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# Seed Treatment with Uniconazole Powder Improves Soybean Seedling Growth under Shading by Corn in Relay Strip Intercropping System

Yanhong Yan<sup>1,2</sup>, Wanzhuo Gong<sup>1</sup>, Wenyu Yang<sup>1</sup>, Yan Wan<sup>1</sup>, Xiaolin Chen<sup>1</sup>,  
Zhongqun Chen<sup>1</sup> and Lanying Wang<sup>3</sup>

<sup>1</sup>College of Agronomy, Sichuan Agricultural University, Ya'an, Sichuan, 625014, China;

<sup>2</sup>College of Animal Science and Technology, Sichuan Agricultural University, Ya'an, Sichuan, 625014, China;

<sup>3</sup>Health Nuclear Institute, Sichuan Academy of Agricultural Science, Chengdu, Sichuan, 610066, China)

**Abstract:** The relay strip intercropping system of wheat-corn-soybean is widely used in southwest China. However, it is hard to obtain a stable production of soybean with this system, since soybean plants grow under shading by corn; the stems are thinner and susceptible to lodging. We examined the effects of seed treatment with uniconazole powder (0, 2, 4 and 8 mg kg<sup>-1</sup> seed) on the growth of soybean seedlings under relay strip intercropping, some morphological characteristics and yield. The seedling height, first internode length, cotyledonary node height and leaf area per plant were decreased, while the stem diameter, root dry weight, shoot dry weight, root volume, leaf greenness and root to shoot dry weight ratio were increased by uniconazole treatment. The root vigor and root active absorption area were also increased significantly by uniconazole treatment. Moreover, 2 and 4 mg kg<sup>-1</sup> uniconazole powder treatment increased shoot dry weight, number of pods per plant, number of seeds per pod and seed yield significantly. Thus, the results suggested that 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 relay strip intercropping system.

**Key words:** *Glycine max* (L.) Merr., Relay strip intercropping, Seed treatment with uniconazole powder, Seedling growth, Shading.

The demand of food is ever increasing with the increase of population. It is particularly important to increase the multiple crop index of land for the development of grain production. Intercropping is an effective means for increasing the income of a farming community as it helps better utilize the limited resources and ensures higher return per unit area and time (Roy et al., 1981). There is generally a trend toward higher yield under intercropping. Even in areas where the yield of the companion crop was substantially reduced, total yield was greater (Evan, 1960; Aggarwal et al., 1992).

In southwest China where the population is large but land resources limited, relay strip intercropping system is widely adopted. Since the 1970s, wheat-corn-sweet potato relay strip intercropping system is dominant in southern China (He et al., 1998), and plays an important role in food production. However, in recent years, with the improvement of living standard and diet of the farmers, the production status of sweet potato has also converted from the staple food to a subsidiary food gradually. Moreover, the

labor of planting, harvesting and transporting is intensive, storage of sweet potato difficult, and the benefit lower, which is easy to exacerbate barren soil and cause problems such as the large area of soil and water loss. Thus, the development of the sweet potato has been greatly restricted. In order to develop the agricultural production more effectively against this situation, we investigated and proposed a new multi-cropping system wheat-corn-soybean (Yang, et al., 2006). In this system, two meter wide strips were prepared in the field. On these strips, the wheat was sown on 1 m wide strip at the beginning of November and the corn was sown on 1 m wide area of the strips at the beginning of April (Figs. 1A, 2). After harvesting wheat in May, soybean was sown in three rows on the strip where wheat had been grown at the end of May or the beginning of June and harvested at the end of October (Figs. 1B, 2). Only soybean grows in the field after harvesting the corn at the beginning of August (Figs. 1C, 2). In the corn-legumes intercropping system, however, the grain yield was increased in corn but was reduced in legumes as compared

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**Abbreviation :** TTC, triphenyl tetrazolium chloride.

