# Brassinosterioids Promote Photosynthetic Parameters of Pigeon Pea Plants under Water Deficit Conditions

**Sujatha Edupuganti, Shahana Tahinyath, Anusha**

***Abstract*: This study was aimed to find the effects of 28-epibrassinolide (28-EBL) and HBL on pigeon pea seedlings subjected to drought stress, either alone and supplemented with 28-EBL and HBL treatments. Supplementation of EBL alone also exhibited the significant improvement on chlorophyll content (44.3% of Chl a and 54.3 % of Chl b) than EBL under drought stress treatments compared to the control plants. Control plants receiving the EBL alone treatment showed the significant effect (by 36%) than the EBL under stress treatment (19.78%) compared to the control for carotenoid levels. supplementation of EBL exhibited the significant improvement of PN (36%; 0.0411, p≤0.05) over their individuals in comparison to control. application of EBL under unstressed was more effective (45%; 0.0341, p≤0.05) over stressed control than their individual applications in enhancing gS in control plants. Plants treated with HBL alone showed a marked increase in Ci (by 25.1%) compared to control plants. application of HBL and was also effective (41.2%; 0.0265, p≤0.05) over unstressed control than their individual applications in enhancing E in control plants. application of EBL and HBL considerably increased the Fv/Fm and ΦPSII in comparison to unstressed control. Control plants treated with EBL exhibited the impact on RuBPcase activity (43.1%; 0.0411, p≤0.05) over stressed control than their individual applications over the control plants. application of EBL was more effective (66.7%; 0.0237, p≤0.05) over unstressed control than their individual applications in enhancing FBPase activity in control plants. Control plants treated with EBL and HBL was found to be less effective on the PGK activity by 66.2% (0.0112, p≤0.05) than their individuals (EBL by 163.9%) compared to control. EBL and HBL has a more significant effect than their individual applications on the improvement of leaf starch and sucrose concentrations in drought stressed and well-watered plants.*

***Keywords*: EBL, HBL, Drought stress, Pigeon pea, Photosynthetic parameters.**

## I. INTRODUCTION

Since brassinolide (BL) was first discovered from Brassicus napus dust 30 years back (Grove et al. 1979), approximately 60 related species have been recognized, which are all referred to as brassinosteroids (BRs). BRs are fundamental for ordinary plant development, reproduction and advancement. Plants hindered in biosynthesis, perception or motioning of BRs display the commonplace phenotypes of dwarf with epinastic leaves, reduced or abrogated fertility and postponed improvement (Bishop and Koncz 2002). In Arabidopsis, the distinguishing proof of BR-inadequate interrupt prompted the cloning of a few critical genes included in BR biosynthesis, for example, constitutive photomorpho genesis what's more, dwarfism (Szekeres et al. 1996), dwarf4 (DWF4; Choe et al. 1998) and detiolated2 (DET2; Li et al. 1996). The CPD and DWF4 genes encode cytochrome P450 monooxygenases. Bolstering explores different avenues regarding BR biosynthetic intermediates uncovered that the C-22 and C-23 places of BRs are progressively hydroxylated by DWF4 what's more, CPD, separately. The DET2 gene encodes a 5-steroid reductase, which catalyzes the initial phase in the BR biosynthesis pathway (Noguchi et al. 1999). The BR-lacking Arabidopsis freak, bul1, is defective in the 7-sterol-C5- desaturation venture in the phytosterol pathway (Catterou et al. 2001). Until this point, the sub-atomic genetics combined with biochemical studies has built up the BR biosynthetic pathway (Yokota 1997; Bishop 2007). Like other plant hormones, BRs effect numerous physiological forms, including cell expansion, cell division, xylem differentiation, proton pump action, ethylene biosynthesis what's more, photosynthesis (Sasse 1997; Clouse and Sasse 1998; Yu et al. 2004). In addition, BRs regulate plant reaction to natural pressure and pathogen contamination (Dhaubhadel et al. 2002; Krishna 2003; Nakashita et al. 2003). Eminently, use of BRs expands the yield of numerous harvests (Khripach et al. 2000). The yield-advancing action of BRs is reliable with their development advancing movement illustrated in Arabidopsis. For instance, in transgenic Arabidopsis overexpressing DWF4, a general increment in BR level outcomes in expanded per-plot seed yield (Choe et al. 2001), though an Arabidopsis freak defective in sterol 7 reductase is deficient in BR biosynthesis and produces aberrantly molded seeds (Choe et al. 2000). There is a

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* Correspondence Author
**Sujatha Edupuganti,** Department of Botany, University College of Science, Osmania University, Hyderabad, Telangana, India.
**Shahana Tahinyath,** Department of Botany, University College of Science, Telangana University, Dichpally, Nizamabad, Telangana, India.
**Anusha,** Department of Botany, University College of Science, Telangana University, Dichpally, Nizamabad, Telangana, India.

