0.04</td>
<td>0.93 ± 0.06</td>
<td>15.25 ± 1.12</td>
<td>0.47 ± 0.05</td>
<td>88.25 ± 4.19</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>T7</td>
<td>16.35 ± 0.55</td>
<td>13.38 ± 0.44</td>
<td>1.79 ± 0.06</td>
<td>0.94 ± 0.09</td>
<td>15.32 ± 1.14</td>
<td>0.45 ± 0.03</td>
<td>89.19 ± 5.17</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>T8</td>
<td>16.48 ± 0.46</td>
<td>13.53 ± 0.24</td>
<td>1.82 ± 0.03</td>
<td>0.98 ± 0.1</td>
<td>15.74 ± 1.38</td>
<td>0.43 ± 0.03</td>
<td>90.73 ± 4.37</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>T9</td>
<td>16.42 ± 0.42</td>
<td>13.49 ± 0.33</td>
<td>1.80 ± 0.06</td>
<td>0.91 ± 0.08</td>
<td>15.45 ± 1.42</td>
<td>0.44 ± 0.04</td>
<td>85.75 ± 4.11</td>
</tr>
</tbody>
</table>

Mean values followed by the same letter in each column show non-significant difference at $P < 0.05$. There was a significant enhancement in TSS, sugar content, carbohydrate, protein and ascorbic acid content, while citric acid content demonstrated a decline with boron treatment. When compared with stage of application, it was quite evident that foliar boron application at 7-day old bud stage resulted in phenomenal variation in the assessed variables. Unlike application at 14-day old bud stage, resulted in subtle enhancement.
in the bio-chemical parameters. It is evident from data that boron level is a deciding factor in harnessing maximal quality attributes. The impact of increasing boron levels on fruit bio-chemical quality parameters ascertained the level 300 mgL\(^{-1}\) as the best concentration when applied at 7-day old bud stage (T4), which registered an upsurge of \(~8\%\), \(~11\%\), \(~1.4\) folds, \(~9\%\), \(~45\%\) and \(~25\%\) in TSS, reducing sugar, sucrose, carbohydrate, protein and ascorbic acid content, respectively in comparison with control. However, the citric acid level declined with imposition of boron treatment. The highest dose of boron (400 mgL\(^{-1}\), T5) did not manifest impressive impact on fruit bio-chemical characteristics.

Boron exhibited significant impact on pigmentation and antioxidative property of dragon fruit (Table 4). In comparison with control, application of boron @300 mgL\(^{-1}\) on 7-day old bud (T4), exhibited the maximum enhancement in betacyanin content (\(~26\%\)), total phenol (\(~25\%\)), and total flavonoid (\(~54\%\)) and FRAP activity (\(~46\%\)) in the fruit. However, further increase in boron concentration (400 mgL\(^{-1}\)), demonstrated a significant reduction in the contents of bio-active compounds. It was conclusive from results that application of boron on 14-day old buds could not exhibit substantial influence on bio-active attributes of dragon fruit.

**Table 4. Influence of boron on pigment and antioxidative property of dragon fruit**

<table>
<thead>
<tr>
<th>Stage of flower bud</th>
<th>Boron level (mgL(^{-1}))</th>
<th>Treatment</th>
<th>Betacyanin (µg g(^{-1}))</th>
<th>Total Phenol (mg GAE 100g(^{-1}))</th>
<th>Total flavonoid (mg QE 100g(^{-1}))</th>
<th>FRAP activity (mM Fe II 100g(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>7-day old</td>
<td>Control</td>
<td>T1</td>
<td>26.07 ± 1.88(^a)</td>
<td>75.34 ± 2.63(^e)</td>
<td>24.50 ± 1.69(^e)</td>
<td>18.96 ± 2.41(^e)</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>T2</td>
<td>26.51 ± 2.16(^e)</td>
<td>81.36 ± 2.56(^e)</td>
<td>31.04 ± 1.93(^e)</td>
<td>23.58 ± 2.51(^e)</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>T3</td>
<td>28.35 ± 1.89(^b)</td>
<td>88.90 ± 2.85(^b)</td>
<td>33.24 ± 2.33(^b)</td>
<td>24.90 ± 2.32(^b)</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>T4</td>
<td>32.86 ± 2.52(^a)</td>
<td>95.26 ± 3.72(^a)</td>
<td>37.65 ± 2.14(^a)</td>
<td>27.71 ± 2.14(^a)</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>T5</td>
<td>27.11 ± 2.11(^a)</td>
<td>84.24 ± 3.11(^a)</td>
<td>30.45 ± 2.65(^a)</td>
<td>21.97 ± 2.22(^a)</td>
</tr>
<tr>
<td>14-day old</td>
<td>100</td>
<td>T6</td>
<td>25.36 ± 2.82(^c)</td>
<td>75.15 ± 2.82(^e)</td>
<td>26.15 ± 2.15(^d)</td>
<td>19.55 ± 2.55(^d)</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>T7</td>
<td>26.70 ± 2.15(^c)</td>
<td>76.79 ± 2.54(^c)</td>
<td>26.50 ± 2.13(^d)</td>
<td>20.50 ± 2.61(^d)</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>T8</td>
<td>27.10 ± 2.78(^d)</td>
<td>81.70 ± 3.22(^d)</td>
<td>27.55 ± 2.33(^d)</td>
<td>21.24 ± 2.62(^d)</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>T9</td>
<td>26.01 ± 2.62(^c)</td>
<td>79.35 ± 3.16(^d)</td>
<td>25.25 ± 2.61(^d)</td>
<td>21.90 ± 2.53(^d)</td>
</tr>
</tbody>
</table>

