Influence of Seed Treatment with Uniconazole Powder on Soybean Growth, Photosynthesis, Dry Matter Accumulation after Flowering and Yield in Relay Strip Intercropping System

Yanhong Yan¹, Yan Wan²,³,⁴, Weiguo Liu²,³, Xiaochun Wang²,³, Taiwen Yong²,³, Wenyu Yang²,³ and Liulan Zhao¹

¹College of Animal Science and Technology, Sichuan Agricultural University, Ya’an, Sichuan, 625014, China; ²College of Agronomy, Sichuan Agricultural University, Wenjiang, Sichuan, 611130, China; ³Key Laboratory of Crop Ecophysiology and Farming System in Southwest China, Ministry of P. R. China, Wenjiang, Sichuan, 611130, China; ⁴College of Biotechnology Industry, Chengdu University, Chengdu, Sichuan, 610106, China

Abstract: The relay strip intercropping system of wheat-corn-soybean is widely used in southwest China. However, it is hard to produce soybean stably with this system, since the growth of soybean plants is slower under shading by corn at the seedling stage, and it is compensated by accelerated growth after the symbiotic stage. Soybean plants show excessive vegetative growth due to more rain during the flowering stage, which results in fallen petals, fallen pods and lower yield. This study investigated whether seed treatment with uniconazole powder (0, 2, 4 and 8 mg kg⁻¹ seed) suppresses excessive vegetative growth of soybean plants during the flowering stage and delays senescence of photosynthetically active leaves at the pod-setting stage. If such events are correlated with changes in photosynthesis, they may affect dry matter accumulation and seed yield in the relay stripping system. Uniconazole promoted biomass accumulation from 31 (R₃) to 61 (R₅) days after flowering (DAF) and seed yield. Seed treatment with uniconazole raised the net photosynthetic rate, stomatal conductance, transpiration rate, and total chlorophyll and chlorophyll a contents. In contrast, uniconazole reduced leaf area index (LAI) from 1 DAF (R₁) to 46 DAF (R₄) with the increase in uniconazole concentration, whereas, uniconazole significantly increased LAI at 61 DAF, and the greatest promotion occurred at 2 mg kg⁻¹ treatment. The study clearly showed that uniconazole effectively suppressed excessive vegetative growth of soybean during flowering stage, delayed senescence of photosynthetically active leaves at pod-setting stage and induced higher yield, which were related to the changes in photosynthetic rate, chlorophyll content, dry matter accumulation and LAI in the relay strip intercropping system.

Key words: Photosynthetic characteristics, Relay strip intercropping, Soybean, Uniconazole, Yield.

The global demand for food is ever increasing with the increase of population (Yan et al., 2010; Godfray et al., 2010; Tilman et al., 2011). Crop yields need to be increased by improving the multiple crop index of land and the use efficiency of resources such as water and nutrients (Awal et al., 2006; Yan et al., 2010), while meeting the demand for diverse agricultural commodities. Intercropping is an effective means for increasing the income of a farming community as it helps better utilize the land and other resources (Rodrigo et al., 2001). Li et al. (2011) indicated that the yield from intercropping was higher than that expected from the yields of component species under sole cropping. In China, because of the large number of soybean imported from foreign countries and the decline of the cultivated area of the main soybean-producing areas such as northeastern China, the soybean industry has been facing an unprecedented crisis of development in recent years (Wan et al., 2013).

The relay strip intercropping system of wheat-maize-soybean has been developed widely and contributes greatly to China's soybean production (Yan et al., 2010; Zhang et al., 2011; Xiang et al., 2012). However, soybean grows slowly when shaded by maize at the seedling stage, and its compensation growth is accelerated after the symbiotic
stage (Xiang et al., 2012). Soybean plants showed excessive vegetative growth due to much rain during the flowering stage, resulting in fallen petals, fallen pods and lower yield (Wan et al., 2012).

