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EFFECT OF HOMOBRASSINOLIDE ON BIO-CHEMICAL ACTIVITIES AND CHLOROPHYLL PIGMENTS OF MUSTARD PLANTS GROWN IN SEMI-ARID TROPICS OF NIZAMABAD

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ABSTRACT
The effects of homobrassinolide (HBL) sprayed in three concentrations viz., 0.5 µM, 1.0 µM and 2.0 µM on biochemical parameters viz., carbohydrate fractions (reducing sugars, non reducing sugars and starch), proteins and nucleic acids (RNA and DNA) and chlorophyll pigments of mustard plants grown in the semi-arid tropics of Nizamabad was studied. The black soil in Nizamabad district is very saline wherein the plants usually experience drought and saline stresses. All the three concentrations of HBL played a very positive role in mitigating the saline stress and enhanced the bio-chemical activities viz., carbohydrate fractions (reducing sugars, total sugars and starch), nucleic acids (DNA and RNA), soluble proteins as well as chlorophyll pigments in mustard plants. HBL at 2.0µM was found most effective in increasing carbohydrates, proteins, nucleic acids and chlorophyll pigments compared to the other treatments as well as control plants. The increase in bio-chemical activities and chlorophyll pigments in mustard plant is an indicator that, the HBL mitigated the negative effect of the semi-arid conditions of the soil.

KEYWORDS: *Homobrassinolide (HBL), carbohydrates, chlorophyll pigments, nucleic acids, proteins.*

INTRODUCTION
Brassinosteroids (BRs) are a new type of polyhydroxy steroidal phytohormones with significant growth-promoting influence.[1,2] Mitchell et al.[3] discovered BRs which were later extracted from the pollen of *Brassica napus L*. by Grove et al.[4] BRs can be classified as C27, C28 or C29 BRs according to the number of carbons in their structure.[5] However, Vardhini et al.[6] reported that brassinolide (BL), 28-homobrassinolide (28-HomoBL/HBL) and 24-epibrassinolide (24-EpiBL/EBL) are the three bioactive BRs being widely used in most physiological and experimental studies. The work with BR biosynthetic mutants in *Arabidopsis thaliana*[7] and *Pisum sativum*[8] have provided strong evidences that BRs are essential for plant growth and development and BR- signaling plays a positive in plant growth and development viz., spatiotemporal control of BR pathways in plant development employing microscope lens turret to study the pleiotropic phenotypes of the BR mutants at a higher magnification. Rao et al.[9] stated that BRs are a new group of plant growth hormones that perform a variety of physiological roles like growth, seed germination, rhizogenesis, senescence, etc. and also confer resistance to plants against various abiotic stresses. BRs have explored for stress-protective properties in plants against a numerous abiotic stresses like high temperature,[10] low temperature in terms of chilling[11] as well as freezing,[12] salt,[13] light,[14] water in terms of drought[15] as well as flooding,[16] heavy metals[17-19] etc.

MATERIALS AND METHODS
Chemicals and Plant Material
Homobrassinolide (HBL: double) which is a commercially available BR was procured from Bahar Agrochem & Feeds Pvt. Ltd, Ratnagiri, Maharashtra State, India. It is marketed by Godrej Agrovet Pvt. Ltd., Hyderabad, Andhra Pradesh, India. HBL (Double) consists of 0.1% of HBL, 2.0% of emulsifier and 97.9% of solvent IPA. The certified seeds of the *Brassica juncea* L. var. Indian mustard "*Tulasi*" were procured from, National Seeds Corporations Limited, Hyderabad, Telangana state, India.

Biochemical analysis
Mustard seeds were sown in clay pots containing fresh sieved black soil mixed with farmyard manure. The plants were grown under natural day length. The HBL was supplied to the plants as foliar spray at three different concentration levels viz.,0.5 µM, 1.0 µM and

2.0 µM on 30th, 40th and 45th day (from the day of sowing). The bio-chemical parameters of the plant such as carbohydrate fractions (i.e. reducing sugars, non reducing sugars and starch), soluble proteins and nucleic acids (RNA, DNA) were analyzed by collecting the leaves from 60 day old plant. The leaves were washed with tap water followed by distilled water and the moisture on the leaf surface was removed with the help of blotting paper. Then, one gram of leaf material was thoroughly homogenized with 70% (v/v) ethyl alcohol. The homogenate was transferred to plastic bottles, labeled the concentrations and stored in deep freezer for further bio-chemical analysis. Fresh mustard plant leaf material was used for determining the chlorophyll pigments on 35th day and 45th day.

