Bulletin of the National Research Centre

RESEARCH

Open Access

Interference with sex expression, estimation of yield and bioactive compounds in bitter gourd under PGRs-induced conditions

Md. Rafikul Islam[1], Shafiquzzaman Siddiquee[2], Md. Mofizur Rahman[3], Noor Muhammad[4,5], Jasim Uddain[3] and Md. Dulal Sarkar[3,5*]

Abstract

Background The bitter gourd fruit yields are not at a desirable level due to maleness. Maleness is one of the biggest difficulties with bitter gourd, reducing fruit yields substantially. On the other hand, plant stages are the most important consideration for PGR application because of their sensitive responsiveness. Therefore, it is necessary to determine how bitter gourd plants respond to plant growth regulators, namely PGR_0 (control: application of water as control treatment), PGR_1 (GA_3-Gibberellic acid, 100 mgL^{-1}), PGR_2 (NAA-Naphthalene acetic acid, 100 mgL^{-1}), and PGR_3 (MH- Maleic hydrazide, 100 mgL^{-1}) at three application phases, S_1: soaking of seeds; S_2: four-leaf; and S_3: flower bud in terms of vegetative development, male–female flower sex alteration, and fruit features including bioactive compounds.

Results Physiological, floral and yield characteristics of bitter gourd exposed significant changes by GA_3 and MH at various stages. The number of leaves $plant^{-1}$, the fresh mass of the plant, and the fresh mass of fruit and fruit dry matter content were enhanced remarkably for MH application at the seed soaking stage except for plant height and the number of branches $plant^{-1}$. MH increased the number of female flowers $plant^{-1}$, the number of fruits $plant^{-1}$ and the yield at the 4-leaf stage of application. PGRs substantially influenced the quantity of sugar, chlorophyll, total carotenoids, protein, and water content at various phases of their application but not on ascorbic acid, TSS, or total phenol concentration.

Conclusions For sex expression and yield attributes, 100 mgL^{-1} MH at the 4-leaf stage would be effective in bitter gourd. Exogenous NAA showed a considerable dual influence on plant development and antioxidant enzyme activity in bitter gourd fruits.

Keywords Secondary metabolites, Maleic hydrazide, Growth promoters, Growth retardants

*Correspondence:
Md. Dulal Sarkar
dulal@sau.edu.bd
Full list of author information is available at the end of the article

Background

The pleading for vegetable cultivation has stamped up rapidly due to accomplishing great financial interest worldwide. People are becoming more concerned about their health and continuously favor requiring vegetables, cucurbitaceous, for their fundamental therapeutic and nutritional qualities (Gayathry and John 2022). Bitter gourd (*Momordica charantia* L.) belongs to the family Cucurbitaceae and is a tendril bearing vine type herbaceous plant and one of the most popular crops in Bangladesh. Fruits are highly nutritive and high in antibiotic, antimutagenic, antioxidant, antiviral, antidiabetic and immune-enhancing properties (Anayat 2020). Its extract, obtained from leaves and fruits, is useful for controlling higher blood sugar and treating infections, wounds and fevers (Ahmad et al. 2019). However, flowering behavior may shift with cultivar, and climatic conditions and sex expression in cucurbits can be modified by hormonal factors (Moniruzzaman et al. 2019; Reddy et al. 2020; Shailendrakumar et al. 2017). Fruit yields are not increasing satisfactorily according to Bangladesh's rising demand. Total production of bitter gourd in 2020–2021 was about 59,313.35 metric tons on around 26,810.75 acres of land (BBS 2022). Like other cucurbits, maleness is one of the major problems in the bitter gourd, significantly reducing fruit yields.

The contemporary farming paradigm, including agricultural advancement, pressures researchers to find novel ways to intensify agricultural production, enhance quality metrics, and minimize adverse environmental consequences (Caradonia et al. 2022). The usage of biostimulants, in accordance with (Mousavi et al. 2022), reduces the adverse impacts of biotic and abiotic stressors. Plant growth regulators, including growth promoters and retardants, are another class of biologically active compounds. These agents work primarily by boosting growth while retaining the inherent ability to produce plants, including improved roots, yield, and chemical properties (Kołodziejc and Gwó 2022). Generally, they are used for enhancing flowering, especially for sex expression. They also enhance the source-sink relationship and stimulate photoassimilate translocation to help better the fruit set (Moniruzzaman et al. 2019; Reddy et al. 2020; Shailendrakumar et al. 2017). The exogenous application of gibberellic acid actively influenced many plants' physiological activities, including cell division, cell elongation and cell expansion, which stimulate plant growth (Sprangers et al. 2020). Altering the flowering sequence and sex ratio is the most important in the sex modification of cucurbits. Maleic hydrazide affected the growth and sex expression in bottle gourd (Sarkar et al. 2019). NAA is also used to change the sex ratio and sequence, which affects a plant's ability to develop and produce traits that contribute to its yield (Gerdakaneh et al. 2018).

Different plant stages are the prime consideration for PGR's application because of their sensitivity. Plant growth regulators significantly enhance early flowering, harvesting, and maximum fruit setting when applied at the 2-leaf and flower initiation stage (Sarkar et al. 2019). Plant growth regulators positively influenced vegetative, flowering, modification of sex expression and fruit traits in bitter gourd when sprayed twice at various stages (Sarkar et al. 2019). So, PGRs might have potentialities to influence plant growth in terms of use in the suitable stage. Although PGRs have been utilized effectively in many crops, their precise effects during critical stages of development, such as seed germination, leaf expansion, and flower bud formation in bitter gourd, are still mostly unclear. Because of their wide spectrum effectiveness on every aspect of plant growth, the present experiment aimed that plant growth regulators have a useful potentiality to determine the sex ratio for increasing the yield and quality of bitter gourd and to evaluate the performances of PGR at various stages.

Methods

Experimental site

The experiment was carried out from February to June in open field provision at the Horticulture Farm of Sher-e-Bangla Agricultural University, Bangladesh, 24.09°N and 90.26°E longitude with an elevation of 8.20 m from sea level. The soil was loamy, and the site was characterized by three distinct seasons: winter (November to February), pre-monsoon (March to April) and monsoon (May to October).

Experimental design and application of PGRs

The experiment was laid out following Randomized Complete Block Design with four replications. Four levels of plant growth regulators were studied as PGR_0: control (0 mgL^{-1}), PGR_1: GA_3 (Gibberellic acid 100 mgL^{-1}), PGR_2: NAA (naphthalene acetic acid100 mgL^{-1}) and PGR_3: MH (maleic hydrazide 100 mgL^{-1}) at three application stages viz. S_1: seed soaking, S_2: 4-leaf and S_3: flower bud. Plant growth regulators were applied at every stage, beginning with the soaking of the seeds and continued until the beginning of the flowering phase. Each plant received roughly 20 mL of solution. A commercial hand-held sprayer was used to completely cover the plant by spraying the abaxial and adaxial surfaces of the leaves.

Growing conditions

Seeds of BARI Karala-1, a variety of bitter gourd, were collected from the Horticulture Research Centre of Bangladesh Agriculture Research Institute and used as

planting material. The plot was opened in the second week of February with a power tiller and displayed to the sun for a week. Pits 45 × 45 × 40 cm sized were ready 1.5 m apart during a single row on the bed. Inorganic fertilizers- N, P, K, S, Zn and B and organic fertilizers were used for commercial production in the form of urea, triple superphosphate, muriate of potash, gypsum, zinc sulfate, boric acid, and cow dung, respectively. Healthy about 20 day-old seedlings were selected, and only those with at least four true leaves were transplanted in the prepared pit on the second week of March. Intercultural operations were done as per requirements. Bitter gourd fruits were harvested while they were still green, just about full size but before the skin began changing color.

