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Investigation Effect of Different Amount and Time of Apply a Bio-Regulator on Growth Indices of *Vicia faba* L.

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ABSTRACT

BACKGROUND: Gibberellins may play a key role in many metabolic pathways affecting these characteristics, such as chlorophyll production and degradation, translocation of assimilates, nitrogen metabolism, and nitrogen redistribution.
OBJECTIVES: The current study was conducted to assess the effect of different amount and time of apply Gibberellin acid on growth curves and crop production of Broad bean.
METHODS: This research was done via factorial experiment based on randomized complete blocks design with three replications along 2010 year. The treatments included different concentration of Gibberellin (nonuse of Gibberellin or control, 5 ppm, 50 ppm and 250 ppm) and time of application Gibberellin (Vegetative growth before flowering, flowering until pod emergence, Pod emergence until grain filing).
RESULT: Result of analysis of variance revealed effects of different amount, time of apply Gibberellin acid and interaction effect of treatments on seed yield was significant at 5% probability level. Compare different level of Gibberellin acid concentration revealed maximum amount of seed yield (2761 $kg.ha^{-1}$), leaf area index (2.43), total dry matter (3750 $gr.m^{-2}$), relative growth rate (0.049 $gr.gr.day^{-1}$), crop growth rate (34 $gr.m^{-2}.day^{-1}$) and net assimilation rate (7.5 $gr.m^{-2}.day^{-1}$) belonged to 50 ppm Gibberellin acid and lowest ones was for control treatment. Also among different time of apply Gibberellin acid, maximum amount of mentioned traits was for Vegetative growth before flowering phase and the lowest one belonged to pod emergence until grain filing.
CONCLUSION: Consume 50 ppm Gibberellin acid concentration at vegetative growth before flowering phase had the highest amount of crop production and growth indices and it can be advised to farmers.

KEYWORDS: *Dry matter, Growth curve, Net assimilation rate, Physiology, Pulse.*

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1. BACKGROUND

Vicia faba L. is one of the most ancient cultivated crops, with high protein content of its seeds that provide rich sources of food for humans and fodder for livestock (Suresh *et al*., 2013). Faba bean is third most important legume after soybean (*Glycine max* L.) and pea (*Pisum sativum* L.) in world, it has important role in the management of soil fertility and crop rotation by fixing 130 to 160 kg N/ha (Singh *et al*. 2013). *Vicia faba* is considered as a rich source of protein and carbohydrate (Khamooshi *et al*., 2012a). Broad bean (Fabaceae) has several benefits: It is important for soil fertility, animal feeding and industry aims (Khamooshi *et al*., 2012b). Growth regulators are organic substances besides nutrients, synthesized in plants, causing alteration in their cellular metabolism. Synthesis of some plant hormones is adversely affected by environmental factors, which causes restriction on physiological processes of the plant and ultimately, limits their growth potential (Copur *et al*., 2010). The application of these hormones in low concentration regulates growth, differentiation and development, either by promotion or inhibition (Naeem *et al*., 2004), and allows physiological processes to occur at their normal rate (Gulluoglu, 2004). Among PGRs, auxin and gibberellin play vital role in regulating developmental processes within plant bodies (Gou *et al*., 2010). Mckenzie and Deyholos (2011) reported that treatment of GA causes stem elongation, expansion and proliferation and cell wall thickening in bast fiber of linseed. Phytohormones have significant roles in plant growth and development and respond to biotic and abiotic stresses. These hormones regulate a variety of developmental processes along with signaling networks in plants under different biotic and abiotic stresses. Improvements in plant molecular biology have revolutionized the involvement of phytohormones in improving the damaging effects posed by abiotic stresses (Khan *et al*., 2013; Masood *et al*., 2012). Numerous phytohormones like abscisic acid (ABA), gibberellins (GAs), ethylene (ET), auxin (indole-3-acetic acid (IAA)), cytokinins (CKs), and brassinosteroids (BRs) that regulate plant development are also involved in controlling a variety of physiological and biological signaling and processes in the sessile plants. These cellular messengers may function as either adjacent or distant molecules from their positions of synthesis to respond against external stimuli or genetically automated progressive variations (Fahad *et al*., 2015). Rady *et al*. (2021) reported Foliar applied Db-H (Diluted bee honey) or GA_3 improved the nutrients status, tissue health, leaf photosynthetic pigments, and photosynthetic efficiency leading to higher growth and productivity (yield and water use efficiency) of drought-stressed Faba bean plants. Therefore, the application of these growth regulators (Diluted bee honey: Db-H and GA_3) was identified to be an effective strategy to mitigate the damage effects of irrigation water deficits for sustainable faba bean production in arid and semi-arid areas.