Carbohydrate fractions

The alcohol homogenate was heated and centrifuged. The supernatant was used for the estimation of reducing sugars[20] and non-reducing sugars were calculated by the formula given by Loomis & Shull.[21] The residue was used for the estimation of starch by McCready et al.[22] method.

Soluble proteins

Soluble proteins in the ethanol homogenate were precipitated by adding 20% (w/v) tricholoroacetic acid. The precipitate was dissolved in 1% (w/v) sodium hydroxide. The method of Lowry et al.[23] was used for protein estimation.

Nucleic acids

DNA and RNA in the alcohol homogenate were separated by Ogur & Rosen[24] method. DNA was estimated by Burton[25] method and RNA was estimated by the procedure of Schneider.[26]

Chlorophyll Pigments

Chlorophyll pigments were extracted and estimated by the procedure described by Arnon[27]. Leaves were homogenized with 80% (v/v) acetone and centrifuged. The acetone extract was used to calculate the chlorophyll a, b and total chlorophylls employing the formula given below.

Chlorophylls a=
[O.D at 663 × 12.7 –O.D at 645 × 2.69] × [v/1000) × w]
Chlorophyll b=
[O.D at 645 × 22.9– O.D. at 663 × 4.68] × [v/1000) × w]
Total chlorophylls =
[O.D at 663 × 8.2+O.D at 645 × 20.2] × [v/1000) × w]
Where: v = volume of acetone extract, w=weight of leaves.

RESULTS

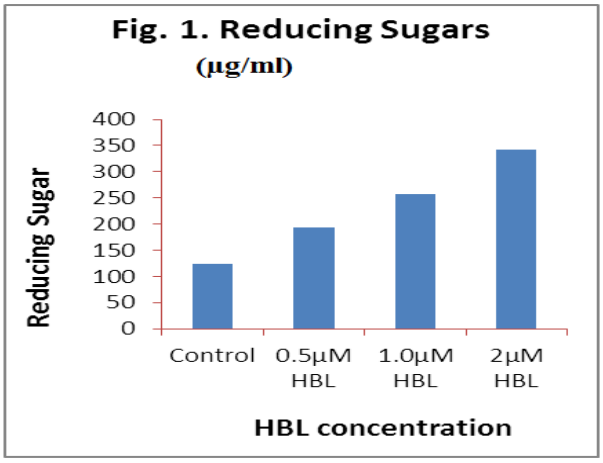
Carbohydrate Fractions

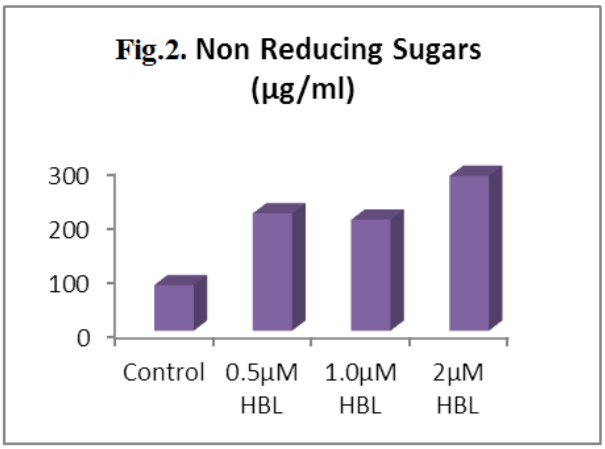
The carbohydrate fractions in terms of reducing sugars, non reducing sugars and starch were elevated by the application of HBL over control plants grown in semi-arid tropics of Nizamabad as shown in Table 1 and Fig. 1-3. The exogenous application of HBL resulted in an average increase around 20 µg/ml in carbohydrate fractions of the three different concentrations, viz. 0.5 µM, 1 µM and 2 µM compared to water treated controls. Exogenous application of 2µM HBL resulted in maximum enhancement of all the carbohydrate fractions over control plants followed by 1 µM and 0.5 µM compared to the untreated control plants.

