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Plant Growth Regulators Increase Soybean Yields by Delaying Leaf Senescence in Maize (*Zea mays* L.)-Soybean [*Glycine max* (L.) Merr] Relay Strip Intercropping System

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ABSTRACT

Background: The shading caused by maize inhibited the growth of soybean, reduced the yield of soybean under maize-soybean relay strip intercropping system. To investigate whether foliar spraying different types of plant growth regulators could improve the growth and yield of intercropped soybean.

Methods: A field experiment was conducted in 2018 to evaluate the effect of foliar spraying 6BA (6-benzylaminopurine), S3307 (uniconazole) and DTA-6 (diethyl aminoethyl hexanoate), at the soybean beginning flowering stage, on the growth and yield of three soybean varieties [*i.e.*, Nandou 25 (ND25), Gongqiu 8 (GQ8) and Guixia 3 (GX3)].

Result: The leaf area index of three soybean varieties were highest in DTA-6 at 35 days after spraying (DAS) and no significant difference ($p<0.05$) were observed among three plant growth regulators. The chlorophyll a and chlorophyll b content under 6BA and DTA-6 treatments were significantly higher than CK and S3307 at 35 DAS. The activities of antioxidant enzymes [*i.e.*, SOD (Superoxide dismutase) and CAT (Catalase)] and antioxidant substances [*i.e.*, GSH (Glutathione)] were increased after spraying 6BA, S3307 and DTA-6 in three soybean varieties at 35 DAS. Independent soybean varieties, foliar spraying 6BA, S3307 and DTA-6 increased the soybean yield, pods per plant, 100-grains weight and grain per plant and the effect of DTA-6 (60 mg L^{-1}) treatment were best than others.

Key words: Maize-soybean relay strip intercropping, Plant growth regulators, Soybean, Yield.

INTRODUCTION

Soybean [Glycine max (L.) Merrill] is an important grains and oil-seed crop, the seeds of which contain rich nutrients, including polyunsaturated fatty acids, proteins, vitamins and oils and can supply a variety of needs of humans and animals (Anuradha *et al.* 2017). Intercropping systems, especially the maize-soybean relay strip intercropping system, have been widely planted in south-west China which supply exogenous nitrogen through the legume symbiotic fixation process and increase the system production with reduce nitrogen fertilizer input (Du *et al.* 2018; Gou *et al.* 2017).

In maize-soybean relay strip intercropping, the soybean is symbiotic with the maize during the vegetative growth period of soybean and the soybean growth was inhibited by maize shading (Liu *et al.* 2016b; Liu *et al.* 2017). The maize shading changes the morphological characteristics, biomass and physiological response of soybean and the chlorophyll a and chlorophyll b contents; the net photosynthesis rates and leaf thickness were significantly decreased under shading conditions (Yang *et al.* 2014). Compared with the sole cropping soybean system, the soybean yield and yield components in this system, especially the pods per plant and grains per plant, were significantly decreased (Fan *et al.* 2018).

Application of suitable plant growth regulators could alleviate the detrimental effect caused by maize shading and increase the yield of soybean (Ramesh and Ramprasad 2015; Kuchlan *et al.* 2019; Cohen *et al.* 2019;). For example, 6BA (6-benzylaminopurine) could improve the soybean pod formation by enhancing the photosynthesis ability of soybean leaves (Nonokawa *et al.* 2012). S3307 (uniconazole) can delay the degradation of photosynthetic pigments by increasing the activities of SOD, CAT and POD enzymes (Ahmad *et al.* 2019). DTA-6 (diethyl aminoethyl hexanoate) could reduce membrane lipid peroxidation to decrease the abscission of soybean flowers and pods (Zhang *et al.* 2016).

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To improve the yield production of soybean in maize-soybean relay strip intercropping system, we suggested that the application of suitable concentration chemical additives could improve the growth of shorter crops by improving the morphological features and photosynthetic capacity of soybean leaves. Therefore, the main objective of the current study was to study the effect of 6BA, S3307 and DTA-6 on the productivity of soybean and select the most effective combination of soybean varieties and plant growth regulators.

MATERIALS AND METHODS

Maize was Denghai 605 (provided by Nanchong Academy of Agricultural Science, Sichuan). Soybean seeds included Nandou25 (provided by Nanchong Academy of Agricultural Science, Sichuan), Gongqiu8 (provided by Zigong Academy of Agricultural Science, Sichuan) and Guixia3 (provided by Guangxi Maize Research Institute), respectively. 6BA, S3307 and DTA-6 were provided by Sichuan Agricultural University. The study was carried out during the period of April 2018 to October 2018, in Ren'shou Modern Agricultural Professional Compound and Sichuan Agricultural University, China.

