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Effect of 28-homobrassinolide on the performance of sensitive and resistant varieties of Vigna radiata

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Abstract  A study was undertaken to examine the morpho-physiological alterations under different concentrations of 28-homobrassinolide (HBL) in two contrasting varieties of Vigna radiata. Sterilized seeds of V. radiata (T-44 and PDM-139) were inoculated with specific Rhizobium and allowed to grow and then 14 day old seedlings were exposed to different concentrations (0, 10^{-10}, 10^{-8}, or 10^{-6} M) of HBL and allowed to grow under natural environmental conditions. At the 15 and 21 day stage, plants were harvested to evaluate various parameters. Results clearly indicated that growth bio-markers, accumulation of proline and activities of various antioxidant enzymes increased significantly in T-44 at a later stage of growth in the presence of HBL whereas, 10^{-8} M showed the most promising response. It is concluded that HBL modifies the physiological functions and biochemical metabolism of V. radiata by increasing photosynthetic efficiency at an early stage of growth and antioxidant system in T-44 at a later stage of plant growth that are manifested in growth at later stages. It is believed that increased accumulation of proline and enhanced antioxidant system provide strength to the plants to withstand environmental cues.

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1. Introduction

Out of the recognized categories of plant hormones, much attention has been focused on auxins, cytokinins, gibberellins, abscisic acid, and ethylene. In 1970, Mitchell and co-workers showed that the growth stimulating activity was found in the organic solvent extract of pollen from Brassica napus and the unidentified active compound was named as brassin. The specific growth promoting effects of brassin have been reflected in many bioassays including the bean second-internode bioassay (Bajguz and Hayat, 2009; Hayat et al., 2012). Based on their ability to cause marked changes in growth and differentiation at low concentrations, Mitchell et al. (1970) proposed that brassins constituted a new family of plant hormones known as brassinosteroids (BRs). They emerged as the steroidal plant hormones required for normal growth and development of plants. Till now, about 69 BRs have been isolated from plants (Bajguz, 2010). They have been implicated in a wide range of physiological and molecular responses in plants, such as stem elongation, pollen tube growth, leaf bending and epinasty, ethylene biosynthesis, proton pump activation,
vascular differentiation, photosynthesis, gene expression, nucleic acid, increased proline production and protein synthesis (Bajguz and Hayat, 2009; Alyemeni and Al-Quwaiz, 2014). Moreover, the identification of biosynthetic BR deficient mutants in Arabidopsis has further elucidated its essential role in plant growth and development (Clouse, 1996) and also increases total biomass and yield. Moreover, Yu et al. (2004) reported that one of the analogs of BRs, epibrassinolide, increased the activity of Rubisco, the maximum quantum yield of photosystem II, and photosynthetic rate in Cochliobolus sativus. In addition to this, BRs showed significant responses in plants due to their involvement in cell elongation (Catterou et al., 2001), vascular differentiation and also the regulation of gene expression involved in xylem development in Zinnia mesophyll cells (Ashraf et al., 2010). BRs also play a key role in xylem formation in soybean epicotyls (Hayat and Ahmad, 2011). In addition to this, further genetic and biochemical approaches have contributed to an impressive progress in our understanding the precise role of BRs in the plant metabolism (Noguchi et al., 2000), and also in BR-induced signaling including, the identification of BR receptors, key signaling elements, and BR-induced gene expression (Geldner et al., 2007).

With these well-established reports of BRs, this experiment was designed with an objective to explore the responses of different concentrations of most stable brassinosteroids (HBL) under stage specific study in sensitive and resistant varieties of Vigna radiata and also assess the physiological and biochemical alterations under different concentrations of HBL.

