Research Article

# Effects of 24-epibrassinolide and 28-homobrassinolide on the growth and antioxidant enzyme activities in the seedlings of *Brassica juncea* L.

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**ABSTRACT**

The present paper deals with the effects of two active forms of brassinosteroids (BRs) as epibrassinosteroid (24-EBL) and homobrassinosteroid (28-HBL) on percentage germination, growth in the form of shoot length, activities of auxinase (IAAO), polyphenol oxidase (PPO), superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APOX) in 10 day old seedlings of *Brassica juncea* L. (RCM 619) under field conditions. Exogenous application of 240-EBL and 28-HBL significantly ameliorate the total protein content as compared to untreated control seedlings. $10^{-8}$ M 28-HBL helps in enhancing the PPO activity very significantly, as compared to all other concentrations of EBL and HBL and also to that of untreated control. Similar trend was observed in IAAO activity. It was observed that all the concentrations of EBL were unable to enhance the APOX activity as compared to untreated control seedlings but $10^{-8}$ M HBL significantly ameliorates APOX activity. CAT and SOD activities ameliorate significantly with exogenous application of EBL and HBL. Out of two active forms of BRs, 28-HBL was more effective at germination stage in scavenging the free radicals, which are produced in greater amount during germination from basic metabolic processes, whereas 28-EBL was effective in the initial growth of seedlings in the form of increase in shoot length. **[Physiol. Mol. Biol. Plants 2009; 15(4) : 335-341]** *E-mail : geetikasir123@gmail.com*

***Keywords :*** *Brassinosteroids, Brassica juncea, Antioxidant enzymes, Polyphenol oxidase, Auxinase, Total Proteins*

***Abbreviations :*** *SOD – Superoxide dismutase; CAT – Catalase; APOX – Ascorbate peroxidase; PPO – Polyphenol oxidase; IAAO – Indole acetic acid oxidase*

## INTRODUCTION

Brassinosteroids (BRs) due to their antistress and immunomodulatory properties are becoming strong candidates for new generation phytohormones. They have been reported to modulate almost every aspect of growth and development and various plant responses to oxidative stress, directly and/or indirectly by scavenging free radicals produced during normal metabolism and also help plants under oxidative stress by scavenging reactive oxygen species (Cao *et al*., 2005; Xiong *et al*., 2002). Reactive oxygen species (ROS) are produced at many places in living cells like chloroplasts and peroxisomes through normal respiration and photorespiration (Maxwell *et al*., 1999; Moller, 2001; Foyer and Noctor, 2003). These species participate actively in signal transduction, but can also modify cellular components and cause damage to lipid membranes, proteins and nucleic acids when they exceed their permissible limits (Wang *et al*., 2003). To scavenge these ROS in plants, the operating antioxidant defense system comprises various enzymes like superoxide dismutase (SOD), guaiacol peroxidase (POD), ascorbate peroxidase (APOX), catalase (CAT), and glutathione reductase (GR) and non-enzymatic metabolites like ascorbic acid, glutathione, proline, α-tocopherol (Asada and Takahashi, 1987; Salin, 1988). Several plant hormones like ethylene, abscisic acid, salicylic acid, auxins, polyphenols, flavenoids and brassinosteroids (BRs) have been reported to help in modulating this antioxidant defense system and thus scavenging the free radicals and help the plant to protect from oxidative

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stress. Brassinosteroids showed a great potential in modulating such stresses by regulating the system at the biochemical level. These compounds, when applied to plants, improve their quality and yield and make them more resistant to various stresses.

