

GROWTH, YIELD AND BIOCHEMICAL RESPONSES OF BARI CHHOLA-9 (*CICER ARIETINUM* L.) TO GA₃ AND RHIZOBIUM APPLICATION

TASNIA JAHAN, FARHANA ISLAM KHAN, AMM GOLAM ADAM*
AND HASNA HENA BEGUM

Department of Botany, Jagannath University, Dhaka-1100, Bangladesh

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Abstract

A field experiment was conducted to investigate the responses of various concentrations of GA₃ (10, 20, 50 ppm) and *Rhizobium* inoculation on growth, yield and biochemical parameters of BARI Chhola-9. Foliar application of 50 ppm GA₃ resulted maximum plant height, number of primary branches per plant, shoot and root length, fresh and dry weight of shoot and root and biomass duration per plant with significant variations in case of fresh and dry weight of shoot and biomass duration. The stimulatory effect of 50 ppm GA₃ on number of pods per plant, fresh and dry weight of pods per plant, number of seeds per pod, number of seeds and straw yield per plant and weight of 1000-seed eventually produced 4.76% higher yield over the control. Yield parameters of BARI Chhola-9 showed almost negative response to *Rhizobium* and 10 ppm treatments with a few exceptions. Pigment content of leaves also increased due to 50 ppm GA₃ treatment at both vegetative and flowering stages where, significantly higher amount of chlorophyll a and b were recorded from flowering stage although statistically similar to 20 ppm GA₃ treatment. However, protein content of leaves increased following *Rhizobium* and 10 ppm GA₃ treatments at vegetative stage and to all treatments at flowering stage but, increase in protein content of seeds was recorded from *Rhizobium* treatment only. Out of four treatments, 50 ppm produced better yield.

Introduction

Chhola (*Cicer arietinum* L.) is one of the important pulse crops for its nutritive seeds. In terms of area covered by pulses it occupies third position in Bangladesh. Seeds contain 25.3 - 28.9 protein, 38 - 59 carbohydrate, 3.0 fibre, 4.8 - 5.5 oil, 0.2 calcium, and 0.3% phosphorus. Digestibility of protein varies from 76 - 78% and carbohydrate from 57 - 60% (Hulse 1991). Leaves also contain 4 - 8% protein. The per capita consumption of pulses in Bangladesh is only 13.29 g per day, while the World Health Organization suggests 45 g per day (BBS 2011). It has been estimated that the demand of pulse requirement will be increased to about 3 million metric tons in the year of 2020 (Golder *et al.* 2014). There are many methods which may help to increase the pulse production where the use of growth promoting substances and organism are considered as one of the best way. Deficiency of these substances at any stage of plant growth may create a barrier in attaining maximum yield. Moreover, these substances can improve the physiological efficiency including photosynthetic ability and can enhance the effective partitioning of accumulates from source and sink in the field crops. Therefore, research on the effect of growth promoting substances as well as organism on economically important crop plants are of primary concern for a large number of plant physiologists and agronomists all over the world.

Investigations all over the world showed that GA₃ at appropriate concentrations had beneficial effect on different parameters of economically important plants *viz.*, green gram (Akbari *et al.* 2008), mungbean (Abdel and Al-Rawi 2011), chickpea (Thakare *et al.* 2011 and Mazid 2014), tomato (Kumar *et al.* 2014), cowpea (Nabi *et al.* 2014), china aster (Kumar *et al.* 2015). On the other hand, plant growth promoting bacteria (PGPB) play pivotal role in plant growth by producing

*Author for correspondence: <adam_du04@yahoo.com>.

phytohormones, fixing N₂ asymbiotically, solubilizing inorganic phosphate, mineralizing organic phosphate and supplying nutrients (Dey *et al.* 2004). Findings of Bashan and de-Bashan (2005) revealed that a good number of bacterial genera *viz.*, *Acinetobacter*, *Alcaligenes*, *Arthrobacter*, *Azospirillum*, *Azotobacter*, *Bacillus*, *Beijerinckia*, *Burkholdria*, *Enterobacter*, *Erwinia*, *Flavobacterium*, *Serroti*, mostly associated with the rhizosphere, are found to be beneficial for crop growth, yield and quality. The stimulating effects of inoculation of seeds with *Rhizobium* alone or in combination with other growth bacteria have also been reported by many authors in various important crops including Chhola (Hoque and Haq 1994, Rabbani *et al.* 2005, Togay *et al.* 2008, Nishita and Joshi 2010, Ogutcu *et al.* 2010).

