Homobrassinolide on Growth, Minerals and Vitamins of *Curcuma longa* L. (turmeric) Rhizome

Abstract

The application of homobrassinolide on the growth, mineral content and vitamin content of *Curcuma longa* L. (turmeric) rhizome grown in Nizamabad, Telangana State, India was studied. Homobrassinolide application as foliar spray to turmeric on the 20^{th}, 40^{th} and 60^{th} day from sowing resulted in enhanced growth of turmeric rhizome in terms of fresh and dry weights. The promotion of growth was associated with increased levels of minerals in terms of calcium (Ca), Manganese (Mn), potassium (K), phosphorus (P), copper (Cu), zinc (Zn) and iron (Fe) of turmeric rhizome. Further, the contents of Vitamin B fractions [Thiamine (Vitamin B_1), Niacin (Vitamin B_2), and Riboflavin (Vitamin B_3)] and Vitamin C/Ascorbic Acid of the turmeric rhizome were also found enhanced indicating the ability of homobrassinolide in positively monitoring nutrient value in turmeric rhizome.

Key words: Homobrassinolide; Minerals; Rhizome; Turmeric; Vitamin B; Vitamin C

Introduction

Plant Growth Regulators (PGRs) organize and help in adjustment of the physiological, atomic, and biochemical instruments to build pressure resilience for development and improvement in crop plants [1,2] (Peleg and Blumwald, 2011; Ha et al., 2012) and among the recently included PGRs, BRs have been the subject of sharp enthusiasm for plant scientists for their role in growth and metabolism of plants under normal as well as stressful environment. Brassinosteroids (BRs) are fundamental low molecular weight PGRs (plant growth regulators) that are omnipresent all through the plant kingdom

(Rao et al. 2002, Vardhini et al., 2012) and were first reported by Mitchell et al. (1970) in 'Nature' depicting the growth promoting activity of *Brassica napus* L. pollen extricates at exceptionally low amounts. The cascades of research on these potential PGRs are gaining much importance and the research studies on BRs clearly depict their ability to mitigate various biotic (bacteria, virus, fungi, nematodes) and abiotic (temperature, salt, drought, metal, water) in plants (Bajguz and Hayat, 2009; Vardhini et al. 2010; Vardhini, 2019).

Turmeric (*Curcuma longa* L.) plant belongs to ginger family (Zingiberaceae). It is an established fact that turmeric plant is often called as a 'wonder spice' capable of acting as an antioxidant and used in all parts of India in most of the dishes. *Curcuma longa* L. is sterile as is incapable of producing seeds. Hence it is widely propagated from its rhizomes. The present research study is undertaken to give an insight on the application of homobrassinolide (HBL) on the yield of turmeric rhizome and contents of minerals (Ca, Mn, K, P, Cu, Zn and Fe) present in the turmeric rhizome. Further, Vitamin B fractions [thiamine (vitamin B_1), niacin (vitamin B_2), and riboflavin (vitamin B_3)] and Vitamin C/ascorbic Acid present in the turmeric rhizome were also analyzed.

MATERIALS AND METHODS

Curcuma longa L. plant popularly called as 'turmeric' plant belongs to the ginger family (Zingiberaceae) is the present research study material. The underground rhizomes of *Curcuma longa* L. variety Acc-79 were purchased from Ashwini Fertilizers Ltd., Nizamabad. Homobrassinolide (HBL) was procured from Godrej Agrovet Pvt. Ltd., Hyderabad, Telangana State, India. Homobrassinolide obtained as Double comprises of 0.04% of Homobrassinolide, 4.0% of water and solvent.

Curcuma longa L. plants were grown under natural day length in Nizamabad district of Telangana State, India. The experiments were conducted in the field plots beds. Each plot bed consisted of 121 square yards in measurement. A total of 5 plot beds were prepared resulting in 605 (121 x 5) square yards of total field sizes. The distance between each bed was around two and a half feet. Care was taken to maintain 1/2 feet distance between each plant. The selected plots were mixed with manure and vermi compost. The rhizomes were sterilized by metaloxin mangozeb (250g/250ml concentration) by soaking for 30 minutes. After 30 minutes, the turmeric rhizomes were dried in shade for around one and a half hour and planted in the plots mentioned above. After 15 days of sowing, the saplings were exogenously treated with 70% thiophanate methyl which is a fungicide.

