Magna Scientia Advanced Research and Reviews
eISSN: 2582-9394
Cross Ref DOI: 10.30574/msarr
Journal homepage: https://magnascientiapub.com/journals/msarr/

(Research Article)

# Homobrassinolide and Seed Germination of *Cleome viscosa* and *Cleome gynandra* species

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Magna Scientia Advanced Research and Reviews, 2026, 17(01), 161–167

Publication history: Received on 15 April 2026; revised on 22 May 2026; accepted on 25 May 2026

Article DOI: https://doi.org/10.30574/msarr.2026.17.1.0088

## Abstract

Brassinosteroids are polyhydroxylated sterol derivatives, identified in the pollen of *Brassica napus* and can control many of the plant morphological and physiological responses. The role of BRs in improving/ modifying/ modulating plant growth and development paved way for betterment of crops cultivated for specific purposes. *Cleome viscosa* and *Cleome gynandra* are shrubs and both are rich in nutrients and bioactive compounds. The present research study is to understand the role of homobrassinolide, a potent brassinosteroid in improving the seed germination and seedling growth of two species of *Cleome* viz., *Cleome viscosa* and *Cleome gynandra* in terms of percentage of seed germination, seedling length, seedling fresh and dry weights.

**Keywords:** *Cleome gynandra*; *Cleome viscosa*; Homobrassinolide; Seed Germination; Seedling Length; Seedling Fresh; Dry Weights

## 1. Introduction

*Cleome viscosa* and *Cleome gynandra* species contain secondary metabolites such as phenolic compounds, alkaloids, essential oils, tannins, terpenoids, saponins, cardiac glycosides and it is an established fact pharmaceuticals, flavor enhancers, and recreational drugs are some of the ways that humans put these secondary metabolites to usage. Traditional and folkloric activities make considerable use of *Cleome viscosa* and *Cleome gynandra*'s leaves, seeds, and roots. The powdered leaf of *Cleome viscosa* qualitative phytochemical screening, revealed the presence of specific compounds (Paul et al., 2012). Ayurveda recognizes the pharmacological significance of *Cleome gynandra* for its possible therapeutic uses in a number of ailments, including *krmiroga* (worm infection), *asthila* (prostate hypertrophy), *kandu* (pruritus), *gu1ma* (tumour, irregularity, or diverticu1osis), and *karnaroga* (ear infections), among others (Bala et a1., 2010).

Brassinosteroids and several plant growth regulators (PGRs) are essential for controlling responses to different stressors, including abscisic acid (Vardhini and Anjum, 2015; Wang et a1., 2019; An et a1., 2023), salicylic acid (Malaga et a1., 2019), ethylene (Betti, 2021), and brassinosteroids (Sirhindi et a1., 2014). The external application of PGRs, whether through seed priming or foliar spray on the aerial portions of the plant, alleviates the detrimental effects induced by abiotic stresses (Kumar et al., 2010; Kumar et a1., 2011). The present research study aims in investigating the effects of homobrassinolide on the seed germination and seed viability of *Cleome viscosa* and *Cleome gynandra* seeds.

## 2. Materials and Methods

*Cleome viscosa* L. and *Cleome gynandra* L. seeds were collected from the surroundings of Ghanpur village, Chandur Mandal, Nizamabad District, Telangana. Homobrassinolide was purchased from Godrej Agrovet Private Limited, India

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The preliminary research was conducted to test the modulated rate of seed germination in both *Cleome viscosa* and *Cleome gynandra* species by supplementation of homobrassinolide and to stabilize the concentrations of homobrassinolide. Seeds of *Cleome viscosa* and *Cleome gynandra* underwent sterilization using 0.5% (v/v) sodium hypochlorite solution and were subsequently placed in a sterile petri dish lined with Whatman No. One filter paper which would be provided along with 5ml of either solution.

- Distilled water (control sample)
- Homobrassinolide at 2 μM, 3 μM, 4 μM, 6μM, and 8 μM concentrations.