Data collection

The number of days it took for seeds to germinate, the final height of each plant, and the total number of leaves and branches were all recorded. Floral data such as days to first blooming, the numbers of male and female flowers and their ratio per plant were recorded. Since PGRs were applied at three different times, namely the seed soaking stage, the 4-leaf stage, and the flower bud stage, floral parameters were only recorded after the flowers had fully blossomed. The roots and fruits were separated, and the fresh and dry biomass weights were determined using a delicate balance. The chlorophyll content of the leaves was determined using the method described in Lee et al. (2000). To summarize, 0.2 g of fresh leaves were mashed in a mortar with 3 mL of acetone (80 percent v/v). After that, the pellet was re-extracted with a 10 mL acetone solution till discoloration appeared. The uppermost phase of the solution was filtered, and the absorbance was measured at 645 and 663 nm with a spectrophotometer to determine chl a and chl b.

Extraction and determination of fruit biochemical composition

After immediate harvesting, fresh fruit samples were taken to evaluate bioactive compounds. Sugar contents were estimated by the Fehling reagent method (AOAC 2005). The carotenoid content of the fruits was determined using the same method for chlorophyll content described earlier (Lee et al. 2000), where the absorbance was measured at 470 nm with a spectrophotometer. The Folin–Ciocalteu method was used to determine total phenolic content, as defined by Jayaprakasha et al. (2001). In brief, a 5 g sample was extracted with 100 mL 80% methanol for 24 h in a shaking bath. Next, 7.9 mL distilled water, 0.1 mL extract, and 0.5 mL Folin–Ciocalteu reagent (1:1 with water) were mixed in a 10 mL tube. Next, 1.5 mL sodium carbonate (10%) was added after 1 min and thoroughly mixed, and the absorbance was measured at 765 nm. The total phenolic content is expressed as mg g^{-1} fresh extract. The Kjeldahl method was used to determine the protein content (Motsara and Roy 2008). The proportion of total nitrogen (%TN) was measured first, followed by the fraction of non-protein (%NPN). The difference between % NPN and % TN was used to calculate the proportion of protein nitrogen (%PN). The actual protein (total N- non-protein N) × 6.25 was calculated. For the determination of total N, 0.5 g sample, 5.0 g sodium sulfate (Na_2SO_4), and 0.5 g copper sulfate ($CuSO_4$) were heated for around 2 h with 11 mL of concentrated sulfuric acid (H_2SO_4) until the color of the digested mixture changed to colorless. After cooling the digestion mixture, around 10 ml of water was added to dissolve the digestion product. 35 ml of sodium hydroxide (40%) was added to that digested result. The ammonia gas was absorbed with 20 mL of 1 M HCL in another big capillary tube. The intensity of HCL was measured with a standard sodium carbonate solution and methyl orange as an indicator. A 0.2 g sample was obtained and dissolved in 20 ml of water and 30 ml of 20% trichloroacetic acid to determine non-protein nitrogen (NPN). The filtrate was then mixed with 5.0 g of sodium sulfate (Na_2SO_4), 0.5 g of copper sulfate ($CuSO_4$), and 8.0 ml concentrated sulfuric acid (H_2SO_4). The NPN was calculated from the change in HCl concentration using the previously stated technique for total nitrogen determination. The indophenol method outlined by Nielsen (2017) was used to estimate vitamin C content. Briefly, using a mortar and pestle, 30 g of fresh fruits were mashed with 10 mL of trichloroacetic acid (6%) (TCA) added. The extract was brought to 100 mL with the TCA mixture after being ground and strained. The samples were titrated separately with the indophenol dye solution until a light rose pink color persisted for 5 s. TSS (°Brix) was measured directly in the juice with a digital hand refractometer (ERMA, Tokyo, Japan) at room temperature in a 58–92% range.

Statistical analysis

Analysis of variance was performed to evaluate the significance of the effect of plant growth regulators and their application stage in bitter gourd's growth physiology and quality. LSD test was used to determine variances among the treatments where $P < 0.05$ was considered significant.

Results

Growth features

Plant growth regulators at different application stages showed significant effects on days to germination (Fig. 1), plant height, number of leaves and number of branches $plant^{-1}$ of bitter gourd (Table 1). The findings on the time needed for germination completion were considerably longer under PGR_3 (7.63 days) conditions applied in the

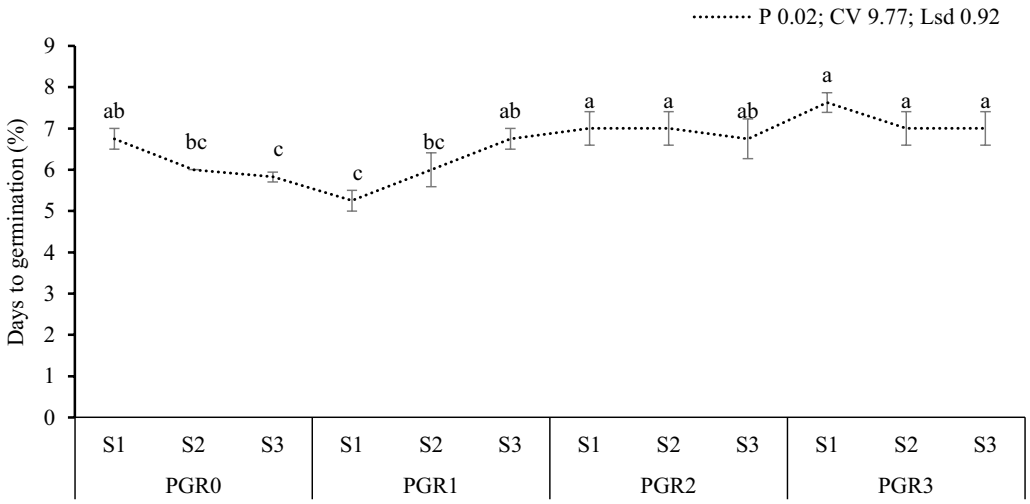

Fig. 1 Effect of plant growth regulators at different stages on days to germination (%). Abbreviations are as follows: PGR_0: 0, PGR_1: 100 mgL^{-1} GA_3, PGR_2: 100 mgL^{-1} NAA and PGR_3: 100 mgL^{-1} MH (maleic hydrazide), S_1: seed soaking stage, S_2: 4-leaf stage and S_3: flower bud stage. Different letters in the same column indicate significant differences between treatments ($p < 0.05$). Vertical bars indicate standard errors

Table 1 Plant height (cm), number of leaves and number of branches per plant as influenced by plant growth regulators at different stages

Growth regulators	Application stage	Plant height	Number of leaves	Number of branches
	S_1	388.75 ± 3.20^{d}	325.25 ± 7.26^{f}	20.75 ± 0.63^{f}
PGR_0	S_2	401.25 ± 3.59^{cd}	344.75 ± 1.89^{ef}	24.25 ± 0.25^{d}
	S_3	388.25 ± 0.48^{d}	351.75 ± 6.06^{de}	22.75 ± 0.48^{e}
	S_1	458.50 ± 2.53^{a}	365.00 ± 7.63^{cde}	25.25 ± 0.48^{cd}
PGR_1	S_2	454.00 ± 3.76^{a}	361.25 ± 2.78^{de}	22.75 ± 0.63^{e}
	S_3	457.00 ± 9.26^{a}	372.75 ± 6.13^{bcd}	26.50 ± 0.29^{b}
	S_1	451.50 ± 6.86^{ab}	374.00 ± 9.16^{bcd}	23.00 ± 0.41^{e}
PGR_2	S_2	409.75 ± 4.33^{c}	388.75 ± 3.45^{bc}	25.50 ± 0.29^{bc}
	S_3	439.25 ± 6.29^{b}	371.50 ± 5.63^{bcd}	24.50 ± 0.29^{cd}
	S_1	353.25 ± 4.64^{f}	426.00 ± 22.00^{a}	17.75 ± 0.25^{g}
PGR_3	S_2	374.25 ± 1.11^{e}	391.25 ± 8.20^{b}	28.00 ± 0.41^{a}
	S_3	360.50 ± 4.35^{ef}	423.75 ± 6.92^{a}	20.00 ± 0.41^{f}
$LSD_{(0.05)}$		13.95	25.59	1.23
CV%		2.36	4.75	3.67
P value		0.00	0.04	0.00