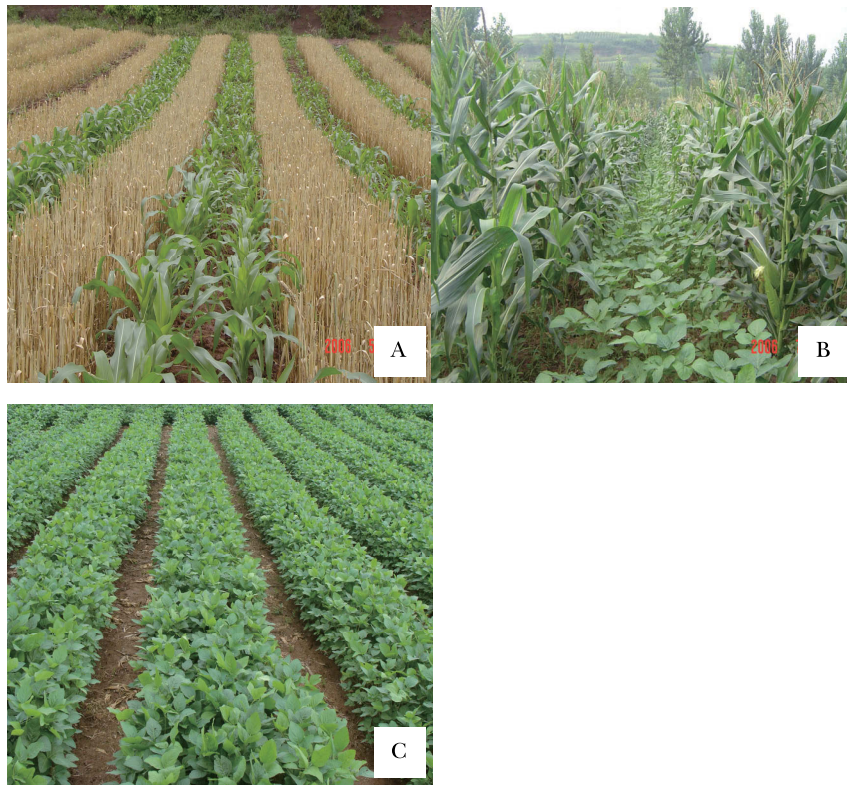


Fig. 1. Photograph of corn with wheat (A), soybean with corn (B) and soybean after harvesting of corn (C) in wheat-corn-soybean relay strip intercropping system in southwest China. In this system, two meter wide strips were prepared in the field. On these strips, the wheat was sown on 1 m wide strip at the beginning of November and the corn was sown on 1m wide area of the strips at the beginning of April (Figs. 1A, 2). After harvesting of wheat in May, the soybean was sown in three rows on the wheat strip at the end of May or the beginning of June and harvested at the end of October (Figs. 1B, 2). Only soybean grows in the field after harvesting the corn at the beginning of August (Figs. 1C, 2).

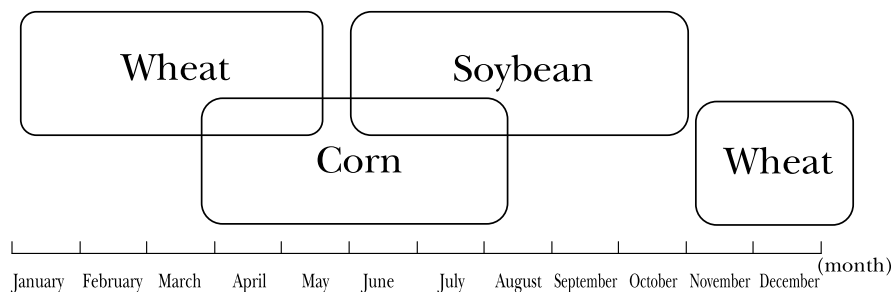


Fig. 2. Growth season of wheat-corn-soybean relay strip intercropping. In this system, wheat was sown at the beginning of November and harvested in May. Corn was sown at the beginning of April and harvested in August. Soybean was sown at the end of May or the beginning of June and harvested at the end of October.

with that in sole cropping. Corn grain yield was increased by 15–20% by intercropping with soybean, blackgram or peanut (Singh et al., 1986). Corn-soybean, corn-mungbean and corn-peanut intercropping reduced the yield of soybean, mungbean and peanut as compared with sole cropping (Chui and Shibles, 1984; Polthanee and Treloges, 2003). This is because the legumes are very sensitive to

shading (Herrera and Harwood, 1973) and they were grown under shading by corn in this system, resulting in thinner stems and easier to lodging.