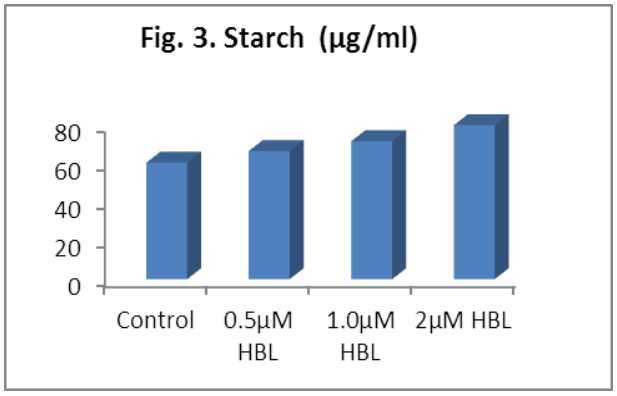
Table 1: Effect of homobrassinolide (HBL) on the carbohydrate fractions of mustard plant.

Treatments	Reducing Sugars (µg/ml)*	Non Reducing Sugars (µg/ml)*	Starch (µg/ml)*
Control	124.89 ± 31.650	83.56 ±4.785	60.06 ± 2.292
0.5µM HBL	193.30 ± 18.350	214.22 ±15.149	66.10 ± 1.690
1.0µM HBL	257.24 ± 15.360	202.93 ± 20.770	71.17 ±1.690
2µM HBL	341.84 ± 5.475	282.77 ± 24.164	79.41± 1.150

*Mean ± S.E (n=3).







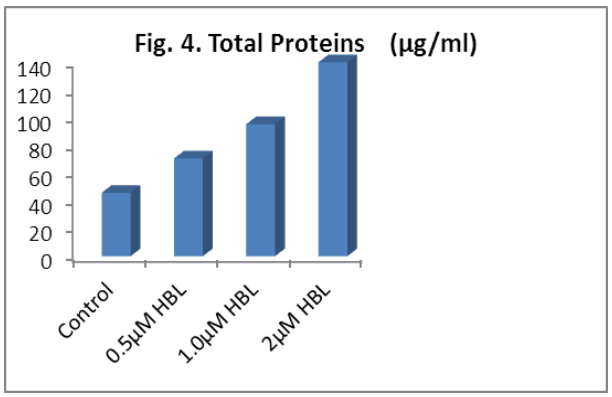
Soluble Proteins

The application of HBL enhanced the contents of soluble proteins present in mustard plants grown in semi-arid tropics of Nizamabad (Table 2 & Fig. 4) over the untreated control plants. 2µM BL was most effective in elevating the levels of soluble proteins compared to the other treatments.

Table 2: Effect of homobrassinolide (HBL) on the soluble proteins of mustard plant.

Treatment	Total Proteins (µg/ml)*
Control	45.707 ±1.57
0.5µM HBL	70.43 ±1.91
1.0µM HBL	95.16 ±1.18
2µM HBL	139.82 ±2.048

*Mean ± S.E. (n =3)



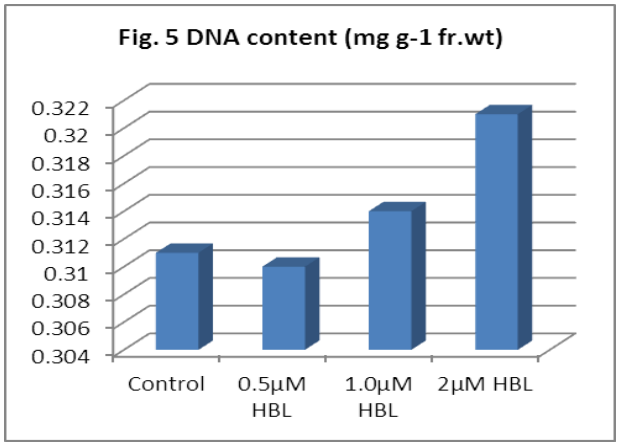
Nucleic Acids

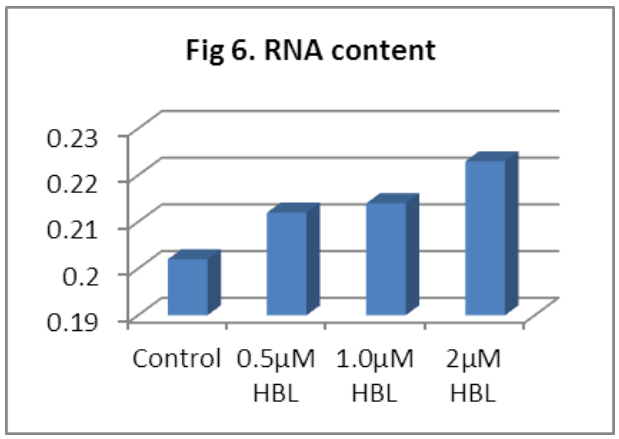
The growth promotion by HBL was associated with increment in the levels of DNA and RNA (Table 3 and Fig. 5 & 6) in the mustard plants grown in semi-arid tropics of Nizamabad. All the three concentrations of HBL enhanced the nucleic acid levels and 2µM HBL was most effective in enhancing the levels of DNA and RNA compared to the other treatments in the mustard plants grown in semi-arid tropics of Nizamabad.