2. OBJECTIVES

The current study was conducted to assess the effect of different amount and time of apply Gibberellin acid on growth curves and crop production of Broad bean.

3. MATERIALS AND METHODS

3.1. Field and Treatments Information

This research was carried out via factorial experiment based on randomized complete blocks design with three replications along 2010 year. Place of research was located in Ahvaz city at longitude 48°40'E and latitude 31°20'N in Khuzestan province (Southwest of Iran). The treatments included different concentration of Gibberellin (nonuse of Gibberellin or control, 5 ppm, 50 ppm and 250 ppm) and time of application Gibberellin (Vegetative growth before flowering, flowering until pod emergence, Pod emergence until grain filing). This experiment had 36 plots. Each plot consisted of 7 lines with a distance of 60 cm and 5 meters length. The distance between the shrubs on every row was 15 cm.

3.2. Farm Management

Base fertilizers (75 $kg.ha^{-1}$ Nitrogen from urea, 100 $kg.ha^{-1}$ phosphorus from ammonium phosphate and 80 $kg.ha^{-1}$ potassium from potassium sulfate) were added to the soil based on soil tests and the recommendations of the Iranian Soil and Water Research Institute at the planting stage. Also 50 $kg.ha^{-1}$ nitrogen added at stem elongation stage. Physical and chemical properties of studied soil were mentioned in table 1.

Table 1. Physical and chemical properties of studied field

Soil depth (cm)	SP (%)	EC ($ds.m^{-1}$)	pH
0-30	50	11.48	7.95
30-60	51	12.1	7.9
Soil depth (cm)	**N (%)**	**P (ppm)**	**K (ppm)**
0-30	0.07	4.6	202
30-60	0.052	4.0	171
Soil depth (cm)	**TNV (%)**	**OM (%)**	**OC (%)**
0-30	36	1.24	0.72
30-60	39	0.95	0.55
Soil depth (cm)	**Sand (%)**	**Silt (%)**	**Clay (%)**
0-30	14	53	33
30-60	12	57	31

To combat weeds during growth, weeding and thinning was done manually.

3.3. Measured Traits

In order to determine the yield two planting lines from each plot harvested and after the removal of marginal effect were carried to the laboratory and were placed in the oven at 75°C for 48 hours and after ensuring that the samples were completely dry, they were weighed and finally the total yield was measured. By measuring three factors including leaf area, leaf dry weight and total dry weight, the physiological parameters of growth including LAI, NAR, CGR and RGR were obtained using the following equations. To determine the leaf area of the linear relationship S= K. L.W was used in which S, L and W were the leaf area, L and W respectively, the maximum length and width of each leaf and K= 0.75 correction coefficient.

The leaf area index was calculated from leaf area ratio to ground level. Crop growth rate, net assimilation rate and relative growth rate were measured according fallowing formula (Buttery, 1970; Enyi, 1962):

Equ.1. CGR ($g.m^{-2}.day^{-1}$) =
TDM_2- TDM_1/T_2-T_1

TDM_1= Primary dry weight (g), TDM_2= Secondary dry weight (g)

T_1= initial sampling time, T_2= Secondary sampling time

Equ.2. NAR ($g.m^{-2}.day^{-1}$) =
CGR× $LnLA_2-LnLA_1/LA_2-LA_1$

CGR = Growth rate in grams per day per square meter

LA_1 = Initial leaf area, LA_2 = Secondary leaf area

Equ.3. RGR ($g.g^{-1}.day^{-1}$) = [Ln (TDM_2) – Ln (TDM_1)]/T_2-T_1

RGR= relative growth rate in gram per gram per day.

3.4. Statistical Analysis

All data analyzed via MSTAT-C software and the means were compared by using LSD test at 5% probability level. All curves were drawn by Excel Software (Ver. 2010).

