Original Research Article

Role of Homobrassinolide and Salicylic Acid on the Vegetative Growth of Groundnut (*Arachis hypogaea*., L) Plants Subjected to Cadmium Stress

ABSTRACT

The present study was carried out to study the role of homobrassinolide (HomoBL) and salicylic acid (SA) on vegetative growth of groundnut (*Arachis hypogaea*., L.) wherein field experiments were conducted with foliar spraying of 2µM and 3µM HomoBL and 1mM and 2mM SA on 20th, 30th, 45th and 60th DAS (Days after sowing). Observations on vegetative parameters like root and shoot length, root and shoot fresh weights, root and shoot dry weights, foliage showed that application of HomoBL and SA were capable of successfully mitigating cadmium stress (100µM) as well as enhancing the vegetative growth of groundnut.

Key Words: Cadmium Stress; Homobrassinolide; Salicylic Acid; Groundnut; Vegetative Growth

Abbreviations: Brassinosteroids (BRs); Cadmium (Cd); Homobrassinolide (HomoBL)Plant growth regulators (PGRs); Salicylic acid (SA)

1. INTRODUCTION

Cadmium (Cd) is considered to be a non-essential trace element, highly phytotoxic at higher concentrations with capabilities of interference with the uptake, transport, and utilization of essential nutrients, water, reduce photosynthesis and modulate enzyme activities [1, 2].

Brassinosteroids (BRs) are a class of essential plant growth regulators (PGRs)with multiple roles with special emphasis in induction of tolerance to various biotic as well as abiotic stresses such as high or low temperature, moisture, drought, salinity and heavy metal stresses [3 - 8]. Salicylic acid is potential PGR that plays a prominent role in stress alleviations of different abiotic and biotic components of the earth [9, 10].

The present research was undertaken to understand the potentiality of two important PGRs (homoBL and salicylic acid) employed in the current era of research in mitigating the metal stress induced by cadmium in groundnut plants.

MATERIAL AND METHODS

Arachis hypogaea L., var. Kadiri-6 (K-1240) was procured from Regional Agricultural Research Station, Polasa, Jagtial, Telangana State, India. Homobrassinolide (HomoBL) was purchased from Godrej Agrovet Ltd., Mumbai (in the form of Godrej Double). Salicylic acid and Cadmium (employed in the form of $CdCl_{2)}$ were purchased from Dwarakamai Enterprises, Hyderabad. Groundnut plants were raised in the fields and sprayed with Homobrassinolide (HomoBL) and Salicylic acid as the following treatments viz., (i) distilled water (control), (ii) Cd 100µM (iii) Cd 100µM supplemented with HomoBL 2µM (iv) Cd 100µM supplemented with HomoBL 3µM (v) Cd 100µM supplemented with SA 1mM (vi) Cd 100µM supplemented with SA 2mM (vii) Cd 100µM supplemented with HomoBL 2µM and SA 1mM (viii) Cd 100µM supplemented with HomoBL 3µM and SA 2mM (ix) Cd 100µM supplemented with HomoBL 2µM and SA 2mM (x) Cd 100µM supplemented with BR 3µM and SA 1mM. Application of HomoBL as foliar spray in two different concentrations viz., 2.0 µM and 3.0 µM to groundnut plants was done on 20th, 30th, 45th and 60th days after sowing. SA was supplied to groundnut plants as foliar spray at two different concentrations viz., 1.0 mM and 2.0 mM on 20th, 30th, 45th and 60th days after sowing.

2. RESULTS

The effect of HomoBL viz., 2µM and 3µM & SA viz., 1mM and 2mM on the growth (shoot, root and leaves) of groundnut plants grown under cadmium stress were accessed in the present study on the 60th day from sowing are represented in Table 1.

3.1. *Shoot and Root Growth of Groundnut Plants*

The effect of different concentrations of HomoBL and SA on the shoot and root growth of groundnut plants subjected to Cd stress was estimated in terms of shoot length and root length. The shoot and root length were measured in centimeters (cm).

