Pak. J. Bot., 52(1): 229-234, 2020. DOI: http://dx.doi.org/10.30848/PJB2020-1(38)

COMPARATIVE PERFORMANCE OF CGR$_3$ AND CGR$_{3-1}$ ON PHOTOSYNTHETIC CHARACTERISTICS AND YIELD OF MUNG BEAN (*PHASEOLUS RADIATA*)

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Abstract

The effects of methyl 1-(3, 3-dimenthyl-2-oxobutyl)-1H-1, 2, 4-triazole-3-carboxylate (CGR$_3$) and 1-(3,3-dimethyl-2-oxobutyl)-1H-1, 2, 4-triazole-3-carboxylic acid (CGR$_{3-1}$), as two new plant growth regulators, on photosynthetic characteristics and mung bean yield were investigated in this study. Both regulators improved the net photosynthetic rate (P_N), stomatal conductance (gs), intercellular CO_2 concentration (Ci) and transpiration rate (E); CGR$_{3-1}$ had a larger impact than CGR$_3$. In terms of photosynthetic efficiency, CGR$_{3-1}$ showed higher effectiveness. Considering photosynthetic pigments, the two regulators enhanced the contents of chlorophyll a, chlorophyll b, chlorophyll (a+b) and carotenoid. The application of CGR$_{3-1}$ (CGR$_3$) significantly increased (decreased) the contents of soluble sugar and starch. The results indicated that CGR$_{3-1}$ was more effective in improving the photosynthetic characteristics and yield of mung bean and can be a widely adopted strategy for producers.

Key words: Photosynthetic characteristics, Plant growth regulator, Yield, Mung bean.

Introduction

Mung bean (*Phaseolus radiate* L.) is an important grain legume for its high nutritive value. It can be boiled, cooked with vegetables or meat, or eaten as a dessert (Anwar *et al*., 2007). However, as a minor coarse crop, little attention has been paid to mung bean crop improvement compared to cereals and major pulses.

Photosynthesis is related to plant growth due to its direct association with net productivity (Zhu *et al*., 2010; Evans, 2013). A range of ways can be adopted to improve crop photosynthesis; for example, by means of selecting high light efficiency varieties (Hao *et al*., 2002) and applying plant growth-promoting rhizobacteria (Zhu *et al*., 2014; Garciaseco *et al*., 2015) or fertilizer (Zhang *et al*., 2013). In addition, the application of plant growth regulator is also a good choice (Wang *et al*., 2016; Ma *et al*., 2017). Plant growth regulators have different roles in agriculture. Some can delay or accelerate the growth of plants, stimulate flowering, suppress weed growth, control the height, etc. (Bonnet-Masimbert & Zaerr, 1987; MacíAs *et al*., 2000; Yan *et al*., 2010). Furthermore, plant growth regulators has been reported to improve the mung bean growth (Mubeen *et al*., 2015), even under adverse environmental conditions (Muthukumarasamy & Panneerselvam, 1997; Ghassemi-Golezani *et al*., 2015).

Triazoles have been classified as plant growth regulators. Our lab has developed methyl 1-(3, 3-dimenthyl-2-oxobutyl)-1H-1, 2, 4-triazole-3-carboxylate (CGR$_3$) and1-(3, 3-dimethyl-2-oxobutyl)-1H-1, 2, 4-triazole-3-carboxylic acid (CGR$_{3-1}$) recently.

CGR$_{3-1}$ is the hydrolyzed material of CGR$_3$. In previous experiment (unpublished), we found CGR$_{3-1}$ can increase the photosynthesis characteristics and yield of soybean, but the effect on mung bean has not been conducted, especially on the study of carbohydrate metabolism. Therefore, the objectives of this study were to 1) understand whether CGR$_3$ and CGR$_{3-1}$ were effective in improving the photosynthetic characteristics and the yield of mung bean and 2) compare their effectiveness by measuring the gas exchange parameters, photosynthetic pigment contents, chlorophyll fluorescence, soluble sugar, starch and yield.