In Bangladesh, although very few experiments had been taken up with chhola using *Rhizobium* inoculation (Bhuiyan *et al.* 2008 and Zaman *et al.* 2011) but, research work using GA₃ on any variety of chhola is also scanty. Thus, an attempt was made to study the effect of GA₃ and *Rhizobium* inoculation on the growth, yield and biochemical responses of BARI Chhola-9.

Materials and Methods

A field experiment was conducted during the year of 2015-2016 at the botanical garden of the Department of Botany, Jagannath University, Dhaka-1100. A high yielding variety, BARI Chhola-9 used for this investigation was released in the year of 2011. The initial properties of soil was analysed following standard methods for the determination of required doses of fertilizers (Murphy and Riley 1962, Jackson 1973). The experiment was laid out in RBD with three replications for each treatment. The total area of the experimental field was 35 m². Seeds and *Rhizobium* strain (*Rhizobium* BARI Rca 259) were collected from BARI. The experiment was consisted of 5 treatments *viz.*, T₀ = Distilled water (Control), T₁ = Seed treated with *Rhizobium* BARI Rca 259, T₂ = 10 ppm GA₃, T₃ = 20 ppm GA₃, T₄ = 50 ppm GA₃. Seeds were sterilized with 0.05% calcium hypochlorite solution and soaked in distilled water for 12 hrs. But, in case of bacterial treatment seeds were surface-sterilized with 95% ethanol and then aseptically germinated in Petri dishes containing 20 ml of deionized water for 5 days at 25°C in a growth chamber. The germinated seeds were treated with 2 ml bacterial culture (10⁸ cell/ml). Seeds were sown on December 14, 2015 maintaining row to row distance of 40 cm and seed to seed distance of 10 cm. Cultural practices (thinning, irrigation, weeding etc.) and fertilizer application were done following Hand Book of Agricultural Technology (Chowdhury and Hassan 2013) and Fertilizer Recommendation Guide (2012). Urea as a source of nitrogen was not used in case of bacterial treatment. Gibberellic acid was applied as foliar spray in sunny early morning at the age of 35 days.

Chlorophyll a, b, carotenoid and protein contents of leaves were determined at vegetative and flowering stages. Protein content of seeds was also determined after harvest. The amount of chlorophyll a and b was determined by using specific absorption co-efficient of Mckinney (1940) and the formulae of Maclachalan and Zalik (1963). The amounts of carotenoid was calculated using the equation of von Wettstein (1957). The method of Lowry *et al.* (1951) was employed for the determination of protein content of leaves and seeds. Plants were harvested at the age of 112 days. Nine plants from each treatment were harvested separately to record data on different growth and yield parameters. Data were analyzed statistically and treatment means were compared by LSD test at 5% level of significance (Steel *et al.* 1997).

Results and Discussion

Results showed that plant height increased due to *Rhizobium* inoculation and 50 ppm GA₃ treatments where, the maximum height was recorded from 50 ppm GA₃ treatment (Table 1).

Decrease in plant height was found in case of 10 and 20 ppm treatments. Results of Hoque and Haq (1994) had revealed that seed inoculated with *Rhizobium* significantly increased plant height of lentil. Similar results of increase had also been reported by Togay *et al.* (2008) in chickpea. By applying 50 ppm GA₃, maximum plant height was recorded in tomato (Kumar *et al.* 2014). However, application of GA₃ decreased plant height in mungbean (Abdel and Al-Rawi 2011). Thus, these results are in agreement with those of previous workers who have observed that height of plant may increase or decrease depending on concentration of GA₃ treatments.

Results also showed that number of primary branches per plant increased due to 50 ppm GA₃ treatment whereas, number of secondary branches per plant responded negatively following all treatments. Previously Kumar *et al.* (2015) reported increased number of primary and secondary branches per plant following GA₃ treatments in China aster. Application of *Rhizobium* inoculation and 50 ppm GA₃ treatments had stimulating effect in shoot length although, length of root was found to decrease due to all treatments except 50 ppm GA₃. Reports of Nishita and Joshi (2010) had revealed that rhizobial inoculated plants gave significantly higher shoot length in chickpea. By applying GA₃ treatment, Thakare *et al.* (2011) had found increased shoot and root length in chickpea.