2. Materials and methods

The surface sterilized seeds of V. radiata cultivar T-44 (Drought-tolerant) and PDM-139 (Drought-sensitive) were surface-sterilized with 0.01% mercuric chloride solution, followed by repeated washing with double-distilled water (DDW). The experiment was conducted in a completely randomized design. Forty earthen pots of 6 inch diameter were divided into 8 sets of 5 pots each (replicates) representing treatment and cultivars. The treatment pattern is as set I: foliage of 14 day old plants sprayed with DDW (served as control); set II: foliage of 14 days old plants sprayed with $10^{-10}$ M of HBL; set III: foliage of 14 days old plants sprayed with $10^{-8}$ M of HBL; set IV: foliage of 14 day old plants sprayed with $10^{-6}$ M of HBL; set V: served as control (-HBL); set VI: foliage of 14 day old plants sprayed with $10^{-10}$ M of HBL; set VII: foliage of 14 day old plants sprayed with $10^{-8}$ M of HBL; set VIII: foliage of 14 day old plants sprayed with $10^{-6}$ M of HBL. The foliage of each plant was sprinkled thrice. The nozzle of the sprayer was adjusted in such a way that it pumped out 1 ml (approx.) in one sprinkle. Therefore, each foliage of plants received 3 ml HBL solution. At 15 (24 h after spray) and the 21 day stage, plants were harvested to evaluate the growth biomarkers, SPAD chlorophyll content, photosynthetic parameters, activities of catalase, peroxidase and superoxide dismutase, and proline content as followed earlier (Hayat et al., 2010). Data were analyzed statistically and analysis of variance (ANOVA) was performed on the data using SPSS (ver. 10.0 Inc., USA) to determine the least significant difference (LSD) to identify difference in the mean of the treatment and cultivars. The treatment means were separated by the LSD test.

3. Result

3.1. Growth biomarkers

The Tables 1–5 showed that treatment with HBL significantly increased the growth traits of mung bean plants. Foliar application of HBL ($10^{-8}$ M) showed a maximum increase in their shoot and root length, fresh and dry mass and leaf area over their respective controls, at the later stage of growth in T-44. The per cent increase in the length, fresh and dry mass of shoot and root by HBL ($10^{-8}$ M) was 57.9%, 59.8%, 45.0%, and 51.6% in T-44 at the 21 day growth stage with respect to their controls. The leaf area was also increased 30.8% by HBL ($10^{-8}$ M) over the control (Table 5). It is clear evident from the Table 5 that T-44 generated a greater growth response than PDM-139 at a later stage.

**Table 1** Effect of BR ($10^{-10}$, $10^{-8}$, or $10^{-6}$ M) on shoot length in two varieties (T-44 and PSM-139) of Vigna radiata at the 15 and 21 day stage of growth.

| Shoot length (cm) | 15 DAS | 21 DAS |
|-------------------|--------|--------|
| **T-44** | **PDM-139** | **Mean** | **T-44** | **PDM-139** | **Mean** |
| Control | 10.05 ± 0.50 | 7.31 ± 0.50 | 8.68 | 11.25 ± 0.80 | 9.22 ± 0.97 | 10.23 |
| BR $10^{-10}$ | 10.30 ± 0.40 | 7.29 ± 0.59 | 8.79 | 16.87 ± 0.71 | 12.90 ± 0.81 | 14.88 |
| BR $10^{-8}$ | 11.00 ± 0.35 | 7.44 ± 0.55 | 9.22 | 17.77 ± 0.69 | 13.64 ± 0.85 | 15.70 |
| BR $10^{-6}$ | 10.71 ± 0.54 | 7.10 ± 0.49 | 8.90 | 15.75 ± 0.70 | 11.84 ± 0.74 | 13.79 |
| Mean | 10.51 | 7.28 | 15.41 | 11.90 |
| LSD @ 5% | V = 0.80 | V = 1.22 | V = 0.69 | V = 1.06 | V × T = 1.49 | V × T = 2.28 |
3.2. Chlorophyll content (SPAD value) and photosynthetic parameters

It is evident from the Table 6, foliar application of HBL (10^{-10}, 10^{-8}, or 10^{-6} M) increased the SPAD level by 14.9%, 24.8%, and 7.8% at the 15 day stage and 17.8%, 29.9%, and 10.9% at the 21 day stage of growth over their respective controls in T-44. Out of the two stages of growth, the 21 day stage of growth showed maximum response for SPAD value irrespective of treatments.