promising prospect to build harvest yield and sustenance generation through utilization of BR-inferred development advancing substances in present day agrosystem (Wu et al. 2008). BRs advance plant development likely through different mechanisms. Various investigations have embroiled BRs in the guideline of cell division and expansion (Hu et al. 2000; Catterou et al. 2001). Also, hindrance of BR biosynthesis by the expansion of brassinazole (Brz) restrains the development of light-developed Chlorella vulgaris cells (Bajguz and Asami 2004). It has been accounted for that BRs increment leaf photosynthesis and this action of BRs may add to expanded harvest yield after BR application (Yu et al. 2004). It has been as of late demonstrated that tissue-specific overexpression of sterol C-22 hydroxylases in stems, leaves and roots, be that as it may, not in the developing spores or endosperms, brings about 15–44% increment in grain yield per plant (Wu et al. 2008). Further examination proposes that upgraded CO2 digestion and amplified sugar pools in the leaves may stimulate assimilate in the seeds and increment grain yields. Expanded paces of CO2 absorption have been watched in plants with expanded BR levels through BR applications (Hayat et al. 2000; Yu et al. 2004). Be that as it may, it is difficult to decide if reduced BR biosynthesis or flagging has a comparing negative effect on CO2 digestion due to the solid and pleiotropic phenotypes of BR-deWcient or on the other hand flagging freaks in Arabidopsis (Müssig et al. 2002). Then again, BR blend changes can be phenocopied by exogenously connected Brz, a speciWc inhibitor of BR biosynthesis (Asami et al. 2000). Brz legitimately ties to DWF4 protein, hinders the hydroxylation of the C-22 position of BR biosynthetic intermediates and effectively decreases the BR content in plants (Asami et al. 2001). By utilizing Brz, we can lessen the BR content inside a short timeframe without causing solid pleiotropic phenotypes in cucumber. Without optional impacts of solid pleiotropic phenotypes, we can decide if BR is straightforwardly associated with the guideline of photosynthesis. Moreover, we have recently exhibited that BR application prompted an expansion in ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) actuation state (Yu et al. 2004).

BR-prompted activation of Rubisco is induced. To address these issues, we investigated the substance of RCA, which is personally associated with guideline of Rubisco movement and the articulation of a few genes associated with the Calvin cycle. Our outcomes show that BRs assume a significant job in directing blend and enactment of an assortment of proteins in the photosynthetic device.

Various investigations have demonstrated that BRs can enhance the capacity of plants to adapt to stresses, for example, heavy metal stress, water stress, salt stress, high and low temperature stress and pathogen assault (Ali et al., 2008; Bajguz and Hayat, 2009; Hayat et al., 2010). Schilling et al. (1991) detailed that homobrassinolide treatment increased tap-root weight, sucrose substance, and yield of sugar beets developed under drought stress. Sairam (1994) found that homobrassinolide application increased water take-up and membrane stability, and decrease the ion leakage in two wheat assortments. Pustovoitova et al. (2001) saw that cucumber splashed with a manufactured BR, 24-epibrassinolide (EBR), had improved protection from dehydration. BR treatment has likewise been accounted to increase germination and seedling development of Sorghum vulgare under osmotic stress (Vardhini furthermore, Rao, 2003) and enhance drought resistance in Phaseolus vulgaris (Upreti and Murti, 2004). Moreover, it has been demonstrated that treatment with EBR increased the survival pace of Arabidopsis thaliana furthermore, Brassica napus seedlings exposed to drought stress (Krishna, 2003; Kagale et al., 2007). Farooq et al. (2009) likewise found that BRs application improved the leaf water economy and CO2 assimilation, furthermore, empowered rice to withstand drought.

## II. MATERIALS AND METHODS

**Determination of photosynthetic pigments: Arnon, (1949)**

Chlorophyll pigments were extracted and estimated according to the method of Arnon, (1949). Fresh leaf material was taken in a clean mortar and homogenized with pestle using 80% (v/v) acetone. The green slurry was centrifuged at 5000 rpm for 10 minutes. The supernatant was transferred to a 25 ml volumetric flask. The residual pigments were re-extracted using small amounts of 80% acetone and centrifuged. The supernatant was transferred to the volumetric flask. The extraction was repeated till complete white residue was obtained. The combined chlorophyll extracts were made up to 25 ml with 80% acetone. The optical density was recorded at 645 nm, 663 nm and 480 nm against 80% (v/v) acetone as blank in UV-Visible Spectrometer (SCHIMADZU UV-1800, Japan).

The amount of pigments present in the pigment extract was determined employing the following formulae:

Chlorophyll ‘a’ = [(OD 663 ×12.7) – (OD 645 × 2.69)] × V / (1000 × W)

Chlorophyll ‘b’ = [(OD 663 × 22.9) – (OD

645 × 4.68)] × V / (1000 × W)

Total chlorophylls = [(OD 663 × 20.2) – (OD 645 × 8.02)] × V/ (1000× W)

Carotenoids = (1000 OD 480 – 3.27 (Chl a) – 104 (Chl b)) / 227

Where,

V-volume of the pigment extract; W -weight of the leaf material in grams.

Chlorophyll and carotenoid contents were expressed in mg g/fresh weight.

**Gas exchange measurements:**

Gas exchange parameters were measured in mature, fully expanded leaves from the upper crown of plants. Gas exchange and chlorophyll fluorescence were measured in the same leaf. Gas exchange parameters such as net photosynthetic rate (Pn), transpiration rate (E), stomatal conductance (Gs) and internal $CO_2$ concentration (Ci) were measured with a Li-Cor model 6400 system (Lincoln, NE, USA). These measurements were carried out on the middle part of the youngest (fully opened second leaf), which avoided the leaf vein. The measurements were conducted from during 8:30 to 10.00 am., during this time the curtain of the greenhouse was shut down to avoid effects of different light conditions. The saturating photosynthetic photon flux density was between 1000 and 1500 µmol $m^{-2}$ $s^{-1}$ in the leaf chamber during the measurement periods, and the temperature, $CO_2$ concentration and relative humidity inside the leaf cuvette were always close to ambient air values.