Materials and Methods

Plant material and experimental treatments: Healthy seeds of the 'lvfeng-5' mung bean cultivar (*Vigna radiata* (L.) Wilczek) were obtained from the Heilongjiang Bayi Agricultural University (China). 2% (v/v) sodium hypochlorite solution was used to surface sterilization for 10 min and then thoroughly washed with distilled water (Scala *et al*., 2004). Then the seeds were sown in plastic pots (upper diameter of 30 cm, bottom diameter of 20 cm, and height of 25 cm). Each pot was filled with nutrient soil (pH = 5.0-6.0, total N = 1%-2.5%, total P_2O_5 = 0.3%, total K_2O = 0.21%, organic matter = 70% ± 5). The mung bean plants were thinned to five per pot. CGR$_3$ and CGR$_{3-1}$ were foliar applied to the mung bean seedlings at a rate of 250mg/L. Three treatments were established in the experiment: (1) Control: The water was applied to the plants when the third trifoliolate leaf fully expanded; (2) and (3): CGR$_3$ treatment and CGR$_{3-1}$ treatment: CGR$_3$ and CGR$_{3-1}$ were sprayed to the mung bean plants at the same time, respectively. The solution was applied to the mung bean plants using a hand-held aerosol sprayer. For each pot, the plants received 10 ml solutions of water or the plant growth regulator only. Completely randomized design was used in the experiment and each treatment had five pots. Fresh leaf samples were collected for immediate determination or frozen in liquid nitrogen and transferred to the -80℃ refrigerator to analyze physiological indexes.

Measurements of photosynthetic gas exchange parameters: Photosynthetic gas exchange parameters were determined on the newly fully-expanded leaf by using a Cl-340 Handheld Photosynthesis System (CID Bio-Science, USA). The leaf net photosynthetic rate (P_N), stomatal conductance (gs), intercellular CO_2 concentration (Ci) and transpiration rate (E) were read from the instrument. The measurements were made on three randomly selected plants for each treatment. The Ci /Ca ratio was calculated as the internal CO_2 concentration (Ci) divided by the ambient CO_2 concentration (Ca) (Singh & Reddy, 2011). Water use efficiency (WUE) was calculated as the ratio of the net photosynthetic rate to transpiration (Brilli *et al*., 2011).

Measurements of photosynthetic pigment contents: Leaf discs (0.1g) were extracted in 95% ethanol in the dark for 24 h (Zaman & Asaeda, 2014). The concentrations of Chl a, Chl b and carotenoid were determined using a spectrophotometer (U-2500, Hitachi). The contents of Chl a, Chl b and carotenoid were calculated using the following equations (Tapia *et al*., 2010):

$$\text{Chlorophyll-a } (mg\cdot L^{-1}) = 13.95\ A_{665} - 6.88A_{649}; \quad (1)$$

$$\text{Chlorophyll-b } (mg\cdot L^{-1}) = 24.96\ A_{649} - 7.32A_{665}; \quad (2)$$

$$\text{Carotenoid } (mg\cdot L^{-1}) = (1000A_{470} - 2.05\ \text{Chla} - 114.8\ \text{Chlb}) / 245; \quad (3)$$

$$\text{Chlorophyll (a+b) } (mg\cdot L^{-1}) = 18.08A_{649} + 6.63A_{665}, \quad (4)$$

Measurements of chlorophyll fluorescence: The chlorophyll fluorescence parameters Fv/Fm and Fv/Fo were measured on four randomly selected plants for each treatment. Chlorophyll fluorescence was measured by the OS-5P+ chlorophyll fluorescence system (Opti-Sciences, USA). Measurements were made on the newly fully-expanded leaf after a 30 min dark adaptation period to obtain steady values of Fv/Fm and Fv/Fo (Jerzykiewicz & Grazyna, 2007).

Determination of soluble sugar and starch: Total soluble sugars were determined using anthrone reagent (Mandal *et al*., 2008) with some modification. Samples leaves (0.1g) were extracted with 4 mL of 80 % ethyl alcohol and kept at 80℃ for 20min. After that, extracted solution was centrifuged at 2000rpm for 5 min. and the supernatants of three successive centrifugations were used for sugar analysis. The residue from the homogenate was used to determine the content of starch, following the procedures of Sakai (1962).

