Evaluation of Soybean (*Glycine max*) Stem Vining in Maize-Soybean Relay Strip Intercropping System

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Abstract: Forming a method of judging the degree of soybean vining in the intercropping system is very important for estimating the shade tolerance of soybean germplasm and choosing the special soybean varieties for intercropping system. Thirty varieties were subjected to two treatments (sole and maize-soybean relay strip intercropping system) with three replications in a complete randomized block design. Light environment characteristics in two cropping systems were measured. At the beginning of the bloom stage of soybean when maize was mature, the soybean stem morphology characteristics stem length, node number, hypocotyl length, internode length, stem diameter, stem breaking strength and stem biomass were measured. The results revealed that the intercropped soybean could capture 13.8% photosynthetic active radiation at the vegetative stage, as compared with the sole crop soybean, with longer soybean main stem, internode and hypocotyl, and lower stem diameter at this stage. The relative value of agronomic traits in the sole and intercropping system could be used to calculate the vining severity index (VI) and the weighted vining index (WVI), and the latter could be used as the comprehensive index of the degree of soybean vining in the relay strip intercropping system. Based on the values of WVI, through the Hierarchical Cluster Analysis, the soybean varieties were divided into the 5 clusters, normal, mild, moderate, severe and extreme vining. Eight of the 30 varieties of soybean were normal vining grade and could be used as the parent plants to breed special varieties for intercropping.

Key words: Lodging resistance, Relay intercropping, Shade, Soybean, Stem.

Intercropping is an important way to increase the land-use efficiency and it is widely practiced to increase the grain yield (Willey, 1990; Li et al., 2009; Lithourgidis et al., 2011). In China, the soybean industry has been facing an unprecedented challenge, the pressures were mainly from declining of domestic main soybean producing areas and large amount of imported soybean from foreign countries (Yang et al., 2008). Maize-soybean relay strip intercropping system has contributed greatly to soybean production and to maintain maize yields. During the past 10 years, this intercropping system has been used in about 2 million hectare in Sichuan province (one of provinces in the southwest of China), and 3600 million kilogram soybean has been produced. The Chinese Ministry of Agriculture has recommended wide use of this system as a main agricultural technology (Yang et al., 2008; Yong et al., 2009; Yan et al., 2010; Zhang et al., 2011).

However, in the maize-soybean relay strip intercropping system, soybean seedlings were generally overshadowed by maize during the co-growth stage. In order to capture more solar energy, the soybean stems were thinner and more vining in the intercropping system than in the sole cropping system, and also the plant height of soybeans was higher in the intercropping population than in the sole population (Kayhan 1999; Wu et al., 2007). Therefore, the quality and yield of intercropping soybean were affected by vining, and soybean was difficult to harvest (Wang et al., 2008).

Vining is a common trait in wild soybean, and the degree of vining is related to the evolution process. This is consistent with the previous reports. Li (1994) indicated that the wild-type soybean stems vine easily, semi-wild type soybean stems sometimes are prone to vining. Liu et al. (2011) found that soybean stem often vined at the seedling stage when soybean were intercropped with maize, and the degree of vining varied with the soybean genotype.

In the practical farming system, coating the shading-
tolerant varieties with Uniconazole powder was very useful way to avoid soybean seedling vining in the relay strip intercropping system (Yan et al., 2010; Yan et al., 2011). However, there is no comprehensive or systematic method to evaluate the vining degree of different soybean varieties.

In this research, the maize-soybean relay strip intercropping system was used, and light environment characteristics and specific characteristics of vining were measured 1) to understand the specific difference in soybean vining traits under maize-soybean relay strip intercropping system, and 2) to build up the evaluating method of soybean vining in the intercropping system.

Materials and Methods

1. Field experiments

Field experiments were conducted at the Experimental Farm, Sichuan Agricultural University, Ya’an (29°59’ N, 103°00’ E), Sichuan Province, China from March to October in 2010 and 2012. The experimental soil texture was clay.