**Chlorophyll fluorescence:**

Chlorophyll fluorescence parameters were determined using a PAM-2500 chlorophyll fluorescence analyser (WALZ, Germany) between 9:00 and 11:00. After a 20 min dark adaptation period, the maximal photochemical efficiency of PSII (Fv/Fm), quantum efficiency of PSII ($\phi_{PSII}$) and Photochemical quenching ($q_P$) were determined. The cuvette of the gas exchange system was modified to accept the fibre optic of the fluorimeter at a 60° angle without significantly interfering with PPFD distribution at the leaf surface. Minimal fluorescence ($F_0$) was measured under a weak pulse of modulating light over a 0.8 s period, and maximal fluorescence ($F_m$) was induced by a saturating pulse of light (8000 µmol $m^{-2}s^{-1}$) applied over 0.8 s. The maximal quantum efficiency of PSII was determined as $F_v/F_m$, where $F_v$ is the difference between $F_0$ and $F_m$ An actinic light source (600 µmol $m^{-2}$ $s^{-1}$) was then applied to achieve steady state photosynthesis and to obtain $F_s$ (steady state fluorescence yield), after which a second saturation pulse was applied for 0.7 s to obtain $F'_m$ (light adapted maximum fluorescence). Fluorescence parameters were calculated by the FMS 2, based on the dark adapted and light adapted fluorescence measurements. The quantum efficiency of PSII ($\phi_{PSII}$) and the efficiency of excitation capture by open PSII centres were calculated as $(F'_m–F_s)/F'_m$ and $F'_v/F'_m$, respectively. Photochemical quenching ($q_P$) was calculated as $(F'_m–F_s)/(F'_m–F_0)$.

**Calvin Cycle Enzymes:**

Makino *et al.*, (1988) Fully expanded trifoliate leaves without petioles were homogenized in ice cold 5 ml of 100 mM Tris-HCl (pH 8) buffer consisting of 10 mM $MgCl_2$, 10 mM $NaHCO_3$, 10 mM ß-mercaptoethanol, 2 mM DTT, 1% (v/v) Triton X-100, 10% (v/v) glycerol, 100 mg insoluble PVP and 1 mM PMSF. The extracts were centrifuged at 16,000 *g*, for 20 min, (4 °C) and the supernatant was used for the enzyme assays, all of which were based on NADH oxidation at 340 nm, at 25 °C, in 1 mL final volume in the cuvette. 100 mg insoluble PVP

Hatch and Kagawa (1973), Ribulose-1,5-bisphosphate carboxylase (RuBPcase, EC 4.1.1.39): Extraction was done as described by Makino *et al.*, (1988). RuBPcase was activated for 20 min at 0°C after preparation of the supernatant in the activation medium that contained 75 mM Hepes–KOH at pH 7.5, 10 mM $MgCl_2$ and 10 mM $NaHCO_3$. To determine the Rubisco activity, a 50 µl of extract was added to 900 µl of reaction mixture consisting of 100 mM bicine at pH 8.2, 5 mM $MgCl_2$, 10 mM $NaHCO_3$, 5 mM creatine phosphate, 1 mM ATP-2 Na, 0.1 mM NADH, 0.3 mM RuBP, 10 units of phosphocreatine kinase, 10 units of glyceraldehydes-3-phosphate dehydrogenase and 10 units of phosphoglycerate kinase, as described by Sawada *et al.*, (1990). The change in absorbance at 340 nm was immediately recorded for every 5 s for 5min. The enzymatic activities were corrected for the decrease in absorbance in a control assay medium prepared without ribulose bisphosphate at 25 ºC.

3- phospho-glycerate kinase (PGK, EC 2.7.2.3): Scheibe *et al.*, (1986), PGK activity was determined according to Hatch and Kagawa, (1973). The reaction mixture consisted of 100mM HEPES-KOH (pH 7.8), 10 mM $MgCl_2$, 1 mM NaF, 1 mM KH2PO4, 1 mM phosphoglyceric acid, 4 units of triose phophate isomerase and 4 units of glyceraldehydes-3-phosphate dehydrogenase. The reaction was started by the addition of 2 mM ATP and 0.1 1 mM NADH. NADH oxidation was determined spectrophotometrically at 340 nm at 25 ºC.

Fructose 1,6-bisphosphatase (FBPase, EC 3.1.3.11): FBPase activity was determined by monitoring the absorption at 340 nm, using an extinction coefficient of 6.2 mM-1 cm-1 (Scheibe *et al.*, 1986). Total activity was assayed after the crude extract had been activated in a 0.1 ml activation mixture containing 100 mM DTT, 2 mM fructose-1,6- bisphosphate (FBP), 10 mM MgCl2, and 0.1 M Tris–HCl (pH 8.0). The initial activity was assayed immediately after homogenization. The assay mixture consisted of 0.1 M HEPES (pH 8.0), 0.5 mM Na2EDTA, 10 mM MgCl2, 0.3 mM NADP+, 0.6 mM FBP, 0.6 units of glucose- 6-phosphate dehydrogenase from baker's yeast, 1.2 units of glucose phosphate isomerase from baker's yeast, and 100 µl of enzyme extract in a final volume of 1 ml. The reaction was initiated by the addition of enzyme extract.

Ribulose 5-phosphate kinase (RuB5PK, EC 2.7.1.19): RuB5PK activity was performed according to Kagawa, (1982). Twenty (20) µl of supernatant was added to the reaction mixture containing 100 mM Tris-HCl, pH 8.0, 8 mM $MgCl_2$, 40 mM KCl, 20 mM phosphoenol pyruvate, 5 mM ATP, 1 mM NADH, 20 mM DTT, 8 units of pyruvate kinase, 10 units of lactate dehydrogenase and 5 units of phosphoriboisomerase. After an incubation period of 15 min, the reaction was initiated by adding 10 µl of 500 mM ribose-5-phosphate and change in absorbance was monitored at 340 nm for every 5 s for 5 min.