PGR_0: 0, PGR_1: 100 mgL^{-1} GA_3, PGR_2: 100 mgL^{-1} NAA (naphthalene acetic acid) and PGR_3: 100 mgL^{-1} MH (maleic hydrazide), S_1: seed soaking stage, S_2: 4-leaf stage and S_3: flower bud stage. Different letters in the same column indicate significant differences between treatments ($p < 0.05$). Values are mean ± SE

seed soaking stage. Maleic hydrazide induces dormancy, whereas gibberellic acid reduces dormancy and accelerates germination. It takes 5.25 days to germinate when the bitter gourd seed is treated at the seed soaking stage (Fig. 1). Different phases of bitter gourd and GA_3 application rates interacted highly with plant height (Table 1). The maximum plant height (458.50 cm) was recorded in the seed soaking stage (Table 1). In comparison, a significant relationship between NAA and various application stages was also noticed in bitter gourd plant height, with a peak in the seed soaking stage (451.50 cm). The maximum number of leaves (426.00) and branches (28.00) $plant^{-1}$ was recorded in PGR_3 (maleic hydrazide) application in the stage of S_1 and S_2, respectively, being significantly superior among different treatment combinations at harvesting (Table 1).

Floral features

The number of female flowers plant^{-1} respond notably in variation by different PGRs when treated at seed soaking, 4-leaf stage and flower bud stage of bitter gourd (Table 2). In any case, days to first flowering, the number of male flower plants^{-1} and the male–female ratio did not respond significantly. The number of female flowers in plant^{-1} increased by around 29.9% and was highest (27.83) with PGR_3S_2 though the lowest (19.5) in PGR_0S_1 (Table 2). No statistically significant difference in the male–female flower ratio was influenced by MH (Table 2).

Physiological attributes

Fresh plant mass, single fruit weight, fruit dry matter content and root dry matter content exhibited significant variation by PGRs at different application stages of bitter gourd except for fresh root mass (Table 3). Plants growing in PGR_1S_2 showed the highest fresh mass of plant (14.08 kg) and fruit dry matter content (10.37%), while the lowest fresh mass of plant (9.11 kg) in PGR_3S_1 and fruit dry matter content (6.80%) was attained in PGR_0S_1 at harvesting.

Yield components

Data regarding the number of fruits plant^{-1}, single fruit weight, fruit length (Table 4) and fruit yield (Fig. 2) of bitter gourd under PGRs conditions at different stages were significantly higher among treatment combinations where fruit setting (%) showed non-significant. However, compared to the control treatment in the 4-leaf stage and flower bud stage, except for the seed soaking phases, fruit diameter in MH (maleic hydrazide) treated plants exhibited reduced diameter (Table 4). The maximum number of fruits plant^{-1} was recorded in PGR_3S_2 at harvest, 35.9% more than PGR0S1, where the minimum number was found (Table 4). GA_3 was distinct from other growth regulator treatments for bitter gourd fruit weight enhancement (Table 4). All growth regulators as treatments differed considerably from the control regarding fruit weight gain. The interaction of GA_3 application and bitter gourd stages was an important factor for fruit weight (Table 4). When 100 mgL^{-1} GA_3 was sprayed in the 4-leaf stage of bitter gourd, the fruit weight increased by 52.74% compared to the control (Table 4). Moreover, the fruit yield ranged between 25.42 t ha^{-1} in PGR_3S_2 to 11.26 t ha^{-1} PGR_0S_1 at harvest, showing 55.7% enhancement (Fig. 2). Present results showed that the number of fruits plant^{-1} and fruit yield was highest in plants receiving MH at the 4-leaf stage. Meanwhile, plants growing under PGR_1S_2 exhibited the topmost fruit length (19.52 cm) and fruit diameter (4.99 cm), respectively, followed by PGR_2S_2 and PGR_3S_2. However, the undermost fruit length (9.97 cm) and fruit diameter (3.35 cm) were attained in PGR_0S_1, respectively, at harvesting (Table 4).

Table 2 Effect of plant growth regulators on days to flowering, number of male and female flowers (plant^{-1}) and their sex ratio at various stages

Growth regulators	Application stage	Days to first flowering	Number of male flowers	Number of female flowers	Male–Female flower ratio
	S_1	43.75 ± 0.48^{a}	55.25 ± 1.18^{a}	19.50 ± 0.20^{h}	2.83 ± 0.04^{a}
PGR_0	S_2	42.25 ± 0.85^{a}	56.75 ± 1.03^{a}	21.32 ± 0.27^{fg}	2.67 ± 0.06^{a}
	S_3	42.75 ± 0.63^{a}	56.25 ± 1.25^{a}	20.83 ± 0.50^{g}	2.70 ± 0.08^{a}
	S_1	39.75 ± 0.85^{a}	61.00 ± 1.08^{a}	22.60 ± 0.36^{e}	2.70 ± 0.04^{a}
PGR_1	S_2	39.25 ± 0.48^{a}	61.75 ± 1.18^{a}	24.40 ± 0.24^{d}	2.53 ± 0.05^{a}
	S_3	39.25 ± 0.48^{a}	62.25 ± 1.10^{a}	24.75 ± 0.25^{cd}	2.52 ± 0.04^{a}
	S_1	40.00 ± 0.41^{a}	59.50 ± 1.04^{a}	22.27 ± 0.50^{ef}	2.68 ± 0.08^{a}
PGR_2	S_2	39.50 ± 0.65^{a}	62.00 ± 1.41^{a}	25.50 ± 0.29^{bc}	2.43 ± 0.07^{a}
	S_3	39.75 ± 0.48^{a}	61.75 ± 0.85^{a}	24.00 ± 0.35^{d}	2.58 ± 0.06^{a}
	S_1	38.75 ± 0.63^{a}	60.00 ± 1.78^{a}	23.75 ± 0.32^{d}	2.53 ± 0.11^{a}
PGR_3	S_2	38.50 ± 0.65^{a}	61.00 ± 1.58^{a}	27.83 ± 0.25^{a}	2.19 ± 0.08^{a}
	S_3	40.00 ± 0.41^{a}	58.25 ± 2.06^{a}	26.25 ± 0.43^{b}	2.22 ± 0.06^{a}
$LSD_{(0.05)}$		1.78	2.48	1.02	0.15
CV%		3.07	2.89	3.01	4.15
P value		0.63	0.31	0.02	0.32

PGR_0: 0, PGR_1: 100 mgL^{-1} GA_3, PGR_2: 100 mgL^{-1} NAA (naphthalene acetic acid) and PGR_3: 100 mgL^{-1} MH (maleic hydrazide), S_1: seed soaking stage, S_2: 4-leaf stage and S_3: flower bud stage. Different letters in the same column indicate significant differences between treatments ($p < 0.05$). Values are mean ± SE

Table 3 Influenced of different plant growth regulators on the fresh mass of plant (kg), fresh root mass (g), fruit dry matter content (%) and root dry matter content (%) at various stages of bitter gourd