Foliar spraying 6BA (20 mg L^{-1}), S3307 (50 mg L^{-1}) and DTA-6 (60 mg L^{-1}) on three soybean varieties (ND25, GQ8, GX3) at the beginning of the flowering stage and with equal amount of water as a control (CK). The amount of water application was 450 kg ha^{-1}. The soybeans were sowed in the maize-soybean relay strip intercropping system, which was performed with a wide-narrow row planting pattern with alternating strips of maize and soybean. The total rate of maize-soybean rows per strip in the relay strip intercropping system was 2:2. The distance between the maize and soybean strips was 0.6 m and both row spaces were 0.4 m under strip intercropping. Each plot included 2 strips of relay intercropping, with the area of an individual strip being 2.0 m×6.0 m. All treatments had three replicates and each plot area was 24 m^2. The plant densities were 58,500 per ha for maize and 117,000 per ha for soybean.

Sampling method and measurement

The leaf area of soybeans was measured by the hole-punching weighting method (Gou *et al.* 2017). Fifteen soybean plants in each plot were collected to investigate agronomic traits, including number of pods $plant^{-1}$ and number of grains $plant^{-1}$, along with the 100-grains weight when the soybean was harvested. The yields of soybean were measured by two adjacent rows of soybean, with 4 m length in each plot.

After 7, 14 and 35 day after spraying (DAS), three soybean plants with similar growth were randomly selected from each plot. The absorbance was measured at 663 nm and 645 nm to calculate the chlorophyll content at 7, 14 and 35 DAS (Zhang *et al.* 2016). Superoxide dismutase (SOD) activity was assayed with the NBT method described by Kukavica and Jovanovic (2004). Catalase (CAT) activity was assayed with the method described by Simonova *et al.* (2020). The content of Glutathione (GSH) was assayed as described by Wu *et al.* (2014).

Microsoft Excel 2016 was used to arrange the data. Significant differences in data were analysed by analysis of variance (ANOVA) in several steps: soybean varieties were considered the fixed factor, PGRs treatments were considered the random factor and data were considered the dependent variable. Multiple comparisons were performed with the least significance difference (LSD, $p<0.05$) method using SPSS (Version 20.0, SPSS, Chicago, IL, USA).

RESULTS AND DISCUSSION

Yield and yield components

Foliar spraying 6BA, S3307 and DTA-6 increase the yields and yield components of three soybean varieties (Table 1). All the PGRs treatments increased the pods per plant, grains per plant and 100-grains weight of soybean cultivars and DTA-6 performed better than the others. The treatment combined with ND25 variety and DTA-6 achieved the highest yield among all the cultivars, where the pods per plant, grains per plant and 100-grains weight increased by 25.4%, 35.6% and 4.2% compared with CK, respectively. The numbers of pods, grains per plant and the hundred-grain weight were highly positive correlation with the yield of soybean (Yu *et al.* 2019). Results in this experiment showed that the pods per plant and grains per plant under DTA-6 were significantly higher than 6-BA and S3307. The increase in pods per plant and grains per plant contribute to soybean yield increase. Similar results were reported that foliar spraying S3307 and DTA-6 were significantly increase the pods per plant of soybean by regulating sucrose and starch accumulation and distribution in soybean leaves (Liu *et al.* 2019).

Leaf area index, chlorophyll content antioxidant systems

PGRs treatments increased the leaf area index (LAI) of three soybean cultivars at 7, 14 and 35 DAS (Fig 1). Independent soybean varieties, the value of LAI in three regulators treatments were significantly higher than CK at 35 DAS and DTA-6 performed better than other treatments. Compared with CK, the value of LAI in GQ8, ND25 and GX3 under DTA-6 treatment were increased by 40.9%, 50% and 43% at 35 DAS, respectively. The leaf area of plants determines the area of light absorbed by plants and affects the efficiency of photosynthesis and organic matter synthesis (Khaswan *et al.* 2016). PGRs treaements incresed the value of LAI of soybenas were favroalbe to improve the light interception rate of plant.