4. RESULT AND DISCUSSION

4.1. *Seed yield*

Result of ANOVA revealed effects of different amount, time of apply Gibberellin acid and interaction effect of treatments on seed yield was significant at 5% probability level (Table 2). Mean comparison result of different level of Gibberellin acid concentration indicated that maximum seed yield (2761 $kg.ha^{-1}$) was noted for 50 ppm Gibberellin acid and minimum of that (1983 $kg.ha^{-1}$) belonged to control treatment (Table 3).

Table 2. Result of analysis of variance effect of treatment on seed yield

S.O.V	df	Seed yield
Replication	2	27630.32^{ns}
Gibberline concentration (GC)	3	844221.83*
Growth stage (GS)	2	87210.47*
GC × GS	6	68911.76*
Error	22	27837.53
CV (%)	-	11.17

ns, * and **: no significant, significant at 5% and 1% of probability level, respectively.

Table 3. Mean comparison effect of Gibberline concentration on seed yield

Gibberline concentration	Seed yield ($kg.ha^{-1}$)
5 ppm	2504b
50 ppm	2761a
250 ppm	2365c
Control	1983d
LSD	55.1

*Similar letters in each column show non-significant difference at 5% probability level.

As for Duncan classification made with respect to different level of time of applies Gibberellin acid maximum and minimum amount of seed yield belonged to Vegetative growth before flowering (2478 $kg.ha^{-1}$) and pod emergence until grain filing (2330 $kg.ha^{-1}$) (Table 4). Assessment mean comparison result of interaction effect of treatments indicated maximum seed yield (2899 $kg.ha^{-1}$) was noted for 50 ppm and vegetative growth before flowering and lowest one (1898 $kg.ha^{-1}$) belonged to nonuse of Gibberellin acid and pod emergence until grain filing (Table 5).

Table 4. Mean comparison effect of time of application Gibberline on seed yield

Time of application Gibberline	Seed yield (kg.ha^{-1})
Vegetative growth before flowering	2478a
Flowering until pod emergence	2399ab
Pod emergence until grain filing	2330b
LSD	59.3

*Similar letters in each column show non-significant difference at 5% probability level.

Rastogi *et al*. (2013) reported that growth hormones, whether alone or in combination, have a major impact in the stimulation of various growth parameters in linseed. They were concluded that plant growth hormones could be successfully employed for enhancement of seed yield, directly or indirectly, through its components. Based on findings, it is recommended use of a combined dose of auxin and gibberellins (1 mg.L^{-1} + 400 mg.L^{-1}) for seed yield, and auxin alone at 0.5 mg.L^{-1} doses for vegetative growth, in order to enhance yield in this important oil seed crop.

4.2. *Leaf area index* (*LAI*)

Compare different level of Gibberellin acid concentration revealed maximum amount of LAI (2.43) belonged to 50 ppm Gibberellin acid and lowest one was for control treatment (1.42) (Fig. 1). Among different time of apply Gibberellin acid, Vegetative growth before flowering had highest amount of LAI (2.01) and the lowest one belonged to pod emergence until grain filing (1.74) (Fig. 2). Haghighi *et al*. (2011) reported humic acid led to increased growth parameters and seed yield in compared to control treatments.

Table 5. Mean comparison interaction effect of treatment on seed yield

Gibberline concentration	Time of application Gibberline	Seed yield (kg.ha^{-1})
5 ppm	Vegetative growth before flowering	2532c
	Flowering until pod emergence	2510c
	Pod emergence until grain filing	2471bc
50 ppm	Vegetative growth before flowering	2899a
	Flowering until pod emergence	2736ab
	Pod emergence until grain filing	2650b
250 ppm	Vegetative growth before flowering	2451d
	Flowering until pod emergence	2345e
	Pod emergence until grain filing	2300e
Control	Vegetative growth before flowering	2030f
	Flowering until pod emergence	2021g
	Pod emergence until grain filing	1898h
LSD		10.5

*Similar letters in each column show non-significant difference at 5% probability level.