Uniconazole \([(E)-(RS)-1-(4-chlorophenyl)-4,4\text{-dimethyl-2-(1H-1,2,4-triazol-1-yl) pent-1-en-3-ol}\], an active member of the triazole family, was developed for use as a plant growth retardant (Zhang et al., 2007). Seed film coating with uniconazole at a suitable concentration can improve rapeseed seedling growth and increase seedling establishment during waterlogging (Qiu et al., 2005). In addition, uniconazole applied as a foliar spray at the three-leaf stage improved plant growth and increased seed and oil yields of waterlogged winter rape compared to untreated plants (Leul and Zhou, 1998). Zhang et al. (2002) reported that foliar application of uniconazole retarded soybean shoot elongation, increased the stem diameter, and the numbers of branches, flowers and pods under net cropping. Previously, we showed that the seed treatment with uniconazole powder at a suitable concentration can improve soybean seedling growth, resist the lodging and also increase the seed yield under shading by corn in the relay strip intercropping system (Yan et al., 2010). In the relay strip intercropping system, uniconazole-treated seed had significantly higher activities of certain enzymes at the seedling stage (Yan et al., 2011a), and they degraded more easily in soil (Zhou and Ye, 1996). However, the effect of uniconazole on excessive vegetative growth is uncertain and available studies have not been reported.

This investigation was conducted to determine whether uniconazole could control soybean excessive vegetative growth, and produce compact plants after flowering in the relay strip intercropping system and whether such improvement is correlated with changes in photosynthesis and yield under field conditions.

Materials and Methods

1. Plant materials and treatments

A field experiment was conducted using the relay strip intercropping system at the farm of Sichuan Agricultural University in 2011 – 2012 on a heavy loam. Two meter wide strips were prepared in the field as reported previously (Yan et al., 2010). On these strips, wheat (cv. Neimai 8) was sown on 1 m wide strips on 3 November 2010 and harvested on 17 May 2011, and corn (cv. Chuandan 418) was sown on another area of 1 m wide strips on 8 April 2011 and harvested on 11 August 2011. After harvesting wheat, soybean was sown in three rows on the strip with 33 cm row spacing and 30 cm intervals, and had been grown at 29 May 2011, and harvested on 25 October. The soil characteristics of 0 – 20 cm were determined according to the methods reported by Dordas and Sioulas (2009). The organic matter content and pH were 35.58 g kg$^{-1}$ and 6.98, respectively. The total N, P, and K were 3.25, 3.02 and 18.86 g kg$^{-1}$, respectively. The readily available N, P, and K were 129.86, 28.85 and 82.85 mg kg$^{-1}$, respectively.

Seeds of soybean \([\text{Glycine max (L.) Merr.}]\) cultivar Gongxuan1, were treated with 5% water-dispersible uniconazole powder at the rate of 0, 2, 4 and 8 mg kg$^{-1}$, in B0 (control), B1, B2 and B3, respectively. The reagent was provided by the United Chemical Factory of Lanyue, Chengdu City, Sichuan Province. The average particle diameter of the powder was 3.0 μm. Seeds coated with uniconazole powder were sown in three rows on every strip where the wheat was grown after wheat harvest. The experiment was laid out in a randomized complete block design with three replications, the plot size was 2 m × 8 m. N, P and K were applied at 16.2, 63 and 52.5 kg ha$^{-1}$, respectively, before seed sowing in the soybean strip, and N at 16.2 kg ha$^{-1}$ was again applied at 1 day after flowering (DAF), which is reproductive stage R$_1$. The light penetration through the corn canopy to soybean was 85% when soybean was sown, 69% at 57 days after sowing (vegetative stage V$_5$), 75% at 1 DAF (reproductive stage R$_1$), and 72% at 16 DAF (R$_5$), which was when corn was at maturity.