The different soybean varieties and two cropping system (maize-soybean relay strip intercropping and soybean sole cropping) were designed with a complete randomized block design, with three replications. In 2010, thirty soybean varieties (Table 1) were used. In 2012, in order to validate the researching results in 2010, ten of the thirty soybean varieties were used with the same experiment design.

The maize variety and the planting methods of the relay strip intercropping system were the same as those described in the technical procedures of relay-cropping system of wheat, maize and soybean, which was issued by Sichuan Province Department of Agriculture in 2009 (Yang et al, 2009). The maize variety, Chuandan 418 was used in both years. Maize was sown into a flat wet seedbed on 26 March and transplanted into the field on 7 April. Soybean seeds were sown on 13 June. Maize was harvested on 5 August, and soybean on 28 October. The co-growth time of maize and soybean was 53 days. In the field, two rows of maize between two rows of soybean were planted in alternating 2.0-m-wide strips including a 0.8-m-wide maize strip (two maize rows with 0.4 m inter-row distance) and a 0.8-m-wide soybean strip (two soybean rows with 0.4 m inter-row distance). The space between maize strip and soybean strip was 0.6 m (Fig. 1). The plant spacing within rows of maize and soybean were 0.2 m and 0.1 m, respectively. The row spacing and hill spacing of sole soybean were 1 m and 0.1 m, respectively. The row length was 7 m. The other cultural practices for growing maize and soybean crops were as recommended in the area.

2. Agronomic traits

At the beginning of the blooming stage of soybean when maize was being harvested, ten plants of soybean were taken randomly from each replication to measure the following agronomic traits: main stem length, average internode length, hypocotyl length, stem diameter, and node number. The 4th internodes were used to measure WVI was calculated referring to the methods of Qi et al. (2012) based on the agronomic traits in 2010. K-Means cluster was used to classify the 30 genotypes according to the WVI. The vining degree of soybean in intercropping system was divided into 5 grades. The numbers 1, 2, 3, 4 and 5 in the column of vining grade represent normal vining, mild vining, moderate vining, severe vining and extreme vining, respectively.

| Number | Varieties          | WVI | vining Grade | Number | Varieties          | WVI | vining Grade |
|--------|--------------------|-----|--------------|--------|--------------------|-----|--------------|
| F01    | Jiuyuhuang         | 0.90| 2            | F16    | WYDongdou          | 0.98| 2            |
| F02    | Zongmaodou         | 1.00| 2            | F17    | LZJiuyuehuang      | 0.93| 2            |
| F03    | XCXiaohuangdou     | 1.08| 3            | F18    | Dahedou            | 0.96| 2            |
| F04    | Nandk 022-2        | 0.94| 2            | F19    | Shiyuehuang        | 1.33| 4            |
| F05    | Nandou 12          | 0.69| 1            | F20    | Gongxuan 5         | 0.79| 1            |
| F06    | LZShiyuehuang      | 0.74| 1            | F21    | PWHuangdou         | 0.93| 2            |
| F07    | TNXiaohuangdou     | 1.05| 3            | F22    | Gong 109           | 0.93| 2            |
| F08    | Dabaimao           | 0.99| 2            | F23    | Gong 378-1         | 1.03| 3            |
| F09    | GXShiyuehuang      | 0.95| 2            | F24    | MYHuangmaodou      | 1.11| 3            |
| F10    | Zhuyaozi           | 0.80| 1            | F25    | Xiaohuangdou       | 0.87| 2            |
| F11    | Huangqiaodou        | 1.57| 5            | F26    | Chihuangdou        | 0.83| 1            |
| F12    | Heqidou            | 0.95| 2            | F27    | Huangdou           | 0.74| 1            |
| F13    | HSXiaohuangdou     | 1.43| 4            | F28    | Texuan 11          | 0.88| 2            |
| F14    | Hepidou            | 1.13| 3            | F29    | Guixia 3           | 0.95| 2            |
| F15    | Xibaimao           | 0.75| 1            | F30    | Gongxuan 1         | 0.64| 1            |