Growth regulators	Application stage	Plant fresh mass	Root fresh mass	Fruit dry matter content	Root dry matter content
	S_1	9.28 ± 0.30^{ef}	9.70 ± 0.24^{a}	6.80 ± 0.09^{h}	11.6 ± 0.62^{cd}
PGR_0	S_2	10.98 ± 0.41^{bc}	9.65 ± 0.28^{a}	8.56 ± 0.05^{d}	12.13 ± 0.38^{bcd}
	S_3	9.81 ± 0.45^{cdef}	9.93 ± 0.42^{a}	7.09 ± 0.06^{g}	11.25 ± 0.40^{d}
	S_1	10.53 ± 0.64^{bcd}	10.97 ± 1.32^{a}	8.22 ± 0.07^{e}	13.30 ± 0.51^{a}
PGR_1	S_2	14.08 ± 0.47^{a}	9.12 ± 0.51^{a}	10.37 ± 0.05^{a}	12.40 ± 0.54^{abc}
	S_3	11.18 ± 0.43^{b}	9.24 ± 0.74^{a}	8.50 ± 0.13^{de}	11.78 ± 0.41^{bcd}
	S_1	10.24 ± 0.36^{bcdef}	10.59 ± 0.58^{a}	8.66 ± 0.01^{d}	12.31 ± 0.08^{bc}
PGR_2	S_2	10.75 ± 0.48^{bc}	10.09 ± 0.48^{a}	9.38 ± 0.14^{b}	12.18 ± 0.37^{bcd}
	S_3	9.14 ± 0.36^{f}	8.55 ± 0.89^{a}	8.99 ± 0.09^{c}	12.58 ± 0.22^{ab}
	S_1	9.11 ± 0.37^{f}	9.44 ± 0.33^{a}	6.96 ± 0.04^{gh}	12.15 ± 0.30^{bcd}
PGR_3	S_2	10.35 ± 0.35^{bcde}	10.09 ± 0.71^{a}	8.67 ± 0.10^{d}	12.53 ± 0.39^{abc}
	S_3	9.44 ± 0.40^{def}	9.88 ± 0.72^{a}	7.81 ± 0.20^{f}	12.53 ± 0.38^{abc}
$LSD_{(0.05)}$		1.19	1.88	0.27	0.95
CV%		7.98	13.40	2.29	5.38
P value		0.02	0.26	0.00	0.05

PGR_0: 0, PGR_1: 100 mgL^{-1} GA_3, PGR_2: 100 mgL^{-1} NAA (naphthalene acetic acid) and PGR_3: 100 mgL^{-1} MH (maleic hydrazide), S_1: seed soaking stage, S_2: 4-leaf stage and S_3: flower bud stage. Different letters in the same column indicate significant differences between treatments ($p < 0.05$). Values are mean ± SE

Table 4 Influenced of different plant growth regulators on fruit setting (%), number of fruits ($plant^{-1}$), single fruit weight (g), fruit length (cm) and fruit diameter (cm) at various application stages in bitter gourd

Growth regulators	Application stage	Fruit setting	Number of fruits	Single fruit weight	Fruit length	Fruit diameter
	S_1	84.64 ± 1.70^{a}	16.50 ± 0.29^{h}	102.30 ± 0.80^{h}	9.97 ± 0.20^{f}	3.35 ± 0.01^{h}
PGR_0	S_2	91.19 ± 1.91^{a}	19.45 ± 0.53^{f}	122.73 ± 0.93^{g}	15.08 ± 0.43^{cd}	$4.18 \pm 0.01d^{e}$
	S_3	86.47 ± 1.24^{a}	18.00 ± 0.41^{g}	119.70 ± 1.41^{g}	13.45 ± 0.51^{e}	4.06 ± 0.01^{e}
	S_1	91.85 ± 1.24^{a}	20.75 ± 0.25^{def}	137.19 ± 3.19^{ef}	14.13 ± 0.43^{de}	4.02 ± 0.03^{ef}
PGR_1	S_2	88.18 ± 2.05^{a}	21.50 ± 0.29^{cd}	156.26 ± 3.33^{a}	19.52 ± 0.31^{a}	4.99 ± 0.16^{a}
	S_3	92.56 ± 3.39^{a}	22.90 ± 0.80^{bc}	148.72 ± 2.29^{bc}	16.42 ± 0.52^{b}	4.14 ± 0.02^{de}
	S_1	89.84 ± 3.03^{a}	20.00 ± 0.71^{ef}	132.47 ± 3.75^{f}	14.07 ± 0.24^{de}	4.32 ± 0.07^{cd}
PGR_2	S_2	91.56 ± 3.33^{a}	23.32 ± 0.68^{b}	149.98 ± 1.99^{b}	16.61 ± 0.24^{b}	4.76 ± 0.02^{b}
	S_3	88.06 ± 2.57^{a}	21.20 ± 0.59^{de}	140.00 ± 2.69^{de}	14.94 ± 0.28^{cd}	4.38 ± 0.06^{c}
	S_1	84.67 ± 3.14^{a}	20.10 ± 0.72^{def}	140.16 ± 1.34^{de}	14.18 ± 1.00^{de}	3.62 ± 0.03^{g}
PGR_3	S_2	92.53 ± 0.94^{a}	25.75 ± 0.25^{a}	147.96 ± 1.51^{bc}	16.59 ± 0.26^{b}	4.31 ± 0.06^{cd}
	S_3	81.10 ± 3.02^{a}	21.25 ± 0.48^{de}	143.67 ± 1.35^{cd}	15.30 ± 0.35^{c}	3.85 ± 0.11^{f}
$LSD_{(0.05)}$		6.79	1.42	5.39	1.06	0.19
CV%		5.33	4.72	2.74	4.88	3.18
P value		0.06	0.00	0.00	0.00	0.00

PGR_0: 0, PGR_1: 100 mgL^{-1} GA_3, PGR_2: 100 mgL^{-1} NAA (naphthalene acetic acid) and PGR_3: 100 mgL^{-1} MH (maleic hydrazide), S_1: seed soaking stage, S_2: 4-leaf stage and S_3: flower bud stage. Different letters in the same column indicate significant differences between treatments ($p < 0.05$). Values are mean ± SE

Biochemical attributes

Non-reducing sugar, total carotenoid, total phenol concentration, ascorbic acid and TSS were not significantly influenced by PGRs at various phases of application (Table 5). The reducing sugar content (Table 5), chlorophyll content (Fig. 3), and water content (Fig. 4) in bitter gourd cultivars is considerably influenced by the interaction between PGRs and the application stages. When treatment PGR_2: 100 mgL^{-1} NAA was sprayed to the flower bud stage in bitter gourd, it significantly enhanced the amount of reducing sugar (1.98 mg) and protein (1.75 mg) compared to control and other treatments (Table 5). The treatment of PGRs at various stages, including seed soaking and flower

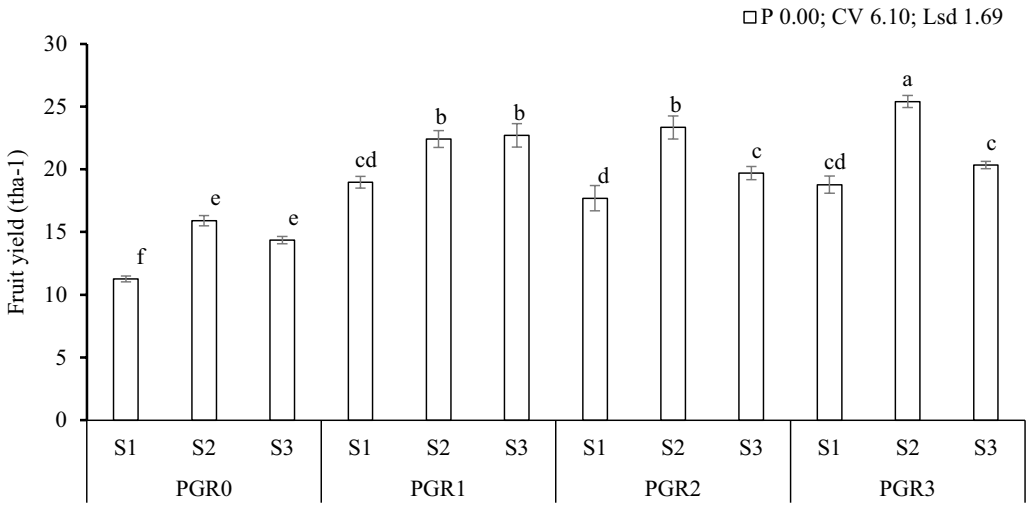

Fig. 2 Effect of plant growth regulators at various application stages on fruit yield (tha^{-1}) in bitter gourd. Abbreviations are as follows: PGR_0: 0, PGR_1: 100 mgL^{-1} GA_3 (gibberellic acid) PGR_2: 100 mgL^{-1} NAA (naphthalene acetic acid) and PGR_3: 100 mgL^{-1} MH (maleic hydrazide), S_1: seed soaking stage, S_2: 4-leaf stage and S_3: flower bud stage. Different letters in the same column indicate significant differences between treatments ($p < 0.05$). Vertical bars indicate standard errors