Determination of crop yield: At maturity, 5 plants per treatment were harvested to determine the grain yield. The number of pods, seeds, grain, weight per plant and hundred-grain weight were measured.

Statistical analysis

All experimental data were analyzed with variance at the 0.05 and 0.01 probability level. SPSS23 software (Chicago, USA) was used for analysis and Origin 9.1 (Microcal, Northhampton, MA) was used to graph. Data presented in table and figures showed mean values with standard error (± SE).

Results

Effect on photosynthetic gas exchange parameters: The photosynthesis of mung bean plants was significantly affected by plant growth regulators. Both of CGR_3 and CGR_{3-1} had favorable effects on photosynthetic gas exchange parameters (Fig. 1A-E). Plants sprayed with CGR_{3-1} displayed the highest photosynthesis activity; net photosynthetic rate (P_N), intercellular CO_2 concentration (Ci), stomatal conductance (gs), transpiration rate (E) and ci/ca ratio were 1.5, 1.2, 1.9, 2.0 and 1.2 times higher than that in the control and 1.4, 1.1, 1.6, 1.7 and 1.1 times higher than the control group in the CGR_3 treatment. However, the water use efficiency declined after the application of two regulators (Fig. 1F). The maximum value of water use efficiency occurred in the control group. The CGR_{3-1} treatment showed lowest value of water use efficiency because of a higher transpiration rate.

Effect on photosynthetic efficiency: Data related to chlorophyll fluorescence efficiency, Fv/Fm and Fv/Fo are shown in Fig. 2. As compared to the control, the application of CGR_3 and CGR_{3-1} had no significant difference in Fv/Fm and Fv/Fo. CGR_3 treatment resulted in a 2.1 and 6.8% reduction and CGR_{3-1} treatment caused a 0.6 and 4.2% increase in Fv/Fm and Fv/Fo, respectively.

Effect on photosynthetic pigments: Data involving the light harvesting pigments, i.e. chl a, chl b and carotenoid are presented in Table 1. CGR_3 and CGR_{3-1} improved the contents of photosynthetic pigments and reduced the ratio of chl a/chl b. The contents of chl a, chl b, chl (a+b) and carotenoid were increased by 16.7 (6.1), 28.0 (12.0), 19.7 (7.9) and 12.2 (7.3) %, respectively, in CGR_3 (CGR_{3-1}) treatment as compared to the control. The chl a/chl b was declined by 10.3 and 6.9 % in CGR_3 and CGR_{3-1} treatments compared to the control group, respectively.

Effect on soluble sugar and starch: As shown in Fig. 3, there are pronounced differences ($p<0.01$) among the three treatments. CGR_{3-1} significantly improved the contents of soluble sugar and starch which were increased by 10.8% and 105.9% as compared to the control, respectively. However, the application of CGR_3 decreased the contents of soluble sugar and starch by 20.9% and 11.4%, respectively.

Effect on crop yield: Data in Table 2 shows an increase in pods, seeds number and grain weight per plant as the application of CGR_3 and CGR_{3-1}. Between the two options, CGR_{3-1} would be the most effective management strategy to increase actual yield. Compared to control, the CGR_3 treatment increased 1.9, 8.6 and 6.3% in pods, seeds and grain weight per plant respectively while the CGR_{3-1} increased 25.0, 31.9 and 42.4%, respectively. The hundred-grain weight had no difference among the three treatments.