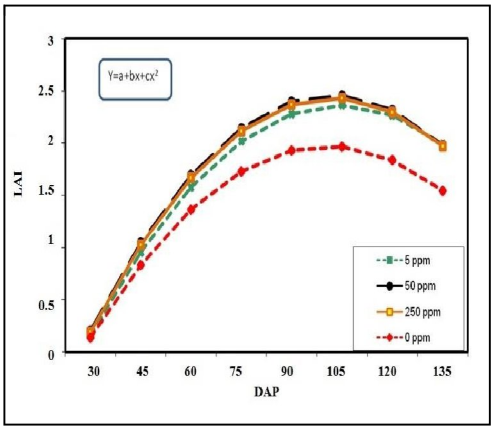

Fig. 1. Effect of different concentration of gibberllin acid on leaf area index (LAI)

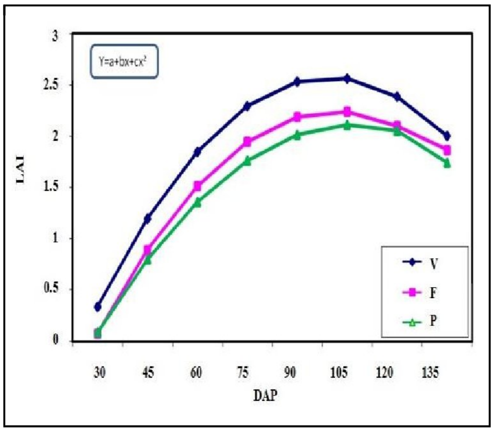

Fig. 2. Effect of different time of application gibberllin acid on leaf area index (LAI) **V:** Vegetative stage**, F:** Flowering stage**, P:** Pod emergence

Leite *et al*. (2003) reported Gibberellin acid led to increase LAI in soybeans. So, gibberellin acid increases vegetative growth by affecting cellular processes such as cell division stimulation and cell elongation (Stuart and Jones, 1977).

4.3. *Total dry matter* (*TDM*)

Compare different level of Gibberellin indicated maximum amount of TDM (3750 gr.m^{-2}) belonged to 50 ppm Gibberellin acid and lowest one was for control treatment (2500 gr.m^{-2}) (Fig. 3).

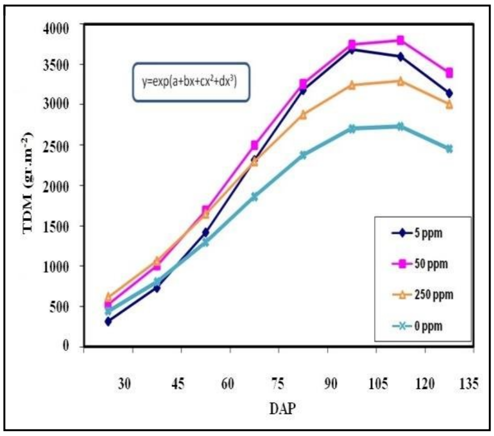

Fig. 3. Effect of different concentration of gibberllin acid on total dry matter (TDM)

Among different time of apply Gibberellin acid, Vegetative growth before flowering had highest amount of TDM (3500 gr.m^{-2}) and lowest one belonged to pod emergence until grain filing (2800 gr.m^{-2}) (Fig. 4). Sadi (2016) reported use different levels of gibberellin and humic magnesium chelate by improving the process of plant dry matter accumulation, increasing leaf area index, crop growth rate, relative growth rate and net assimilation rate led to improve crop production of cowpea. Also in amount of 250 ml.h^{-1} humic magnesium chelate and 150 ppm gibberellin, Due to the greater canopy and photosynthesis in the plant, less leaf fall in the final stages of growth, the maximum crop growth rate was achieved in the middle of the growth period. After these conditions, the crop growth rate had a decreasing trend as well as a less negative trend. With increasing concentration of humic magnesium chelate (450 ml.h^{-1}) and regulator hormone (300 ppm gibberellin) led to increase leaf area index and less crop growth rate in compare lower concentrations.

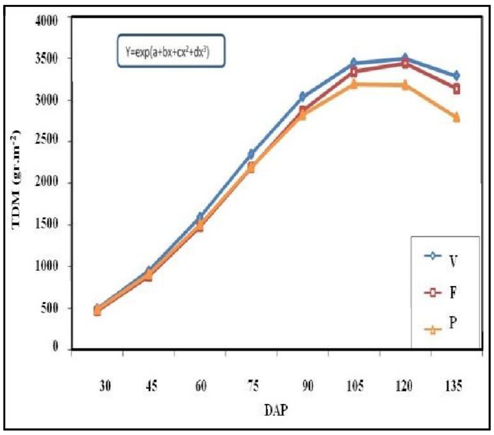

Fig. 4. Effect of different time of application gibberllin acid on total dry matter (TDM)