The turmeric plants were grown in the field conditions in the semi-arid tropics of Nizamabad. Homobrassinolide was supplied as foliar spray where four different concentrations viz., 0.5µM, 1.0µM, 2.0µM, and 3.0µM were taken based on the experimental studies conducted by Vardhini and Rao (1998; 2003). Ten plants were chosen for foliar application of each concentration. The first homobrassinolide spray was given as foliar spray when the turmeric plants were twenty - day old. Homobrassinolide was exogenously applied as foliar spray three times to the turmeric plants viz., on the 20^{th}, 40^{th} and 60^{th} day from sowing. The control turmeric plants were treated with distilled water on the same days.

The rhizome is the main consumable part of the turmeric plants. The rhizome yield of turmeric plants in terms of its fresh weight and dry weights were recorded. The minerals calcium (Ca), Manganese (Mn), potassium (K), phosphorus (P), copper (Cu), zinc (Zn) and iron (Fe) present in the turmeric rhizome were estimated. Further, Vitamin B fractions [Thiamine (Vitamin B_1), Niacin (Vitamin B_2), and Riboflavin (Vitamin B_3)] and Vitamin C/Ascorbic Acid present in the turmeric rhizome were also analyzed.

Homobrassinolide and Rhizome Fresh Weight of Turmeric Plants

The fresh weights of the rhizome of turmeric plants were recorded on 55^{th} and 65^{th} days. A meter balance (Shimadzu Top Loading Balance –BL Series) was used for this purpose. The rhizome fresh weights were recorded and expressed in grams.

Homobrassinolide and Rhizome Dry Weight of Turmeric Plants

The turmeric rhizome was oven dried at 110^{o}C for 24 hours and their dry weights were recorded on 55th and 65th days using a meter balance (Shimadzu Top Loading Balance –BL Series). The rhizome dry weights were expressed in terms of grams.

Homobrassinolide and Minerals in Turmeric Rhizome

The Method of AOAC (1990) was used to determine the mineral contents of the Rhizome of turmeric. The dried turmeric rhizomes were made moisture free. One gram of each sample was weighed out and ashed at a controlled temperature of 550^{0}C. The ash obtained was treated with hydrochloric acid and concentrated nitric acid before filtering and taken for Ca, Mn, K, P, Cu, Zn and Fe mineral determination using a UV Visible Spectrometer of Schimadzu UV- 1800, Japan.

Homobrassinolide and of Vitamin B Fractions [Thiamine (Vitamin B1), Niacin (Vitamin B2), Riboflavin (Vitamin B3)] in Turmeric Rhizome

Vitamin B fractions of turmeric rhizome were estimated following the procedures adopted by Deepak (2011).

Thiamine or Vitamin B1:

Five grams of turmeric rhizome was homogenized in 50 ml of ethanolic sodium hydroxide. Ten ml of the filtrate was added to ten ml of potassium dichromate and the absorbance was recorded at 360 nm in a UV Visible Spectrometer of Schimadzu UV- 1800, Japan after development of color.

Riboflavin or Vitamin B2:

Five grams of turmeric rhizome was extracted with 100 ml of ethyl alcohol for 1 hour. Ten ml of 5% potassium permanganate and ten ml of 3% hydrogen peroxide was added. The solution was allowed to stand on hot water bath for 30 minutes. Two ml of 40 % sodium sulfate was added to this reaction mixture. The volume was made up to 50 ml and the absorbance was recorded at 510 nm in a UV Visible Spectrometer of Schimadzu UV- 1800, Japan.

Niacin or Vitamin B3:

Five grams of turmeric rhizome was treated with 50 ml of 1N sulfuric acid for 30 minutes. 0.5 Milliliters of ammonia solution was added to this and then filtered using Whatman Filter Paper. Ten ml of this filtrate was taken, and five ml of potassium cyanide was added and then acidified with five ml of 0.02 N sulfuric acid. The absorbance was recorded at 420 nm in a UV Visible Spectrometer of Schimadzu UV- 1800, Japan.