PGRs treatments have no significant effects on the chlorophyll content under GQ8 at 35 DAS (Fig 2). The chlorophyll a and chlorophyll b content under S3307 treatment were significantly higher than CK at 35 DAS in Nandou 25 and increased by 16.2% and 22.1% compared to CK. For GX3, chlorophyll a and chlorophyll b content in 6BA was higher than in other treatments, which increased by 21.6% and 21.4% compared to CK at 35 DAS. Chlorophyll

Table 1: Effect of spraying plant growth regulators on the yield parameter of soybean.

Varieties	Treatment	Pods per plant (pods plant $^{-1}$)	Grains per plant (grains plant $^{-1}$)	100-grians weight (g)	Yield (Kg ha^{-1})
GQ8	CK	55.22 b	87.95 c	18.05 b	1641.48 c
	6BA	58.38 a	99.23 b	18.07 b	1762.91 b
	DTA-6	63.23 a	113.33 a	19.90 a	2255.04 a
	S3307	61.97 a	103.38 ab	18.96 a	1959.89 ab
ND25	CK	45.27 c	76.25 c	24.78 b	2285.73 c
	6BA	50.82 b	87.52 b	25.02 b	2439.71 b
	DTA-6	56.77 a	103.37 a	25.84 a	2929.12 a
	S3307	52.30 ab	89.13 b	25.46 ab	2523.60 b
GX3	CK	53.62 b	91.37 c	15.96 c	1458.12c
	6BA	55.17 b	96.80 b	16.30 b	1577.68b
	DTA-6	69.30 a	110.10 a	16.63 a	1830.78a
	S3307	65.40 a	104.90 ab	16.31 b	1710.75ab

GQ8 (Gongqiu 8), ND25 (Nandou 25); GX3 (Guixia 3), CK (Water control); 6BA (6-Benzylaminopurine); DTA-6 (2-N, N-diethylamino ethyl caproate); S3307(Uniconazole). Different letters in the column indicate statistical difference according to a least significant difference test (LSD P<0.05).

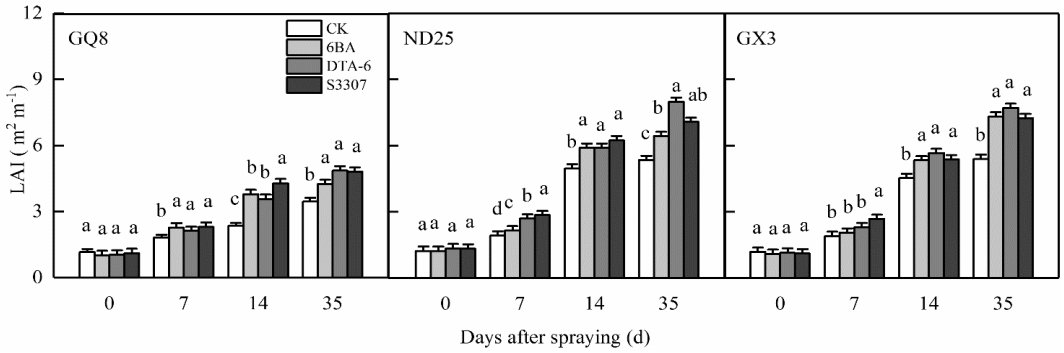

Fig 1: Effect of foliar praying 6BA, S3307 and DTA-6 on Leaf area index of soybean.

GQ8(Gongqiu 8), ND25(Nandou 25); GX3(Guixia 3), CK (Water control), 6BA (6-Benzylaminopurine), DTA-6(2-N, N-diethylamino ethyl caproate); S3307(Uniconazole). Different letters in the column indicate statistical difference according to a least significant difference test (LSD P<0.05). The followed are same.