**Carbohydrate Fractions:**

Samples for enzyme assays and chemical analyses were fully expanded leaves frozen in liquid $N_2$ and stored at-80$^{o}$C and used for soluble sugar analysis. The residue left after extracting soluble sugars was used for determination of starch content. Ethanol homogenate (2.5 ml) was taken into centrifuge tubes. The tubes were kept in a boiling water bath for 5 minutes. After cooling, the contents were centrifuged at 4,000 rpm for 10 minutes. The supernatant was collected. The residue was re-extracted with 5 ml of 70% (v/v) ethanol and was centrifuged again. This procedure was repeated 3 times. The ethanol supernatants were pooled and made up to 10 ml. This was used for the estimation of total sugars and reducing sugars.

**Estimation of total sugars:**

Total sugars were estimated according to the method of Yoshida *et al.*, (1976). 5 ml of alcohol extract was evaporated to dryness in a clean beaker in a water bath at 60 ºC. The lipids and pigments were removed by washing the evaporated residue repeatedly with diethylether. Then the residue was dissolved in 5 ml of 40 % (v/v) ethanol. This was used for the estimation of total sugars by anthrone reagent.

Anthrone reagent: 200 mg of anthrone dissolved in 100 ml of concentrated sulphuric acid.

One ml of extract was taken and to it 5 ml of anthrone reagent was added. The tubes were heated for 7½ minutes in a boiling water bath. The tubes were cooled and the intensity of brown colour developed was recorded at 630 nm in UV Visible Spectrometer (SCHIMADZU UV-1800, Japan) using blank. The blank consisted of 1 ml of 40 % (v/v) ethanol and 5 ml of anthrone reagent. The total sugars were estimated as D-glucose equivalents. The amount of glucose was found out from a glucose standard curve. The amount of total sugars was expressed as mg $g^{-1}$fr.wt.

**Estimation of reducing sugars:**

Reducing sugars were determined according to Nelson, (1944) method. Nelson reagent was used for the estimation of reducing sugars (Glucose and Fructose, using standard graphs).

Nelson reagent: Nelson reagent was prepared by mixing reagents A and B prior to their use as follows:

Reagent A: 2.5 g of sodium carbonate, 2.5 g of sodium potassium tartarate, 2 g of sodium bicarbonate and 400 mg of copper sulphate were dissolved in distilled water and then the volume was made up to 100 ml in a volumetric flask with distilled water.

Reagent B: Reagent B contains solution 1 and 2.

Solution 1: 2.5g of ammonium molybdate was dissolved in 90 ml of distilled water and to this 2.1 ml of concentrated sulphuric acid was added.

Solution 2: 300 mg of sodium arsenate was dissolved in 7.9 ml of distilled water.

Just before use, solution 1 and solution 2 were mixed and heated gently to obtain light yellowish Reagent B.

One ml of Nelson reagent A was added to 1 ml of the sample. A blank was prepared with 1 ml of 70% ethanol instead of sample and 1 ml of reagent A. The colour of the mixture turns to light green. The contents were heated in a water bath for 15 minutes till the green color disappears. It was cooled to room temperature and to this 1 ml of Nelson reagent B was added. Soon after the addition of Nelson reagent B, the mixture turned to thick blue color. The contents were diluted by adding 5 ml of distilled water.

The absorbance was recorded in at 550 nm

against the blank in UV Visible Spectrometer (SCHIMADZU UV-1800, Japan).

Estimation of Non reducing sugars:
The amount of non-reducing sugars was calculated by the following formulae as given by Loomis and Shull, (1937):

Non reducing sugars = (total sugars – free reducing sugars) ×0.95

The amount of non-reducing sugars was expressed as glucose equivalents in terms of mg $g^{-1}$ fresh weight.

**Estimation of Starch:**
Starch was estimated from the residue left after alcohol extraction of the sugar by employing the method of Mc. Cready *et al.*, (1950). The starch was solubilized from the residue for 1 hour with 5 ml of 52% perchloric acid. The contents were centrifuged at 3000 rpm for 15 minutes. The supernatant was collected. 1 ml of perchloric acid extract was diluted to 3 ml with distilled water. To this 5 ml of freshly prepared anthrone reagent was added. The mixture was heated in a water bath for 7 ½ minutes at 100ºC. The contents were cooled and were thoroughly shaken. The absorbance of the contents was measured at 630 nm in a UV Visible Spectrometer (SCHIMADZU UV-1800, Japan) against blank, which was made without the starch extract. The amount of glucose was calculated from a standard curve prepared by using known amount of glucose. The starch content was calculated by multiplying the glucose equivalents present in the sample with 0.9. The content of starch was expressed as mg $g^{-1}$ fresh weight.

## III. RESULTS AND DISCUSSION

**Photosynthetic pigments**:
Effect of BRs treatment on the chlorophyll (Chl) a, b and carotenoid contents in normal and drought stressed plants of pigeon pea plants are presented in Table1.

Sign of chlorosis was observed in pigeon pea plants under water limited conditions (25% soil moisture content) after one week. The Chla and total Chl content were significantly improvement by 44.3% and 54.3% under drought stress whilst the decrease of Chl b content was insignificant (19.78%, p=0.0623) compared to control. Drought stress also found to be reduced the carotenoid levels significantly by 36%. In many plant species, accumulation of soluble sugars occurs to counteract stressful environment through osmotic alterations (Rosa *et al.*, 2009; Neeta and Shitole, 2010).However, drought stressed plants fed with BRs, inhibitory effect of drought on the photosynthetic pigments was reduced. Exogenous HBL accounted for the significant improvement in the Chl a, Chl b and total Chl contents by 39.5, 21.6 (at 1µM HBL) and 38.2% respectively over the stressed control. The enzyme sucrose phosphate synthase (SPS) catalyses sucrose biosynthesis in the plant tissues whereas sucrose synthase (SS) and acid invertase (AI) involved in sucrose-cleavage in vivo and translocating the assimilates to diverse pathways in plant storage cells (Rosa *et al.*, 2009; Ruan, 2014).
Similarly, stressed plants receiving EBL application also showed significant increase in the photosynthetic pigments with maximum effect being at 2µM concentration by 47.1, 27.1 and 45.9% respectively. Carotenoid levels were also increased by 14.16% and 12.6% upon EBL and HBL treatment to stressed plants respectively. BRs alone treatments marginally increased the Chl b content, whereas Chl a (20% at 2µM EBL), total Chl (24.2 at 2µM EBL) and carotenoids levels (14.48% at 1µM EBL) were increased significantly compared to the control.