3.1.1.1. Shoot Length of Groundnut Plants

Treatment of Cd (100µM) caused maximum decline in shoot length (50.79%). Treatment of 2µM HomoBL and 3µM HomoBL improved shoot length around 51.35% and 43.09%, respectively compared to Cd stress –treated plants. Similarly, treatment of 1mM SA and 2mM SA also improved length around 38.89% and 65.42% compared to Cd –treated plants. All the Co-applications of HomoBL and SA increased shoot length. Combined application of 3µM HomoBL and 1mM SA not only ameliorated the inhibitory effect of Cd, but also promoted the shoot length compared to individual treatments as well as untreated controls.

3.1.1.2. Root Length of Groundnut Plants

Cadmium at 100µM caused maximum decline in root length (46.9%) of groundnut plants. Treatment of 2µM HomoBL and 3µM HomoBL improved shoot length around 19.68% and 29.53%, respectively compared to Cd stress - imposed plants. Similarly treatment of 1mM SA and 2mM SA also improved root length around 25.46% and 47.6%

compared to only Cd supplemented plants. All the Co-applications of HomoBL and SA increased root length. Combined application of 3µM HomoBL and 1mM SA not only ameliorated the inhibitory effect of Cd, but also promoted the shoot length compared to individual treatments as well as untreated controls.

3.1.2. *Shoot and Root Fresh Weights of Groundnut Plants*

The effect of different supplementations of HomoBL and SA on the growth of groundnut plants subjected to cadmium stress estimated in terms of shoot and root fresh weights (g) were recorded on 60th day from sowing and are shown in Table – 2.

3.1.2.1. Shoot Fresh Weight of Groundnut Plants

All the treatments of HomoBL and SA increased the shoot fresh weight of groundnut. Cd at 100µM caused maximum decline in shoot fresh weight (35.5%) of groundnut plants. Application of 2µM and 3µM HomoBL improved the shoot fresh weight up to 11.24% and 13.16% respectively. Similarly, treatment of SA (1mM and 2mM) increased the shoot fresh weight around 10.75% and 22.92% over Cd stress – treated groundnut plants. All the Co-applications of HomoBL and SA increased shoot fresh weight. Co-application of 3µM HomoBL and 1mM SA not only mitigated the inhibitory effect of Cd, but also promoted the shoot fresh weight compared to individual treatments as well as untreated controls.

3.1.2.2. Root Fresh Weight of Groundnut Plants

All the treatments of HomoBL and SA increased the root fresh weight of groundnut. Cadmium at 100µM caused maximum decline in root fresh weight (52.14%) of groundnut plants. Application of 2µM and 3µM HomoBL improved the root fresh weight around 24.05% and 36.79%. Similarly, treatment of SA (1mM and 2mM) enhanced the root fresh weight around 27.35% and 50.94% over only Cd stress treated plants. All the Co-applications of HomoBL and SA increased root fresh weight. Co-application of 3µM HomoBL and 1mM

SA not only mitigated the inhibitory effect of Cd stress, but also promoted the shoot fresh weight compared to all the individual treatments as well as untreated controls.

3.1.3. Shoot and Root Dry Weight of Groundnut Plants

The effect of different supplementations of HomoBL and SA on the growth of groundnut plants subjected to cadmium stress estimated in terms of shoot and root fresh weights (g) were recorded on 60th day from sowing and are shown in table (3).

3.1.3.1. Shoot Dry Weight of Groundnut Plants

The exogenous application of HomoBL and SA substantially increased the shoot dry weight of groundnut under cadmium stress. Cadmium (100µM) caused maximum decline in shoot dry weight (42%). Application of 2µM and 3µM HomoBL caused an increase of 28.48% and 32% compared to cadmium stress subjected plants. Similarly, application of 1mM and 2mM SA caused an increase of 29.85% and 53.02% compared to Cd stress – treated plants. Combined application of both HomoBL and SA not only mitigated the negative impact of Cd toxicity, but also promoted the shoot dry weight with respect to individual treatments as well as untreated controls.