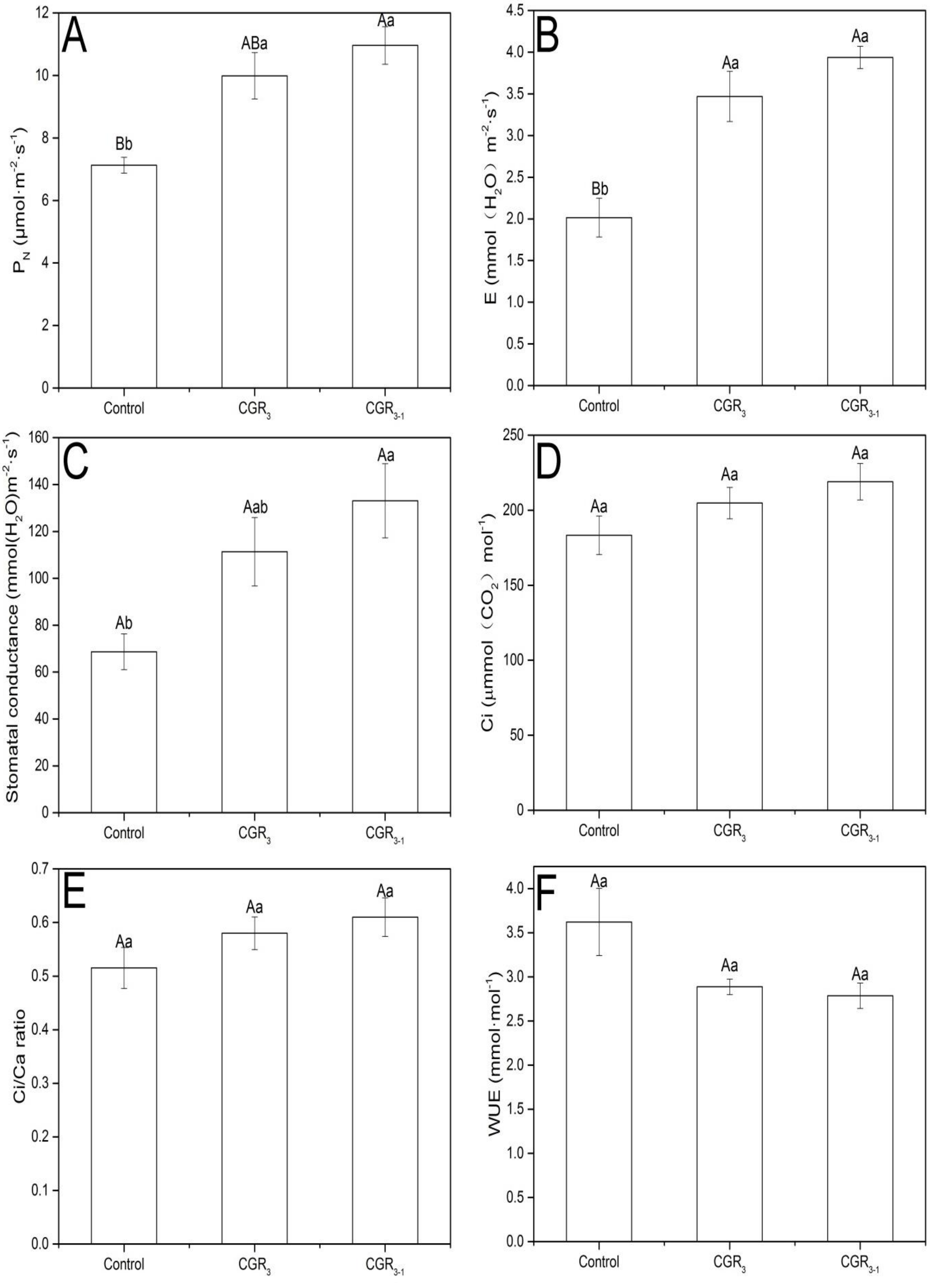

Fig. 1. Effect of $CGR_3$and CGR_{3-1}on net photosynthetic rate (A), the intercellular CO_2 concentration (B) ,stomatal conductance (C) transpiration rate (D), ci/ca ratio (E),water use efficiency(F). a, b, c indicates significant difference at the 0.05 level according to Duncan's new multiple range test; A, B, C shows significant difference at 0.01 level. The following letters have same meaning.