The parameters of photosynthesis in 21 day old plants were high on receiving HBL in both the varieties (T-44/PSM-139; Tables 6-9). The maximum increase in net photosynthetic rate of about 41.7% was recorded in the leaves of T-44 sprayed with 10^{-8} M of HBL at the 15 day stage of growth, whereas, 37.9% increase was noted at the 21 day stage of growth. The other photosynthetic parameters (stomatal conductance, internal CO2 concentration, and transpiration rate) exhibited a trend similar to that of net photosynthetic rate. The per cent increase in stomatal conductance (41.6%), internal CO2 concentration...
3.3. Antioxidant enzymes [catalase (CAT), peroxidase (POX), superoxide dismutase (SOD)]

Brassinosteroid (BR), at three concentrations (10⁻¹⁰, 10⁻⁸, or 10⁻⁶ M) when applied to the plant foliage improved activity of antioxidant enzymes (CAT, POX, and SOD) to a significant level (Tables 11–13). HBL (10⁻⁸ M) increased the value of CAT, POX, and SOD activity by 21.8%, 43.9%, 30.5% at the 15 day stage and 26.9%, 47.9%, 35.8% at the 21 day stage of growth, respectively in comparison to their control plants in T-44. Of the two stage study, 21 day stage of growth proved to be the best and variety T-44 excelled over the PDM-139 under all concentrations of HBL.

Table 5 Effect of BR (10⁻¹⁰, 10⁻⁸, or 10⁻⁶) on leaf area in two varieties (T-44 and PDM-139) of *Vigna radiata* at the 15 and 21 day stage of growth.

|                | 15 DAS          | 21 DAS          |
|----------------|-----------------|-----------------|
|                | T-44            | PDM-139         | Mean | T-44            | PDM-139         | Mean |
| Leaf area (cm²) |                 |                 |      |                 |                 |      |
| Control        | 6.85 ± 0.33     | 5.82 ± 0.30     | 6.33 | 7.67 ± 0.42     | 6.46 ± 0.33     | 7.06 |
| BR 10⁻¹⁰       | 6.87 ± 0.37     | 5.80 ± 0.27     | 6.33 | 9.20 ± 0.40     | 7.36 ± 0.39     | 8.28 |
| BR 10⁻⁸        | 6.90 ± 0.30     | 5.82 ± 0.31     | 6.36 | 10.04 ± 0.41    | 7.94 ± 0.41     | 8.99 |
| BR 10⁻⁶        | 6.81 ± 0.25     | 5.78 ± 0.30     | 6.29 | 8.74 ± 0.37     | 7.10 ± 0.45     | 7.92 |
| Mean           | 6.85            | 5.80            | 6.29 | 8.91            | 7.21            |      |
| LSD @ 5%       | V = 0.56        | V = 0.72        |      | V × T = 1.06    | V × T = 1.35    |      |

Table 6 Effect of BR (10⁻¹⁰, 10⁻⁸, or 10⁻⁶) on chlorophyll content (SPAD value) in two varieties (T-44 and PDM-139) of *Vigna radiata* at the 15 and 21 day stage of growth.