3.1.3.2. Root Dry Weight of Groundnut Plants

The exogenous application of HomoBL and SA substantially increased the root dry weight of groundnut grown under cadmium stress. Cadmium (100µM) caused maximum decline in root dry weight (43.2%) over other treatments. Application of 2µM and 3µM HomoBL caused an increase of 4.96% and 18.93% in root dry weight compared to Cd applied groundnut plants. Similarly, application of 1mM and 2mM SA caused an enhancement of 19.83% and 48.76% compared to groundnut plants subjected to only Cd stress. Combined application of both HomoBL and SA alleviated the negative impact of Cd and promoted the root dry weight compared to individual treatments as well as untreated controls.

4. *Foliage of Groundnut Plants*

The effect of different applications of HomoBL and SA on the foliage of groundnut plants grown under cadmium stress was estimated in terms of number of leaves/plant and leaf area of plants.

4.1. Number of Leaves per Groundnut Plant

All the different treatments of HomoBL and SA elevated the number of leaves/groundnut plant grown under cadmium stress. These readings were recorded on 60th day from sowing and are shown in Table – 4.

Cadmium (100µM) caused a steep decline in number of leaves/ groundnut plant (50.2%). 3µM HomoBL concentration caused an elevation of 39.84% followed by 2µM HomoBL which caused an elevation of 18.11%. Similarly, 1mM concentration of SA caused an elevation of 21% followed by 2mM SA which caused an elevation of 41.41%. All the Co-applications of HomoBL and SA increased number of leaves/groundnut plant. The co-application of 3µM HomoBL and 1mM SA proved the best wherein it not only removed the inhibitory effect of Cd, but also promoted the number of leaves compared to individual treatments as well as untreated controls.

4.2. Average Leaf Area of Groundnut Plant

The effect of HomoBL and SA on leaf area of groundnut grown under cadmium stress is shown in table (4).

The exogenous application of HomoBL and SA increased the leaf area of groundnut. Cd treatment at 100µM caused maximum decline in leaf area (44.08%). Application of 2µM HomoBL and 3µM HomoBL improved the leaf area around 15.34% and 39.57% compared to cadmium stress treated groundnut plants. Similarly, application of 1mM SA and 2mM SA also improved the leaf area around 39.57% and 55.98% compared to only cadmium stress treated groundnut plants. Combined application of both the plant growth regulators (3µM HomoBL and 1mM SA) not only decreased the inhibitory effect of Cd, but also promoted the average leaf area compared to individual treatments as well as untreated controls.

5. DISCUSSION

5.1. *Shoot and Root Length*

Treatment of Cd (100µM) caused maximum decline in shoot length and root length in groundnut plants. Treatment of HomoBL (2µM and 3µM) as well as SA (1mM and 2mM) improved the shoot length and root length compared to only Cd stress –applied plants. All the Co-applications of HomoBL and SA increased shoot and root lengths. Earlier researchers found that application of brassinosteroids (BRs) improved the shoot and root length of *Brassica juncea* cultivated under copper stress [11, 12]. Sharma et al. [13] also observed that rape seeds pre-soaked with 24-epibrassinolide and germinated under nickel stress showed mitigated stress toxicity and enhanced root/shoot length. Drazic and Mihailovic [14] reported the pre-sowing seed-soaking treatment with lower concentration of SA enhanced the growth of shoot and root of alfalfa plants subjected to cadmium stress

The present research work is in tune with the earlier researches employing BRs and SA in mitigating stresses caused by various metals like copper, nickel as well as cadmium (combined application of 3µM HomoBL and 1mM SA was found to most effective as it not only ameliorated the inhibitory effect of Cd, but also promoted the shoot and root length compared to individual treatments as well as untreated controls).