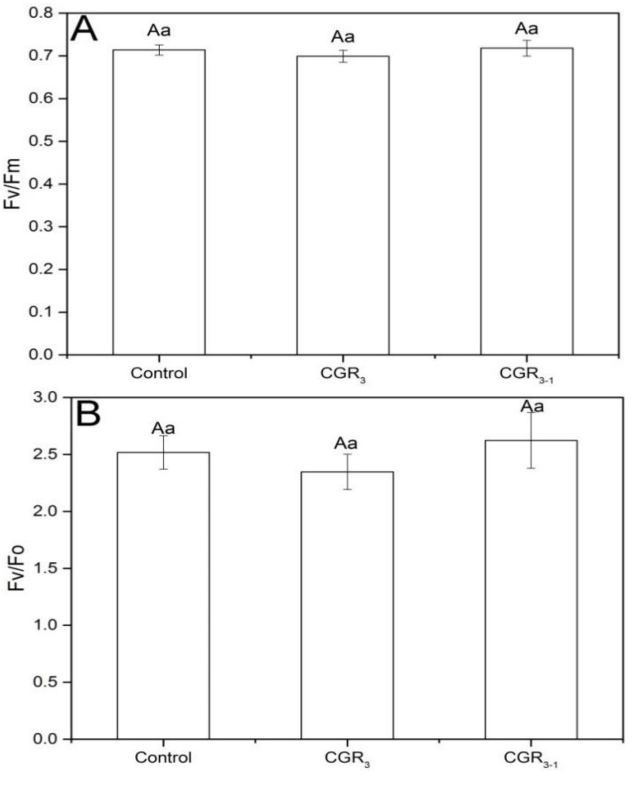

Fig. 2. Effect of CGR_3 and CGR_{3-1} on Fv/Fm and Fv/Fo.

Discussion

Ensuring leaf photosynthetic capacity is very important to obtain higher grain yield (Huang *et al*., 2016; Yamori *et al*., 2016). In this study, both regulators improved the gas exchange parameters andCGR_{3-1} increased more than that in CGR_3 treatment which indicated CGR_{3-1} was more effective in improving photosynthetic characteristics. Moreover, the variation trend of stomatal conductance was similar with net photosynthetic rate. That also confirmed the viewpoint of Athar *et al*., (2015) who thought the enhancement in net photosynthetic rate was correlated with stomatal conductance.

Photosynthetic capacity is related to photosynthetic pigments and the efficiency of light captured to drive photosynthesis (Sikuku & Onyango, 2012). Furthermore, Huang *et al*., (2016) attributed the enhanced leaf photosynthetic parameters to the improvement in light harvesting, photosystem II photochemistry and CO_2 assimilation capacity. These factors exhibited in CGR_{3-1} treatment with higher values of chlorophyll contents and fluorescence parameters. Yobo *et al*., (2009) reported the optimum value of Fv/Fm was about 0.8. CGR_{3-1} treatment had the maximum value of Fv/Fm (0.72) in this study. Light harvesting pigments play an important role in the absorption and transmission of light energy. Hamid *et al*., (2009) indicated a super-elevated CO_2 concentration would resulted in a 64% increase in orchid in chlorophyll concentration, which can permit greater light harvesting for photosynthesis. As is shown in Table 1 that CGR_{3-1} and CGR_3 improved the contents of chl a, chl b and carotenoid in mung bean which can be regarded as the result of increased chlorophyll volume or increased synthesis rate (Qi *et al*., 2013; Wang *et al*., 2016).

The majority of photoassimilates in mung beans are stored in the leaves as the form of starch during the daytime. Starch can degrade and convert to sugar at night (Stitt & Zeeman, 2012). Luo & Huang (2011) also reported that soluble sugar is the substrate for starch synthesis. In this study, CGR_3 decreased the contents of soluble sugar and starch whereas they were increased substantially by CGR_{3-1}. In view of the performance in gas exchange parameters, soluble sugar and starch, the data overall suggested that CGR_{3-1} could improve the photosynthesis to produce more photosynthate.

Table1. Effect of CGR3 and CGR3-1 on contents of chla, chlb, chl (a+b), carotenoid and a/b ratio.