Uniconazole [(E)-(RS)-1-(4-chlorophenyl)-4, 4-dimethyl-2-(1H-1, 2, 4-triazol-1-yl) pent-1-en-3-ol], which belongs to a group of triazoles, is a plant growth retardant that inhibits the elongation of the coleoptiles and stem, decreases

plant height, increases leaf chlorophyll content. In addition, uniconazole protects plants from various stresses (Upadhyaya et al., 1990; Kraus et al., 1991; Leul and Zhou, 1998). Chen et al. (2000) and Zhang et al. (2002) reported that foliar application of uniconazole retarded soybean shoot elongation, and increased the stem diameter, and the numbers of branches, flowers and pods under net cropping.

In the multi-cropping system of wheat-corn-soybean, soybean grows under the corn shade in the seedling stage, resulting in easy lodging due to longer internodes. The lodging risk is higher particularly in the plants with a longer internode on cotyledonary and first nodes (Mancuso and Caviness, 1991). Uniconazole might improve the lodging problem by inhibiting the internode elongation. However, Umezaki et al. (1991) did not find marked effects on the length and diameter of the first internode when they sprayed uniconazole at the floral differentiation stage, the beginning of flowering and the end of the vegetative growth stages, because the elongation of the first internode stopped before the treatment (Umezaki and Matsumoto, 1989). Our previous studies showed that uniconazole spraying at the vegetative stage ( $V_5$ ) increased the dry weight of the leaf and root after blooming, and dry weight of pod at maturity, while it had no effect on the seedling growth, especially on the growth of the lower internode (Yan et al. 2008, 2009).

The dry seed treatment with uniconazole powder is a simple low-cost technology, which requires only a small amount of reagent. Yang et al. (2005) reported that wheat seed treatment with uniconazole powder could improve the physiological function of root and leaf, increase the photosynthetic capacity of leaf, and promote the root capacity of absorbing and transporting, which are propitious to high yield. However, no such approach has been reported with soybean seeds. The objective of the present investigation was to determine the effects of seed treatment with uniconazole powder on the seedling growth and yield of soybean under shading by corn in relay strip intercropping system.

## Materials and Methods

### 1. Plant material and treatments

The experiment was conducted in a relay strip intercropping system at the farm of Sichuan Agricultural University in 2006–2007 and 2007–2008. The same soil, fertilizer and pots were used. Seeds of soybean [*Glycine max* (L.) Merr.] cultivar Gongxuan1, was treated with 5% water-dispersible uniconazole powder at the rate of B0, 0; B1, 2; B2, 4; and B3, 8 mg kg<sup>-1</sup> seeds. The reagent was provided by the United Chemical Factory of Lanyue, Chengdu City, Sichuan Province. The average particle diameter of the powder is 3.0  $\mu\text{m}$ . Seeds treated with uniconazole powder then were sown in the pots, 28.5 cm in height and 24 cm in

diameter, which contained 10 kg soil with fertilizer consisting of N=0.263 g, P<sub>2</sub>O<sub>5</sub>=0.512 g and K<sub>2</sub>O=0.426 g. Only two plants were allowed to grow per pot. Fertilizer containing 32.4 kg N ha<sup>-1</sup>, 63 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> and 52.5 kg K<sub>2</sub>O ha<sup>-1</sup>, was thoroughly mixed in the soil of each pot. Each treatment was conducted with three replicates, and each replicate had 30 pots.