Table 5 Biochemical composition in bitter gourd fruits under different growth regulators conditions at various stages

Growth regulators	Application stage	Reducing sugar	Non-reducing sugar	Total carotenoid	Total phenol	Protein content	Ascorbic acid	TSS (%)
PGR_0	S_1	1.64 ± 0.03^{e}	3.71 ± 0.02^{a}	2.54 ± 0.02^{a}	9.55 ± 0.21^{a}	1.33 ± 0.02^{fg}	106.50 ± 2.36^{a}	4.73 ± 0.16^{a}
	S_2	1.73 ± 0.04^{de}	3.69 ± 0.04^{a}	2.52 ± 0.04^{a}	9.45 ± 0.18^{a}	1.23 ± 0.01^{g}	104.68 ± 0.24^{a}	4.75 ± 0.10^{a}
	S_3	1.78 ± 0.01^{cd}	3.78 ± 0.05^{a}	2.58 ± 0.02^{a}	9.55 ± 0.21^{a}	1.28 ± 0.01^{g}	102.50 ± 2.47^{a}	4.88 ± 0.05^{a}
PGR_1	S_1	1.90 ± 0.04^{ab}	3.85 ± 0.06^{a}	2.66 ± 0.02^{a}	9.58 ± 0.18^{a}	1.48 ± 0.01^{cde}	109.75 ± 0.85^{a}	4.88 ± 0.08^{a}
	S_2	1.78 ± 0.01^{cd}	3.80 ± 0.06^{a}	2.62 ± 0.02^{a}	10.13 ± 0.30^{a}	1.36 ± 0.04^{efg}	105.00 ± 1.22^{a}	4.90 ± 0.06^{a}
	S_3	1.83 ± 0.01^{bcd}	3.89 ± 0.01^{a}	2.72 ± 0.02^{a}	9.83 ± 0.09^{a}	1.43 ± 0.01^{def}	104.50 ± 1.66^{a}	4.90 ± 0.06^{a}
PGR_2	S_1	1.90 ± 0.04^{ab}	3.83 ± 0.04^{a}	2.68 ± 0.01^{a}	9.88 ± 0.08^{a}	1.64 ± 0.03^{ab}	109.00 ± 1.22^{a}	4.93 ± 0.02^{a}
	S_2	1.83 ± 0.05^{bcd}	3.88 ± 0.01^{a}	2.72 ± 0.01^{a}	9.90 ± 0.07^{a}	1.52 ± 0.01^{bcd}	107.00 ± 1.22^{a}	4.95 ± 0.03^{a}
	S_3	1.98 ± 0.09^{a}	4.03 ± 0.02^{a}	2.75 ± 0.03^{a}	9.94 ± 0.03^{a}	1.75 ± 0.02^{a}	107.50 ± 0.50^{a}	4.93 ± 0.05^{ab}
PGR_3	S_1	1.93 ± 0.05^{ab}	3.96 ± 0.04^{a}	2.73 ± 0.02^{a}	9.78 ± 0.09^{a}	1.59 ± 0.01^{bc}	111.25 ± 1.25^{a}	4.98 ± 0.05^{a}
	S_2	1.75 ± 0.03^{cde}	3.78 ± 0.01^{a}	2.62 ± 0.02^{a}	9.85 ± 0.06^{a}	1.45 ± 0.03^{def}	109.50 ± 0.96^{a}	4.98 ± 0.02^{a}
	S_3	1.85 ± 0.04^{bc}	3.98 ± 0.06^{a}	2.72 ± 0.02^{a}	9.94 ± 0.41^{a}	1.65 ± 0.06^{ab}	107.25 ± 1.31^{a}	5.10 ± 0.04^{a}
$LSD_{(0.05)}$		0.12	0.12	0.06	0.55	0.08	3.46	0.21
CV%		4.43	2.16	1.62	3.91	3.74	2.25	2.98
P value		0.03	0.12	0.07	0.73	0.01	0.63	0.89

Sugar content and total phenol are expressed as mg g^{-1} fresh extract, and total carotenoids, protein content and ascorbic acid as mg 100 g^{-1} fresh extract. Abbreviations are as follows: PGR_0: 0, PGR_1: 100 mgL^{-1} GA_3, PGR_2: 100 mgL^{-1} NAA (naphthalene acetic acid) and PGR_3: 100 mgL^{-1} MH (maleic hydrazide), S_1: seed soaking stage, S_2: 4-leaf stage and S_3: flower bud stage. Different letters in the same column indicate significant differences between treatments ($p < 0.05$). Values are mean ± SE

bud development, affects these biochemical compositions. Reducing sugar (Table 5) and chlorophyll (Fig. 3) content were higher when NAA was treated during the seed soaking stage and flower bud stage. In contrast, protein content was higher during the flower bud stage (Table 5). Chlorophyll levels (chl a 1.51 and chl b 0.55 mg) were higher in the PGR_1S_2 treatment than in the other treatments (Fig. 3). Although water content was substantially higher in control, there were

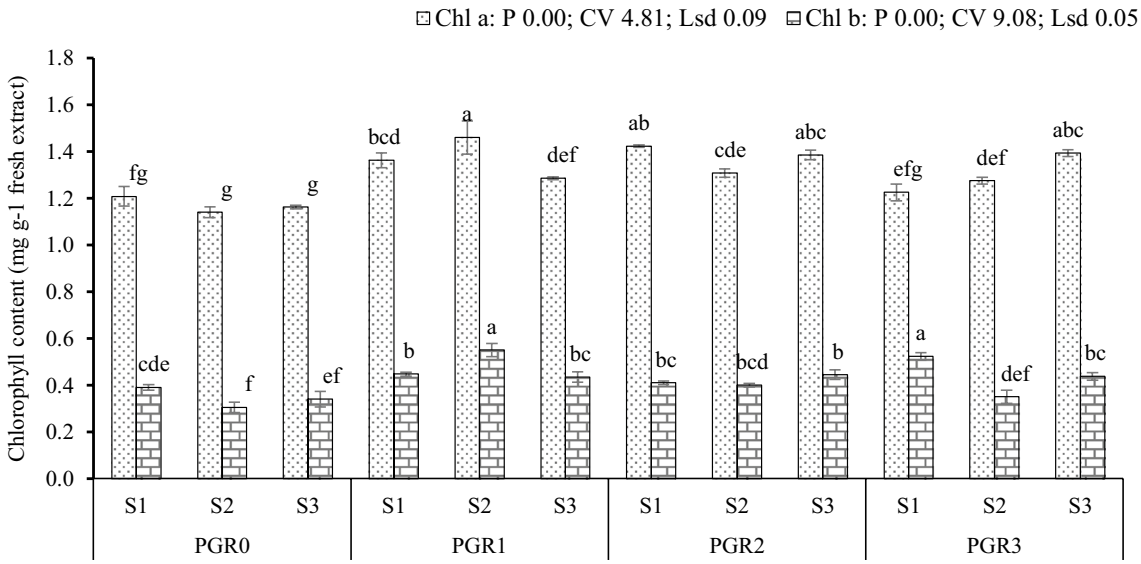

Fig. 3 Interaction effect of plant growth regulators and application stage on chlorophyll content of leaves. Chlorophylls are expressed as mg g^{-1} fresh extract. Abbreviations are as follows: PGR_0: 0, PGR_1: 100 mgL^{-1} GA_3, PGR_2: 100 mgL^{-1} NAA and PGR_3: 100 mgL^{-1} MH (maleic hydrazide), S_1: seed soaking stage, S_2: 4-leaf stage and S_3: flower bud stage. Different letters in the same column indicate significant differences between treatments ($p < 0.05$). Vertical bars indicate standard errors