*Brassica juncea* L. is an important oilseed crop known for its oil content, edible and medicinal uses. The seed is a warming stimulant with antibiotic effects. The chemical constituents of *B. juncea* include glucosinolates, ascorbate, foliate, myrosinase, sterols (brassicasterol, sitosterol and brassinosteroids). Brassinosteroids have been first isolated from brassica species by Grove *et. al.* (1979) and till now more than 70 types of BRs have been reported from a large number of plants. 24-epibrassinolide (24-EBL) and 28-homobrassinolide (28-HBL) are the two most active forms of BRs, which are also available commercially. Stress-protective properties of brassinosteroids have been reported by various researchers in plants against a number of stresses like chilling (Dhaubhadel *et al.,* 1999), salt (Özdemir *et al.,* 2004), heat (Dhaubhadel *et al*., 2002) and heavy metals (Bajguz, 2000; Janeczko *et al.,* 2005). The influence of BRs on the antioxidative defense system of plants under oxidative stress conditions has been studied recently by Cao *et al.* (2005) and Hayat *et al.,* (2007a) The available data shows that the changes induced in the activities of antioxidative enzymes by BRs differed with plant species and with stress conditions (Ozdemir *et al.,* 2004; Almeida *et al.,* 2005 and Hayat *et al.,* 2007b*).* In different plants like barley, tomato, radish, sugar beet and winter rape, appropriate dose of BRs at an appropriate stage of development reduces stress significantly (Volynets *et al.,* 1997*).* Scanty information is available regarding the role of BRs at different stages of growth and development and with special reference to oxidative stress. Keeping this in mind, the present work was undertaken to study the effects of 24-EBL and 28-HBL on seedling growth and antioxidant enzyme activities in *B. juncea.*

## MATERIALS AND METHODS

### Study materials

The seeds of *Brassica juncea* L. cv. RCM 619 were procured from Department of Plant Breeding, Punjab Agriculture University, Ludhiana, India. The seeds were surface sterilized with 0.01 % $HgCl_2$ and rinsed 5-6 times with double distilled water. The sterilized seeds were soaked for 8 h in different concentrations of 24-epiBL and 28- homoBL ($10^{-6}$, $10^{-8}$ and $10^{-10}$ M). The treated seeds were sown in soil under field conditions in three replicas. Morphological data in terms of percent seed germination and seedling growth (shoot length) was noted on 10$^{th}$ day after sowing and seedlings were harvested for analysis of biochemical and antioxidant enzyme activities.

### Enzymatic analysis

Estimation of various antioxidant enzyme activities and protein content was done in 10 day old seedlings by homogenizing 1g fresh plant material in 3 ml of 100 mM phosphate buffer (pH 7.0). The homogenate was centrifuged at 4 ºC for 20 min at 15,000 g. The supernatant was used for estimation of total protein content and various biochemical and antioxidant enzyme activities (PPO, IAAO SOD, CAT, APOX).

Activity of SOD was estimated as increase in absorbance of superoxide nitro blue tetrazolium at 540 nm using the method of Kono (1978). CAT activity was measured according to Aebi (1983) by taking 3 ml reaction mixture containing 1.5 ml of 100 mM phosphate buffer (pH 7.0), 0.05 ml of 75 mM $H_2O_2$ and 0.05 ml enzyme extract. The reaction was started by addition of $H_2O_2$ and CAT activity was measured as decrease in absorbance at 240 nm for 1 min. Enzyme activity was computed by calculating the amount of $H_2O_2$ decomposed per minute. Activity of APOX was measured following the method of Nakano and Asada (1981) by monitoring the rate of decrease in absorbance at 290 nm for 1 min. The reaction mixture contained 100 mM phosphate buffer (pH 7.0), 5 mM ascorbate, 5 mM $H_2O_2$ in enzyme extract. PPO activity was measured using the method of Bastin and Unleur (1972). The reaction mixture contained 60 mM phosphate buffer (pH 7.0) and 0.01 M chlorogenic acid, which was incubated at 30 ºC±2 ºC for one hour. OD was measured at 430 nm. IAAO activity was measured according to the method of Gordon and Weber (1951) Reaction mixture contained Salkowski reagent and 0.01 % IAA solution. Enzyme extract was incubated at 40 ºC for 20 min in the dark. The mixture was cooled and OD was taken at 535 nm. Estimation of total protein content was done using the method of Lowry *et al.* (1951).