Soybean pots were placed in three rows in every strip where the wheat was grown with 30 cm hole spacing after wheat harvest. In the experiment of 2006–2007, wheat (cv. Neimai 8) was sown on 3 November 2006 and harvested on 17 May 2007. Corn (cv. Chundan 418) was sown on 8 April 2007 and harvested on 11 August 2007. Soybean was sown on 29 May 2007 and harvested on 25 October 2007. In the experiment of 2007–2008, wheat was sown on 1 November 2007 and harvested on 11 May 2008. Corn (cv. Chundan 418) was sown on 3 April 2008 and harvested on 13 August 2008. Soybean was sown on 29 May 2008 and harvested on 25 October 2008. The light penetration through the corn canopy to the soybean was 85% when the soybean was sown, 69% at the vegetative stage  $V_5$ , 75% at the reproductive stage  $R_1$ , and 72% when the soybean plant in reproductive stage  $R_2$ , when corn was at maturity.

### 2. Measurement techniques

Six plants (two plants from each row) were taken randomly from each plot at 21, 28, 35 and 42 d after sowing and full pod stage for the morphological and physiological analysis.

#### (1) Morphological measurement

Seedling height, the first internode length, cotyledonary node height from the soil surface, and the stem diameter were measured. Roots and shoots were dried to a constant weight at 80°C after exposure to 105°C for 0.5 hr. Then the dry weight of the roots and shoots were measured, respectively. Root volume was determined by displacement of the entire root system with water in a fine graduated flask. Leaf area per plant was measured by the method of stiletto weigh. The leaf greenness of the 3rd leaf blades from the top was measured by using “SPAD-502” chlorophyll instrument (Konica Minolta Sensing, Inc.).

#### (2) Root vigor and root active absorption area

Root vigor was measured according to the TTC reduction method (Xiong, 2003). Root apex (200 mg) was soaked at 37°C in 5 mL of 0.4% TTC solution and 5 mL of 0.1 mol L<sup>-1</sup> phosphate buffer for 1 hr, then add 2 mL of 1 mM sulfuric acid to terminate reaction. Root tissue was taken out and homogenized in 5 mL of ethyl acetate containing a little of quartz sand with a mortar and pestle. The extract was put in a 10 mL volumetric flask, then the volume was constant. The absorbance of the extract was then measured at 485 nm, and the root vigor was calculated.

Root active absorption area was measured according to methylene blue trihydrate reduction method (Xiong,



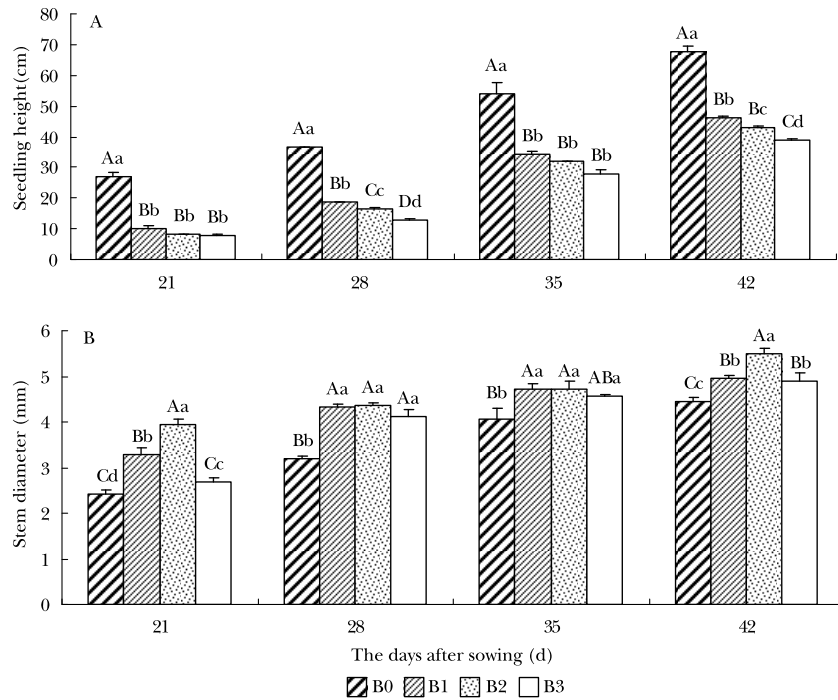


Fig. 3. The effect of uniconazole on plant height (A) and stem diameter (B) of relay strip intercropping soybean. Heavy striped, thin striped, dotted and open columns show uniconazole treatment at B0, B1, B2 and B3, respectively. Different small and capital letters mean significant differences among concentrations treatments in the same day after sowing at  $\alpha=0.05$  and  $\alpha=0.01$ . Values represent a mean of 3 replicates with SE. Tukey's Multiple Test. See Table 1 for B0, B1, B2 and B3.