Homobrassinolide and Vitamin C / (Ascorbic Acid) in Turmeric Rhizome

'Vitamin C' or ascorbic acid present in the turmeric rhizome was estimated according to the method of Sadasivan and Balasubramanian (1987).

One Gram of turmeric rhizome was blended with ten ml of 4% oxalic acid. The slurry was centrifuged, the supernatant collected, and the volume was made up to 20 ml with 4% oxalic acid. Five ml of sample was pipette into 100 ml conical flask. To this, ten ml of 4% oxalic acid was added and titrated against the dye (V2 ml) taken in a burette until the appearance of pink color which persisted for a few seconds. Similarly, five ml of working standard solution was pipetted and ten ml of oxalic acid was added to it. This was also titrated against the dye (V1 ml). The amount of dye consumed was considered equivalent to the amount of Vitamin C present in the turmeric rhizome.

The amount of Vitamin C in the turmeric rhizome = 0.5/V1 x V2/5 ml x 100ml/weight of the sample x 100. Vitamin C was represented as mg/100g turmeric rhizome fresh weight.

Statistical analysis: The results expressed in the present research study are the mean 5 replicates. The analyses were carried out employing One-Way Analysis of Variance (ANOVA). The ANOVA values were followed by Post Hoc Test (Multiple Comparisons) using SPSS (SPSS Inc., Chicago, IL, USA).

The differences were considered significant only when the p was ≤ 0.05.

Results

Application of homobrassinolide resulted in enhanced qualitative as well as quantitative growth of turmeric rhizome grown in Nizamabad.

Rhizome Fresh Weight and Dry Weight of Turmeric Plants

The results of fresh and dry weights of the rhizomes of turmeric plants recorded on the 55th day and 65th day treated by homobrassinolide (HBL) are presented in the Tables 1. Application of HBL (0.5µM, 1.0 µM, and 2.0 µM and 3.0 µM) to turmeric plants resulted in stimulation of rhizome fresh weight around 20% for 0.5µM HBL, 42.33% for 1.0µM HBL, 74.21% for 2.0µM HBL and 90% for 3.0µM HBL concentrations when compared with control on 55th day and around 20.21% for 0.5µM HBL, 42.3% for 1.0µM HBL, 74.06% for 2.0µM HBL and 90% for 3.0µM HBL concentrations when compared with control on the 65th day.

Further, application of HBL increased dry weights of turmeric rhizome around 14.11% for 0.5µM HBL, 23.01% for 1.0µM HBL, 48.4% for 2.0µM HBL and 81.4% for 3.0µM HBL concentrations on 55th day and increased around 14.11% for 0.5µM HBL, 23.01% for 1.0µM HBL, 48.4% for 2.0µM HBL and 61.89% for 3.0µM HBL concentrations on 65th day.

Minerals in Turmeric Rhizome

Foliar spraying of homobrassinolide resulted in increased contents in all the minerals studied in turmeric rhizome grown in Nizamabad District.

Calcium in Turmeric Rhizome

Foliar application of HBL to turmeric plants improved the mineral calcium content in the turmeric rhizome (Table 2.). It was observed that calcium content was increased up to 4.8% for 0.5µM HBL application, 5.4 % for 1.0 µM HBL application, 6.2 % for 2.0 µM HBL application and 6.8% for 3.0µM HBL application over untreated control plants.

Manganese Content in turmeric Rhizome

Exogenous application of HBL to turmeric plants improved the mineral manganese content in the turmeric rhizome (Table 2.). Application of HBL increased up to 5 % for 0.5µM HBL treatment, 8 % for 1.0 µM HBL treatment, 14 % for 2.0 µM HBL treatment and 17.5 % for 3.0µM HBL treatment over untreated control plants.