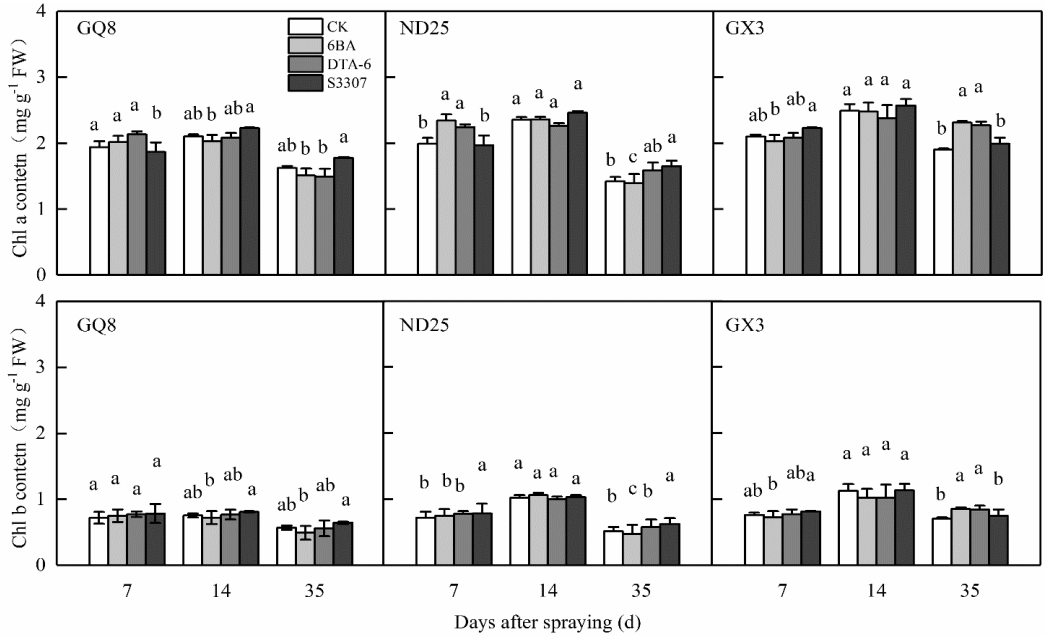

Fig 2: Effect of foliar praying 6BA, S3307 and DTA-6 on Chlorophyll content of soybean.

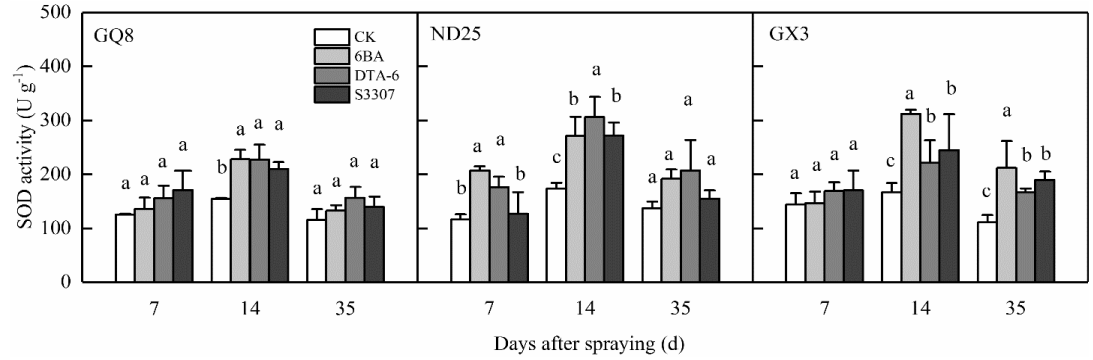

Fig 3: Effect of foliar praying 6BA, S3307 and DTA-6 on SOD activity of soybean.

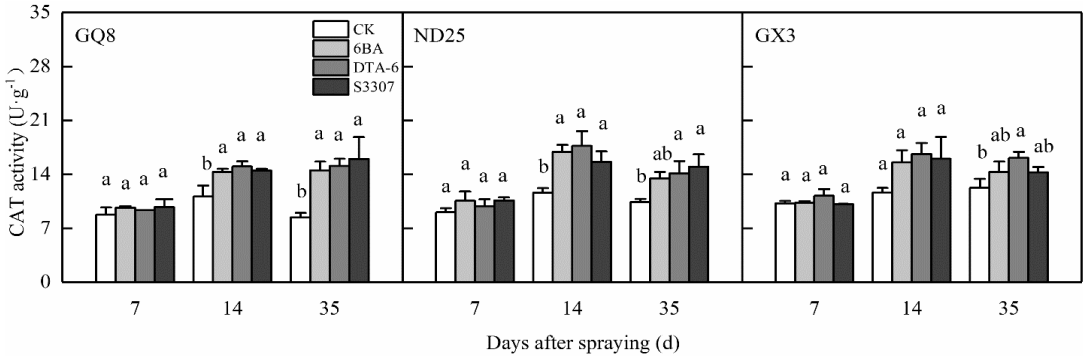

Fig 4: Effect of foliar praying 6BA, S3307 and DTA-6 on CAT activity of soybean.