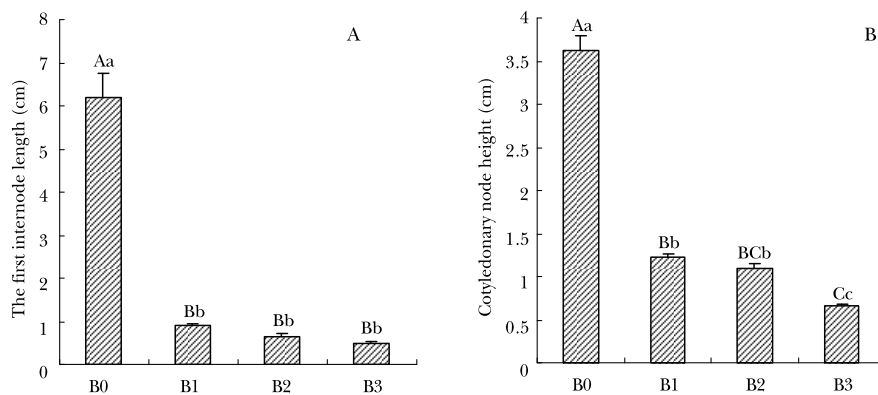


Fig. 4. The effect of uniconazole on the first internode length (A) and cotyledonary node height (B) at 21 d after sowing of relay strip intercropping soybean. See Fig. 3 for details.

2003). First, root volume was determined by displacement of the entire root system with water in a fine graduated flask;  $0.064 \text{ mg L}^{-1}$  methylene blue trihydrate liquor was put into 3 small beakers, the solution volume of each beaker was adjusted to about 10 times of root volume, and the volume of each solution in the beaker was measured accurately. Then the root was soaked in methylene blue solution for 1.5 min and finally, 1 mL solution from each beaker was diluted 10 times separately, and the absorbance at 660 nm was measured. The root active absorption area was calculated from the absorbance according to the

reduction of methylene blue trihydrate.

### (3) Dry matter moment

Dry matter moment at full pod stage was calculated by multiplying the shoot dry weight by plant height.

### (4) Yield and yield components

Twelve plants (four plants from each row) in each replication were harvested at maturity. Number of pods per plant, number of seeds per pod, 100-seed weight, and seed yield were examined.

### (5) Statistical analysis

Mean and standard error (SE) of three replications were

Table 1. Effects of uniconazole on leaf area and leaf greenness of soybean seedling under relay strip intercropping.

	Leaf area (cm <sup>2</sup> plant <sup>-1</sup> )				Leaf greenness			
	Days after sowing (d)				Days after sowing (d)			
	21	28	35	42	21	28	35	42
B0	149.4 Aa	291.8 Aa	409.9 Aa	1030.6 Aa	32.3 Bb	35.1 Bb	29.8 Ab	29.0 Cc
B1	69.9 ABb	99.3 Bb	325.1 Bb	601.2 Bb	44.6 Aa	35.4 Bb	31.7 Aab	30.5 BCbc
B2	44.1 ABb	93.3 Bb	290.9 BCbc	565.0 Bb	45.6 Aa	35.9 Bb	33.7 Aa	31.8 Bb
B3	29.7 Bb	91.7 Bb	256.4 Cc	429.4 Cc	45.5 Aa	39.1 Aa	34.7 Aa	39.0 Aa

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. Tukey's multiple tests. B0: control, B1: 2 mg kg<sup>-1</sup> seed, B2: 4 mg kg<sup>-1</sup> seed, B3: 8 mg kg<sup>-1</sup> seed.

Table 2. Effect of uniconazole on root vigour, root volume and root active absorption area of soybean seedling under relay strip intercropping.