	Chla [mg g^{-1}]	Chlb [mg g^{-1}]	Chl (a+b) [mg g^{-1}]	Carotenoid [mg g^{-1}]	a/b ratio
CK	1.80 ± 0.024Ab	0.50 ± 0.017Bc	2.29 ± 0.040Bb	0.41 ± 0.0092Aa	0.29 ± 0.0058Aa
CGR_3	2.10 ± 0.094Aa	0.64 ± 0.019Aa	2.74 ± 0.11Aa	0.46 ± 0.021Aa	0.26 ± 0.0043Bb
CGR_{3-1}	1.91 ± 0.019Aab	0.56 ± 0.013ABb	2.47 ± 0.032ABb	0.44 ± 0.013Aa	0.27 ± 0.0036ABab

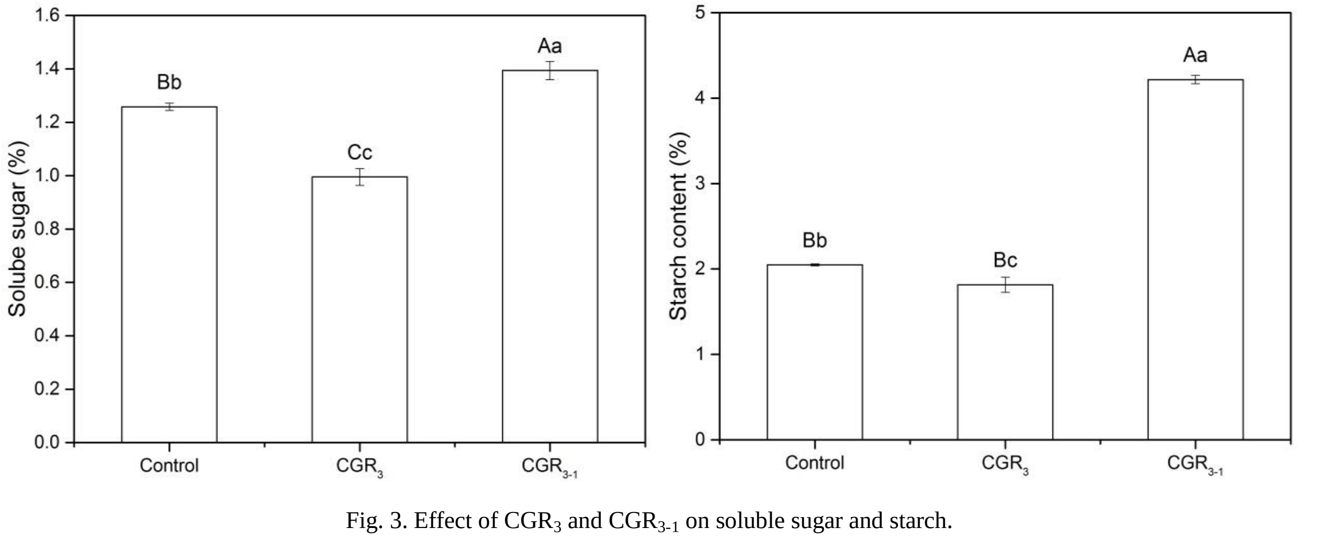

Fig. 3. Effect of CGR_3 and CGR_{3-1} on soluble sugar and starch.

Table 2. Effect of CGR_3 and CGR_{3-1} on pods per plant, seeds number per plant, grain weight per plant grain weight per plant.

	Pods per plant	Seeds number per plant	Hundred-grain weight	Grain weight per plant
CK	10.4 ± 0.4Aa	99.8 ± 6.45Aa	3.77 ± 0.095 Aa	3.68 ± 0.25Aa
CGR_3	10.6 ± 0.98Aa	108.4 ± 9.25Aa	3.86 ± 0.083 Aa	3.91 ± 0.32Aab
CGR_{3-1}	13.0 ± 2.00Aa	131.6 ± 16.38Aa	3.78 ± 0.068 Aa	5.24 ± 0.70Ab

Conclusion and Recommendation

The present study determined the effectiveness of CGR_3 and CGR_{3-1} in improving the photosynthetic characteristics and yield of mung bean. The results revealed that CGR_{3-1} could improve photosynthesis, increase the contents of photosynthetic pigments, promote the accumulation of photosynthate and, as a result increase the mung bean yield. CGR_{3-1} could be popularized as a new and effective plant growth regulator as compared to the CGR_3. Further, comprehensive research is needed to conduct to explore the effects of CGR_{3-1} on the activities of related enzymes, such as RuBP carboxylase activity and dynamic changes of gas exchange parameters to gain a better understanding of the mechanisms.