Twenty-five seeds of *Cleome viscosa* and *Cleome gynandra* were placed in each petriplate. Six replicates were maintained for each treatment. Five ml of homobrassinolide at 2, 3, 4, 6, and 8 micromolar (μM) concentrations were administered to both *Cleome* species. Controls were administered with distilled water. Another five ml of homobrassinolide/distilled was administered on the 6th day. The emergence of the radicle is considered a criterion for germination. The number of seed germinated were recorded on 5 days, 7 days, and 12 days. The various aspects of seed germination studies of *Cleome viscosa* and *Cleome gynandra* are as

### 2.1. Percentage of Seed germination of *Cleome viscosa* and *Cleome gynandra*

The percentage of seed germination of the two species, *Cleome viscosa* and *Cleome gynandra* was noted down on the 5$^{th}$, 7$^{th}$ and 12$^{th}$ days from seed germination and determined by the formula: Number of seeds germinated / total number of seeds) x 100.

### 2.2. Seedling Length of *Cleome viscosa* and *Cleome gynandra*

The seedling length of the two species, *Cleome viscosa* and *Cleome gynandra* was measured on 10$^{th}$, 12$^{th}$ and 15$^{th}$ day and written in centimeters.

### 2.3. Seedling Fresh Weight of *Cleome viscosa* and *Cleome gynandra*

The seedling fresh weight of the two species, *Cleome viscosa* and *Cleome gynandra* was measured on 10$^{th}$, 12$^{th}$ and 15$^{th}$ day and written in grams.

### 2.4. Seedling Dry Weight of *Cleome viscosa* and *Cleome gynandra*

The seedling dry weight of the two species, *Cleome viscosa* and *Cleome gynandra* was measured on 10$^{th}$, 12$^{th}$ and 15$^{th}$ day and written in grams.

## 3. Results

### 3.1. Percentage of seed germination of *Cleome gynandra* and *Cleome viscosa*

The application of 2, 3, 4, 6, and 8 micromolar (μM) concentrations of homobrassinolide resulted in the highest percentage of seed germination in *Cleome gynandra* and *Cleome viscosa* compared to the untreated control group on the 5$^{th}$, 7$^{th}$ and 12$^{th}$ day after germination. In comparison of the two seedlings, *Cleome gynandra* exhibited superior germination relative to *Cleome viscosa* wherein the 12$^{th}$ day showed the highest percentage of seed germination.

Among the homobrassinolide concentrations, there was an increase in the rate of germination percentage from 2 (μM) to 4 (μM) on all days. But the percentage of seed germination reduced from 6 (μM) and 8 (μM) on all days though it was higher than controls.

The present research study showed that homobrassinolide at 4 (μM) on all days exhibited the best germination percentage rate as represented in the given data (Table 1).

### 3.2. Homobrassinolide and Seedling length of *Cleome gynandra* and *Cleome viscosa*

The application of 2, 3, 4, 6, and 8 micromolar (μM) concentrations of homobrassinolide resulted in the highest percentage of seedling length in *Cleome gynandra* and *Cleome viscosa* compared to the untreated control group in 10 days, 12 days and 15 days after germination. In comparison of the two seedlings, *Cleome gynandra* exhibited superior seedling length relative to *Cleome viscosa* wherein the 15$^{th}$ day showed the highest seedling length. Among the homobrassinolide concentrations, there was an increase in the seedling length from 2 (μM) to 4 (μM) on all days. But the seedling length reduced from 6 (μM) and 8 (μM) on all days though it was higher than controls. The present research study showed that homobrassinolide at 4 (µM) on all days exhibited the best seedling length as represented in the given data (Table 2).