**Leaf gas exchange responses:**
Effect of BRs on net photosynthetic rate (*Pn)*, stomatal conductance ($G_S$), inter cellular $CO_2$ concentration (Ci) and transpiration rate (E) under drought stress are presented in Table 2.

Perception of drought drastically reduced the Pn and $G_S$by 25.1% and 42.76% respectively compared to control. However, exogenous application of BRs was able maintained the statistically significantly higher levels of Pn and $G_S$ under drought stress. Foliar spray of EBL significantly improved the Pn and $G_S$ by 31.4% and 77.1% respectively over the stressed control. Similarly, 2µM HBL treatment also exhibited a significant 29.2% and 72.8% of Pn and $G_S$ levels compared to the stressed control plants. BRs treatments alone also maintained the significant levels of Pn (p=0.027) and $G_S$ (p=0.033) in pigeon pea plants compared to the control.

A significant reduction (31.7%) in intracellular $CO_2$ concentration (Ci) was noted for drought stressed plants when compared with controls. Foliar application of BRs to drought stressed plants significantly improved the Ci under water limited conditions. Foliar spray of EBL to drought stressed plants accounted for significant increase in Ci levels by 43.3% at 2µM concentration. HBL treatment at 1 µM concentration increased the Ci levels by 36.9% (p=0.044) in drought stressed plants. Unstressed plants receiving the BRs exhibited the considerable rise in Ci levels at 2 µM concentration.

Compared to control, transpiration rate (E) was significantly decreased by 56.8% in drought stressed pigeon pea plants. However, follow up treatment withBRsreversed the trend and enhanced the E in to drought stressed plants over the stressed controls. The transpiration rate (E) was significantly increased by 122.3% and 124.7% upon EBL and HBL treatments respectively in drought stressed plants. Plants exhibited a significant increase in E value by 35.5% treated with 2 µM EBL alone over the control.

**Chlorophyll fluorescence responses:**

Effect of BRs on the changes of chlorophyll fluorescence parameters in pigeon pea leaves under drought stress are shown in Table 3.

Chlorophyll fluorescence dynamics revealed that Fv/Fm, ΦPSII, and qP were substantially reduced by 39.6, 42.58 and 39.3% ($p \leq 0.05$) under water limited conditions compared with controls.The addition of exogenous BRs alleviated these effects. Exogenous application of EBL to drought stressed plants protected the quantum yield of PSII. Exogenous EBL, at 2 µM concentration able increase the Fv/Fm, ΦPSII and qP significantly by 64.5, 75.9 and 65.3% over stressed control. Similarly, HBL also enhanced the Fv/Fm, ΦPSII and qP by 58.9, 70 and 59.7% compared with stress control. In addition, BRs alone treatments also enhanced the photochemical yield of PSII as evidenced by significant enhancement of Fv/Fm (28.5% by EBL and 12.5% by HBL) and ΦPSII (37% by EBL and 19.4% by HBL). Exogenous application of BRs slightly increased the qP (6.8%, p=0.178) however the effect was insignificant.

**Calvin cycle enzyme activities:**

Effect of BRs on the Ribulose-1,5-bisphosphate carboxylase (RuBPcase), 3- phosphor-glycerate kinase (PGK), Fructose 1,6-bisphosphatase (FBPase) and Ribulose 5-phosphate kinase (RuB5PK) enzyme activities in pigeon pea leaves under drought stress are shown in Table 4.

Ribulose-1,5-bisphosphate carboxylase (RuBPcase): Drought stress caused the significant reduction (43.1%) in RuBPcase activity in pigeon pea plants. Restoration of the RuBPcase activity near to the control was noted in drought stressed plants treated with BRs. Exogenous EBL significantly increased the RuBPcase activity to the tune of 66.7% at 2µM concentration. A significant enhancement (by 61.2%) of RuBPcase activity was observed in *Cajanus cajan* under drought treated with 2µM HBL. BRs alone treatments also showed a considerable increase RuBPcase activity. Treatment with HBL alone increased the RuBPcase activity by 16.4% at1µM concentration over the control. Similarly, EBL alone treatment registered a 25.2% (p=0.027) increase in RuBPcase activity at 2µM concentration over the control.

3-Phosphor-Glycerate Kinase (PGK): A significant reduction (37.46%) in PGK activity was noticed in pigeon pea plants under water limited conditions. However, exogenous EBL and HBL removed the toxic effects of drought and improved the PGK activity by 53.4% (p=0.028) and 50.4% (p=0.019) respectively, at 2µM concentration compared with control. Plants receiving BRs alone treatments also registered the statistically significantly increase in PGK activity ($p \leq 0.05$) at 2µM concentration compared with control.