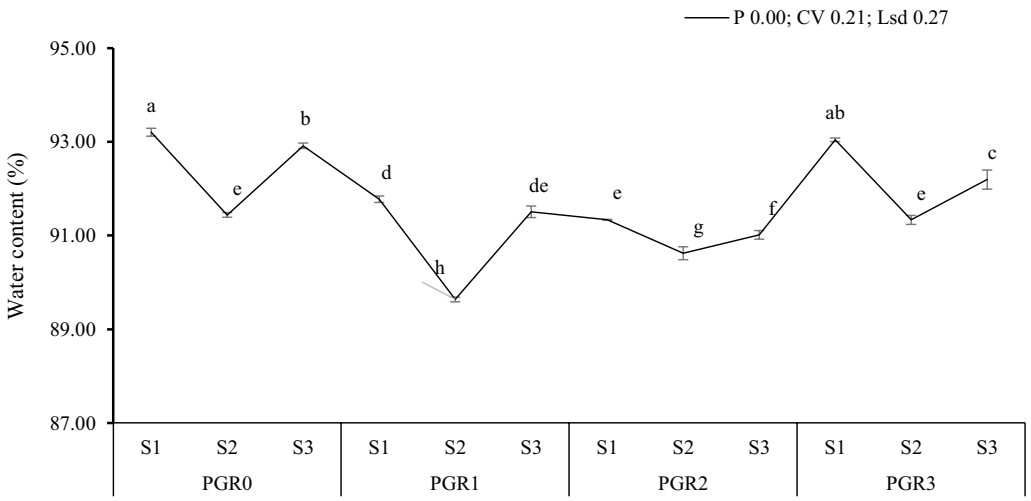

Fig. 4 Interaction effect of plant growth regulators and application stage on the water content of fruit. Abbreviations are as follows: PGR_0: 0, PGR_1: 100 mgL^{-1} GA_3, PGR_2: 100 mgL^{-1} NAA and PGR_3: 100 mgL^{-1} MH (maleic hydrazide), S_1: seed soaking stage, S_2: 4-leaf stage and S_3: flower bud stage. Different letters in the same column indicate significant differences between treatments ($p < 0.05$). Vertical bars indicate standard errors

considerable reductions (89.64%) when treatment with 100 mgL^{-1} NAA was used during the 4-leaf stage (Fig. 4).

Discussion

Exogenous plant hormones may potentially influence the germination process. Maleic hydrazide (MH) acts as a plant growth inhibitor and has activities that oppose gibberellic acid. MH modestly suppresses the sprouting process of the seed of the broomrapes plant (Venezian et al. 2017). Maleic hydrazide may serve as an antimitotic agent, inhibiting cell division but not expansion. As a result, the bitter gourd seed may have delayed cellular proliferation and taken longer to germinate (Fig. 1). The result is supported by Haber and White (1960) previous studies, which suggested that MH influences mitosis in a system where GA does not and that MH does not affect cell growth in a system where GA is active. On the

other hand, the significant influence of GA_3 (Fig. 1) on seed germination may be linked to its role in the functioning of alpha-amylase, which catalyzes the breakdown of starches into simple CHO and releases energy stored that is required in embryonic stimulation. The external application may have accelerated plant development by enhancing cell proliferation and extension with internal gibberellin generated by the seed embryo, leading in fast plant growth (Guzmán et al. 2021). The results are in line with those of Chen et al. (2020), who claimed that cell division or cell growth patterns are commonly altered to generate alterations in tomato fruit morphology and ripening.

The fast and quick emergence may have contributed to the seedlings' strong development throughout the succeeding growth phase. The highest plant height in GA_3 could have come about due to cellular differentiation and expansion, which could have boosted the internodal length of the bitter gourd plant (Table 1). The results are consistent with the statement that GA positively increases plant height (Han et al. 2018). Varying plant growth regulators considerably impact the number of branches and foliage. The increase in the number of leaves might be due to the reduced plant height, which upgraded side branching. Results indicated that maleic hydrazide application at seed soaking hindered plant height (Table 1) but contributed to the maximum number of leaves. MH application marginally limits the advancement of apical tissue and causes diminishment in plant height. Similar findings in cucumber were also associated (Sarkar et al. 2019).

Maleic hydrazide treatment differed considerably from other treatments for altering sex expression in bitter gourd flowers (Table 2). It happened due to the response of MH that equivalenced the sex by reducing respiration and advancing photosynthates accumulation in plants. The results were following the findings of Sarkar et al. (2019), who observed that the application of MH enhanced female flowers in cucumber by lowering respiration and increasing photosynthates in plants. These results also corresponded with the statement of Gosai et al. (2020), where pistillate flowers of cucumber increased with MH application at 450 µM/l concentrations. Plant growth regulators did not significantly influence the male–female flower ratio (Table 2). The reduction of the sex ratio following MH treatment might be attributable to the formation of additional branches on which female flowers bloomed in large numbers.

The effectiveness of plant growth regulators on physiological characteristics, including the fresh plant mass and dry matter content of fruit and root, varied considerably between phases of the bitter gourd plant (Table 3). The increased number of branches and leaves might be attributed to PGRs assisting in the exuberance of plant metabolic processes and the stimulatory action of chemicals in forming new leaves more quicker. The mobilization of nutrients and water moved faster, which may have enhanced more photosynthesizing products and translocation to various plant parts, resulting in better seedling growth and, thus, more fresh and dry weight (Table 3). Exogenous use of PGRs has also been demonstrated to support source-sink relationships with increased fresh biomass and dry matter buildup in bitter gourd fruit production. PGRs can translocate and partition nutrients from sources to sinks (Jan et al. 2023). Each treatment's biomass may indicate the potential of certain hormones to improve photosynthesis rate and photosynthates translocation effectiveness (Mbandlwa et al. 2019). Gibberellic acid has been focused on many plant species to enhance biomass, yields, and dry matter accumulation (Prajapati et al. 2021; Whitehead and Edwards 2015). However, a significant increase in fruit's fresh biomass after applying GA_3 might be attributed to both improved genetic features and GA_3-mediated increased nutritional intake, which would then support the leaves' ability for photosynthetic respiration (Saleem et al. 2021).

Fruit weight responds to the PGRs variedly at different stages of bitter gourd (Table 4). The increase in single fruit weight with GA_3 100 mgL^{-1} at the 4-leaf stage might be attributed to auxins' propensity to trigger physiological changes in plants, primarily increased fruit weight and enhanced photosynthetic activity, synthesis, and metabolite translocation from source to sink sites (Table 4). It was validated by the findings, stating that the favorable effects of growth regulators were evident in cucurbits growth, fruit productivity, and its characteristics (Moniruzzaman et al. 2019; Reddy et al. 2020; Sarkar et al. 2019; Shailendrakumar et al. 2017). Plant growth regulators are recognized to have an impact on crop phenology and yield, with gibberellic acid (GA_3) and NAA being the most notable PGRs that have an impact on the yield component in the bitter gourd in the current experiment (Fig. 2). GAs improves plant growth, floral organs and yield (Hifny et al. 2017). It also affects the antioxidant enzyme activities in fruits (Anwar et al. 2018). Fruit production increased because plants produced more fruit. Another cause for higher fruit productivity owing to MH treatments might be a boost in the number of branching, which is linked to increased production of pistillate flowers in cucumber in bitter gourd (Sarkar et al. 2019). In this experiment, the number of female flowers (Table 2) was increased by MH application, which might be the reason for accelerating the number of fruits (Table 4). These results are in accordance with the statement of (Sarkar et al. 2019), who stated that fruit number and their percentage depend on the number of

female flowers plant^{-1}. Likewise, Ries and Stutte (1985) concluded that the application of MH enhanced female flowering and fruit yield by regulating C:N ratio in plants. Yield, the actual economic trait of any crop, is primarily decided by the number of fruits plant^{-1}. The highest fruit length and diameter were found from plants sprayed with GA_3 at the 4-leaf stage (Table 4). Exogenous application of GA_3 enhanced fruit length and diameter due to the stimulation of the metabolic activity of plants along with cell division and cell enlargement. Similar results were reported by Ahmad et al. (2019, Chen et al. (2020).