Findings of this investigation indicated that the maximum fresh and dry weight of shoot was obtained due to 50 ppm GA₃ treatment, however lowest was found in *Rhizobium* treatment. Similarly fresh and dry weight of root was also increased due to all GA₃ treatments and maximum was found in case of 50 ppm treatment (Table 1). Application of GA₃ treatment had produced higher fresh and dry weight of shoot and root in chickpea (Iqbal *et al.* 2001). Results of Ogutcu *et al.* (2010) had revealed that bacterial inoculations significantly increased dry weight of root under both non-saline and saline conditions in chickpea. Results revealed that highest biomass duration per plant was obtained from the plants of 50 ppm which was statistically at par with control (Table 1). Afrin (2015) obtained maximum value of biomass duration from 50 and 100 ppm GA₃ treatments at early stage of tomato plant.

Table 2 shows that seeds treated with *Rhizobium* resulted maximum number of seeds per pod and 1000-seed weight although, the effect was rather negative in rest of the yield parameters due to the same treatment. Application of 10 ppm GA₃ did not show any positive effect on yield contributing parameters. However, GA₃ at 20 ppm concentration resulted significantly higher number of pod, fresh weight of pods and number of seeds per plant than *Rhizobium* inoculation and 10 ppm GA₃ treatment. Results also indicated that foliar application of 50 ppm GA₃ had stimulating effect on number of pods per plant, fresh and dry weight of pods per plant, number of seeds per pods, number of seeds and straw yield per plant with significant responses in most of the cases. Here, plants obtained from 50 ppm GA₃ treatment produced significantly higher fresh and dry weight of pods and straw yield per plant than *Rhizobium* and 10 ppm GA₃ treatments. Increase in number of pods per plant following GA₃ treatments had also been reported by Mazid (2014) in chickpea. Nabi *et al.* (2014) had recorded higher number of pods, fresh and dry weight of pods per plant in cowpea. Zaman *et al.* (2011) had found increased number of seeds per pod in chickpea when soil treated with *Rhizobium*. Increase in 1000-seed weight by using GA₃ had also been reported by Akbari *et al.* (2008) in green gram.

Yield of BARI Chhola-9 obtained from 50 ppm GA₃ treatment was 4.76% higher over the control. Increases in yield per plant following different concentrations of GA₃ have also been noticed by many workers on various plants *viz.*, chickpea (Choudhury *et al.* 2013), mungbean (Hoque and Haque 2002), green gram (Akbari *et al.* 2008), cowpea (Nabi *et al.* 2014). Harvest index was found to decrease due to *Rhizobium* and GA₃ treatments which is not in agreement with the findings of other workers (Hoque and Haque 2002 and Nabi *et al.* 2014).

Table 1. Effects of GA₃ and *Rhizobium* on growth parameters of BARI Chhola-9 at harvest.

Treatments	Height (cm)	No. of primary branches/plant	No. of secondary branches/plant	Length of shoot (cm)	Length of root (cm)	Fresh wt. of shoot (g)	Dry wt. of shoot (g)	Fresh wt. of root (g)	Dry wt. of root (g)	Biomass duration (g/day)
T ₀	52.53	6.56	8.89	55.64	8.36	8.16 b	4.10 ab	0.56	0.14	110.75ab
T ₁	53.49	5.00	6.22	55.72	5.57	3.74 b	2.27 b	0.75	0.08	73.95 b
T ₂	50.32	5.44	6.33	52.51	7.49	6.50 b	2.83 b	1.27	0.19	111.95ab
T ₃	49.48	6.00	5.11	50.63	7.36	5.79 b	3.83 ab	1.00	0.27	81.22 b
T ₄	57.08	7.00	7.78	60.14	9.61	14.75 a	6.24 a	1.58	0.39	145.25 a
CV (%)	14.49	7.99	14.98	10.86	12.58	9.02	16.66	13.08	12.28	15.44
LSD (0.05)	NS	NS	NS	NS	NS	5.51	2.58	NS	NS	49.28

Mean in a vertical column followed by same letter or without letter do not differ significantly at 5% level. NS indicates not significant.

Table 2. Effects of GA₃ and *Rhizobium* on yield attributes and yield of BARI Chhola-9 at harvest.