2. Measurement techniques

(1) Photosynthesis and related parameters

Leaf area index (LAI) was measured using the LAI-2000 Plant Canopy Analyzer (LI-COR Inc., Lincoln, NE, USA) at 1 (R$_1$), 16 (R$_5$), 31 (R$_5$), 46 (R$_5$) and 61 (R$_5$) DAF.

Table 1. Some physiological characteristics of relay strip intercropping soybean leaves as influenced by uniconazole.

| Treatment | Photosynthetic rate (μmol m$^{-2}$ s$^{-1}$) | Stomatal conductance (mol m$^{-2}$ s$^{-1}$) | Transpiration rate (mmol m$^{-2}$ s$^{-1}$) |
|-----------|-------------------------------------|-------------------------------|-------------------|
|           | Days after flowering (d) | Days after flowering (d) | Days after flowering (d) |
| B0 | 12.9 Gd | 18.1 Bb | 7.5 Bc | 0.35 Cc | 0.44 Dd | 0.18 Dd | 3.69 Bc | 4.29 Bb | 2.51 Bd |
| B1 | 17.6 ABB | 20.2 Aa | 11.4 Aab | 0.38 Bb | 0.60 Bb | 0.31 Bb | 3.94 Aab | 4.74 Aa | 3.50 Ab |
| B2 | 19.0 Aa | 20.4 Aa | 12.4 Aa | 0.41 Aa | 0.64 Aa | 0.34 Aa | 4.06 Aa | 4.82 Aa | 3.78 Aa |
| B3 | 16.4 Bc | 19.7 Aa | 11.0 Ab | 0.38 Bb | 0.50 Cc | 0.21 Cc | 3.87 AAb | 4.42 Abc | 2.74 Bc |

Within columns, means followed by the same small and capital letters are not significantly different at the 0.05 and 0.01 levels of probability, respectively. Least significance difference (LSD) test. B0: control, B1: 2 mg kg$^{-1}$ seed, B2: 4 mg kg$^{-1}$ seed, B3: 8 mg kg$^{-1}$ seed.
Immediately following LAI measurement, photosynthetic rate, stomatal conductance and transpiration rate in the third upper leaves of the soybean plants were measured with a LI-6400 portable photosynthesis system (LI-COR, Lincoln, NE) at 1, 31 and 61 DAF. The LI-6400 was operated in the open mode. CO$_2$ was removed from the air, which was then mixed with pure CO$_2$ to give a reference concentration of 400 $\mu$mol mol$^{-1}$. The flow rate was 200 $\mu$mol s$^{-1}$, photosynthetically active irradiance was 1200 $\mu$mol m$^{-2}$ s$^{-1}$ and the external humidity 70%-75%. All measurements were performed in the morning between 0930 and 1200, and four to six individual plants were analyzed for each measurement.

For pigment assessment, samples of the third upper leaves were frozen in liquid nitrogen and 50 mg of fresh weight of these samples were homogenized in a mortar with 10 mL of 80% acetone. The homogenate were placed for 1 hr at 4°C, then it was centrifuged for 5 min at 5000 rpm., and aliquots of the supernatant were subjected to spectrophotometry (Travaglia et al., 2009).

(2) Agronomic parameters

Six plants (two plants from each row) were taken randomly from each treatment in three replicates at 1, 16, 31, 46 and 61 DAF, the shoot organs were dried to a constant-weight at 80°C for 72 hr after they were fixed at 105°C for 1 hr. Then dry matter weight of shoot was measured. Plant height and stem diameter were measured at the full pod stage. Yield components and grain yield were also measured at maturity. Twelve plants (four plants from each row) in each replication were harvested. The number of pods per plant, number of seeds per pod, 100-seed weight, and seed yield were recorded.

(3) Statistical analyses

Means of three replications were calculated. All data were analyzed using one-way analysis of variance (ANOVA) and the least significance difference (LSD) test at $p = 0.05$ (Yan et al., 2014) for comparison using the SAS program version 9.1 (SAS Institute, Cary, NC).