|                | 15 DAS          | 21 DAS          |
|----------------|-----------------|-----------------|
|                | T-44            | PDM-139         | Mean | T-44            | PDM-139         | Mean |
| SPAD chlorophyll content |               |                 |      |                 |                 |      |
| Control        | 6.51 ± 0.19     | 5.53 ± 0.15     | 6.02 | 7.22 ± 0.19     | 6.20 ± 0.19     | 6.71 |
| BR 10⁻¹⁰       | 7.48 ± 0.18     | 6.13 ± 0.20     | 6.8  | 8.51 ± 0.20     | 7.06 ± 0.18     | 7.78 |
| BR 10⁻⁸        | 8.13 ± 0.16     | 6.52 ± 0.19     | 7.32 | 9.38 ± 0.17     | 7.75 ± 0.21     | 8.56 |
| BR 10⁻⁶        | 7.03 ± 0.17     | 5.80 ± 0.18     | 6.41 | 8.01 ± 0.19     | 6.69 ± 0.21     | 7.35 |
| Mean           | 7.28            | 5.99            |      | 8.28            | 6.92            |      |
| LSD @ 5%       | V = 0.19        | V = 0.11        |      | V × T = 0.45    | V × T = 0.38    |      |

Table 7 Effect of BR (10⁻¹⁰, 10⁻⁸, or 10⁻⁶) on net photosynthetic rate in two varieties (T-44 and PDM 139) of *Vigna radiata* at the 15 and 21 day stage of growth.

|                | 15 DAS          | 21 DAS          |
|----------------|-----------------|-----------------|
|                | T-44            | PDM-139         | Mean | T-44            | PDM-139         | Mean |
| Net photosynthetic rate (µ mol CO₂ m⁻² s⁻¹) |               |                 |      |                 |                 |      |
| Control        | 8.01 ± 0.11     | 6.40 ± 0.11     | 7.20 | 8.97 ± 0.15     | 7.98 ± 0.12     | 8.47 |
| BR 10⁻¹⁰       | 10.41 ± 0.10    | 7.68 ± 0.10     | 9.04 | 11.93 ± 0.13    | 10.21 ± 0.14    | 11.07 |
| BR 10⁻⁸        | 11.05 ± 0.09    | 8.32 ± 0.09     | 9.68 | 12.73 ± 0.14    | 10.77 ± 0.13    | 11.75 |
| BR 10⁻⁶        | 9.61 ± 0.07     | 7.29 ± 0.08     | 6.95 | 10.94 ± 0.11    | 9.33 ± 0.11     | 10.13 |
| Mean           | 9.77            | 7.42            |      | 11.14           | 9.57            |      |
| LSD @ 5%       | V = 0.11        | V = 0.15        |      | V × T = 0.30    | V × T = 0.36    |      |
Table 8  Effect of BR (10^{-10}, 10^{-8}, or 10^{-6}) on stomatal conductance in two varieties (T-44 and PDM-139) of Vigna radiata at the 15 and 21 day stage of growth.

|          | 15 DAS | 21 DAS |
|----------|--------|--------|
|          | T-44   | PDM-139| Mean | T-44   | PDM-139| Mean |
| Stomatal conductance (mol H_{2}O m^{-2} s^{-1}) |        |        |      |        |        |      |
| Control  | 0.011 ± 0.001 | 0.009 ± 0.002 | 0.010 | 0.012 ± 0.001 | 0.010 ± 0.002 | 0.011 |
| BR 10^{-10} | 0.013 ± 0.002 | 0.011 ± 0.001 | 0.012 | 0.015 ± 0.002 | 0.012 ± 0.001 | 0.013 |
| BR 10^{-8}  | 0.015 ± 0.001 | 0.014 ± 0.002 | 0.014 | 0.017 ± 0.002 | 0.013 ± 0.001 | 0.015 |
| BR 10^{-6}  | 0.011 ± 0.002 | 0.009 ± 0.002 | 0.010 | 0.013 ± 0.002 | 0.011 ± 0.002 | 0.012 |
| Mean       | 0.012 | 0.010 | 0.014 | 0.011 |        |        |      |
| LSD @ 5%   | V = 0.001 |        | V = 0.002 |        |        |      |
|           | T = 0.003 |        | T = 0.003 |        |        |      |
|           | V × T = NS |        | V × T = NS |        |        |      |