5.2. *Shoot and Root Fresh and Dry Weights*

Cadmium at 100µM caused maximum decline in shoot fresh weight (35.5%), root fresh weight (52.14%), shoot dry weight (42%) and root dry weight (43.2%) of groundnut plants grown in Nizamabad. Application of HomoBL and SA improved the shoot as well as root fresh and dry weights over cadmium stress – treated groundnut plants. All the Co-applications of HomoBL and SA increased shoot and root fresh weight as well as shoot and root dry weights compared to individual treatments as well as untreated controls. Application of brassinosteroids (BRs) improved the shoot and root fresh and dry masses of *Brassica juncea* cultivated under copper stress [11, 12]. Similarly, exogenous application of SA to barley plants increased the fresh mass of roots and shoots [15].

5.3. *Leaves/Plant and Leaf Area/Plant*

All the different treatments of HomoBL and SA elevated the number of leaves/groundnut plant as well as leaf area of groundnut grown under cadmium stress in Nizamabad. Cd (100µM) caused a steep decline in number of leaves/ groundnut and leaf area of groundnut. All the Co-applications of HomoBL and SA increased number of leaves/groundnut and leaf area of groundnut.

Brassinosteroids have the ability to increase the foliar growth of various plants as reflected in numerous studies. Hasan et al. [16] also reported that spraying of 0 or 10^{-8} M of 28-homobrassinolide/24-epibrassinolide at 59 day stage to two tomato cultivars (K-25 and *Sarvodya*) subjected to cadmium stress, nullified the damaging effect of metal and improved photosynthetic machinery and antioxidant defense system in both the cultivars. Pre-treatment of *Brassica juncea* seeds with epibrassinolide before germination showed improved shoot emergence and plant biomass production, but reduced uptake and accumulation of Cu uptake subjected to Cu stress [17].

Salicylic acid and its close analogues enhanced the leaf area, dry mass production and seedling growth in wheat, when the grains were subjected to pre-sowing seed-soaking treatment of salicylic acid [18]. Sakineh Moradkhani et al. [19] reported that the fresh weight of leaves in sunflower was decreased (66.5%) under the influence of 200 µmol Cd compared to control plants and treatment of 500 µmol SA mitigated the stress and increased the leaf fresh weight is similar to the present research where the co-application of 3µM HomoBL and 1mM SA proved the best in not only removing the inhibitory effect of Cd, but also promoting the number of leaves and leaf area of groundnut compared to individual treatments as well as untreated controls.

6.0. Conclusion

HomoBL (HomoBL) viz., 2µM and 3µM & Salicylic Acid (SA) viz., 1mM and 2mM substantially increased the growth (shoot, root and leaves) of groundnut plants grown under cadmium (100µM) stress emphasizing on the capabilities of PGRs in overcoming metal stresses and also paving way for the employment of PGRs to improve plant growth when subjected to different metal stresses [20].

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.

7. References

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Table 1. Effect of HomoBL and SA on the Shoot and Root Length of Groundnut Plants Grown under Cadmium Stress (N=9)*

Treatments	Shoot Length of Groundnut Plants (cm)*	Root Length of Groundnut Plants (cm)*
Control	36.03 ±1.75	15.30 ±0.85
Cd 100µM	17.73 ±1.27	8.13 ±0.45
HBL 2 µM + Cd 100µM	26.63 ±0.57	9.73 ±0.49
HBL 3 µM + Cd 100µM	25.37 ±1.15	10.53 ±0.71
SA 1mM + Cd 100µM	24.47 ±0.45	10.20 ±0.51
SA 2mM + Cd 100µM	29.33 ±1.20	12.00 ±0.64
HBL 2 µM + SA 1mM + Cd 100µM	33.53 ±1.45	13.57 ±0.21
HBL 3 µM + SA 2mM + Cd 100µM	35.87 ±1.29	13.87 ±0.94
HBL 2 µM + SA 2mM + Cd 100µM	36.77 ±0.47	14.13 ±0.38
HBL 3 µM + SA 1mM + Cd 100µM	41.13 ±1.12	16.37 ±0.64