	Root vigor ( $\mu\text{gTPF g}^{-1}\text{FW.h}^{-1}$ )				Root volume (cm <sup>3</sup> plant <sup>-1</sup> )				Root active absorption area (cm <sup>2</sup> plant <sup>-1</sup> )			
	Days after sowing (d)				Days after sowing (d)				Days after sowing (d)			
	21	28	35	42	21	28	35	42	21	28	35	42
B0	79.3 Bb	110.9 Cc	378.5 Cc	343.7 Cc	1.4 Cd	1.5 Cc	3.0 Cc	4.5 Dd	1.06 Bb	1.61 Bc	2.87 Bc	3.27 Cc
B1	82.2 Bb	162.4 Bb	452.3 Bb	434.3 Bb	1.8 ABb	2.5 Bb	4.6 Bb	6.8 Bb	1.31 Aa	1.97 Ab	3.07 Bb	3.64 Bb
B2	118.6 Aa	206.6 Aa	505.8 Aa	478.1 Aa	2.0 Aa	3.2 Aa	5.6 Aa	7.9 Aa	1.32 Aa	2.22 Aa	4.27 Aa	5.00 Aa
B3	31.9 Cc	93.7 Cd	178.6 Dd	313.4 Dd	1.6 BCc	2.2 Bb	4.5 Bb	5.5 Cc	0.95 Bc	0.98 Cd	1.95 Cd	2.98 Cd

See Table 1 for details.

calculated. The mean values were compared using one-way ANOVA followed by Tukey's multiple tests at the 5% and 1% level.

## Results

### 1. Seedling growth

Seedling height at 21 to 42 d after sowing significantly decreased with the increase in uniconazole concentration (Fig. 3A). The first internode length and cotyledonary node height were significantly reduced as compared with the control (B0) (Fig. 4) and leaf area per plant at 21 to 42 d after sowing was also reduced by the uniconazole treatment (Table 1).

However, uniconazole significantly increased stem diameter and leaf greenness at 21 to 42 d after sowing (Fig. 3B, Table 1). The stem diameter was increased most markedly by the treatment with uniconazole at B2. The results showed that the treatment at B2 increased the stem diameter by 23.6%, but that at B1 only 11.8%, and that at B3 10.6% for 42 d after sowing. However, leaf greenness increased as uniconazole concentration increased.

Root volume (Table 2) and root to shoot dry weight ratio (Fig. 5) were increased by the treatment with uniconazole powder (except at B3 at 21 d after sowing). The most remarkable promotion occurred at B2, followed by B1.

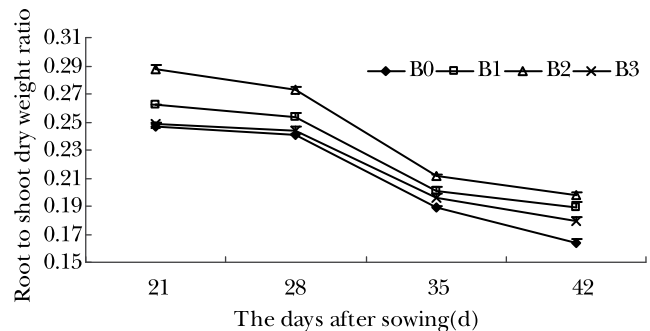


Fig. 5. The effect of uniconazole on root to shoot dry weight ratio of relay strip intercropping soybean. See Fig. 3 for details.

### 2. Root and shoot dry weight

Uniconazole treatment at B1 and B2 significantly increased root dry weight and shoot dry weight from 28 to 42 d after sowing, and the most strikingly at B2 (Table 3). However, root dry weight and shoot dry weight at 21 d after sowing were reduced by treatment at B1, although those at 42 d after sowing were significantly increased by the treatment at B3.

### 3. Root vigor and root active absorption area

Uniconazole treatment improved root vigor and root active absorption area (except at B3) under relay strip intercropping. From 21 to 42 d after sowing, the B2 and

Table 3. Effects of uniconazole on root dry weight and shoot dry weight of soybean seedling under relay strip intercropping.