Results

1. Photosynthesis and related parameters after flowering

The photosynthetic rate, stomatal conductance and transpiration rate were significantly affected by the rate of uniconazole application (Table 1). The highest photosynthetic rate, stomatal conductance and transpiration rate were observed in plants treated with uniconazole in B2. The results showed that the treatment in B2 increased the photosynthetic rate by 12.7%, but that in B1 11.6%, and that in B3 only 8.8% at 31 DAF.

The change in the total chlorophyll and chlorophyll a contents presented a single peak curve after flowering (Fig. 1). The peaks of the total chlorophyll and chlorophyll a contents were about at 46 DAF. Interestingly, the treatments with uniconazole powder did not change the time of the peak, whereas they increased the total chlorophyll and chlorophyll a contents. Maximum total chlorophyll content was observed in B3 treatment, followed by B2 and B1. However, the greatest promotion
in chlorophyll a content was observed in B2 treatment, followed by B1 and B3, which were 14.7%, 11.0% and 5.7% higher than that in the control at 46 DAF, respectively.

The change in LAI also showed a single peak curve with the peak at about 46 DAF (Fig. 2). LAI from 1 to 46 DAF considerably reduced with the increase in uniconazole concentration, and application of 2, 4 or 8 mg uniconazole powder per kilogram seed brought 18.6, 20.3 and 25.3% reduction compared to the control at 46 DAF, respectively. However, uniconazole significantly increased LAI at 61 DAF (Fig. 2), and the greatest promotion occurred in B2, followed by B1 and then B3, which were 61.0%, 48.2% and 10.3%, higher than the control, respectively.

2. Shoot dry matter accumulation after flowering

Uniconazole significantly affected the dry matter accumulation in the leaf, stem, leaf stalk, pod and the total above-ground part (Table 2). Compared to the control, uniconazole treatment greatly reduced the dry matter accumulation in leaf, stem, leaf stalk, pod and the total above-ground part with increasing concentrations from 1 to 16 DAF. On the contrary, the dry matter accumulation

![Diagram](image.png)

Fig. 2. Effect of uniconazole on LAI of relay strip intercropping soybean.

| Change in LAI during 1 to 61 DAF | B0: control, B1: 2 mg kg⁻¹ seed, B2: 4 mg kg⁻¹ seed, B3: 8 mg kg⁻¹ seed. Different small letters mean significant differences among treatments with uniconazole at different concentration at α=0.05. Values represent a mean of 3 replicates. LSD Test. |
|-----------------------------|---------------------------------------------------------------------------------------------------------------|

### Table 2. Effect of uniconazole on the total above-ground biomass of relay strip intercropping soybean after flowering (g plant⁻¹).