Fructose 1,6-bisphosphatase (FBPase): FBPase activity reduced significantly by 66.2% in pigeon pea plants subjected to drought stress. BRs alleviated this effect and improved the FBPase activity under drought stress. Foliar spray of EBL to stressed plants registered the significant enhancement of FBPase activity by 163.9% over the stressed control. At 2µM concentration of HBL had higher FBPase activity (130.9%) in stressed plants. BRs alone treatments also caused considerable increase in the FBPase activity. The maximum increase of 41.1% ($p \leq 0.05$) in FBPase activity was found in plants treated with EBL alone at 2µM concentration. HBL alone treatments also enhanced the FBPase activity with highest being recorded at 2µM concentration (27.5% ; $p \leq 0.05$ over the control).

Ribulose 5-phosphate kinase (RuB5PK): RuB5PK activity was sharply reduced (23.9%) in drought stressed plants compared with control. Application of BRs to drought stressed pigeon pea plants was reversed the hampered RuB5PK activity. Exogenous application of EBL enhanced the RuB5PK activity gradually with increasing supplemented concentration (0.5 µM through 2 µM EBL). At 2 µM concentration of EBL, RuB5PK activity completely restored to normal values, with little increase as well. Similarly, supplementation of HBL to pigeon pea under drought stress resulted in restoration of RuB5PK activity close to normal levels (by 92.9 % compared to control; by 22.1 % compared with stressed control). BRs alone treatment also improved the RuB5PK activity significantly by 29.5% (p=0.0211; at 2 µM EBL) and 22.1% (p=0.0412; at 2 µM HBL) compared with control. Findings of present study are in coherence with the observations of Gengmao *et al.*, (2014), where carbohydrates were reported to increase in *Salvia miltiorrhiza* plants under NaCl toxicity. Similarly, elevated levels of glucose, fructose and sucrose were observed in *Brassica juncea* plants under Cd toxicity.

Glucose and fructose are involved in maintaining osmotic potential and scavenging free radicals in *Oryza sativa* (Pattanagul and Thitisaksakul, 2008). Furthermore, soluble sugars are also involved in ROS anabolism and catabolism, such as the oxidative pentose phosphate pathway associated with ROS scavenging (p *et al.*, 2006).

**Carbohydrate fractions:**
Effect of BRs on the levels of carbohydrate fractions in pigeon pea plants under stress and stress free conditions are presented in Table 5. Carbohydrate fractions were sharply declined in pigeon pea plants subjected to water limited conditions (25% SMC). However, exogenous application of BRs to stressed plants reversed the inhibitory effect of drought stress and improved the carbohydrate pools.

**Reducing sugars:** The levels of reducing sugars decreased significantly by 54.5% under drought stress in pigeon pea plants. Supplementation of both BRs increased the reducing sugar concentrations in stressed plants dose dependently. At 2µM concentration reducing sugars were restored to 77.7% and to 91% by HBL and EBL respectively in pigeon pea plants compared to stress control. Plants receiving BRs alone treatments also exhibited the significant ($p \leq 0.05$) levels of reducing sugars over the control plants.

**Non-reducing sugars:** Drought stress decreased the non-reducing sugars levels significantly by 55.2% in pigeon pea. Exogenous application of BRs restored the non-reducing sugar levels in drought stressed plants near to control, with maximum restoration being at 2µM concentration. Compared to the EBL, HBL accounted for the higher significant levels of non-reducing levels (56.9% vs 45.8%, $p \leq 0.05$). Both, BRs alone treatments also improved the non-reducing levels considerably as compared to control.

Starch: Drought stress was found to reduce the starch levels by 59.3% in pigeon pea plants compared to control. However, exogenous application of EBL and HBL enhanced the starch levels significantly by 95.5% and 124% over the stressed control respectively. BRs alone (0.5 µM to 2 µM) treatments also triggered the starch levels considerably compared to the unstressed control.

Table 1. Effect of Brassinosteroids on the levels of photosynthetic pigments in Cajanus cajan plants under drought stress and stress free conditions.

| Treatments | Chlorophyll a (mg $g^{-1}$ FW) | Chlorophyll b (mg $g^{-1}$ FW) | Total Chlorophyll (a +b) | Carotenoids (mg $g^{-1}$ FW) |
|---|---|---|---|---|
| **Control** | 1.779 ±0.634 c | 0.854 ±0.057 f | 2.463 ±0.724 e | 0.718 ±0.044 e |
| **0.5 µM EBL** | 1.691 ±0.387 d | 0.857 ±0.012 e | 2.548 ±0.527 d | 0.772 ±0.057 d |
| **1 µM EBL** | 1.723 ±0.408 c | 0.901 ±0.077b | 2.624 ±0.864 c | 0.822 ±0.079 a |
| **2 µM EBL** | 2.138 ±0.211 a | 0.923 ±0.067a | 3.061 ±0.727 a | 0.792 ±0.085 b |
| **0.5 µM HBL** | 1.689 ±0.325 e | 0.854 ±0.074 e | 2.543 ±0.625 d | 0.753 ±0.088 d |
| **1µM HBL** | 1.745 ±0.214 bc | 0.917 ±0.092 a | 2.662 ±0.826c | 0.766 ±0.072 c |
| **2 µM HBL** | 1.876 ±0.448 b | 0.893 ±0.064 c | 2.769 ±0.923b | 0.789 ±0.077 b |
| **Drought Stress (DS)** | 0.991 ±0.079 l | 0.685 ±0.073 j | 1.596 ±0.422 l | 0.459 ±0.087 k |
| **DS +0.5µM EBL** | 1.229 ±0.280 j | 0.683 ±0.054 h | 1.992 ±0.643 j | 0.485 ±0.064 hi |
| **DS +1µM EBL** | 1.458 ±0.370 f | 0.871 ±0.094 d | 2.329 ±0.428 f | 0.511 ±0.077 g |
| **DS +2µM EBL** | 1.326 ±0.278 h | 0.838 ±0.092 f | 2.164 ±0.621 h | 0.524 ±0.072 f |
| **DS +0.5µM HBL** | 1.218 ±0.218 k | 0.648 ±0.083 i | 1.866 ±0.512 k | 0.476 ±0.024 j |
| **DS +1µM HBL** | 1.266 ±0.261 i | 0.833 ±0.091 f | 2.099 ±0.748 i | 0.491 ±0.050 h |
| **DS +2µM HBL** | 1.383 ±0.374 g | 0.824 ±0.075 g | 2.207 ±0.488 g | 0.517 ±0.083 g |

The values are means ±SE ($n$ = 5); mean followed by the same alphabet in a column is not significantly different at $p \leq 0.05$ according to Post Hoc test.