Acknowledgments

This research was supported by National Science-technology Support Plan Projects of China (2014BAD07B05; 2014BAD07B05-H07. We are thankful to Seth Kutikoff for improving English.

References

Anwar, F., S. Latif, R. Przybylski, B. Sultana and M. Ashraf. 2007. Chemical composition and antioxidant activity of seeds of different cultivars of mungbean. *J. Food Sci*., 72: 503-10.

Athar, H., Z.U. Zafar and M. Ashraf. 2015. Glycinebetaine improved photosynthesis in canola under salt stress: evaluation of chlorophyll fluorescence parameters as potential indicators. *J. Agron. Crop Sci*., 201: 428-442.

Bonnet-Masimbert, M. and J.B. Zaerr. 1987. The role of plant growth regulators in promotion of flowering. *Hormonal Control of Tree Growth*. Springer Netherlands, pp. 13-35.

Brilli, F., L. Hörtnagl, A. Hammerle, A. Haslwanter, A. Hansel, F. Loreto and G. Wohlfahrt. 2011. Leaf and ecosystem response to soil water availability in mountain grasslands. *Agri. Forest Meteorol*., 151: 1731-1740.

Evans, J.R. 2013. Improving photosynthesis. *Plant Physiol*., 162: 1780-1793.

Garcia-Seco, D., Y. Zhang, F.J. Gutierrez-Mañero, C. Martin and B. Ramos-Solano. 2015. Application of pseudomonas fluorescens to blackberry under field conditions improves fruit quality by modifying flavonoid metabolism. *Plos One*., 10: e0142639.

Ghassemi-Golezani, K., R. Lotfi and N. Najafi. 2015. Some physiological responses of mungbean to salicylic acid and silicon under salt stress. *Adv. Biores*., 64: 7-13.

Hamid, N., F. Jawaid, and D. Amin. 2009. Effect of short-term exposure to two different carbon dioxide concentrations on growth and some biochemical parameters of edible beans (*Vigna radiata and Vigna unguiculata*). *Pak. J. Bot*., 41:1831-1836.

Hao, N., W. Du, Q. Ge, G. Zhang, W. Li, W. Man, D. Peng, K. Bai and T. Kuang. 2002. Progress in the breeding of soybean for high photosynthetic efficiency. *Acta Bot. Sin*., 44: 253-258.

Huang, M., S. Shan, X. Zhou, J. Chen, F. Cao, L. Jiang and Y. Zou. 2016. Leaf photosynthetic performance related to higher radiation use efficiency and grain yield in hybrid rice. *Field Crop Res*., 193: 87-93.

Jerzykiewicz, J. and K. Grazyna. 2007. The effect of triazine - and urea - type herbicides on photosynthetic apparatus in cucumber leaves. *Acta Soc. Bot. Pol*., 76: 43-47.

Luo, X. and Q. Huang. 2011. Relationships between leaf and stem soluble sugar content and tuberous root starch accumulation in cassava. *J. Agri. Sci*., 3: 64-72.

Ma, N., C. Hu, L. Wan, Q. Hu, J. Xiong and C. Zhang. 2017. Strigolactones improve plant growth, photosynthesis, and alleviate oxidative stress under salinity in rapeseed (*Brassica napus* L.) by regulating gene expression. *Front Plant Sci*., 8: 1671.

MacíAs, F.A., J.C.G. Galindo, J.M.G. Molinillo and D. Castellano. 2000. Dehydrozaluzanin c: a potent plant growth regulator with potential use as a natural herbicide template. *Phytochem*., 54: 165.

Mandal, K., R. Saravanan and S. Maiti. 2008. Effect of different levels of n, p and k on downy mildew (*Peronospora plantaginis*) and seed yield of isabgol (*Plantago ovata*). *Crop Prot*., 27: 988-995.

Mubeen, B.R., M.D. Shamim, A.H. Khan, A.K. Singh, S.P. Singh, R.K. Yadav and U. Singh. 2015. Response of mungbean (*Vigna radiata* L.) to rhizobium inoculation and growth regulators on biochemical changes and yield. *Environ. Ecol*., 33: 105-109.