Treatments	No. of pod bearing branches/plant	No. of pods/plant	Fresh wt. of pods/plant (g)	Dry wt. of pods/plant (g)	No. of seeds/pod	No. of seeds/plant	Straw yield/plant (g)	1000-seed weight (g)	Yield/plant (g)	Harvest index (%)
T ₀	7.00 a	11.20 a	3.95 ab	2.33 ab	1.09	12.22 a	6.57 ab	153.59	1.89	21.72 a
T ₁	3.22 c	4.33 b	1.44 bc	0.93 bc	1.40	5.44 b	3.28 b	162.63	0.83	21.05 a
T ₂	3.00 c	4.00 b	1.24 c	0.70 c	1.01	4.33 b	3.72 b	145.98	0.56	13.28 b
T ₃	6.76 ab	13.78 a	4.15 a	2.02 abc	0.99	13.78 a	6.13 ab	134.02	1.43	20.97 a
T ₄	4.78 bc	11.22 a	4.79 a	2.47 a	1.20	13.22 a	9.12 a	157.00	1.98	13.79 b
CV (%)	16.74	13.46	10.39	10.39	13.44	15.74	17.57	7.57	10.81	18.86
LSD (0.05)	2.21	5.87	2.70	1.42	NS	6.69	3.72	NS	NS	6.95

Mean in a vertical column followed by same letter or without letter do not differ significantly at 5% level. NS indicates not significant.

Results presented in Table 3 shows that chlorophyll a, b and carotenoids content of leaves increased due to 50 ppm GA₃ treatment at both vegetative and flowering stages where, significantly higher amount of chlorophyll a and b were recorded from flowering stage although statistically similar to 20 ppm GA₃ treatment. Chlorophyll a content of leaves was also found to increase due to *Rhizobium* inoculation and 20 ppm GA₃ treatments but statistically at par with control. Results also showed that inoculation of *Rhizobium* and 10 ppm GA₃ treatments had almost negative responses to pigment content of leaves at both stages. Previously the positive effects of GA₃ on the photosynthetic pigments had been reported in chickpea (Mazid 2014).

Table 3. Effects of GA₃ and *Rhizobium* on pigment content (mg/g) of leaves of BARI Chhola-9 at two different stages.

Treatments	Vegetative stage			Flowering stage		
	Chl.a	Chl.b	Carotenoids (mg/g)	Chl.a	Chl.b	Carotenoids
T ₀	0.14 ab	0.57a	7.58	0.04b	0.12b	5.94a
T ₁	0.17 ab	0.47ab	6.25	0.04b	0.10b	2.94b
T ₂	0.07 b	0.25b	5.49	0.04b	0.08b	3.49b
T ₃	0.14 ab	0.48ab	6.59	0.09ab	0.21ab	5.16a
T ₄	0.24 a	0.67a	8.49	0.14a	0.27a	6.12a
CV (%)	51.81	40.84	22.44	82.79	63.94	32.09
LSD (0.05)	0.10	0.26	NS	0.05	0.11	1.14

Mean in a vertical column followed by same letter or without letter do not differ significantly at 5% level. NS indicates not significant.

Protein content of leaves increased following *Rhizobium* inoculation and 10 ppm GA₃ treatments at vegetative stage and due to all treatment at flowering stage (Table 4). Foliar application of GA₃ had stimulatory effect on protein content of leaves at vegetative and flowering stages of tomato (Afrin 2015). Increase in protein content of seeds was recorded from *Rhizobium* treatment only. By applying *Rhizobium* inoculation, Rabbani *et al.* (2005) had obtained increased protein content in pea seeds.

Table 4. Effects of GA₃ and *Rhizobium* on protein content (mg/g) of leaves and seeds of BARI Chhola-9 at different stages.

Treatments	Protein content of leaves (mg/g)		Protein content of seeds (mg/g)
	Vegetative stage	Flowering stage	At harvest
T ₀	0.93	31.88	127.56
T ₁	1.02	32.19	137.63
T ₂	1.13	36.75	125.75
T ₃	0.82	33.94	127.44
T ₄	0.76	44.56	64.75
CV(%)	27.05	41.82	15.91
LSD(0.05)	NS	NS	NS

Mean in a vertical column followed by same letter or without letter do not differ significantly at 5% level. NS indicates not significant.

Findings of the present experiment indicate *Rhizobium* treatment substantially increase protein content of both leaves and seeds but reduced the yield considerably. On the other hand 50 ppm GA₃ treatment increased the yield significantly but reduced the protein content of seeds drastically.

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