**Table 1** Homobrassinolide and Percentage of Seed Germination of *Cleome gynandra* and *Cleome viscosa*

| S.No. | Treatments | Percentage of seeds germinated in 5 days* | Percentage of seeds germinated in 7 days* | Percentage of seeds germinated in 12 days* |
|---|---|---|---|---|
| 1 | Control (Distilled water) | 55 ± 0.002 | 65 ± 2.254 | 75 ± 0.219 |
| 2 | Homo BL 2.0 µM on *C. gynandra* | 35 ± 3.291 | 45 ± 2.241 | 65 ± 2.254 |
| 3 | Homo BL 3.0 µM on *C. gynandra* | 55 ± 1.251 | 65 ± 2.101 | 85 ± 1.363 |
| 4 | Homo BL 4.0 µM on *C. gynandra* | 75 ± 2.012 | 85 ± 1.265 | 100 ± 12.114 |
| 5 | Homo BL 6.0 µM on *C. gynandra* | 65 ± 2.087 | 70 ± 2.047 | 75 ± 2.009 |
| 6 | Homo BL 8.0 µM on *C. gynandra* | 55 ± 1.010 | 60 ± 1.232 | 65 ± 2.021 |
| 7 | Control (Distilled water) *C. viscosa* | 40 ± 1.432 | 45 ± 1.201 | 55 ± 1.105 |
| 8 | Homo BL 2.0 µM on *C. viscosa* | 55 ± 2.021 | 65 ± 2.010 | 70 ± 1.636 |
| 9 | Homo BL 3.0 µM on *C. viscosa* | 65 ± 2.020 | 75 ± 2.120 | 95 ± 1.145 |
| 10 | Homo BL 4.0 µM on *C. viscosa* | 70 ± 2.012 | 85 ± 1.363 | 100 ± 1.564 |
| 11 | Homo BL 6.0 µM on *C. viscosa* | 55 ± 2.214 | 60 ± 2.102 | 65 ± 2.254 |
| 12 | Homo BL8.0 µM on *C. viscosa* | 40 ± 2.231 | 45 ± 1.271 | 50 ± 1.245 |

*The mean +/- SE (n is 6.); the Post Hoc test revealed that the Mean would not differ significantly at a p value is 0.05 if it the same alphabet after that in each column

**Table 2** Homobrassinolide and Seedling length (cm) of *Cleome gynandra* and *Cleome viscosa*.

| S.No. | Treatments | Seedling length in 10 days | Seedling length in 12 days | Seedling length in 15 days |
|---|---|---|---|---|
| 1 | Control<br>(Distilled water) | 1.95 ± 0.12 | 2.15± 0.16 | 3.69± 0.27 |
| 2 | Homo BL 2.0 µM on *C. gynandra* | 3.98± 0.28 | 4.09± 0.28 | 5.26± 0.37 |
| 3 | Homo BL 3.0 µM on *C. gynandra* | 7.09± 0.49 | 9.08± 0.63 | 10.11± 0.53 |
| 4 | Homo BL 4.0 µM on *C. gynandra* | 10.09± 0.63 | 12.63± 0.61 | 13.10± 0.58 |
| 5 | Homo BL 6.0 µM on *C. gynandra* | 9.08± 0.63 | 11.66± 0.72 | 12.01± 0.48 |

| | | | | |
|---|---|---|---|---|
| 6 | Homo BL 8.0 µM on *C. gynandra* | 7.39± 0.49 | 9.08± 0.65 | 10.76± 0.61 |
| 7 | Control (Distilled water) *C. viscosa* | 1.81 ± 0.14 | 2.18 ± 0.16 | 2.93± 0.25 |
| 8 | Homo BL 2.0 µM on *C. viscosa* | 4.01 ± 0.35 | 4.59 ± 0.24 | 5.02± 0.54 |
| 9 | Homo BL 3.0 µM on *C. viscosa* | 5.11 ±0.31 | 7.97 ± 0.33 | 8.03 ± 0.34 |
| 10 | Homo BL 4.0 µM on *C. viscosa* | 9.05 ± 0.69 | 11.91 ± 0.64 | 12.63 ± 0.55 |
| 11 | Homo BL 6.0 µM on *C. viscosa* | 7.97 ± 0.33 | 9.14± 0.34 | 10.11± 0.59 |
| 12 | Homo BL 8.0 µM on *C. viscosa* | 5.13 ± 0.29 | 6.81 ± 0.87 | 7.48 ± 0.35 |

*The mean +/- SE (n is 6.); the Post Hoc test revealed that the Mean would not differ significantly at a p value is 0.05 if it the same alphabet after that in each column