Table 1. Local soybean varieties across Southwest of China used in this experiment
system diameter and breaking strength according to the procedure described by Kokubo et al. (1989). The instrument used for measuring breaking strength was a digital force tester (YFD-1, Zhejiang Top Instrument, China). Then the stems were dried to a constant weight at 80°C after exposure to 105°C for 0.5 hr, and the dry weight of the stem was measured as the stem biomass. The mean values were compared using two-way ANOVA followed by Duncan’s multiple range test at the 5 and 1% levels, three replications were calculated.

3. Photosynthetic parameters of the soybean strip

The light environment characteristics in the maize-soybean relay strip intercropping system and soybean sole cropping system were measured using AvaField (Avantes Corporation, Netherlands). The measuring wavelength was between 200 and 1000 nm with 2.4 nm spectral resolution. The sensors were placed above the soybean rows, and the spectral characteristic was measured at 9-10 a.m. every 7 to 10 d from soybean emergence to maize harvest on sunny days. The 10 horizontal points with 10 cm spacing in each plot of three replications were measured every time. The light spectral data in the same cropping system were averaged and analyzed with Ava-Soft 7.0 (Yang, 2012). Photosynthetically active radiation was estimated by integrating the spectral energy between 400 nm and 700 nm. The ratio of red light (R) to far red light (FR) was defined as follows (Franklin and Whitelam, 2005):

\[
R:FR \text{ ratio} = \frac{\text{spectral energy integral between } 655 \text{ and } 665 \text{ nm}}{\text{spectral energy integral between } 725 \text{ and } 735 \text{ nm}}
\]

Based on the measured soybean agronomic traits in the maize-soybean relay strip intercropping system and in the soybean sole cropping system, the following equation was used to calculate Vining Index (VI):

\[
VI = \left| 1 - \frac{X_r}{X_s} \right| \div \left| 1 - \frac{X_{ar}}{X_{as}} \right|
\]

where \(X_r\) is the agronomic trait value of every variety measured in the intercropping system, and \(X_s\) is that measured in the sole cropping system, \(X_{ar}\) is the average agronomic trait value of all varieties in intercropping system, and \(X_{as}\) is that in the sole cropping system (Qi et al., 2012).

Microsoft Excel 2007 and SPSS (19.0) analysis software were used to calculate the Weighted Vining Index (WVI) as following equation:

\[
WVI = \sum_{i=1}^{n} \left( \frac{VI \times \left| r_i \right|}{\sum_{i=1}^{n} \left| r_i \right|} \right)
\]

\(r_i\) is the related coefficient between the VI of single

Fig. 1. Diagram showing the arrangement of the rows of maize (○) and soybean (●) in the maize-soybean relay strip intercropping system.

Fig. 2. Photosynthetic parameters (spectral energy) in sole cropped canopy and maize-soybean relay strip intercropped canopy.
agronomic characters and the average VI of all agronomic characters. $\sum_{i=1}^{n} |ri|$ is the Index Weight, which means the importance degree of the number $i$ index (Qi et al., 2012).

### Results

1. **Photosynthetic parameters in relay strip intercropped canopy and sole cropped canopy**

   In the maize-soybean relay strip intercropping system shown in Fig. 1, soybean was co-grown with maize in the field from sowing to flowering. Because of the shading by maize, the intercropped soybean cannot absorb sufficient light in the seedling stage (Fig. 2). Photosynthetically active radiation, estimated by integrating the spectral energy from 400 nm to 700 nm, was 4776.4 μwatt m$^{-2}$ in the sole cropped soybean, but it dropped to 662.8μwatt m$^{-2}$, in the relay intercropped soybean strip. Compared with the sole cropped soybean, intercropped soybean could capture only 13.8% of the photosynthetic active radiation at vegetative stage. In addition, the R: FR ratio in the sole cropped soybean row was 1.14. In the maize-soybean intercropped soybean row, radiation in the FR region was poorly absorbed by maize and, consequently, the light that was transmitted through, or reflected, from maize was depleted in R and significantly enriched in FR wavelengths. The ration of R : FR above the canopies of intercropped soybean strip dropped to 0.64.