Muthukumarasamy, M. and R. Panneerselvam. 1997. Amelioration of Nacl stress by triadimefon in peanut seedlings. *Plant Growth Regul*., 22: 157-162.

Qi, R.J., W.R. Gu, J. Zhang, H. Ling, M.C. Zhang, L.S. Duan and Z.H. Li. 2013. Exogenous diethyl aminoethyl hexanoateenhanced growth of corn and soybean seedlings through altered photosynthesis and phytohormone. *Aust. J. Crop Sci*., 7: 2021-2028.

Sakai, A. 1962. Studies on the frost-hardiness of woody plants. i. the causal relation between sugar content and frost-hardiness. *Contributions from the Institute of Low Temperature Science*, 11: 1-40.

Scala, A., L. Pazzagli, C. Comparini, A. Santini, S. Tegli and G. Cappugi. 2004. Cerato-platanin, an early-produced protein by *Ceratocystis fimbriata* f.sp. *platani*, elicits phytoalexin synthesis in host and non-host plants. *J. Plant. Pathol*., 86: 27-33.

Sikuku, P. and J. Onyango. 2012. Physiological and biochemical responses of five nerica rice varieties (*Oryza sativa* L.) to water deficit at vegetative and reproductive stage. *ABJNA*., 3: 93-104.

Singh, S.K. and K.R. Reddy. 2011. Regulation of photosynthesis, fluorescence, stomatal conductance and water-use efficiency of cowpea (*Vigna unguiculata* [L.] Walp.) under drought. *J. Photoch. Photobio. B*., 105: 40-50.

Stitt, M. and S.C. Zeeman. 2012. Starch turnover: Pathways, regulation and role in growth. *Curr. Opin. Plant Biol*., 15: 282.

Tapia, F.M.L., A.G. Toro, R.B. Parra and E.A. Riquelme. 2010. Photosensitivity of cucumber (*Cucumis sativus* L.) seedlings exposed to ultraviolet-b radiation. *Chil. J. Agr. Res*., 70: 16-25.

Wang, Y., W. Gu, T. Xie, L. Li, Y. Sun, H. Zhang, J. Li and S. Wei. 2016. Mixed compound of DCPTA and CCC increases maize yield by improving plant morphology and up-regulating photosynthetic capacity and antioxidants. *Plos One*., 11: e0149404.

Yamori, W., E. Kondo, D. Sugiura, I. Terashima, Y. Suzuki and A. Makino. 2016. Enhanced leaf photosynthesis as a target to increase grain yield: Insights from transgenic rice lines with variable rieske fes protein content in the cytochrome b6 /f complex. *Plant Cell Environ*., 39: 80.

Yan, Y., W. Gong, W. Yang, Y. Wan, X. Chen, Z. Chen and L. Wang. 2010. Seed treatment with uniconazole powder improves soybean seedling growth under shading by corn in relay strip intercropping system. *Plant Prod. Sci*., 72: 367-374.

Yobo, K.S., M.D. Laing and C.H. Hunter. 2009. Effects of single and dual applications of selected *Trichoderma* and *Bacillus* isolates on performance of dry bean seedlings grown in composted pine bark growth medium under shadehouse conditions. *J. Plant Nutr*., 32: 1271-1289.

Zaman, T. and T. Asaeda. 2014. Assessment of macro-micro element accumulation capabilities of elodea nuttallii under gradient redox statuses with elevated nh4-n concentrations. *Acta Bot. Croat*., 73: 215-231.

Zhang, X., G. Huang, X. Bian and Q. Zhao. 2013. Effects of root interaction and nitrogen fertilization on the chlorophyll content, root activity, photosynthetic characteristics of intercropped soybean and microbial quantity in the rhizosphere. *Plant Soil Environ*., 59: 80-88.

Zhu, X.G.,S.P. Long and D.R. Ort. 2010. Improving photosynthetic efficiency for greater yield. *Annu. Rev. Plant Biol*., 61: 235.

Zhu, Y., Z. Wang, J. Wang and J. Zhou. 2014. Plant growth-promoting rhizobacteria improve shoot morphology and photosynthesis in dryland spring wheat. *Wit Trans. on the Built Environ.*, 145: 343-350.

(Received for publication 17 June 2018)