Table 9  Effect of BR (10^{-10}, 10^{-8}, or 10^{-6}) on internal CO_{2} concentration in two varieties (T-44 and PDM-139) of Vigna radiata at the 15 and 21 day stage of growth.

|          | 15 DAS | 21 DAS |
|----------|--------|--------|
|          | T-44   | PDM-139| Mean | T-44   | PDM-139| Mean |
| Internal CO_{2} concentration (ppm) |        |        |      |        |        |      |
| Control  | 150 ± 1.95 | 126 ± 1.79 | 138 | 171 ± 1.75 | 145 ± 1.90 | 158 |
| BR 10^{-10} | 190 ± 2.01 | 151 ± 1.88 | 170 | 222 ± 1.95 | 179 ± 2.11 | 200 |
| BR 10^{-8}  | 202 ± 1.91 | 163 ± 1.92 | 182 | 239 ± 2.01 | 192 ± 1.99 | 215 |
| BR 10^{-6}  | 183 ± 1.75 | 147 ± 2.12 | 165 | 215 ± 2.12 | 188 ± 1.88 | 201 |
| Mean       | 181 | 146 | 211 | 176 |        |        |      |
| LSD @ 5%   | V = 2.62 |        | V = 3.09 |        |        |      |
|           | T = 2.20 |        | T = 2.26 |        |        |      |
|           | V × T = 4.82 |        | V × T = 5.35 |        |        |      |

Table 10  Effect of BR (10^{-10}, 10^{-8}, or 10^{-6}) on transpiration rate in two varieties (T-44 and PDM-139) of Vigna radiata at the 15 and 21 day stage of growth.

|          | 15 DAS | 21 DAS |
|----------|--------|--------|
|          | T-44   | PDM-139| Mean | T-44   | PDM-139| Mean |
| Transpiration rate (ppm) |        |        |      |        |        |      |
| Control  | 1.08 ± 0.07 | 0.96 ± 0.06 | 1.02 | 1.19 ± 0.08 | 1.07 ± 0.09 | 1.13 |
| BR 10^{-10} | 1.29 ± 0.05 | 0.82 ± 0.05 | 1.05 | 1.47 ± 0.07 | 1.28 ± 0.11 | 1.37 |
| BR 10^{-8}  | 1.40 ± 0.07 | 1.19 ± 0.07 | 1.29 | 1.58 ± 0.09 | 1.39 ± 0.10 | 1.48 |
| BR 10^{-6}  | 1.24 ± 0.09 | 1.05 ± 0.05 | 1.14 | 1.42 ± 0.10 | 1.17 ± 0.09 | 1.29 |
| Mean       | 1.25 | 1.00 | 1.41 | 1.22 |        |        |      |
| LSD @ 5%   | V = 0.018 |        | V = 0.021 |        |        |      |
|           | T = 0.011 |        | T = 0.013 |        |        |      |
|           | V × T = NS |        | V × T = NS |        |        |      |

3.4. Proline content

The spray of HBL (10^{-10}, 10^{-8} or 10^{-6} M) increased the proline accumulation, irrespective of the stage dependent study. HBL (10^{-8} M) was more efficient over the other two concentrations and significantly increased the proline accumulation at both stages of growth in T-44 and the value was 39.8% (15 day stage) and 44.0% (21 day stage) over their control plants. T-44 performs significantly in accumulation of proline over the PDM-139 at a later stage (21 days stage) of growth.