2. **Soybean stem traits in relay intercropping and sole cropping**

   The direct performance of soybean stem vining in intercropping system was slender (Fig. 3). The results showed that the main stem, internode and hypocotyl of soybean were longer by 125.5%, 164.7% and 62.9%, respectively, in maize-soybean relay strip intercropping system than in the sole cropping system, respectively (Table 2). The node number and stem diameter in intercropping system were 85% and 56.1% of those in sole cropping, respectively. The stem breaking strength and stem biomass in relay strip intercropping were 56.1% and 38.2% of those in the sole crop soybean, respectively.

   In the two cropping systems, all variation coefficients of the 7 agronomic traits in the soybean varieties were over 13% (Table 2). The two-way ANOVA showed significant differences between the varieties and cultivations in the 7 agronomic traits. The variety × cultivation interactions were significant for all the traits.

3. **Correlation analysis of stem characteristics in soybean**

   In the two cropping systems, there was a significant positive correlation between main stem length and node numbers, between main stem length and internode length, and between stem diameter and stem breaking strength.

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**Table 2. Morphological traits and stem biomass of different soybean varieties in the relay strip intercropping system and sole cropping system.**

| Treatment          | Statistical parameter | Main stem length (cm) | Node numbers | Hypocotyl length (cm) | Internode length (cm) | Stem diameter (cm) | Stem breaking strength (N) | Stem biomass (g/plant) |
|--------------------|-----------------------|-----------------------|--------------|-----------------------|-----------------------|----------------------|----------------------------|------------------------|
| Sole cropping      | Average               | 56.8                  | 15.8         | 3.3                   | 3.6                   | 0.7                  | 310.9                      | 8.4                    |
|                    | Standard deviation    | 18.7                  | 2.5          | 0.6                   | 1.0                   | 0.1                  | 114.4                      | 4.4                    |
|                    | Coefficient of variation (%) | 33.0                  | 15.6         | 18.2                  | 28.1                  | 15.3                  | 36.8                       | 52.7                   |
| Intercropping      | Average               | 128.6                 | 13.4         | 5.4                   | 9.5                   | 0.4                  | 136.6                      | 5.2                    |
|                    | Standard deviation    | 36.4                  | 1.8          | 0.8                   | 1.9                   | 0.1                  | 62.7                       | 2.8                    |
|                    | Coefficient of variation (%) | 28.3                  | 13.3         | 14.4                  | 19.9                  | 16.0                  | 45.9                       | 54.0                   |
|                    | Relative value (%)    | 226.4                 | 84.8         | 163.6                 | 263.9                 | 57.1                  | 43.9                       | 61.9                   |

$F$ value

| $F$ value | V         | 2.4**    | 6.6**    | 3.9**    | 2.2*     | 3.0**    | 4.2**    | 9.3**    |
|-----------|-----------|----------|----------|----------|----------|----------|----------|----------|
|           | C         | 159.2**  | 68.7**   | 328.4**  | 383.9**  | 367.1**  | 144.8**  | 57.0**   |
|           | VxC       | 3.4**    | 3.4**    | 1.6*     | 2.6**    | 3.4**    | 3.3**    | 1.8*     |

$F$ value is the result from two-way ANOVA; V represents soybean variety, C represents cropping system, V × C represents the interaction between variety and cropping system. *: 0.01 < $P$ < 0.05; **: $P$ < 0.01.
On the other hand, the cropping system somewhat changed the correlation between the stem characteristics and node number and internode length. There was a significant negative correlation between main stem length and diameter, and between internode length and stem biomass. In the intercropping system, however, the correlations mentioned above were not significant. The results showed that the light environment in intercropping could change the growth of soybean stem.