### Statistical Analysis

Each growth experiment was conducted three times taking three replicas of each BR treatment, under natural field condition. The data presented in the graphs are means of three values. The data was analyzed statistically by using one-way analysis of variance and

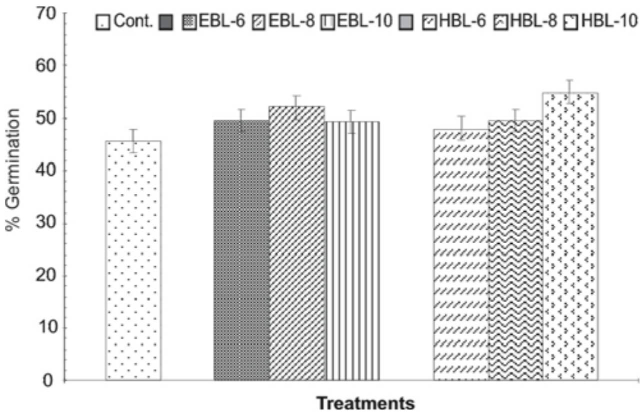


**Fig. 1.** Effect of 24-EBL and 28-HBL on the germination of seeds of *B. juncea* L. under natural field condition. Cont. indicates control and EBL and HBL indicate epibrassinolide and homobrassinolide respectively, and the numbers indicate the concentration (e.g. EBL-6 = $10^{-6}$ M). Data are means of three replicates. The treatments are significantly different from control at $P<0.05$.

standard error and comparisons of P-value at 0.05 were considered significantly different from control.

## RESULTS

It was observed that seed germination and seedling growth was enhanced slightly but significantly with treatments of different concentrations ($10^{-6}$, $10^{-8}$ and $10^{-10}$ M) of 24-EBL and 28-HBL as compared to untreated control (Fig. 1). Increase in seed germination was observed to be maximum in $10^{-10}$ M 28-HBL (55.66±0.52) as compared to untreated control seeds (46.67±2.18). $10^{-8}$ M 24–EBL was found to be the best concentration for seed germination (52.12±0.47). Treatment of $10^{-8}$ M 24–EBL (6.38±0.26 cm) was found to be the best concentration for shoot growth as compared to control (4.78±0.14 cm) and $10^{-8}$ M 28–HBL (4.98±0.29 cm) (Fig. 2).

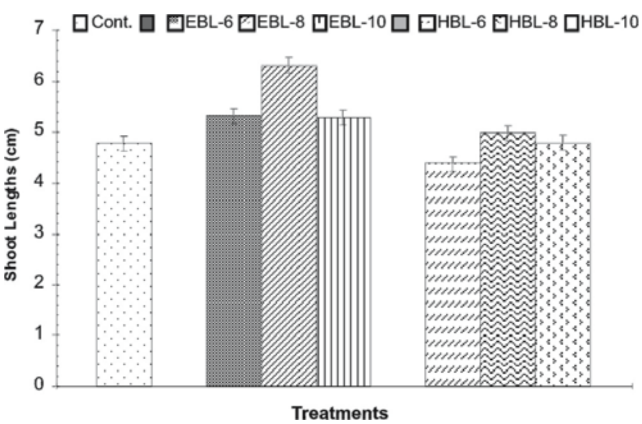


**Fig. 2.** Effect of 24-EBL and 28-HBL on shoot length of 10 days old seedlings of *B. juncea* L.under natural field condition. Cont. indicates control and EBL and HBL indicate epibrassinolide and homobrassinolide respectively, and the numbers indicate the concentration (e.g. EBL-6 = $10^{-6}$ M). Data are means of three replicates. The treatments are significantly different from control at $P<0.05$.

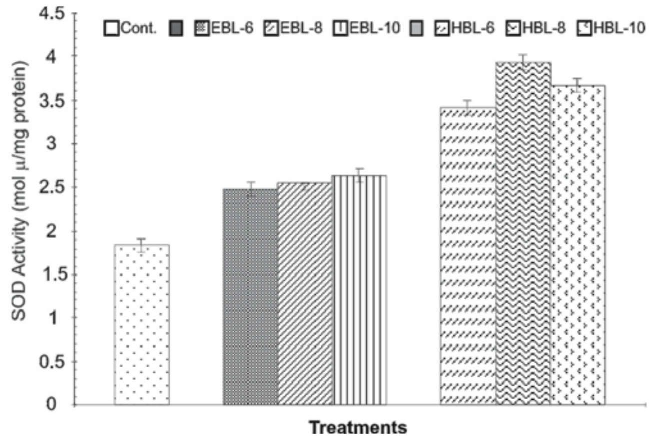


**Fig. 3.** Effect of 24-EBL and 28-HBL on SOD activity of 10 days old seedlings of *B. juncea* L. under natural field condition. Cont. indicates control and EBL and HBL indicate epibrassinolide and homobrassinolide respectively, and the numbers indicate the concentration (e.g. EBL-6 = $10^{-6}$ M). Data are means of three replicates. The treatments are significantly different from control at $P<0.05$.