Table 3: Effect of homobrassinolide (HBL) on the nucleic acids (DNA and RNA) of mustard plant.

Treatment	DNA (mg g^{-1} fr. wt.)*	RNA (mg g^{-1} fr. wt.)*
Control	0.311± 0.003	0.205±0.002
0.5µM HBL	0.310 ± 0.310	0.212± 0.002
1.0µM HBL	0.314 ± 0.180	0.214 ±0.002
2µM HBL	0.321 ±0.001	0.22 ± 0.002

*Mean ± S.E. (n =3)





Chlorophyll Pigments

Exogenous application of HBL resulted in a significant increase in chlorophyll levels in mustard plants (Table: 4) estimated on 35th as well as 45th day in mustard plants grown in semi-arid tropics of Nizamabad. All the three concentrations of HBL enhanced the chlorophyll pigments in terms of chlorophyll 'a', chlorophyll 'b' and total chlorophylls and 2µM HBL was most effective in enhancing the all the different chlorophyll pigments compared to the other treatments as well as control plants.

Table 4: Effect of homobrassinolide (HBL) on the chlorophylls (35th and 45th day) of mustard plant.

Treatments	Chlorophyll 'a' (µg.g^{-1} f.w.)*		Chlorophyll 'b'(µg.g-1 f.w.)*		Total chlorophylls (µg.g-1 f.w.)*	
	35th Day	45th Day	35th Day	45th day	35th Day	45th day
Control	17.96 ±1.56	23.26 ±2.64	8.20 ± 0.83	10.90 ± 1.69	26.82 ± 2.53	33.02 ± 3.63
0.5µM HBL	18.0 ± 3.73	25.97 ± 1.82	11.01 ± 2.55	14.60 ± 0.67	29.86 ± 2.12	35.86 ± 9.20
1.0µM HBL	19.19 ±1.44	31.85 ±1.64	14.53 ± 6.09	15.37 ± 0.72	32.54 ± 4.05	39.98 ± 1.10
2µM HBL	25.41 ± 6.35	33.44 ± 6.08	14.72 ± 2.24	16.67 ± 2.27	36.78 ± 9.32	48.17 ± 8.17

*Mean ± S.E. (n =3)

DISCUSSION

Carbohydrate Fractions

Foliar application of HBL caused sharp rise in the levels of all the three carbohydrate fractions viz., reducing sugars, non reducing sugars and starch present in mustard plants grown in semi-arid tropics of Nizamabad. The increase might be due to enhanced photosynthetic capacity of the plants as influenced by the HBL. In fact increase in CO_2 fixation and levels of reducing sugars were reported in wheat and mustard plants by the application of HBL.[28] Similarly increase in photosynthesis in wheat treated with 28-HBL was obtained by Sairam.[29] Exogenously applied 24-EBL reduced the lignification and altered the cell wall carbohydrate biosynthesis in the secondary xylem of *Liriodendron tulipifera*.[30] Hasan et al.[31] reported that spraying of 28- HBL protected seeds of *Cicer arietinum* (L.) cv. Uday from Cd (50, 100 or 150 micro M) by increasing plant fresh mass, dry mass, number of nodules, leghemoglobin content, nitrogen, carbohydrate content, leaf chlorophyll content, proline content, NR, CA and activities of antioxidant enzymes (CAT, POD and SOD). Application of 10^{-8} M BL to *C. vulgaris* cultures reduced the accumulation of heavy metals stress on growth, prevented monosaccharides loss, and increased phytochelatins content.[32] The increase in the carbohydrate fractions might be due to the enhanced photosynthetic capacity of the plants and an efficient source –sink translocation by the foliar application of BRs. Similarly, soaking the seeds of *Triticum aestivum* for around one day in HBL significantly enhanced the soluble sugars in the seedlings.[33]