4. Evaluation of soybean vining in relay strip intercropping system

In order to measure the vining parameters in different soybean varieties in maize-soybean relay strip intercropping system, soybean stem traits in the two cropping systems were used to calculate the vining severity index (VI) and the weighted vining index (WVI). The high WVI value means that the relay strip intercropping environment had a heavy impact on soybean stem which was easy to vine. The WVI values ranged from 0.64 to 1.57, and the average value was 0.96, the standard deviation was 0.21.

Based on the value of WVI, k-Means cluster was used to classify the 30 genotypes. The vining degree of soybean in intercropping system was divided into 5 grades, which were normal vining, mild vining, moderate vining, severe vining and extreme vining (Table 1).

Eight of the 30 soybean varieties were classified into normal vining, accounting for 26.7% of the varieties. There were 14 varieties which were classified to mild vining, accounting for 46.7% of the total varieties. The 5 varieties XChaoxiangdou, TXiaoouhoudou, Hepidou, Gong 378-1 and MY Huangmaodou were moderate vining and the 2 varieties HSxiaoouhongdou, Shiyuehuang were severe vining.

5. Regression analysis of WVI and agronomic traits

In order to apply the WVI to quantitatively describe the vining of different soybean varieties in intercropping system, the regression equation was built through stepwise multiple regression analysis based on the traits relative values of the main stem length, node number, hypocotyl length, average internode length, stem diameter, stem breaking strength and stem biomass. The relative trait value was the ratio of agronomic trait value in the intercropping system to that in the sole cropping system. The regression equation was:

\[ Y = 0.875 + 0.070X_1 - 0.678X_2 + 0.396X_3 + 0.125X_4 - 0.381X_5 - 0.195X_6 - 0.144X_7 \]

\( F = 17360.01, R^2 = 0.998 \).

\( Y \) was the WVI, \( X_1 \), \( X_2 \), \( X_3 \), \( X_4 \), \( X_5 \), \( X_6 \) and \( X_7 \) represented the relative trait values of main stem length, node number, hypocotyl length, average internode length, stem diameter, stem breaking strength and stem biomass, respectively.

In the maize-soybean relay intercropping system and soybean sole cropping system in 2012, we planted 10 of the 30 soybean varieties, which were studied and classified into different vining grades in 2010 (Table 1), to validate the evaluation method of the vining of soybean in the intercropping system (Table 4). Calculation of the WVI by the regression equation showed that 3 of the 10 varieties were normal vining and moderate vining, two were mild vining, one was severe vining and one extreme vining (Table 4). Although there were differences in WVI between the values in two years, the relative value of the WVI and the classifying results of vining were the same in both years.

Discussion

The vining of soybean in the intercropping system is the result of shade-avoidance. The mass of evidence shows...
that, shade-avoidance responses were typically initiated in advance of canopy closure and light became limiting (Franklin and Whitelam, 2005). Thus, plants respond predominantly to the reduction in R: FR ratio of light reflected from surrounding vegetation (a proximity signal) and therefore initiate escape responses in anticipation of being shaded.

Rapid shoot elongation is one of the typical shade avoidance responses (Schmitt et al., 2003). However, the degree of response varies with the variety or ecotype (Sasidharan et al., 2008). In soybean in the intercropping system, shade avoidance responses lead to vining, and the degree of vining is related to the shade endurance of soybean varieties.

In the maize-soybean relay strip intercropping system, the vining is an important indicator of the shade endurance of soybean varieties. Chen et al. (2003) and Lang (2000) chose plant height, pod length, biomass, pod number, grain weight and hundred seed weight to evaluate the shade endurance of soybean germplasm at harvest stage. In their studies, maize and soybean were sown at the same time. The soybean was shaded by the maize after the stem morphology has formed. Therefore, the stem of soybean would not be vining, and it was feasible to use the agronomic traits at harvest stage to evaluate shade endurance.