Potassium Content in Turmeric Rhizome

Foliar spraying of HBL to turmeric plants improved the mineral potassium content in the turmeric rhizome (Table 2) and it was observed that potassium increased up to 18.4 % for 0.5μM HBL treatment, 20 % for 1.0 μM HBL treatment, 22 % for 2.0 μM HBL treatment and 24.8 % for 3.0μM HBL treatment over untreated control plants.

Phosphorus Content in turmeric Rhizome

Exogenous treatment of HBL to turmeric plants improved the total phosphorus content in the turmeric rhizome (Table 2.) Supplementation of HBL (0.5μM, 1.0 μM, and 2.0 μM and 3.0μM) to turmeric plants increased phosphorus content increased up to 36.5 % for 0.5μM HBL, 47.71 % for 1.0 μM HBL, 44.25 % for 2.0 μM HBL and 47.79 % for 3.0μM HBL over untreated control plants.

Copper Content in Turmeric Rhizome

Foliar treatment of HBL to turmeric plants improved the mineral copper content in the turmeric rhizome (Table 2.) and increments were observed that as the concentrations increased from 0.5 μM HBL to 3.0 μM HBL. The observation made for copper content was that it increased up to 8 % for 0.5μM HBL treatment, 16 % for 1.0 μM HBL treatment, 20 % for 2.0 μM HBL treatment and 28 % for 3.0μM HBL treatment over untreated control plants.

Zinc Content in Turmeric Rhizome

Exogenous supplementation as foliar spray of HBL to turmeric plants improved the zinc content in the turmeric rhizome (Table 2.). It was observed that HBL increased zinc content up to 9.4 % when supplied at 0.5μM, 13.86 % when supplied at 1.0 μM, 16.38 % when supplied at 2.0 μM and 20.79 % when supplied at 3.0μM compared to untreated control plants.

Iron Content in Turmeric Rhizome

Foliar application of homobrassinolide to turmeric plants improved the mineral iron content in the turmeric rhizome (Table 2.) HBL – supplementation increased iron content up to 18.8 % for 0.5µM, 20.4 % for 1.0 µM, 23.2% for 2.0 µM and 24.8 % for 3.0µM HBL supplementations over untreated control plants.

Vitamin B Fractions (Thiamine, Riboflavin, Niacin) in Turmeric Rhizome

Supplementation of all four concentrations of HBL resulted in enhanced three fractions of Vitamin B in the turmeric rhizome grown in Nizamabad.

Thiamine (Vitamin B_1) Content in Turmeric Rhizome

Foliar spraying of HBL to turmeric plants improved the thiamine or Vitamin B_1 content in the turmeric rhizome (Table 3). Exogenous application of HBL (0.5µM, 1.0 µM, and 2.0 µM and 3.0µM) as foliar spray to turmeric plants resulted in increased thiamine or Vitamin B_1 content up to 33.6 % for 0.5µM HBL- treatment, 40 % for 1.0 µM HBL- treatment, 60.8% for 2.0 µM HBL- treatment and 83.2 % for 3.0µM HBL- treatment over untreated control plants.

Riboflavin (Vitamin - B_2) Content in Turmeric Rhizome

Exogenous application of homobrassinolide to turmeric plants improved the riboflavin or Vitamin B_2 content in the turmeric rhizome (Table 3) and it was observed that HBL – treatment increased up to 46.2 % for 0.5µM HBL, 70.8 % for 1.0 µM HBL, 79.65 % for 2.0 µM HBL and 99.71 % for 3.0µM HBL over untreated control plants.

Niacin (Vitamin B_3) Content in Turmeric Rhizome

Exogenous application of HBL to turmeric plants improved the niacin or Vitamin B_3 content in the turmeric rhizome (Table 3.) and it was observed that niacin or Vitamin B_3 content increased up to 56.35 % for 0.5µM HBL- application, 63.94 % for 1.0 µM HBL- application, 69.46% for 2.0 µM HBL- application and 73.37 % for 3.0µM HBL- application over untreated control plants.