	Root dry weight (g plant <sup>-1</sup> )				Shoot dry weight (g plant <sup>-1</sup> )			
	Days after sowing (d)				Days after sowing (d)			
	21	28	35	42	21	28	35	42
B0	0.082 Bc	0.112 Cc	0.204 Cc	0.233 Dd	0.331 Cc	0.464 Dd	1.08 Cc	1.42 Dd
B1	0.080 Bc	0.212 Bb	0.262 Bb	0.525 Bb	0.307 Cc	0.837 Bb	1.30 Bb	2.78 Bb
B2	0.186 Aa	0.347 Aa	0.384 Aa	0.767 Aa	0.647 Aa	1.268 Aa	1.82 Aa	3.88 Aa
B3	0.113 Bb	0.119 Cc	0.207 Cc	0.337 Cc	0.451 Bb	0.489 Cc	1.06 Cc	1.82 Cc

See Table 1 for details.

Table 4. Effects of uniconazole on plant growth, shoot dry weight and dry weight moment at full pod stage.

	Plant height (cm)	Stem diameter (cm)	The first internode length (cm)	Shoot dry weight (g plant <sup>-1</sup> )	Dry weight moment
B0	120.0 Aa	0.98 Bb	8.6 Aa	37.24 Bc	4467.0 Aa
B1	102.6 Bb	1.08 ABb	2.3 Bb	42.33 Aab	4344.1 Ab
B2	97.8 Cc	1.21 Aa	1.8 BCb	44.20 Aa	4321.6 Ab
B3	89.6 Dd	0.98 Bb	1.1 Cc	39.62 ABbc	3552.2 Bc

See Table 1 for details.

Table 5. Effects of uniconazole on yield and yield component of relay strip intercropping soybean in 2006–2007.

	Number of pods (plant <sup>-1</sup> )	Number of seeds (pod <sup>-1</sup> )	100-seed weight (g)	Yield (kg ha <sup>-1</sup> )
B0	58.8 Cc	1.31 Dd	23.7 Aab	1819.8 Bbc
B1	68.1 Bb	1.61 Aa	23.4 Ab	2570.2 Aa
B2	73.0 Aa	1.54 Bb	23.3 Ab	2618.6 Aa
B3	55.7 Dd	1.46 Cc	24.0 Aa	1954.9 Bb

See Table 1 for details.

Table 6. Effects of uniconazole on yield and yield component of relay strip intercropping soybean in 2007–2008.

	Number of pods (plant <sup>-1</sup> )	Number of seeds (pod <sup>-1</sup> )	100-seed weight (g)	Yield (kg ha <sup>-1</sup> )
B0	61.6 Cc	1.70 ABa	24.25 Bc	2533.4 Bc
B1	63.6 Bb	1.71 Aa	24.68 ABbc	2688.6 Bb
B2	75.6 Aa	1.70 Aa	24.88 ABab	3196.8 Aa
B3	60.5 Dd	1.67 Bb	25.28 Aa	2557.8 Bc

See Table 1 for details.

B1 increased significantly root vigor and root active absorption area over that of the control. The root vigor was significantly increased by the treatment at B2 as compared with that at B1. There was no significant difference in the root active absorption area between the treatments at B2 and B1 until 35 d after sowing (Table 2).

#### 4. Plant growth, shoot dry weight and dry weight moment at full pod stage

Plant height, the first internode length and dry weight moment at the full pod stage significantly decreased with

the increase in concentration of uniconazole (Table 4). However, uniconazole treatment increased stem diameter and shoot dry weight, and those of B2 treatment were increased significantly.

#### 5. Yield and yield components of relay strip intercropping soybean

In the plants treated with uniconazole at B2, the number of pods per plant, number of seeds per pod and seed yield were 24.1, 17.6 and 43.9% higher than those in the controls respectively (Table 5). However, in those

treated at B3, the number of pods per plant was decreased significantly; 100-seed weight and seed yield were not different from the control in 2006–2007. In 2007–2008, the number of pods per plant, number of seeds per pod and seed yield in the treatment at B1 and B2 were significantly higher than those in the control. However, in the treatment at 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 (Table 6).