Mean values followed by the same letter in each column show non-significant difference at P < 0.05.

The correlation studies between pollen germination and fruit quality parameters (Figure 1), demonstrated that bio-chemical attributes of fruit such as TSS, reducing sugar, Sucrose, carbohydrate, protein, ascorbic acid, betacyanin, total phenol, total flavonoid content and FRAP activity established significant positive correlation with pollen germinability (r = 0.98**, 0.96**, 0.93**, 0.68*, 0.87**, 0.95**, 0.95**, 0.96** and 0.96**, respectively). Likewise, these parameters were also significantly positively correlated with fruit weight and seed weight. Conversely, the citric acid in fruit substantially correlated positively with seed number (r = 0.71*). The bioactive compounds established significant positive correlation with sugar content (r = 0.97**, 0.92**, 0.89** and 0.91**, respectively). Additionally, the betacyanin, total phenol and total flavonoid correlated significantly with the FRAP activity (r = 0.87**, 0.95** and 0.95**, respectively).

**Enzyme activity**

The data clearly illustrate influence of boron on carbohydrate metabolising and cell wall degrading enzymes (Figure 2). The assessment of carbohydrate metabolizing enzyme indicated that boron application significantly enhanced the activities of invertase (~2-fold), α-amylase (~24%) and sucrose synthase (~2.3-fold) over control when boron was applied @300 mgL\(^{-1}\) on 7-day old bud. However higher dose resulted in declining the enzymatic activity. It is also evident that application of boron on 14-days old bud stage did not result in appreciable enhancement of enzyme activities. On contrary, the cell wall degrading enzymes exemplified a reverse trend, wherein the control manifested the highest enzymatic activities. With increasing boron levels, the enzymatic activities depicted reducing trend. Activity of cellulase, polygalacturonase and pectin methyl
esterase was reduced by ~50%, ~47% and ~54%, respectively when boron was applied @400 mgL⁻¹ on 14-day old bud (T9).

Figure 2. Influence of boron on enzyme activity of dragon fruit
Bars show standard errors (±). Within a bar, different lowercase shows a significant difference (P < 0.05) between treatments.

The correlation studies (Figure 3) illustrated significant correlation between carbohydrate metabolizing enzymes and TSS, sugar content in fruit. The TSS established positive correlation with the activities of invertase, α-amylase and sucrose synthase (r = 0.89**, 0.91** and 0.93**, respectively). Positive correlation existed between reducing sugar and invertase, α-amylase activities (r = 0.88** and 0.91**, respectively). The correlation appeared to be significant between sucrose and sucrose synthase activity (r = 0.88). The firmness of fruit pulp correlated negatively with the activities of cell wall degrading enzyme (r = -0.83**, -0.89** and -0.83**, with cellulase, pectin methyl esterase and polygalacturonase, respectively).
Figure 3. Correlation matrix depicting relationship among enzymes and physico-chemical attributes of dragon fruit
INV-Invertase, Amy-α-amylase, Susy-sucrose synthase, Cel-cellulase, PG-polygalacturonase, PME-pectin methyl esterase, TSS, sugar content, PF - pulp firmness.