Vitamin C (Ascorbic Acid) in Turmeric Rhizome:

Foliar supplementation of HBL to turmeric plants improved the Vitamin C or Ascorbic Acid content in the turmeric rhizome (Table 3) and it was observed that the ascorbic acid contents were found increased as the concentrations increased from 0.5 µM HBL to 3.0 µM HBL.

Discussion

Homobrassinolide and Fresh and Dry Weights of Turmeric Rhizome:

Application of HBL substantially increased the fresh and dry weights of turmeric rhizome. Schilling et al. (1991) observed that application homobrassinolide enhanced the tap - root mass and yield of sugar beets grown under drought stress conditions. Plant growth regulators (PGRs) like brassinolide (0.1 ppm), paclobutrazol - pp333 (250 ppm) and salicylic acid (100 ppm) applied as foliar spray on the third and fifth month to ginger (*Zingiber officinale* Rosc.) increased the fresh and dry weight of rhizomes (Velayutham and Parthiban, 2013) which is tune with the results obtained in the present study wherein foliar application of HBL resulted in increased rhizome fresh and dry weights of turmeric plants.

Homobrassinolide and Minerals in Turmeric Rhizome:

Application of HBL led to significant raises of minerals viz., calcium (Ca), Manganese (Mn), potassium (K), phosphorus (P), copper (Cu), zinc (Zn) and iron (Fe) present in the turmeric rhizome.

Application of Plant growth regulators (PGRs) like mefluidide and chlormequat to alfalfa (*Medicago sativa* 'Algonquin') on 28 and 49 days after initiation of spring growth showed increased calcium and phosphorus contents (Buck et al. 1988). Vardhini et al. (2011) and Vardhini et al. (2012) observed that foliar application of 24-epibrassinolide and 28- homobrassinolide modulated the qualitative changes in the storage roots of radish by increased levels of minerals like phosphorous, potassium, calcium, iron, and sodium indicating their ability to improve the nutritive value of storage roots of radish. Further, Sosnowski et al. (2018) studied that application of a combination of auxins and cytokinins to alfalfa (*Medicago x varia T. Martyn*) showed enhanced contents of phosphorus, calcium, potassium, magnesium, and titanium in alfa alfa biomass.

Homobrassinolide and Vitamin B Fractions [Thiamine (Vitamin B_1), Niacin (Vitamin B_2), Riboflavin (Vitamin B_3)] and Vitamin C in Turmeric Rhizome:

Application of homobrassinolide markedly increased all the three fractions of Vitamin B like Thiamine (Vitamin B_1), Niacin (Vitamin B_2) and Riboflavin (Vitamin B_3)] as well as contents of vitamin C or ascorbic acid in turmeric rhizome. 24-Epibrassinolide and 28-homobrassinolide modulated the qualitative changes in the storage roots of radish by increased levels of niacin and vitamin C or ascorbic acid in the radish roots indicating their ability to improve the quality of storage roots of radish (Vardhini et al. 2012). The content of antioxidant (ascorbic acid) was increased in the seeds of pea and lupine after application of brassinosteroids and 24-epibrassinolide (Kościelniak et al., 2014). Further, Hasan et al. (2011) also observed that two native (to India) varieties of *Lycopersicon esculentum* (tomato) - K-25 and *Sarvodya* showed increased ascorbic acid levels when treated with two BR analogues (28- homobrassinolide and 24-epibrassinolide) by two different modes of application (foliar spray and root dipping) which is similar to the results observed in the present study indicating the ability of HBL to improve the growth, mineral content and vitamins in turmeric rhizome.

Conclusion

The present research study gave a clear insight that foliar of homobrassinolide resulted in enhanced growth of turmeric rhizome in terms of fresh and dry weights. The growth promotion was reflected in enhanced levels of minerals in terms of calcium (Ca), Manganese (Mn), potassium (K), phosphorus (P), copper (Cu), zinc (Zn) and iron (Fe) of turmeric rhizome. Further the contents of Vitamin B fractions [Thiamine (Vitamin B_1), Niacin (Vitamin B_2), and Riboflavin (Vitamin B_3)] and Vitamin C/Ascorbic Acid of the turmeric rhizome were also found enhanced clearly proving the growth promoting nature of these 6^{th} group of PGRs (homobrassinolide).