### Discussion

Lodging and shading reduce root and shoot dry weight, number of pods per plant, number of seeds per plant, 100-seed weight and yield, the quality of grain, and the efficiency of mechanical harvesting (Cooper, 1971; Shapiro and Flowerday, 1987; Mancuso and Caviness, 1991; Zheng et al., 2009). Harvest loss due to lodging has been reported to be 22% of potential yield in soybean (Noor and Caviness, 1980) and 21% in barley (Briggs, 1990). Moreover, the yield of legumes is reduced by shading when they are intercropped with corn (Chui and Shibles, 1984; Polthanee and Trelo-ges, 2003). Many reports indicate that shading or reduced light intensity conditions increase the plant height, the first internode length and leaf area per plant; and reduce the stem diameter (Mancuso and Caviness, 1991; Nagasuga and Kubota, 2008; Hossain et al., 2009; Oh et al., 2009). These morphological traits directly correlate with lodging resistance (Zuber et al., 1999; Kashiwagi et al., 2005). A significant yield reduction was caused by lodging at the full pod stage (Noor and Caviness, 1980; Huang et al., 2008); and plant height, the first internode length, stem diameter, and dry weight moment were directly correlated with lodging resistance (Huang et al., 2008). In the wheat-corn-soybean relay strip intercropping system, treatment of soybean seeds with uniconazole powder significantly decreased seedling height, the first internode length, cotyledonary node height and leaf area per plant, and the consequently increased stem diameter and leaf greenness at the seedling stage, indicating that soybean seed treatment with uniconazole powder effectively improved the seedling growth of soybean, and it increases the lodging resistance under relay strip intercropping. Similarly, Zhou and Ye (1996) reported that uniconazole can significantly reduce seedling height, and increase stem width and chlorophyll content of rape seedling. In the present study, we also found that leaf area per plant was significantly decreased by uniconazole dose-dependently, and the greenness was increased. However, shoot dry weight was the heaviest at the full pod stage in the plants treated with 4 mg kg<sup>-1</sup> uniconazole, which was consistent with seed yield. This indicates that increase of greenness is effective for photosynthesis in the plant community, but only up to the optimum degree. In our study, uniconazole

treatment decreased plant height, the first internode length and dry weight moment at the full pod stage, significantly, which also increases lodging resistance.

Root growth is another important key factor in lodging resistance (Yang et al. 2005). A stronger root system will also increase the lodging resistance, and consequently improve the vegetative and reproductive growth of soybean plants. Seed treatment with uniconazole powder significantly increased the root volume, root vigor and the root active absorption area of these seedlings under relay strip intercropping (Table 2). This suggested that the treatment of seeds with uniconazole powder enhanced shading tolerance of soybean seedlings via an improved root system mass. The higher ratio of root to shoot dry weight of seedlings in the plants treated with uniconazole powder, indicating that uniconazole greatly retarded elongation growth of the soybean shoot rather than root growth. Similarly, Ye and Zhou (1995) and Leul and Zhou (1998) reported that uniconazole can improve the root to shoot dry weight ratio of rape seedling. This was consistent with our results that uniconazole decreased seedling height, the first internode length, and cotyledonary node height.

In this study, treatment of soybean seeds with 2 and 4 mg kg<sup>-1</sup> uniconazole powder increased shoot dry weight, number of pods per plant and seed yield. However, shoot dry weight and seed yield were not significantly influenced by treatment with 8 mg kg<sup>-1</sup> uniconazole (Tables 4, 5, 6), because the plant height was seriously shortened by 8 mg kg<sup>-1</sup> uniconazole treatment. Flintham et al. (1997) reported that there is an optimum plant height for maximum photosynthetic capacity within a vegetation canopy, and reducing plant height below this level may reduce crop yields. In conclusion, seed treatment with uniconazole powder can improve seedling growth and yield, and the concentration which showed the greatest effect was 4 mg kg<sup>-1</sup> seed.

### Acknowledgements

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\* In Chinese.

\*\* In Chinese with English abstract.

\*\*\* In Japanese with English abstract.