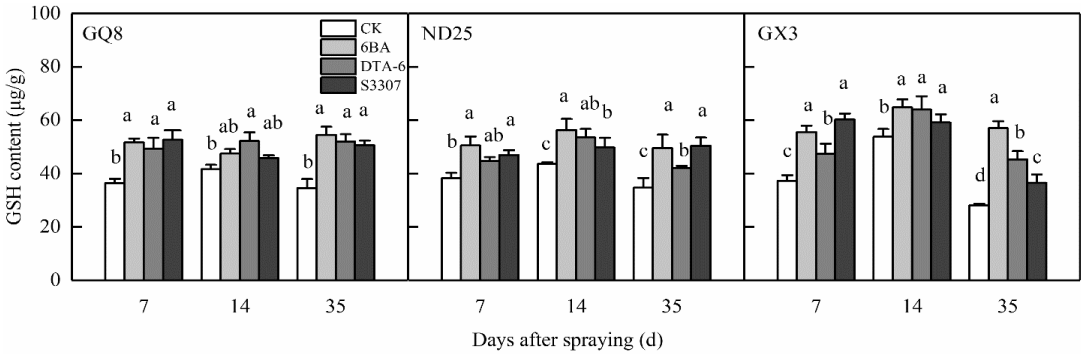

Fig 5: Effect of foliar praying 6BA, S3307 and DTA-6 on soybean GSH content.

content and leaf area are essential parameters to determine soybean's capacity to absorb light, which could affect the photosynthesis and the synthesis of organic materials of plants (Man *et al.* 2017). This experiment showed that 6BA, S3307 and DTA-6 treatments increased the leaf chlorophyll a and chlorophyll b content of soybean leaves at 35 DAS. We suggested that PGRs treatments increased the photosynthesis ability of soybean by increasing content of photosynthesis pigments. Similar results were founded that increase the plants chlorophyll content were contributed to improving the photosynthesis ability, light capture and transformation of soybean leaves (Fan *et al.* 2018). Previous research founded that application of PGRs could upregulate the enzymes involved in the chlorophyll biosynthesis pathway and improve the antioxidant system to delay the degradation of photosynthetic pigments to increase the leaf chlorophyll content and improve the plant photosynthesis rate (Zhang *et al.* 2016; Zhang *et al*. 2018).

The SOD activity of ND25 under 6BA, S3307 and DTA-6 were significantly higher than CK and increased by 76%, 56.5% and 56% compared to CK at 14 DAS, respectively (Fig 3). PGRs treatments increased the CAT activity in three soybean varieties and the CAT activity of GQ8 under 6BA, S3307 and DTA-6 were increase by 73%, 80% and 90% compared to CK at 35 DAS, respectively (Fig 4). Independent soybean varieties, PGRs treatments increased the GSH content and performed better under 6-BA treatment. The GSH content in GQ8, ND25 and GX3 under 6BA treatment were increased by 57.2%, 42.7% and 102.4% compared to CK at 35 DAS, respectively (Fig 5). PGRs treatments delayed the leaf senescence of soybean leaves at the later growth

stage by increasing the SOD and CAT activities and the GSH content in soybeans leaves. The results were similar with Liu *et al.* (2016a) who found that spraying cytokinin could increase the yield of rice by mediating chlorophyll degradation membrane deterioration and senescence associated genes expression in rice flag leaf. Zhao *et al.* (2019) also showed that foliar spraying S3307 (50 mg L^{-1}) significantly increased the yield of soybean by modulating photosynthesis, photoinhibition and activating oxygen metabolism system.

CONCLUSION

The insufficient soybean growth caused by maize shading during co-growth stage decrease the yield of soybean in maize-soybean relay strip intercropping system. Therefore, we are celebrating to select the suitable soybean varieties and plant growth regulators to increase the soybean yield under maize-soybean relay strip intercropping system which were favorable to stabilize domestic food security. Results showed that foliar spraying 6-BA, S3307 and DTA-6 increased the yield and yield components of soybean, especially the pods per plant, by increasing the photosynthate parameters of soybean leaves (*e.g.*, leaf area, chlorophyll content). The increases in the activities of antioxidant enzymes (*e.g.*, SOD and CAT) and the antioxidant materials (*e.g.*, GSH) were favorable for maintain the leaf photosynthate efficiency of soybean leaves at the later growth stage. The highest yields of soybean in this experiment were obtain with foliar spraying DTA-6 (60 mg L^{-1}) on Nandou 25 at the flowering stage in maize-soybean relay strip intercropping system.

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