**Table 3** Homobrassinolide and Seedlings Fresh weight (g) of *Cleome gynandra* and *Cleome viscosa*

| S. No. | Treatments | Seedling Fresh Weight ($10^{th}$ Day) * | Seedling Fresh Weight ($12^{th}$ Day) * | Seedling Fresh Weight ($15^{th}$ Day) * |
|---|---|---|---|---|
| 1 | Control (Distilled water) | 0.708 ± 0.12 | 0.905± 0.16 | 1.43 ± 0.27 |
| 2 | Homo BL 2.0 µM on *C. gynandra* | 0.802± 0.28 | 1.08± 0.28 | 1.89 ± 0.37 |
| 3 | Homo BL 3.0 µM on *C. gynandra* | 0.978 ± 0.49 | 1.137± 0.63 | 2.43± 0.53 |
| 4 | Homo BL 4.0 µM on *C. gynandra* | 2.07± 0.63 | 2.89 ± 0.61 | 3.95 ± 0.58 |
| 5 | Homo BL 6.0 µM on *C. gynandra* | 1.87± 0.63 | 2.26± 0.72 | 2.96± 0.48 |
| 6 | Homo BL 8.0 µM on *C. gynandra* | 1.130± 0.49 | 1.92 ± 0.65 | 2.32± 0.61 |
| 7 | Control (Distilled water) *C. viscosa* | 0.621 ± 0.14 | 0.811 ± 0.16 | 1.13± 0.25 |
| 8 | Homo BL 2.0 µM on *C. viscosa* | 0.698 ± 0.35 | 0.816 ± 0.24 | 1.34± 0.54 |
| 9 | Homo BL 3.0 µM on *C. viscosa* | 0.943 ±0.31 | 1.06± 0.33 | 2.09 ± 0.34 |
| 10 | Homo BL 4.0 µM on *C. viscosa* | 1.78 ± 0.69 | 2.54 ± 0.64 | 3.12 ± 0.55 |
| 11 | Homo BL 6.0 µM on *C. viscosa* | 1.22± 0.33 | 1.95± 0.34 | 2.21± 0.59 |
| 12 | Homo BL 8.0 µM on *C. viscosa* | 0.986 ± 0.29 | 1.23 ± 0.87 | 1.79 ± 0.35 |

*The mean +/- SE (n is 6.); the Post Hoc test revealed that the Mean would not differ significantly at a p value is 0.05 if it were the same alphabet after that in a given column

**Table 4** Homobrassinolide and Seedling Dry Weight (g) of *Cleome gynandra* and *Cleome viscosa*.

| S.No. | Treatments | Seedling Dry Weight (10th Day )* | Seedling Dry Weight (12th Day )* | Seedling Dry Weight (15th Day )* |
|---|---|---|---|---|
| 1 | Control (distilled water) | 0.557 ± 0.14 | 0.732± 0.18 | 0.812 ± 0.17 |
| 2 | Homo BL 2.0 µM on *C. gynandra* | 0.609± 0.20 | 0.845 ± 0.17 | 0.998 ± 0.40 |
| 3 | Homo BL 3.0 µM on *C. gynandra* | 0.778 ± 0.54 | 0.937± 0.76 | 1.14± 0.63 |
| 4 | Homo BL 4.0 µM on *C. gynandra* | 1.23 ± 0.43 | 1.67 ± 0.76 | 2.19 ± 0.83 |
| 5 | Homo BL 6.0 µM on *C. gynandra* | 0.856 ± 0.69 | 0.976± 0.70 | 1.67± 0.76 |
| 6 | Homo BL 8.0 µM on *C. gynandra* | 0.779 ± 0.39 | 0.918 ± 0.65 | 1.04± 0.67 |
| 7 | Control (distilled water) *C. viscosa* | 0.198 ± 0.11 | 0.312± 0.14 | 0.445± 0.21 |
| 8 | Homo BL 2.0 µM on *C. viscosa* | 0.498 ± 0.38 | 0.616 ± 0.24 | 0.779± 0.56 |
| 9 | Homo BL 3.0 µM on *C. viscosa* | 0.643 ±0.38 | 0.798 ± 0.31 | 0.934 ± 0.37 |
| 10 | Homo BL 4.0 µM on *C. viscosa* | 0.945 ± 0.66 | 1.04 ± 0.60 | 1.67 ± 0.58 |
| 11 | Homo BL 6.0 µM on *C. viscosa* | 0.732 ± 0.36 | 0.812 ± 0.31 | 0.998± 0.58 |
| 12 | Homo BL 8.0 µM on *C. viscosa* | 0.569 ± 0.29 | 0.725 ± 0.76 | 0.876 ± 0.34 |

*The mean +/- SE (n is 6.); the Post Hoc test revealed that the Mean would not differ significantly at a p value is 0.05 if it the