V: Vegetative stage, F: Flowering stage, P: Pod emergence

4.4. *Relative growth rate* (*RGR*)

The relative growth rate changes with changes in photosynthesis and plant respiration, and for this reason, over time, plant growth decreases to zero with increasing respiration at the end of the growing season. Relative growth rate indicates the amount of dry matter accumulated in the plant per unit time. The relative growth rate of crops during the plant life cycle has a decreasing trend. The reason for the downward trend in relative growth rate can be found in the accumulation of dry matter, which is mainly allocated to undifferentiated tissues. Also, the phenomenon of leaf shading on each other can be considered effective in this reduction (Sadi, 2016). Compare different level of Gibberellin acid concentration revealed the highest amount of the RGR (0.049 gr.gr.day^{-1}) belonged to 50 ppm Gibberellin acid and lowest one was for control treatment (0.031 gr.gr.day^{-1}) (Fig. 5).

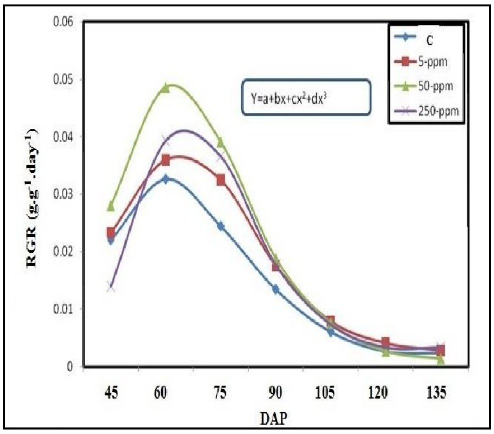

Fig. 5. Effect of different concentration of gibberllin on relative growth rate (RGR)

It seems Gibberellin may accelerate deceasing trend of relative growth rate, possibly due to accelerating the formation of mature tissues and reducing the rate of meristems tissue formation. Among different time of apply Gibberellin acid, Vegetative growth before flowering had highest amount of RGR (0.044 gr.gr.day^{-1}) and the lowest one belonged to pod emergence until grain filing (0.035 gr.gr.day^{-1}) (Fig. 6).

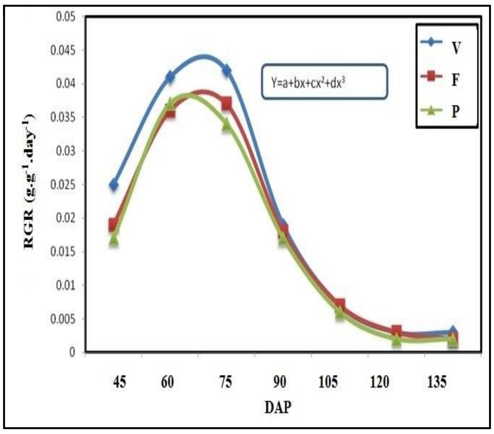

Fig. 6. Effect of different time of application gibberllin acid on relative growth rate (RGR)

V: Vegetative stage, **F**: Flowering stage, **P**: Pod emergence

4.5. *Crop growth rate* (*CGR*)

Compare different level of Gibberellin acid concentration revealed maximum amount of CGR (34 $gr.m^{-2}.day^{-1}$) belonged to 50 ppm Gibberellin acid and lowest one was for control treatment (24 $gr.m^{-2}.day^{-1}$) (Fig. 7). Among different time of apply Gibberellin acid, Vegetative growth before flowering had highest amount of CGR (35 $gr.m^{-2}.day^{-1}$) and the lowest one belonged to pod emergence until grain filing (26 $gr.m^{-2}.day^{-1}$) (Fig. 8).

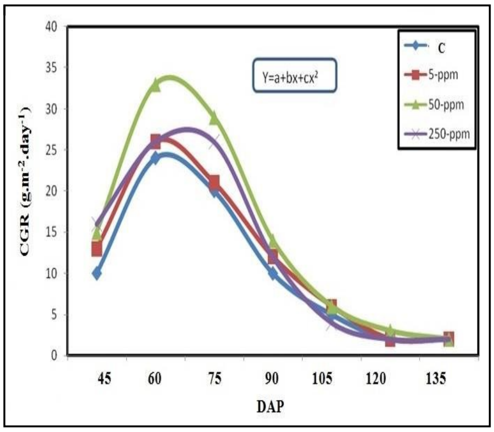

Fig. 7. Effect of different concentration of gibberllin acid on crop growth rate (CGR)