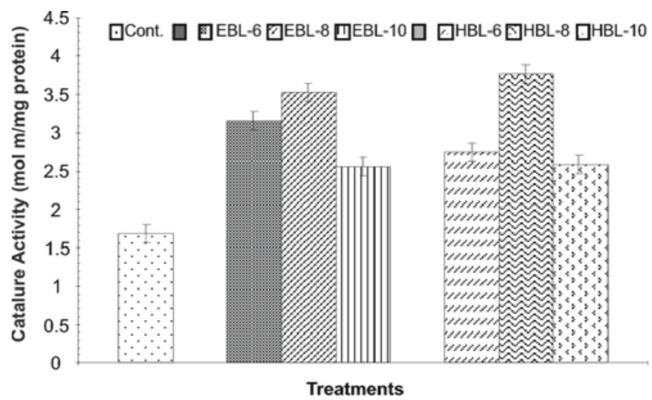


**Fig. 4.** Effect of 24-EBL and 28-HBL on the CAT activity of 10 days old seedlings of *B. juncea* L. under natural field condition. Cont. indicates control and EBL and HBL indicate epibrassinolide and homobrassinolide respectively, and the numbers indicate the concentration (e.g. EBL-6 = $10^{-6}$ M). Data are means of three replicates. The treatments are significantly different from control at $P<0.05$.

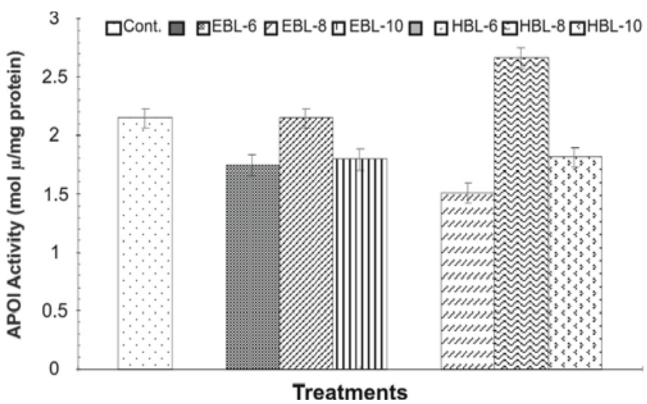


**Fig. 5.** Effect of 24-EBL and 28-HBL on the APOX activity of 10 days old seedlings of *B. juncea* L. under natural field condition. Cont. indicates control and EBL and HBL indicate epibrassinolide and homobrassinolide respectively, and the numbers indicate the concentration (e.g. EBL-6 = $10^{-6}$ M). Data are means of three replicates. The treatments are significantly different from control at P<0.05.

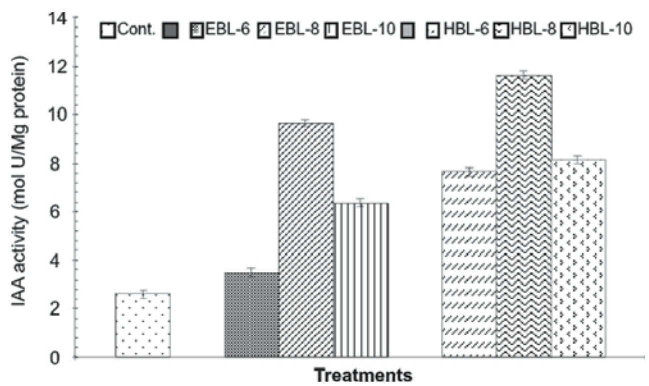


**Fig. 7.** Effect of 24-EBL and 28-HBL on the IAAO activity of 10 days old seedlings *B. juncea* L. under natural field condition. Cont. indicates control and EBL and HBL indicate epibrassinolide and homobrassinolide respectively, and the numbers indicate the concentration (e.g. EBL-6 = $10^{-6}$ M). Data are means of three replicates. The treatments are significantly different from control at P<0.05.