### 3.3. Homobrassinolide and Seedling Fresh Weight of *Cleome gynandra* and *Cleome viscosa*

The application of 2, 3, 4, 6, and 8 micromolar (µM) concentrations of homobrassinolide resulted in the highest percentage of seedling fresh weight in *Cleome gynandra* and *Cleome viscosa* compared to the untreated control group in 10 days, 12 days and 15 days after germination. In comparison of the two seedlings, *Cleome gynandra* exhibited superior seedling fresh weight relative to *Cleome viscosa* wherein the 15th day showed the highest seedling fresh weight. Among the homobrassinolide concentrations, there was an increase in the seedling fresh weight from 2 (µM) to 4 (µM) on all days. But the seedling fresh weight reduced from 6 (µM) and 8 (µM) on all days though it was higher than controls. The present research study showed that homobrassinolide at 4 (µM) on all days exhibited the best seedling fresh weight as represented in the given data (Table 3).

### 3.4. Homobrassinolide and Seedling Dry Weight of *Cleome gynandra* and *Cleome viscosa*

The application of 2, 3, 4, 6, and 8 micromolar (µM) concentrations of homobrassinolide resulted in the highest percentage of seedling dry weight in *Cleome gynandra* and *Cleome viscosa* compared to the untreated control group in 10 days, 12 days and 15 days after germination. In comparison of the two seedlings, *Cleome gynandra* exhibited superior seedling dry weight relative to *Cleome viscosa* wherein the 15th day showed the highest seedling dry weight. Among the homobrassinolide concentrations, there was an increase in the seedling dry weight from 2 (µM) to 4 (µM) on all days. But the seedling dry weight reduced from 6 (µM) and 8 (µM) on all days though it was higher than controls. The present research study showed that homobrassinolide at 4 (µM) on all days exhibited the best seedling dry weight as represented in the given data (Table 4).

## 4. Discussion

Seed germination is considered as the first and primary physiological aspect on the growth of plants. Seed germination includes, imbibition of water or solutes, the activation of certain enzymes and finally the emergence of the embryonic root called as the radicle. In the present research study, the various aspects of seed germination studies of *Cleome gynandra* and *Cleome viscosa* were the length of the seedling, the fresh weight, the dry weight, and the percentage of seed germination recorded on the fifth, seventh, and twelfth days from seed germination. Brassinosteroids (BRs) and spermine (SPM) primed seeds of two distinct rice cultivars (CY927; sensitive, YLY689; tolerant) cultivated under Cr-stress significantly reduced Cr-induced phytotoxicity by increasing the seed germination ratio. (Basit et al., 2022).

All the research indicates that supplication of PGRs may always result in the seed germination of various crops. When cotton seeds were pre-soaked in 1 µM of epibrassinolide and grown under 200 mM of sodium chloride, there was more than double enhancement of seed germination over the non-treated controls (Chakma et al., 2021). Supplementation of 24-epibrasinoloide and 28- homobrassinolide significantly increased the percentage of germination and seedling growth of all the three varieties of sorghum under osmotic stress wherein the growth promotion was associated with enhanced levels of soluble proteins and free proline (Vardhini and Rao, 2003). Recently, Deepak and Vardhini (2023) observed that supplementation of homobrassinolide to *Plumbago zeylanica* L improved the seed germination percentage with respect to the parameters studied like germinability (% G), germination rate index (GRI), coefficient velocity of germination (CVG) and relative seed germination (RSG). The potentiality of brassinosteroids in increasing the seed germination and seedling growth of plants like sorghum, groundnut is well documented (Vardhini and Rao1996; Vardhini and Rao, 2005).

All the above aspects are in tune with the present research study wherein the supplication of 2, 3, 4, 6, and 8 micromolar (µM) concentrations of homobrassinolide resulted in the highest percentage of seedling growth, seedling length, seedling fresh and seedling dry weights in *Cleome gynandra* and *Cleome viscosa* compared to the untreated control group and homobrassinolide at 4 (µM) exhibited the best percentage of seedling growth, seedling length, seedling fresh and seedling dry weights proving the ability of homobrassinolide to act a typical plant growth regulator (PGR).

## Compliance with ethical standards

### *Disclosure of conflict of interest*

No conflict of interest to be disclosed.

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