Table 1. Effect of Homobrassinolide (HBL) on the Turmeric Rhizome Weight on 55th Day and 65th Day

Rhizome Weight on 55th Day*			Rhizome Weight on 65th Day*	
Treatment	**Rhizome Fresh Weight (gm/fr.wt)***	**Rhizome Dry Weight (gm/dry.wt)***	**Rhizome Fresh Weight (gm/fr.wt)***	**Rhizome Dry Weight (gm/dry.wt)***
Control	100.20±0.31	75.10±0.10	120.05±0.31	95.10±0.19
0.5µM HBL	120.19±0.126	89.21±1.262	140.29±0.162	109.21±1.289
1.0µM HBL	142.33±1.028	98.2±0.081	162.35±1.025	118.2±0.075
2µM HBL	174.21±0.596	123.5±0.327	194.11±0.247	143.5±0.341
3.0µM HBL	201.43±0.214	156.5±0.118	221.31±0.142	171.1±0.002

HBL = Homobrassinolide

*Mean ± SE (*n* = 5); The Mean if followed by the same alphabet in a column was found not significantly different at *p*=0.05 according to Post Hoc test.

Table 2. Effect of Homobrassinolide (HBL) on the Minerals Contents in Turmeric Rhizome

Treatments	**Calcium Content (g/fr.wt)***	**Manganese Content (g/fr.wt)***	Potassium **(g/fr.wt)***	**Phosphorus Content (g/fr.wt)***	**Copper Content (g/fr.wt)***	**Zinc Content (g/fr.wt)***	**Iron Content (g/fr.wt)***
Control	0.20± 0.002	0.05± 0.002	0.4 ± 0.002	0.59± 0.002	0.2 ± 0.002	0.21 ± 0.002	0.040± 0.0002
0.5µM HBL	0.24 ± 0.002	0.10± 0.002	0.46 ± 0.004	0.62± 0.002	0.4 ± 0.004	0.45 ± 0.004	0.047± 0.001
1.0µM HBL	0.27± 0.002	0.16± 0.002	0.5 ± 0.00	0.69± 0.002	0.8 ± 0.00	0.66 ± 0.00	0.051± 0.0005
2.0µM HBL	0.31 ± 0.00	0.28± 0.005	0.55± 0.005	0.75± 0.005	0.10± 0.005	0.78± 0.005	0.058± 0.0002
3.0µM HBL	0.34± 0.002	0.35± 0.006	0.62 ± 0.01	0.81± 0.006	0.14 ± 0.01	0.99 ± 0.01	0.062± 0.0004

HBL = Homobrassinolide

*Mean ± SE (*n* = 5); The Mean if followed by the same alphabet in a column was found not significantly different at *p*=0.05 according to Post Hoc test.

Table 3. Effect of Homobrassinolide on the Vitamins in Turmeric Rhizome

Treatments	Thiamine (Vitamin B_1) Content (mg/gm. fr. wt.)*	Riboflavin (Vitamin - B_2) Content (mg/gm. fr. wt.)*	Niacin (Vitamin B_3) Content (mg/gm. fr. wt.)*	Ascorbic Acid (Vitamin C) levels (nmol g -1)*
Control	0.16 ± 0.002	0.59 ± 0.001	2.30 ± 0.002	450.2 ±10.17
0.5µM HBL	0.21 ± 0.004	0.78 ± 0.006	2.45 ± 0.004	572.6±8.64
1.0µM HBL	0.25 ± 0.001	1.20 ± 0.02	2.78 ± 0.00	609 ±7.42
2.0µM HBL	0.38± 0.005	1.35± 0.005	3.02± 0.005	682 ±6.19
3.0µM HBL	0.52 ± 0.01	1.69 ± 0.03	3.19 ± 0.01	769±0.230

HBL = Homobrassinolide

*Mean ± SE (*n* = 5); The Mean if followed by the same alphabet in a column was found not significantly different at *p*=0.05 according to Post Hoc test.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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