Naphthalene acetic acid and Gibberellic acid in bitter gourd boosted biochemical substances as their exogenous application in various phases of plant growth and development (Table 5). Fruit biochemical attributes of bitter gourd might be improved as a consequence of more active food utilization, higher photosynthesized product, enhanced nutrients and water ingestion, lowered transpiration, and higher translocation due to the application of plant growth regulators. According to earlier research, either singly or in combination, plant growth regulators control the transcription of genes, enhancing the synthesis of a particular hormone to create proteins (Zhao et al. 2023). However, by stimulating the formation of phenolic compounds, GA_3 treatment can increase the antioxidant activity of plants (Didi et al. 2022). Gibberex, when applied exogenously and at higher concentrations, has two effects on bitter gourd plants: first, it improves plant growth and yield (Hifny et al. 2017; Rajashree and Deepanshu 2022), and second, it affects the antioxidant enzyme activities in fruits (Abbas et al. 2020; Anwar et al. 2018). Chlorophyll concentration may rise or decrease depending on the application stage of plant growth regulators (Fig. 3). The increased photosynthesis rate conferred by GA_3 treatment might be linked to improved plastid ultra-structural morphogenesis and increased rubisco activity (data not taken). The result is supported by the previous findings that fluctuation in chlorophyll concentration owing to growth regulator treatment is attributable to less chlorophyll degradation and/or enhanced chlorophyll synthesizing (Mbandlwa et al. 2019; Talal and Al-Chalabi 2020).

Conclusions

Plant growth regulators, which influence the process from seed germination to fruit development, including fruit quality through multiple physiological mechanisms, can modify the bitter gourd plant's growth pattern. Growth, yield and fruit dry matter content in bitter gourd were significantly enhanced by PGRs application at seed soaking, 4-leaf, and flower bud stage. Under different PGRs, MH application at the seed soaking stage showed significant differences in the number of leaves plant^{-1}, the fresh mass of the plant, the fresh mass of fruit, and the fruit dry matter content of the bitter gourd. Besides, MH application also exhibited remarkable differences in sex expression and fruit yield at the 4-leaf stage. Meanwhile, MH had no significant effect on the biochemical attributes of bitter gourd in terms of various application stages. Thus, among different PGRs, 100 mgL^{-1} MH application at the 4-leaf stage would be the better option for sex expression and yield enhancement of bitter gourd. The application of GA_3 resulted in a considerable increase in bitter gourd leaf chlorophyll content and individual fruit weight. Naphthalene acetic acid significantly impacted both plant growth and antioxidant enzyme activity in bitter gourd fruits. Further study on PGR levels and stages of application on bitter gourd will indeed be required to have a better knowledge of how plants respond to growth regulators or retardants.

Abbreviations

%	Percentage
µM/L	Micromoles per liter
AOAC	Association of Official Agricultural Chemists
B	Boron
BARI	Bangladesh Agricultural Research Institute
BBS	Bangladesh Bureau of Statistics
Chl	Chlorophyll
CHO	Carbohydrate
cm	Centimeter
$CuSO_4$	Copper sulfate
CV	Coefficient of variation
g	Gram
GA_3	Gibberellic acid
H_2SO_4	Sulfuric acid
HC	Hydrochloric acid
K	Potassium
Kg	Kilogram
LSD	Least Significant Difference
mgL^{-1}	Milligram per liter
MH	Maleic hydrazide
N	Nitrogen
Na_2SO_4	Sodium sulfate
NAA	Naphthalene acetic acid
NPN	Non-protein
P	Phosphorus
P	Probability
PGR	Plant growth regulator
PN	Protein nitrogen
S	Sulfur
SE	Standard error
TCA	Trichloroacetic acid
tha^{-1}	Ton per hectare
TN	Total nitrogen
TSS	Total soluble solids
Zn	Zinc

Acknowledgements

The authors express their profound gratitude to the Ministry of Science and Technology, Bangladesh, for their research support during this experiment.

Author contributions
M.D.S. proposed the research concept. M.R.I. designed and conducted the experiment. M.D.S. and M.R.I. analyzed and discussed the data. M.R.I. and M.M.R. wrote the first draft of the manuscript. The manuscript was edited by S.S., J.U., N.M. and M.D.S., who also substantially analyzed and finalized the entire manuscript. All authors have read and approved the final version of the manuscript.

Funding
This research received no external funding.

Availability of data and materials
The datasets used and/or analyzed during the present study are accessible online and upon request from the corresponding author.

Declarations

Ethics approval and consent to participate
This study was carried out strictly in compliance with the relevant Bangladeshi laws, regulations, and guidelines, as well as World Health Organization (WHO) standards for the integrity of scientific research.

Consent for publication
Not applicable.

Competing interests
The authors have confirmed that there are no conflicting interests.

Author details
[1]Department of Agriculture Extension, Ministry of Agriculture, Chhatak, Sunamganj 3080, Bangladesh. [2]Biotechnology Research Institute, Universiti Malaysia Sabah, JLN UMS, 88400 Kota Kinabalu, Sabah, Malaysia. [3]Department of Horticulture, Sher-e-Bangla Agricultural University, Dhaka 1207, Bangladesh. [4]Rural Development Academy, Ministry of Local Government, Rural Development and Cooperatives, Bogura 5842, Bangladesh. [5]Department of Agriculture, King Abdulaziz University, Jeddah 80208, Saudi Arabia.

Received: 24 January 2023 Accepted: 19 April 2023
Published online: 26 April 2023

References

Abbas M, Imran F, Khan RI, Zafar-Ul-hye M, Rafique T, Khan MJ, Taban S, Danish S, Datta R (2020) Gibberellic acid induced changes on growth, yield, superoxide dismutase, catalase and peroxidase in fruits of bitter gourd (*Momordica charantia* L.). Horticulturae 6:1–12. https://doi.org/10.3390/HORTICULTURAE6040072

Ahmad T, Muhammad Zia-ul-Hassan S, Iqbal Q, Awais Ghani M, Muhammad Sabir Tariq R (2019) Effect of PGRs on vegetative and reproductive traits of bitter gourd (*Momordica charantia* L.): a field assessment. J Hortic Sci Technol 2:2664–9489

Anayat R (2020) Effect of gibberllic acid and cycocel on yield and quality of bitter gourd. Indian J Pure Appl Biosci 8:402–406. https://doi.org/10.18782/2582-2845.8289

Anwar A, Yan Y, Liu Y, Li Y, Yu X (2018) 5-Aminolevulinic acid improves nutrient uptake and endogenous hormone accumulation, enhancing low-temperature stress tolerance in cucumbers. Int J Mol Sci 2018, vol 19, 19:3379. https://doi.org/10.3390/IJMS19113379

AOAC (2005) Association of official analytical chemists (AOAC). Van Nostrand's Encycl Chem GD Considine (ed). https://doi.org/10.1002/0471740039.VEC0284

BBS (2022) Yearbook of Agricultural Statistics-2021, 33rd series

Caradonia F, Ronga D, Tava A, Francia E (2022) Plant biostimulants in sustainable potato production: an overview. Potato Res 65:83–104. https://doi.org/10.1007/S11540-021-09510-3/METRICS

Chen S, Wang XJ, Tan GF, Zhou WQ, Wang GL (2020) Gibberellin and the plant growth retardant Paclobutrazol altered fruit shape and ripening in tomato. Protoplasma 257:853–861. https://doi.org/10.1007/S00709-019-01471-2

Didi DA, Su S, Sam FE, Tiika RJ, Zhang X (2022) Effect of plant growth regulators on osmotic regulatory substances and antioxidant enzyme activity of nitraria tangutorum. Plants (Basel, Switzerland) 11:. https://doi.org/10.3390/PLANTS11192559