Soluble Proteins

Soluble protein content in HBL treated mustard plants grown in semi-arid tropics of Nizamabad was greater than untreated control plants. 24-EpiBL, at concentrations of 10^{-7} M and 0.5×10^{-9} M attenuated the negativity of salinity in plants of *C. cajan* (L.) Millsp., cultivar C11 by increasing the levels of proteins, amino acids, nitrate, nitrate reductase of roots and the composition of xylem sap amino acids in the shoots.[34] The growth promotion in groundnut by BRs was found to be associated with improved nitrogen fixation[35] and soluble protein content.[36] Exogenous application of BL alleviated the detrimental effects of drought in maize by enhancing proteins and enzymatic antioxidants.[37] Raghu et al.[38] reported that application of BRs to *Raphanus sativus* seedlings inhibited the negative effect of arsenic by increasing proteins, proline, SOD, CAT and reducing the lipid peroxidation. Further, Arora et al.[39] observed that application of 24-epiBL enhanced growth, protein content and antioxidative defense system of *Brassica juncea* L. subjected to cobalt ion toxicity. Application of 10^{-8} M BL to *C. vulgaris* cultures reduced the accumulation of heavy metals (cadmium, lead and copper) stress on growth, prevented protein loss, and increased phytochelatins content.[32] Exogenous application of 24-EBL and 28-HBL enhanced the total protein contents in the seedlings of *Brassica juncea*[40] which is in tune with the experiments on effect of HBL on the soluble proteins present in the mustard plants grown in semi – arid tropics of Nizamabad.

Nucleic Acids

BRs influence the growth by regulating nucleic acid synthesis. The increase in the levels of nucleic acids might be due to enhanced synthesis and reduced degradation as reflected in the present study wherein the foliar application of HBL resulted in elevated levels of nucleic acids in mustard plants. Elevated activity of RNA polymerase and lowered activity of RNase and DNase were observed in mung bean seedlings when treated with EBL.[41] The promotion of growth by BR under salt stress conditions was associated with enhanced levels of nucleic acids and soluble proteins.[42]

Chlorophyll Pigments

Exogenous application of HBL resulted in a significant increase in chlorophyll pigment levels viz., chlorophyll 'a', chlorophyll 'b' and total chlorophylls in mustard plants grown in semi-arid tropics of Nizamabad (Table: 4). Particularly at 2µM concentration, HBL was found to be highly effective in increasing the contents of chlorophyll pigments. Similarly, the amount of chlorophyll a, b and total chlorophyll were increased under BR treatments in *Gossypium* and *Vigna mungo*.[43] Sonjaroon et al.[10] reported that exogenous application of 7,8-dihydro-8a-20-hydroxyecdysone, a BR improved the photosynthesis, and yield in rice under high-temperature condition. Application of 10^{-8} M BL to *C. vulgaris* cultures reduced the accumulation of heavy metals stress on growth, prevented chlorophyll, monosaccharides, and protein loss, and increased phytochelatins content.[32] Filova et al.[44] also reported that BRs eliminated the toxic effect of Cu in 6 sunflower cultivars (*Helianthus annuus* L. cv. Belinda, cv. Codiwer, cv. ESPrim, cv. MAS 95, cv. MAS 97 and cv. Spirov) by decreasing the lipid peroxidation (MDA), enhancing chlorophylls, proline and relative water content (RWC).

4. CONCLUSION

The present study clearly demonstrated the positive influence of HBL on the growth of mustard plants grown in semi-arid tropics of Nizamabad. HBL- application caused substantial increase in carbohydrate fractions (reducing sugars, non reducing sugars and starch), soluble sugars, nucleic acids (DNA and RNA) and chlorophyll pigments which have crucial bearing on growth and development. The soils of Nizamabad are saline and dry in nature inhibiting the growth of plants. BRs have the ability to promote biochemical activity and contents of chlorophyll pigments of mustard plants grown under stressful conditions.

Recently Wang et al.,[11] reported that exogenous application of BL ameliorated chilling stress in *Leymus chinensis* (Trin.) Tzvel. by modulating the morphological, physiological and biochemical traits. BRs are involved in regulatory processes, which are more specific to plant growth, including photomorphogenesis and skotomorphogenesis and spraying of 24-epiBL to soyabean seedlings resulted in improved chlorophyll, carotenoid and anthocyanin contents in the hypocotyl, cotyledon and 1st internode kept both in light and dark in the growth chamber for 12 days.[45] The present study reveals a new insight that application of HBL overcame the negative effect of the semi-arid conditions of the soil (reflected in the control plants) and biochemical activity and chlorophyll pigments (reflected in the HBL-treated plants) of mustard.

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