The studies done on biochemical parameters revealed significant effects of BR treatments. Seedlings treated with 28-HBL showed an increase in the activities of antioxidant enzymes (SOD, CAT, APOX) along with biochemical activities of IAAO and PPO at higher levels as compared to 24-EBL seedlings (Figs. 3-6). The treatment of seedlings with $10^{-8}$ M 28-HBL resulted in the maximum protein content (25.85±0.78 mg $g^{-1}$ FW) when compared to all concentration of 24-EBL and untreated control (18.51±0.30 mg $g^{-1}$ FW) (Fig. 8). SOD activity was increased significantly in all the treatments of 28-HBL and 24-EBL as compared to untreated control seedlings (1.83±0.08 mol U $mg^{-1}$ protein) which was maximum in $10^{-8}$ M 28–HBL (3.94±0.70 mol U $mg^{-1}$ protein) (Fig. 3). CAT showed maximum activity (3.77±0.015 mol U $mg^{-1}$ protein) in case of seedlings treated with $10^{-8}$ M 28-HBL in comparison to 24-EBL (3.50±0.048 mol U $mg^{-1}$ protein) and control (1.68±0.012 mol U $mg^{-1}$ protein) (Fig. 4). The activity of APOX also increased under the influence of $10^{-8}$ M

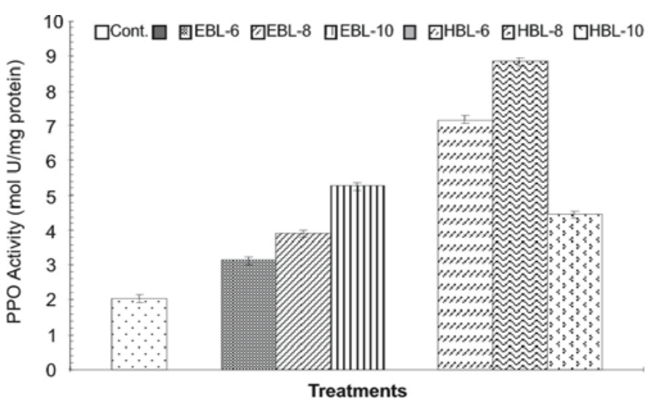


**Fig. 6.** Effect of 24-EBL and 28-HBL on the PPO activity of 10 days old seedlings of *B. juncea* L. under natural field condition. Cont. indicates control and EBL and HBL indicate epibrassinolide and homobrassinolide respectively, and the numbers indicate the concentration (e.g. EBL-6 = $10^{-6}$ M). Data are means of three replicates. The treatments are significantly different from control at P<0.05.

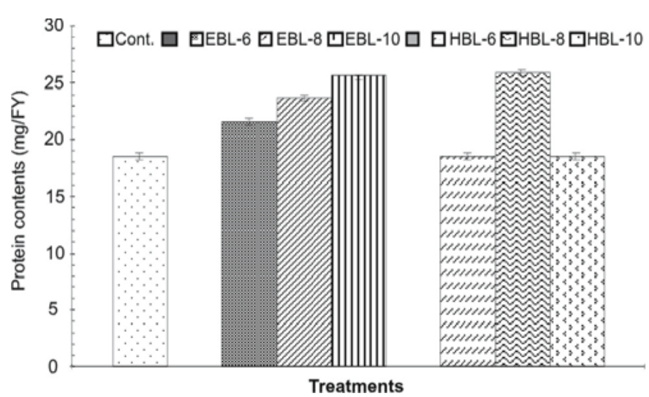


**Fig. 8.** Effect of 24-EBL and 28-HBL on the protein content of seedlings of *B. juncea* L. under natural field condition. Cont. indicates control and EBL and HBL indicate epibrassinolide and homobrassinolide respectively, and the numbers indicate the concentration (e.g. EBL-6 = $10^{-6}$ M). Data are means of three replicates. The treatments are significantly different from control at P<0.05.