Gayathry KS, John JA (2022) A comprehensive review on bitter gourd (*Momordica charantia* L.) as a gold mine of functional bioactive components for therapeutic foods. Food Prod Process Nutr 4:1–14. https://doi.org/10.1186/S43014-022-00089-X/TABLES/6

Gerdakaneh M, Hoseini F, Eftekharinasab N (2018) Effect of paclobutrazol and NAA on sex determination and seed yield of medicinal pumpkin (*Cucurbita pepo* L.). Int J Hortic Sci Technol 5:209–217. https://doi.org/10.22059/IJHST.2018.258581.242

Gosai S, Adhikari S, Khanal S, Poudel PB (2020) Effects of plant growth regulators on growth, flowering, fruiting and fruit yield of cucumber (*Cucumis sativus* L.): a review. Arch Agric Environ Sci 5:268–274. https://doi.org/10.26832/24566632.2020.050306

Guzmán Y, Pugliese B, González C V., Travaglia C, Bottini R, Berli F (2021) Spray with plant growth regulators at full bloom may improve quality for storage of "Superior Seedless" table grapes by modifying the vascular system of the bunch. Postharvest Biol Technol 176:111522. https://doi.org/10.1016/J.POSTHARVBIO.2021.111522

Haber AH, White JD (1960) Action of maleic hydrazide on dormancy, cell division, and cell expansion. Plant Physiol 35:495. https://doi.org/10.1104/PP.35.4.495

Han R, Khalid M, Juan J, Huang D (2018) Exogenous glycine inhibits root elongation and reduces nitrate-N uptake in pak choi (*Brassica campestris* ssp. Chinensis L.). PLoS One 13:e0204488. https://doi.org/10.1371/JOURNAL.PONE.0204488

Hifny HA, Khalifa SM, Hamdy AE, Abd El-Wahed AN (2017) Effect of GA3 and NAA on growth, yield and fruit quality of Washington navel orange. Egypt J Hortic 44:33–43. https://doi.org/10.21608/EJOH.2017.950.1003

Jan R, Lubna, Asaf S, Khan MA, Kim K-M (2023) Effect of mineral nutrition and PGRs on biosynthesis and distribution of secondary plant metabolites under abiotic stress. Med Plants 287–314. https://doi.org/10.1007/978-981-19-5611-9_11

Jayaprakasha GK, Singh RP, Sakariah KK (2001) Antioxidant activity of grape seed (Vitis vinifera) extracts on peroxidation models in vitro. Food Chem 73:285–290. https://doi.org/10.1016/S0308-8146(00)00298-3

Kołodziejc M, Gwó K (2022) Effect of plant growth regulators on potato tuber yield and quality. Plant Soil Environ 68:375–381. https://doi.org/10.17221/127/2022-PSE

Lee H, Sun Q, Zhao S ZW (2000) Principles and techniques of plant physiological biochemical experiment. High Edu, Beijing 195–197

Mbandlwa NP, Fotouo-M H, Maboko MM, Sivakumar D (2019) Stomatal conductance, leaf chlorophyll content, growth, and yield of sweet pepper in response to plant growth regulators. Int J Veg Sci 26:116–126. https://doi.org/10.1080/19315260.2019.1610925

Moniruzzaman M, Khatoon R, Moniruzzaman M, Qamruzzaman A (2019) Influence of plant growth regulators on vegetative growth, sex expression and yield of summer bottle gourd. Bangladesh J Agric Res 44:577–590. https://doi.org/10.3329/BJAR.V44I4.45693

Motsara MR, Roy RN (2008) Guide to laboratory establishment for plant nutrient analysis. FAO Fertil Plant Nutr Bull 19:219

Mousavi SB, Sayfzadeh S, Jabbari H, Valadabadi SA, Hadidi Masouleh E (2022) Effect of auxin foliar application on seed yield and fatty acids composition of two safflower genotypes under late-season drought. Plant Soil Environ 68:82–88. https://doi.org/10.17221/329/2021-PSE

Nielsen SS (2017) Food Analysis Laboratory Manual, Third. Springer, New York

Prajapati L, Yadav PC, Bharose R (2021) Physiological as well as growth parameters of basmati rice (*Oryza sativa* L.) as affected by growth regulators. Curr J Appl Sci Technol 40:95–100. https://doi.org/10.9734/CJAST/2021/V40I531308

Rajashree V, Deepanshu (2022) Growth and yield of bitter gourd as influenced by gibberellic acid and naphthalene acidic acid (*Momordica charantia* L.). Int J Plant Soil Sci IJPSS, 809–817. https://doi.org/10.9734/IJPSS/2022/V34I2131335

Reddy AM, Saravanan DS, Bahadur DV (2020) Effects of different plant growth regulators on plant growth, yield and fruit quality of Bitter gourd (*Momordica charantia* L.) cv. Kashi Mayuri. Int J Chem Stud 8:1573–1575. https://doi.org/10.22271/CHEMI.2020.V8.I4O.9836

Ries SK, Stutte CA (1985) Regulation of plant growth with triacontanol. CRC Crit Rev Plant Sci 2:239–285. https://doi.org/10.1080/07352688509382197
Saleem MH, Wang X, Ali S, Zafar S, Nawaz M, Adnan M, Fahad S, Shah A, Alyemeni MN, Hefft DI, Ali S (2021) Interactive effects of gibberellic acid and NPK on morpho-physio-biochemical traits and organic acid exudation pattern in coriander (*Coriandrum sativum* L.) grown in soil artificially spiked with boron. Plant Physiol Biochem PPB 167:884–900. https://doi.org/10.1016/J.PLAPHY.2021.09.015
Sarkar MD, Moniruzzaman M, Alam S, Rahman J, Quamruzzaman, Rojoni RN, Subramaniam S (2019) Growth, sex expression and nutrient composition of cucumber (Cucumis sativus) as influenced by maleic hydrazide. Pakistan J Bot 51:117–123. https://doi.org/10.30848/PJB2019-1(9)
Shailendrakumar VM, Valia RZ, Sitapara HH (2017) Effect of plant growth regulators on growth, sex expression and yield of sponge gourd (*Luffa cylindrica* L.) Cv. Pusa Chikni. Ann Hortic 10:243. https://doi.org/10.5958/0976-4623.2017.00044.5
Sprangers K, Thys S, van Dusschoten D, Beemster GTS (2020) Gibberellin enhances the anisotropy of cell expansion in the growth zone of the maize leaf. Front Plant Sci 11:. https://doi.org/10.3389/FPLS.2020.01163
Talal A, Al-Chalabi M (2020) Effect of gibberellic acid (GA3) on growth and some characteristics of ornamental plants. Futur J Agric 3:1–4. https://doi.org/10.37229/fsa.fja.2020.07.03
Venezian A, Dor E, Achdari G, Plakhine D, Smirnov E, Hershenhorn J (2017) The INFLUENCE OF THE PLANT GROWTH REGULATOR MALEIC HYDRAZIDE ON EGYPTIAN BROOMRAPE EARLY DEVELOPMENTAL STAGES AND ITS CONTROL EFFICACY IN TOMATO UNDER GREENHOUSE AND FIELD CONDITIons. Front Plant Sci 8:. https://doi.org/10.3389/FPLS.2017.00691
Whitehead D, Edwards GR (2015) Assessment of the application of gibberellins to increase productivity and reduce nitrous oxide emissions in grazed grassland. Agric Ecosyst Environ 207:40–50. https://doi.org/10.1016/J.AGEE.2015.03.019
Zhao H, Liu X, Sun Y, Liu J, Waigi MG (2023) Effects and mechanisms of plant growth regulators on horizontal transfer of antibiotic resistance genes through plasmid-mediated conjugation. Chemosphere 318:137997. https://doi.org/10.1016/J.CHEMOSPHERE.2023.137997

Publisher's Note