Table 2. Effect of exogenous Brassinosteroids on net photosynthetic rate (Pn), stomatal conductance (Gs), intercellular CO2 concentration (Ci), and transpiration rate (E) in Cajanus cajan plants grown under drought stress and stress free conditions

| Treatments | Pn ($\mu$mol $CO_2$ $m^{-2}$ $s^{-1}$) | Ci ($\mu$mol $CO_2$ $m^{-2}$ $s^{-1}$) | Gs (mmol $H_2O$ $m^{-2}$ $s^{-1}$) | E (mmol $H_2O$ $m^{-2}$ $s^{-1}$) |
|---|---|---|---|---|
| **Control** | 9.18 ±0.81d | 274 ±11.021cd | 52.15 ±1.33d | 1.97 ±0.54cd |
| **0.5 µM EBL** | 11.27 ±0.64b | 287 ±12.317c | 55.25 ±0.98cd | 2.22 ±0.11 bc |
| **1 µM EBL** | 11.89 ±0.87b | 291 ±11.477c | 59.77 ±1.12a | 2.67 ±0.67 a |
| **2 µM EBL** | 12.84±0.68 a | 354 ±10.147a | 57.05 ±1.41 c | 2.15 ±0.41 c |
| **0.5µM HBL** | 10.87 ±1.02c | 294 ±9.627c | 57.24 ±1.57c | 2.10 ±0.92 c |
| **1µM HBL** | 11.54 ±0.57b | 307 ±10.107bc | 57.61 ±0.72c | 2.12 ±0.73 c |
| **2 µM HBL** | 11.96 ±0.86 b | 321 ±9.187b | 58.36 ±1.54b | 2.36 ±0.33b |
| **Drought Stress (DS)** | 6.87 ±0.78g | 187 ±9.875 i | 29.85 ±0.98h | 0.85 ±0.98 i |
| **DS +0.5µM EBL** | 7.58 ±0.77f | 213 ±7.954h | 43.55 ±1.21g | 1.21 ±0.57 h |
| **DS +1µM EBL** | 8.77 ±0.74e | 251 ±11.904e | 48.74 ±1.01f | 1.61 ±0.66 f |
| **DS +2µM EBL** | 9.01 ±0.81d | 268 ±9.555d | 52.87 ±0.87d | 1.89 ±0.25 e |
| **DS +0.5µM HBL** | 7.22±0.74f | 221 ±11.887g | 49.37±1.24f | 1.42±0.49g |
| **DS +1µM HBL** | 8.67 ±1.01ef | 256 ±10.091e | 50.68 ±0.98de | 1.78 ±0.93e |
| **DS +2µM HBL** | 8.88 ±0.96e | 244 ±8.931ef | 51.58 ±1.03 d | 1.91 ±0.57d |

The values are means ±SE ($n$ = 5); mean followed by the same alphabet in a column is not significantly different at $p \leq 0.05$ according to Post Hoc test.

Table 3. Effect of Brassinosteroids on the maximal photochemical efficiency of PSII (Fv/Fm), quantum efficiency of PSII ($\phi_{PSII}$) and photochemical quenching ($q_P$) in *Cajanus cajan* plants grown under drought stress and stress free conditions

| Treatments | Fv/Fm | ΦPSII | *qP* |
|---|---|---|---|
| **Control** | 0.875 ±0.014c | 0.674 ±0.009cd | 0.918 ±0.015c |
| **0.5 µM EBL** | 0.895 ±0.015c | 0.691 ±0.005c | 0.927 ±0.015c |
| **1 µM EBL** | 0.912 ±0.011b | 0.685 ±0.008c | 0.962 ±0.021b |
| **2 µM EBL** | 0.995 ±0.013a | 0.924 ±0.014a | 0.981 ±0.016a |
| **0.5µM HBL** | 0.925 ±0.019b | 0.688 ±0.006c | 0.933 ±0.010c |
| **1µM HBL** | 0.888 ±0.013c | 0.698 ±0.019c | 0.988 ±0.013a |
| **2 µM HBL** | 0.905 ±0.011bc | 0.805 ±0.010b | 0.971 ±0.011ab |
| **Drought Stress (DS)** | 0.528 ±0.003h | 0.387 ±0.007i | 0.557 ±0.021h |
| **DS +0.5µM EBL** | 0.627 ±0.010g | 0.467 ±0.009h | 0.758 ±0.024f |
| **DS +1µM EBL** | 0.776 ±0.017f | 0.598 ±0.014g | 0.866 ±0.016d |
| **DS +2µM EBL** | 0.869 ±0.011cd | 0.681 ±0.011c | 0.921 ±0.011c |
| **DS +0.5µM HBL** | 0.793 ±0.008f | 0.525 ±0.010gh | 0.693 ±0.018g |
| **DS +1µM HBL** | 0.839 ±0.009e | 0.604 ±0.006f | 0.857 ±0.017de |
| **DS +2µM HBL** | 0.829 ±0.013e | 0.658 ±0.007e | 0.890 ±0.018d |

The values are means ±SE ($n$ = 5); mean followed by the same alphabet in a column is not significantly different at $p \leq 0.05$ according to Post Hoc test.