28–HBL (2.66±0.16 mol U $mg^{-1}$ protein) as compared to 24-EBL and control (2.15±0.087 mol U $mg^{-1}$ protein) (Fig. 5). Similarly IAAO and PPO activities were also enhanced by applications of different concentrations of 28-HBL and 24-EBL (Fig. 6-7). Maximum IAAO activity was observed in $10^{-8}$ M 28-HBL (11.62±0.194 mol U $mg^{-1}$ protein) as compared to control (2.61±0.17 mol U $mg^{-1}$ protein) and in 24-EBL maximum IAAO activity was observed in $10^{-8}$ M (9.65±1.91 mol U $mg^{-1}$ protein). Similarly PPO activity was also enhanced in seedlings treated with different concentrations of 28-HBL and 24-EBL which was maximum in $10^{-8}$ M 28–HBL (8.83±0.094 mol U $mg^{-1}$ protein) and $10^{-10}$ M 24-EBL (5.27±0.09 mol U $mg^{-1}$ protein) as compared to untreated seedlings (2.03±0.11 mol U $mg^{-1}$ protein).

## DISCUSSION

Brassinosteroids have been reported to control various development functions such as promotion of cell division, cell elongation, photomorphogenesis, seed germination and promotion of ethylene biosynthesis (Mussig *et al.*, 2002). The potential applications of brassinosteroids in agriculture are based on their ability to increase crop yields (Khripach *et al.*, 2000). Brassinosteroids also have strong potential to protect plants from various environmental stresses, including drought, extreme temperatures, heavy metals, herbicidal injury and salinity (Bhardwaj *et al.*, 2006; Krishana, 2003; Haubrick and Assmann, 2006; Janeczko *et al.*, 2008). Present study showed that under field conditions when temperature conditions were favorable with high day temperature (25 ºC±2 ºC) and low night temperature (18 ºC±2 ºC), both brassinolides stimulated germination and seedling growth. Low concentration of 28-HBL ($10^{-10}$ M) worked better to significantly enhance the percentage of seed germination. Our results are in accordance with Takeuchi *et al.* (1995); Hayat and Ahmad (2003) and Ali *et al.* (2005) who reported that $10^{-10}$ M or $10^{-8}$ M homobrassinolide treatment increased the percent germination in witch weed, clover broom-rape seeds, grain seeds and *Cicer arietinum* seeds when presoaking treatment was given. Brassinostreoids affect a broad spectrum of physiological functions of plant growth, differentiation and development such as stem elongation (Tanaka *et. al.*, 2003). In the present study, it was observed that $10^{-8}$ M epibrassinolide presoaking treatment enhanced the shoot length significantly as compared to control 10 day old seedlings. Exogenous application of brassinolide in micro molar concentrations enhanced morphological features of light grown plants i.e. short hypocotyls, expanded cotyledons and true leaves lead to the hypothesis that BRs play a mediatory role in phytochrome regulatory functions (Nagata *et. al.*, 2000; Bhardwaj *et. al.*, 2006). Homobrassinolide at $10^{-10}$ M concentration ameliorated SOD activity significantly as compared to other BRs and control seedlings. Sharma and Bhardwaj (2007) reported maximum increase in SOD activity in seedlings treated with $10^{-7}$ M 28-HBL under Cu metal stress. Correspondingly, CAT also showed enhanced activity in presoaking treatment of BRs. Nuñez *et al.* (2003) reported an increase in CAT activity in rice cultivar that was induced by BR analogue whereas Mazorra *et. al.* (2002) reported that increase in CAT activity depended on the structure of BR, dose and temperature in tomato. Similar results were observed for CAT and other antioxidant enzymes activity during seed germination and seedling growth under various types of stresses by Sharma *et al.* (2007); Hayat *et al.* (2007a) and Devi and Prasad (2005) in *B. juncea*. Increase in IAAO activity in the present study under the influence of BR treatment illustrated the influence of brassinolide on IAAO activity. This augmentation in IAAO activity was more significant in $10^{-8}$ M 28-HBL than all other treatments of BRs. Several reports are available in literature regarding the cross talk between BRs with other phytohormones. IAA and BRs perform one common function of cell elongation along with other physiological responses. Presoaking treatment of BRs enhanced the protein content significantly. These results were similar to that of Bajguz (2000) who reported that BRs treatment stimulated and enhanced the content of nucleic acids and proteins in alga *C. vulgaris.*

The present study revealed that both BRs are effective in ameliorating the oxidative stress as compared to untreated control seedlings and thus make the plant perform better in terms of seed germination and seedling growth. Out of the two active forms, 28-HBL was more effective than 24-EBL, indicating that BR induced responses depend on the structure of BR, dose and also on the stage of growth and development.

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