4. Discussion

Application of HBL as foliar spray, improved the growth biomarkers (shoot and root length, fresh and dry mass of shoot and leaf area) V. radiata plants (Tables 1–5). BR gener-
ated such a response because of their involvement in cell elongation (Catterou et al., 2001), regulation of genes encoding XTHs (xyloglucan endotransglycosylase/hydrolase) i.e. enzymes responsible for the modification of cell wall activity and enlargements, cellulose synthase, and sucrose synthase (Ashraf et al., 2010). Beside this, BR caused an increase in transcript levels of gene encoding cyclin-D3, a regulatory protein of cell cycle in Arabidopsis (Ashraf et al., 2010). Leaves of HBL treated plants possessed a larger leaf area (Table 5) which could mainly be an expression of activated cell division and cellular enlargement (Bajguz and Tretyn, 2003). The increases in leaf area by BRs have also been reported by others (Pipattanawong et al., 1996). It is also evident from the present study (Table 6) that the chlorophyll content (SPAD level) was increased in the leaves of HBL-treated plants at both the stages of the plant. HBL treated plants significantly increased the pigment content in various crops (Hayat et al., 2001; Fariduddin et al., 2003, 2014; Yusuf et al., 2011). The reason that sounds best in improving the content of chlorophyll (SPAD level) by BR seems to be due to its involvement in improving transcrip-

### Table 11 Effect of BR (10^{-10}, 10^{-8}, or 10^{-6}) on catalase activity in two varieties (T-44 and PDM-139) of *Vigna radiata* at the 15 and 21 day stage of growth.

|                | 15 DAS       | 21 DAS       | Mean  |
|----------------|--------------|--------------|-------|
|                | T-44 PDM-139 | T-44 PDM-139 | Mean  |
| Control        | 261 ± 8.01   | 214 ± 8.88   | 237   |
| BR 10^{-10}    | 302 ± 7.05   | 257 ± 7.98   | 269   |
| BR 10^{-8}     | 318 ± 8.88   | 250 ± 7.75   | 284   |
| BR 10^{-6}     | 297 ± 9.87   | 231 ± 7.71   | 264   |
| Mean           | 294          | 233          |       |
| LSD @ 5%       | V = 10.01    | V = 11.47    |       |
|                | T = 10.58    | T = 11.01    |       |
|                | V x T = 20.59| V x T = 22.47|       |

### Table 12 Effect of BR (10^{-10}, 10^{-8}, or 10^{-6}) on peroxidase activity in two varieties (T-44 and PDM-139) of *Vigna radiata* at the 15 and 21 day stage of growth.

|                | 15 DAS       | 21 DAS       | Mean  |
|----------------|--------------|--------------|-------|
|                | T-44 PDM-139 | T-44 PDM-139 | Mean  |
| Control        | 7.89 ± 0.32  | 6.31 ± 0.40  | 7.1   |
| BR 10^{-10}    | 10.88 ± 0.35 | 8.20 ± 0.38  | 9.54  |
| BR 10^{-8}     | 11.36 ± 0.37 | 8.51 ± 0.37  | 9.93  |
| BR 10^{-6}     | 10.65 ± 0.39 | 7.82 ± 0.29  | 9.23  |
| Mean           | 10.19        | 7.71         |       |
| LSD @ 5%       | V = 0.34     | V = 0.38     |       |
|                | T = 0.52     | T = 0.35     |       |
|                | V x T = 0.86 | V x T = 0.73 |       |

### Table 13 Effect of BR (10^{-10}, 10^{-8}, or 10^{-6}) on superoxide dismutase activity in two varieties (T-44 and PDM-139) of *Vigna radiata* at the 15 and 21 day stage of growth.

|                | 15 DAS       | 21 DAS       | Mean  |
|----------------|--------------|--------------|-------|
|                | T-44 PDM-139 | T-44 PDM-139 | Mean  |
| Control        | 121 ± 2.99   | 104 ± 2.61   | 112   |
| BR 10^{-10}    | 150 ± 2.75   | 122 ± 3.01   | 136   |
| BR 10^{-8}     | 158 ± 3.13   | 128 ± 2.28   | 143   |
| BR 10^{-6}     | 145 ± 2.66   | 120 ± 2.81   | 132   |
| Mean           | 143          | 118          |       |
| LSD @ 5%       | V = 4.96     | V = 5.63     |       |
|                | T = 4.26     | T = 4.70     |       |
|                | V x T = 9.32 | V x T = 10.33|       |
tion and/or translation machinery (Bajguz, 2000), more efficiently for the synthesis of photosynthetic pigments. To support this statement, a positive response was generated in algae when BRs induced the expression of specific genes involved in the synthesis of enzymes for chlorophyll biosynthesis (Bajguz and Asami, 2005). BR are also known to activate Rubisco (Yu et al., 2004) and CA activity (Yusuf et al., 2011), the key enzymes of photosynthesis. Moreover, high CA activity increases the capacity of CO₂ assimilation in the Calvin cycle which is mainly attributed to efficient functioning of Rubisco (Bajguz and Asami, 2005) thereby improving the net photosynthetic rate and related attributes (Tables 8-10).