| Organs       | Treatment | Days after flowering (d) | Days after flowering (d) | Days after flowering (d) | Days after flowering (d) |
|--------------|-----------|--------------------------|--------------------------|--------------------------|--------------------------|
|              |           | 1           | 16          | 31          | 46          | 61          |
| Leaf         | B0        | 2.84 Aa     | 5.75 Aa     | 6.90 Dd     | 10.92 Cc    | 13.78 Bb    |
|              | B1        | 2.46 Ab     | 5.28 ABb    | 11.94 Bb    | 14.23 Aa    | 21.69 Aa    |
|              | B2        | 1.64 Bc     | 3.91 Cc     | 13.43 Aa    | 14.48 Aa    | 22.98 Aa    |
|              | B3        | 1.35 Bd     | 3.19 Dd     | 9.44 Cc     | 13.27 ABb   | 12.94 Bbc   |
| Stem         | B0        | 1.73 Aa     | 3.81 Aa     | 4.66 Cd     | 11.28 Ab    | 12.74 Cc    |
|              | B1        | 1.72 Aa     | 3.72 Aa     | 8.35 Aab    | 11.33 Ab    | 18.23 Bb    |
|              | B2        | 1.13 Bb     | 3.71 Aa     | 8.77 Aa     | 11.96 Aa    | 22.99 Aa    |
|              | B3        | 0.65 Cc     | 2.84 Bb     | 6.71 Bc     | 11.28 Ab    | 13.35 Cc    |
| Leaf petiole | B0        | 0.98 Aa     | 1.51 Aa     | 2.00 Cd     | 3.87 Cc     | 5.75 Cc     |
|              | B1        | 0.63 Bb     | 1.41 Aa     | 3.31 ABb    | 4.27 AAb    | 6.27 Bb     |
|              | B2        | 0.47 Cc     | 1.24 ABb    | 3.80 Aa     | 4.67 Aa     | 7.65 Aa     |
|              | B3        | 0.36 Dd     | 0.93 Cc     | 3.08 Bc     | 3.90 Cc     | 6.21 Bb     |
| Pod          | B0        | –           | –           | 1.06 Cc     | 11.18 Cc    | 27.11 Cc    |
|              | B1        | –           | –           | 1.73 Bb     | 14.50 AAb   | 40.32 AAb   |
|              | B2        | –           | –           | 2.59 Aa     | 16.09 Aa    | 45.24 Aa    |
|              | B3        | –           | –           | 1.63 Bc     | 11.18 Cc    | 27.48 Cc    |
| Total above-ground biomass | B0        | 5.54 Aa     | 11.07 Aa    | 14.61 Dd    | 37.24 Dd    | 59.37 Cc    |
|              | B1        | 4.81 Ab     | 10.41 Aab   | 25.33 Bb    | 44.33 Bb    | 86.50 Bb    |
|              | B2        | 3.23 Bc     | 8.85 Ab     | 28.58 Aa    | 47.20 Aa    | 98.85 Aa    |
|              | B3        | 2.36 Cd     | 6.96 Bc     | 20.86 Cc    | 39.62 Cc    | 59.97 Cc    |

Within columns, means followed by the same small and capital letters are not significantly different at the 0.05 and 0.01 levels of probability, respectively. Least significance difference (LSD) test. B0: control, B1: 2 mg kg⁻¹ seed, B2: 4 mg kg⁻¹ seed, B3: 8 mg kg⁻¹ seed.
uniconazole after blooming. A positive correlation between and crop yield depends on the amount of dry matter plants (Yan et al., 2011b). The observed positive correlations photosynthetic rate and bean yield was observed in soybean

3. Plant height, stem diameter, yield and yield components of relay strip intercropping soybean

Plant height at the full pod stage significantly decreased with the increase in concentration of uniconazole (Table 3). However, uniconazole treatment increased stem diameter, and the increase was significant in B2 treatment.

The number of pods per plant and seed yield in the treatments in B2 were 22.7, and 26.2% higher than those in the control, respectively, and that in B1 were 3.2% and 6.1% higher than those in the control, respectively (Table 3). However, in B3, the number of pods per plant and number of seeds per pod were decreased significantly; 100-seed weight were increased significantly, but seed yield was not different from that of the control.

Discussion

Photosynthesis is the basic process of matter accumulation, more than 95% of the dry matter is supplied by photosynthesis, and crop yield depends on the amount of dry matter accumulation after blooming. A positive correlation between photosynthetic rate and bean yield was observed in soybean plants (Yan et al., 2011b). The observed positive correlations between photosynthetic rate and total chlorophyll content \( r = 0.89^* \) as well as photosynthetic rate and chlorophyll a content \( r = 0.94^* \) indicated that promotion of photosynthetic rate was ascribed to the higher total chlorophyll and chlorophyll a contents. Our results indicated that seed treatment with uniconazole increased the content of total chlorophyll and chlorophyll a. This shows that uniconazole-treated plants exhibit an intense dark green color due to enhanced chlorophyll synthesis (Tekalign and Hammes, 2004) or more densely packed chloroplasts per unit leaf area (Khalil, 1995). The present results showed that the highest photosynthetic rate, stomatal conductance, transpiration rate and chlorophyll a content were observed in plants treated with uniconazole in B2, followed by B1, whereas, the highest total chlorophyll content was observed in plants treated with uniconazole in B3 (Table 1; Fig. 1). Because chlorophyll a is the center pigment of photosynthesis, if its content is increased, more light energy will be utilized, resulting in higher photosynthetic rate and increased more dry matter accumulation.