Principal component analysis
The loading plots of principal components illustrate the contribution of variables to principal components and the relationships among variables and treatments (Figure 4). The biplot clearly illustrates the proximity of physical attributes with T4 indicating the most effective treatment (Figure 4a). Overlapping of fruit weight and seed test weight indicates a strong degree of positive correlation between the variables. Furthermore, most of the biochemical attributes exhibited proximity with T4 (Figure 4b). The categorical demarcation of T4 in one distinct quadrant, manifest that no treatment matches its influences with respect to bio-chemical and bio-active attributes. The clustering of variables viz., FRAP, total flavonoid and total phenol, indicate that total phenol and flavonoid contents, contribute significantly to fruit anti-oxidant property. Furthermore, the relative impact of each treatment on the positive and negative regulation of enzymatic activities is also depicted (Figure 4c). The carbohydrate metabolizing enzyme were huddled around T4, inferring a similar trend of boron effect. The distribution of treatments in various segments of each bi-plot indicates a substantial dissimilar treatment effect with respect to the variability obtained in the studied attributes. The clustering of majority variables near T4, exemplifies it as the best treatment.

Discussion
Boron plays crucial role in reproductive development of crop by influencing pollen germination and pollen tube growth (Dell et al., 2002). It may be interpreted that application of boron in optimum concentration (300 mg L⁻¹) as well as at optimal stage (7-day old bud) ensured effective pollen germination. Boron contributes to the formation of sugar–borate complexes, which promotes absorption, translocation and the metabolism of sugars in the pollen (Stino et al., 2011).
Figure 4. Principal component biplots representing the relationship among physical attributes (a), biochemical attributes (b) and enzyme activity (c) as well as their relationship with the treatments PG- pollen germinability, PC- pulp content, PF-pulp firmness, PT-peel thickness, RS-reducing sugar, Suc-sucrose, CHO-carbohydrate, Pro-protein, CA-citric acid, AA-ascorbic acid, BT- betacyanin, TP – total phenol, Flavo– total flavonoid, INV-Invertase, Amy-α-amylase, Susy-sucrose synthase, Cel-cellulase, PG-polygalacturonase, PME-pectin methyl esterase.

Additionally, boron helps in hydration of pollen which in turn activates enzymes such as ATPase and esterase that are associated with the growth of pollen tube (Blevins and Lukaszewski, 1998; Nyomora et al., 2000). Probably, high enzymatic activity might have occurred when boron was applied at optimized stage and concentration. On the other hand, higher concentration (400 mgL⁻¹) might have exhibited inhibitory effect on pollen germinability due to excessive cross linking with pollen cell walls (Hu and Brown, 1994). It was also observed that boron at optimal concentration was effective in improving seed development which was substantiated with higher test weight. Increase in the size of seed with boron application might be due to effective fertilization of ovules as well as stimulation in the auxin biosynthesis which in turn plays important role in seed development (Pfeffer et al., 1998). It may be interpreted that not only an optimized dose of boron but also a specific stage of application plays crucial role in pollen germination and consequent seed development as the effect was insignificant when applied on 14-day bud stage. Application of boron significantly influenced the fruit weight and a positive correlation between fruit weight and pollen germinability indicates the probable effect of high degree of pollen germination in enhancing fruit weight in dragon fruit. Kamiloglu (2011) and Camacho-Cristóbal et al. (2008) have also reported that pollen with a high germinability promotes fruit
growth. The enhancement in fruit size with boron application may also be associated with the increase in cell wall and membrane integration, cell division, carbohydrate metabolism, sugar and hormonal transport, protein biosynthesis and nucleic acid metabolism (Shireen et al., 2018). The firmness of fruit is a significant determinant for postharvest quality and improvement in fruit firmness with boron application can be associated with the functional role of boron in improving plasma membrane integrity and cell wall formation (Khalaj et al., 2016). Additionally, it can also be interpreted that the increasing levels of boron application might have contributed in enhancing fruit firmness through excessive cross-linking with pectin in the middle lamella of cell wall (Chormova et al., 2014).