In the relay intercropping system, lodging of soybean occurs usually because of vining at the vegetative stage. However, the feature of lodging is entirely different from what we assume. Usually, with the growth and development of leaves and pod, the stem cannot bear the weight of canopy, and lodging occurs (Weber and Fehr, 1966). Therefore, the gradient of main stem was used to judge the degree of lodging at harvest time. The fresh matter moment (plant height × above ground fresh weight), dry matter moment (plant height × above ground dry weight), fresh weight moment per unit of stem broken strength dry weight moment per unit stem broken strength (PDS), and height of the center of gravity were used to evaluate the lodging resistance indices in soybean (Huang et al., 2008; Zhou et al., 2009). However, in the relay intercropping system, because of the vining, the soybean main stem are curve or voluble, and the gradient of main stem cannot reflect the occurrence of lodging. In the same way, the above characters, such as height of the center of gravity, are lacking in biological concepts. So, it is infeasible to use the conventional methods to study the soybean lodging in the relay intercropping. It must be connected with the vining. The relationship between the lodging ratio and the weighted vining index should be studied further.

Table 4. Regression equation used to descript the vining of different soybean varieties in intercropping system quantitatively

| Number | X1  | X2  | X3  | X4  | X5  | X6  | X7  | Y     | Grade of vining |
|--------|-----|-----|-----|-----|-----|-----|-----|-------|----------------|
| F15    | 1.81| 0.85| 1.35| 2.15| 0.52| 0.74| 0.76| 0.69  | 1              |
| F05    | 1.52| 0.88| 1.65| 1.72| 0.61| 0.64| 0.50| 0.72  | 1              |
| F06    | 1.99| 0.95| 1.81| 2.11| 0.74| 0.70| 0.54| 0.75  | 1              |
| F04    | 2.38| 0.85| 1.01| 2.98| 0.44| 0.23| 0.50| 0.89  | 2              |
| F12    | 2.15| 0.76| 1.59| 2.86| 0.71| 0.65| 0.66| 0.91  | 2              |
| F17    | 3.12| 0.95| 1.58| 3.45| 0.68| 0.27| 0.62| 1.01  | 3              |
| F03    | 2.10| 0.77| 1.90| 2.79| 0.66| 0.44| 0.57| 1.07  | 3              |
| F07    | 3.58| 0.85| 1.83| 4.33| 0.50| 0.86| 1.02| 1.20  | 3              |
| F19    | 1.63| 0.66| 2.71| 2.41| 0.44| 0.21| 0.68| 1.45  | 4              |
| F11    | 3.82| 0.85| 2.80| 4.53| 0.49| 0.24| 0.47| 1.77  | 5              |

Y is the WVI computed by the regression equation. X1, X2, X3, X4, X5, X6 and X7 represent relative trait values of main stem length, node number, hypocotyl length, internode length, stem diameter, stem breaking strength and stem biomass, respectively. The relative trait value is the ratio of agronomic trait value in the intercropping system to that in the sole cropping system. The agronomic trait values are taken from the experiment of 2012.

Conclusion

In the maize-soybean relay strip intercropping system,
the low photosynthetically active radiation and the R: FR ratio lowered by maize canopy were the primary cause of soybean vining. The typical trait of vining in the intercropping system was long and slender stem. WVI could be a comprehensive index of the degree of soybean vining in the relay strip intercropping system. Through the hierarchical cluster analysis based on the values of WVI, the soybean varieties could be divided into the 5 clusters normal, mild, moderate, severe and extreme vining. By validating the regression equation between the WVI and the relatively agronomic traits in sole cropping and intercropping system WVI could be calculated, and the degree of soybean vining in the intercropping system determined accurately. The above method is beneficial for estimating the shade tolerance of soybean germplasm and choosing the special soybean varieties for the intercropping system.

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