Table 4. Effect of Brassinosteroids on Benson-Calvin cycle enzyme activities of Cajanus cajan plants under drought stress and stress free conditions.

| Treatments | RuBPcase ($\mu$mol $s^{-1}$ mg protein$^{-1}$) | PGK ($\mu$mol $s^{-1}$ mg protein$^{-1}$) | FBPase ($\mu$mol mg protein$^{-1}$ $s^{-1}$) | RuB5PK ($\mu$mol mg protein$^{-1}$ $s^{-1}$) |
|---|---|---|---|---|

| | | | | |
|---|---|---|---|---|
| **Control** | 0.677 ±0.005d | 0.323 ±0.008c | 2.87 ±0.74 d | 22.14 ±0.67e |
| **0.5 µM EBL** | 0.696 ±0.003d | 0.367 ±0.002bc | 3.16 ±0.85 c | 25.35 ±0.33b |
| **1 µM EBL** | 0.723 ±0.005c | 0.391 ±0.005b | 3.39 ±0.68 bc | 28.67 ±0.97 a |
| **2 µM EBL** | 0.748 ±0.004a | 0.410 ±0.011a | 4.05 ±0.38 a | 24.05 ±0.41 d |
| **0.5µM HBL** | 0.704 ±0.004c | 0.351 ±0.009c | 2.98 ±0.51cd | 27.14 ±0.58 a |
| **1µM HBL** | 0.788 ±0.007b | 0.398 ±0.005b | 3.26 ±0.92 c | 25.51 ±0.72 bc |
| **2 µM HBL** | 0.715 ±0.006c | 0.405 ±0.006ab | 3.66 ±0.7 b | 26.36 ±0.66b |
| **Drought Stress (DS)** | 0.385 ±0.003h | 0.202 ±0.007f | 0.97 ±0.26 h | 16.85 ±0.98 h |
| **DS +0.5µM EBL** | 0.498 ±0.009g | 0.267 ±0.008d | 1.68 ±0.73 g | 18.21 ±0.48g |
| **DS +1µM EBL** | 0.526 ±0.007f | 0.298 ±0.007d | 2.11 ±0.97be | 20.61 ±0.58 f |
| **DS +2µM EBL** | 0.642 ±0.008de | 0.310 ±0.006d | 2.56 ±0.59 d | 23.88 ±0.87de |
| **DS +0.5µM HBL** | 0.493 ±0.008g | 0.255 ±0.005e | 1.22±0.37g | 19.37±0.65g |
| **DS +1µM HBL** | 0.539 ±0.006f | 0.304 ±0.003d | 1.98 ±0.81f | 20.78 ±0.93f |
| **DS +2µM HBL** | 0.621 ±0.007e | 0.298 ±0.007e | 2.24 ±0.58d | 20.58 ±0.43 f |

The values are means ±SE ($n$ = 5); mean followed by the same alphabet in a column is not significantly different at $p \leq 0.05$ according to Post Hoc test.

Table 5. Effect of Brassinosteroids on carbohydrate levels of *Cajanus cajan* plants under drought stress and stress free conditions.

| Treatment | Reducing Sugars ($mg^{-1}$ g FW) | Non Reducing Sugars ($mg^{-1}$ g FW) | Starch ($mg^{-1}$ g FW) |
|---|---|---|---|
| **Control** | 0.99 ±0.007g | 1.61 ±0.033f | 2.78 ±0.044g |
| **0.5µM EBL** | 1.14 ±0.056f | 1.72 ±0.029e | 2.94 ±0.067e |
| **1µM EBL** | 1.52 ±0.068d | 1.80 ±0.022d | 3.17 ±0.042c |
| **2µM EBL** | 1.78 ±0.022b | 1.99 ±0.044b | 3.24 ±0.045a |
| **0.5µM HBL** | 1.23 ±0.033e | 1.72 ±0.028e | 2.82 ±0.074f |
| **1µM HBL** | 1.65 ±0.023c | 1.82 ±0.034c | 3.21 ±0.043d |
| **2µM HBL** | 1.83 ±0.032a | 2.06 ±0.097a | 2.97 ±0.050b |
| **Drought Stress (DS)** | 0.45 ±0.017n | 0.72 ±0.006m | 1.13 ±0.042n |
| **DS +0.5µM EBL** | 0.54 ±0.015m | 0.84 ±0.009l | 1.70 ±0.041l |
| **DS +1µM EBL** | 0.63 ±0.021k | 0.96 ±0.010i | 1.83 ±0.051j |
| **DS +2µM EBL** | 0.80 ±0.037i | 1.05 ±0.027h | 2.21 ±0.026i |
| **DS +0.5µMH BL** | 0.57 ± 0.023l | 0.85 ±0.009k | 1.24 ± 0.030m |
| **DS +1µM HBL** | 0.69 ±0.007j | 0.95 ±0.027j | 1.62 ±0.043jk |
| **DS +2µM HBL** | 0.86 ±0.016h | 1.13 ±0.044g | 2.53 ±0.037h |

The values are means ±SE ($n$ = 5); mean followed by the same alphabet in a column is not significantly different at $p \leq 0.05$ according to Post Hoc test.

## IV. CONCLUSION

The present study shows that Pigeon pea plants under water stress, photosynthetic activity was reduced by effecting enzymes associated with it. But 2µM 28-epibassinolide and 2µM HBL were applied to the crop under drought stress increased photosynthetic activity and carbohydrate content even under this stress condition. Exogenous application of EBL and HBL promotes the growth and development of pigeon pea plants under different stress conditions. Further research is required for the detailed analysis.

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