Table 2. Effect of HomoBL and Salicylic Acid on the Shoot and Root Fresh Weight of Groundnut Plants Grown under Cadmium stress (N=9)*

Treatments	Shoot Fresh Weight of Groundnut Plants (g)*	Root Fresh Weight of Groundnut Plants (g)*
Control	66.30 ±1.32	4.43 ±0.39
Cd 100µM	42.77 ±1.88	2.12 ±0.29
HBL 2 µM + Cd 100µM	47.58 ±0.45	2.63 ±0.18
HBL 3 µM + Cd 100µM	48.40 ±0.37	2.90 ±0.17
SA 1mM + Cd 100µM	47.37 ±0.39	2.70 ±0.11
SA 2mM + Cd 100µM	52.57 ±1.66	3.20 ±0.14
HBL 2 µM + SA 1mM + Cd 100µM	56.67 ±1.00	3.67 ±0.12
HBL 3 µM + SA 2mM + Cd 100µM	58.43 ±1.20	3.90 ±0.27
HBL 2 µM + SA 2mM + Cd 100µM	59.16 ±0.72	4.10 ±0.19
HBL 3 µM + SA 1mM + Cd 100µM	64.41 ±1.21	4.93 ±0.22

Table 3. Effect of HomoBL and Salicylic Acid on the Shoot and Root Dry Weight of Groundnut Plants Grown under Cadmium stress (N=9)*

Treatments	Shoot Dry Weight of Groundnut Plants (g)*	Root Dry Weight of Groundnut Plants (g)*
Control	31.59 ±1.58	2.13 ±0.05
Cd 100µM	18.33 ± 1.02	1.21 ±0.01
HBL 2 µM + Cd 100µM	23.55 ±0.59	1.27 ±0.03
HBL 3 µM + Cd 100µM	24.20 ±0.99	1.45 ±0.04
SA 1mM + Cd 100µM	23.80 ±1.11	1.35 ±0.04
SA 2mM + Cd 100µM	28.05 ±1.45	1.80 ±0.07
HBL 2 µM + SA 1mM + Cd 100µM	30.18 ±2.10	1.93 ±0.11
HBL 3 µM + SA 2mM + Cd 100µM	32.45 ±2.47	2.03 ±0.04
HBL 2 µM + SA 2mM + Cd 100µM	31.52 ±2.14	2.10 ±0.14
HBL 3 µM + SA 1mM + Cd 100µM	34.16 ±2.80	2.43 ±0.08

Table 4. Effect of HomoBL and Salicylic Acid on Number of Leaves and Average Leaf Area/ Groundnut Plants Grown under Cadmium Stress (N=9)*

Treatments	No. of leaves/Groundnut Plant*	Average Leaf Area of Groundnut Plant (cm^2)*
Control	92.33 ±1.78	11.66 ±0.40
Cd 100µM	46.00 ±1.87	6.52 ±0.45
HBL 2 µM + Cd 100µM	54.33 ±2.86	7.52 ±0.17
HBL 3 µM + Cd 100µM	64.33 ±2.16	9.10 ±0.12
SA 1mM + Cd 100µM	55.67 ±2.27	8.39 ±0.18
SA 2mM + Cd 100µM	65.05 ±2.12	10.17 ±0.57
HBL 2 µM + SA 1mM + Cd 100µM	76.67 ±2.04	10.66 ±0.14
HBL 3 µM + SA 2mM + Cd 100µM	86.67 ±2.04	11.11 ±0.24
HBL 2 µM + SA 2mM + Cd 100µM	92.33 ±1.78	10.70 ±0.19
HBL 3 µM + SA 1mM + Cd 100µM	97.00 ±0.71	11.32 ±0.33