Positive impact of boron on soluble solids and acidity may be ascribed to enhanced transportation of assimilates into fruit tissues (Ganie et al., 2013). Westmark et al. (1996) reported that boron facilitates sugar transport to actively growing regions and also in developing fruits through plasma membrane. The increase in ascorbic acid content with boron application may be credited to the association of boron in ascorbate metabolism through influence on electron transport reactions and enzymatic activity of ascorbate synthesis (Barr et al., 1993). In accordance with the present study, Korkmaz et al. (2016) also reported a similar finding of enhancement in vitamin C content through boron application. Boron also facilitates pigment biosynthesis as reported in beetroot and pomegranate (El-Tantawy et al., 2017). Gilani et al. (2021) reported an increase in phenols, flavonoids and antioxidants in fruit crops with boron application as the nutrient plays a substantial role in phenol and flavonoid metabolism through stimulation of polyphenol oxidase enzyme. Additionally, it can also be construed that boron application might have enhanced the nitrogen content in fruit, thus promoting the synthesis of phenols and betacyanin pigment, which are nitrogen-containing compounds (Dorais et al., 2008).

Boron played important role in carbohydrate metabolizing and cell wall degrading enzyme activity in dragon fruit. The results are in agreement with the findings of Shi et al. (2012) who reported role of boron in activity of amylase and sucrose synthase. Similar findings have also been reported by Han et al. (2009) who stated that invertase activity increased to a larger degree in B-excess leaves than in B-deficient ones. The higher sugar content can be attributed with significant induction of invertase and α-amylase activity in fruits of Boron treated plants compared to control plants (Elwan et al., 2015). The influence of boron treatment on cell wall degrading enzyme illustrates that increasing boron levels significantly reduced the enzyme activities of cellulase, pectin methyl esterase and polygalacturonase. The effect was more pronounced when applied on advanced bud stage (14-day old bud stage). The enzymes polygalacturonase and pectin methyl esterase categorically degrade the pectin of middle lamella of cell. The structural domains of pectin interact with molecules such as calcium, borate, polyamines, phenolic compounds, hemicellulose and cellulose (Popper et al., 2008). It may be interpreted that increasing boron level might have maintained the structural strength of pectin and made it less accessible to the pectin degrading enzyme. Thus, it can be construed that enhanced firmness in boron treated fruits is likely due to reduced activities of thses enzymes. Hence, boron application can magnify the overall fruit quality attributes in dragon.

**Conclusions**

Boron demonstrated significant influence on fruit quality attributes of dragon fruit. Application of boron @ 300 mgL−1 on 7-day old bud resulted in maximal pollen germination, seed test weight, fruit weight, pulp content, pulp firmness, soluble solid contents, sugar, total carbohydrate, protein, betacyanin, total phenol, total flavonoid content and anti-oxidative property. Additionally, the same treatment also enhanced the enzymatic activities such as invertase, α-amylase and sucrose synthase. The activities of cell wall degrading enzymes were significantly lowered with boron application. The principal component analysis also validated the outright efficacy of treatment T4 in producing a desirable effect. Thus, the present study concludes that
application of boron @300 mgL-1 on 7-day old flower bud is beneficial in improving fruit quality attributes in dragon fruit. Considering the potential of the dragon fruit in Southeast Asian countries, optimization of boron application will enhance the fruit quality and marketability which in turn will foster economic profitability of growers.

**Authors' Contributions**


All authors read and approved the final manuscript.

**Ethical approval** (for researches involving animals or humans)

Not applicable.

**Acknowledgements**

Authors are thankful to the Head, Central Horticultural Experiment Station (ICAR-IIHR), Bhubaneswar for extending help during experimentation. Credits are also given to Central Instrumentation Facility, OUAT, Bhubaneswar for providing the basic amenities in carrying out the mineral analysis.

**Conflict of Interests**

The authors declare that there are no conflicts of interest related to this article.

**References**


The journal offers free, immediate, and unrestricted access to peer-reviewed research and scholarly work. Users are allowed to read, download, copy, distribute, print, search, or link to the full texts of the articles, or use them for any other lawful purpose, without asking prior permission from the publisher or the author.

License - Articles published in Notulae Botanicae Horti Agrobotanici Cluj-Napoca are Open-Access, distributed under the terms and conditions of the Creative Commons Attribution (CC BY 4.0) License. © Articles by the authors; Licensee UASVM and SHST, Cluj-Napoca, Romania. The journal allows the author(s) to hold the copyright/to retain publishing rights without restriction.

Notes:

- Material disclaimer: The authors are fully responsible for their work and they hold sole responsibility for the articles published in the journal.
- Maps and affiliations: The publisher stay neutral with regard to jurisdictional claims in published maps and institutional affiliations.
- Responsibilities: The editors, editorial board and publisher do not assume any responsibility for the article’s contents and for the authors’ views expressed in their contributions. The statements and opinions published represent the views of the authors or persons to whom they are credited. Publication of research information does not constitute a recommendation or endorsement of products involved.