The treatment of plants with HBL as a foliar spray enhanced the activity of antioxidant enzymes (CAT, POX and SOD) as well as that of the proline (Tables 11-14). BR regulates the activity of antioxidant enzymes in the tissues where accumulation of free radicals is very high (Ashraf et al., 2010). Due to this peculiarity to manage cells in dual conditions; to provide defense and to promote growth, BRs are considered as novel regulators in plants (Sun et al., 2010). BR treatment conferred tolerance mediated through the induced expression of both regulatory genes, such as RBOH (Respiratory burst oxidase homolog), MAPK1 (Mitogen-activated protein kinase), and MAPK3 (Mitogen-activated protein kinase), and genes involved in defense, antioxidant responses and also those elevated H₂O₂ levels resulting from enhanced NADPH oxidase activity involved in the BR-induced stress tolerance (Xia et al., 2009). On the other hand, proline serves as a persuasive inhibitor of PCD (Gill and Tuteja, 2010) and also acts as a non-enzymatic antioxidant that is known to stabilize the sub cellular structures such as those of proteins and cell membranes, scavenging free radicals and buffering redox potential under stress conditions and also have the ability of molecular chaperones that protect the integrity of protein and enhances the activity of different enzymes, such as protection of nitrate reductase during stresses (Szabados and Savoure, 2010). In addition to this, among various compatible solutes, proline is the only molecule that has been shown to protect plants against singlet oxygen and free radical induced damages resulting from stress (Alia et al., 1997; Alyemeni and Al-Quwaiz, 2014). It has also been reported earlier that BRs induce the expression of biosynthetic genes of proline (Ozdemir et al., 2014).

| Proline content (μ mol g⁻¹ FM) | 15 DAS | 21 DAS |
|-------------------------------|--------|--------|
|                              | T-44   | PDM-139 | Mean | T-44   | PDM-139 | Mean |
| Control                       | 5.14 ± 0.22 | 4.30 ± 0.18 | 4.72 | 5.75 ± 0.19 | 4.89 ± 0.20 | 5.32 |
| BR 10⁻¹⁰                      | 6.63 ± 0.20 | 5.23 ± 0.15 | 5.93 | 7.76 ± 0.22 | 6.35 ± 0.28 | 7.05 |
| BR 10⁻⁸                       | 7.19 ± 0.19 | 5.75 ± 0.17 | 6.47 | 8.28 ± 0.25 | 6.74 ± 0.21 | 7.51 |
| BR 10⁻⁶                       | 6.21 ± 0.11 | 4.97 ± 0.12 | 5.59 | 7.24 ± 0.19 | 5.86 ± 0.19 | 6.55 |
| Mean                          | 6.29    | 5.06    |      | 7.25    | 5.96    |      |

LSD @ 5% V = 0.21 T = 0.34 V × T = NS

5. Conclusions

The present study concluded that different concentrations of HBL enhanced the efficiency of plant metabolism though various physiological and biochemical traits, however, at the early stage of growth, biochemical parameters showed more effective response in comparison to growth biomarkers. At early stage of growth, T-44 showed maximum antioxidant system and proline accumulation that could strengthen the plants to withstand various environmental cues at later stages of plant growth.

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Effect of 28-HBL on performance of Vigna radiata

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