On the contrary, we found that seed treatment with uniconazole reduced the dry matter accumulation in soybean plants concentration dependently from 1 to 16 DAF, but increased it from 31 to 61 DAF except in B3 (Table 2). These results show that seed treatment with uniconazole inhibited seedling height, leaf area per plant and dry matter accumulation at the seedling stage (Yan et al., 2010). The LAI is a key variable for the diagnosis and prediction of crop growth and yield (Jin et al., 2013). This study showed that seed treatment with uniconazole powder significantly decreased LAI from 1 to 46 DAF, but significantly increased LAI at 61 DAF (Fig. 2). Uniconazole powder has been found to effectively suppress growth in a wide range of plant species and the treated plants tend to be more compact in appearance (Kamoutsis et al., 1999; Starman and Williams, 2000; Sebastian et al., 2002), which provides good ventilation, photopermeability and high yield, and uniconazole could delay senescence of soybean leaves at the pod-setting stage. The higher chlorophyll content and delayed senescence of uniconazole-treated soybean leaves may be related to its influence on the endogenous cytokinin content. Uniconazole has been suggested to stimulate cytokinin synthesis, which enhances chloroplast differentiation and chlorophyll biosynthesis, and prevents chlorophyll degradation (Fletcher et al., 1982). Previous investigations revealed that the onset of senescence in several plant species was considerably delayed by triazoles (Davis et al., 1991; Binns, 1994). Uniconazole increased the rate of net leaf photosynthesis at pod-setting stage. This could be attributed to the higher chlorophyll content (especially for chlorophyll a content) and more compact plants in response to uniconazole treatment.

Uniconazole treatment increased soybean yield considerably and this may be due to the interplay of pod shading at the soybean seed formation stage, increased chlorophyll content,
enhanced rate of net photosynthesis, and retaining longer the photosynthetically active leaves. A strong positive correlation (0.99**) was observed between the pod number and seed yield indicating that the substantial increase in pod number was responsible for the yield increment. In agreement with our study, uniconazole treatment promoted soybean seed yield per plant in the trials of Zhang et al. (2007). However, it is not clear whether the observed yield increment was a consequence of the increase in pod number, seed number or 100-seed weight.

It is concluded that uniconazole is an effective plant growth regulator to increase soybean yield by increasing photosynthetic capacity (net photosynthesis), making compact plants and delaying senescence of photosynthetically active leaves in the relay strip intercropping system. The results are of specific importance to increase the productivity of soybean in the relay strip intercropping system. In our study, treatment of soybean seeds with 2 and 4 mg kg$^{-1}$ uniconazole powder produced more compact plants, increased chlorophyll content, number of pods per plant and seed yield. However, soybean yield was not significantly increased by the treatment of seeds with 8 mg kg$^{-1}$ uniconazole powder compared with the control, because the plant growth (plant height, LAI) was severely decreased by 8 mg kg$^{-1}$ uniconazole treatment. Flintham et al. (1997) reported that there is an optimum plant height and LAI for maximum photosynthetic capacity within the vegetation canopy, and crop yields may be reduced by reducing plant height and LAI below this level. In conclusion, seed treatment with uniconazole powder can improve the growth of soybean after flowering and yield of soybean, and the concentration which showed the greatest effect was 4 mg kg$^{-1}$ seed.

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