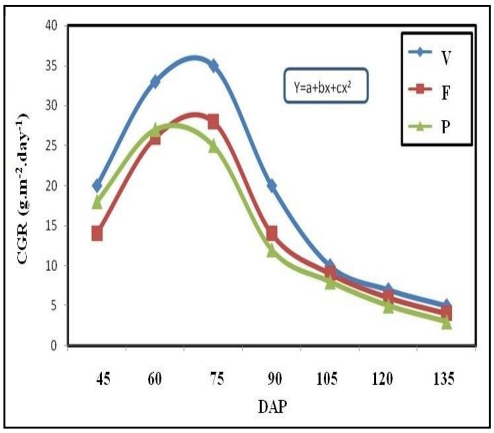

Fig. 8. Effect of different time of application gibberllin acid on crop growth rate (CGR)

V: Vegetative stage, **F**: Flowering stage, **P**: Pod emergence

4.6. *Net assimilation rate* (*NAR*)

Compare different level of Gibberellin acid concentration revealed maximum amount of NAR (7.5 $gr.m^{-2}.day^{-1}$) belonged to 50 ppm Gibberellin acid and lowest one was for control treatment (5.8 $gr.m^{-2}.day^{-1}$) (Fig. 9). Among different time of apply Gibberellin acid, Vegetative growth before flowering had highest amount of NAR (7.8 $gr.m^{-2}.day^{-1}$) and the lowest one belonged to pod emergence until grain filing (6.4 $gr.m^{-2}.day^{-1}$) (Fig. 10).

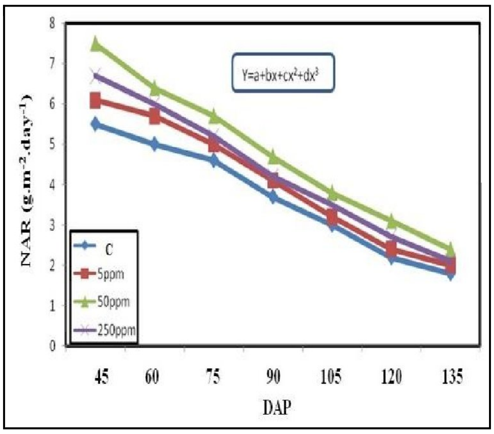

Fig. 9. Effect of different concentration of gibberllin on net assimilation rate (NAR)

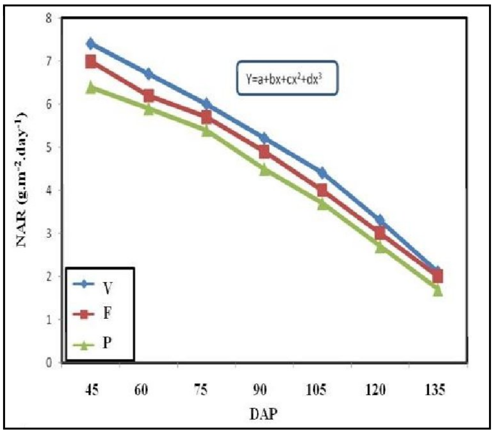

Fig. 10. Effect of different time of application gibberllin acid on net assimilation rate (NAR)

V: Vegetative stage, **F**: Flowering stage, **P**: Pod emergence

Research on tomatoes has shown that leaf application of gibberellin increases photosynthesis, which can also be due to increased leaf area or increased photosynthetic rate per unit area of leaf area. Gibberellin has been shown to increase the activity of the enzyme ribulose bisphosphate carboxylase oxygenase (Rubisco), which is the major enzyme of photosynthesis in plants (Fatek, 2019).

5. CONCLUSION

Generally result of current study revealed between level of Gibberellin acid concentration 50 ppm treatment had highest amount in seed yield, total dry matter, leaf area index, crop growth rate, net assimilation rate and relative growth rate. So among different time of apply Gibberellin acid the highest amount of mentioned traits belonged to consume Gibberellin acid at vegetative growth before flowering phase and it can be advice to farmers.

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FOOTNOTES

AUTHORS' CONTRIBUTION: All authors are equally involved.

CONFLICT OF INTEREST: Authors declared no conflict of interest.

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