



Relationship between cellulose accumulation and lodging resistance in the stem of relay intercropped soybean [*Glycine max* (L.) Merr.]



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ABSTRACT

Stem lodging is the most important constraint for soybean at seedling stage in maize–soybean intercropping in China. This study was conducted to determine whether cellulose accumulation and lodging resistance of soybean was affected by different cropping systems and soybean varieties with different shading tolerance at seedling stage. Three soybean varieties – Nandou 032–4 (shade susceptible), Jiuyue-huang (moderately shade tolerant) and Nandou 12 (shade tolerant) – were used to investigate the effect of intercropping on soybean lodging behavior and cellulose accumulation in stems and its relationship to lodging resistance. Shading by maize significantly reduced cellulose accumulation and the stem breaking strength of the soybean, which were significantly negatively correlated with the lodging rate. In the maize–soybean intercropping system, shading inhibited sucrose transportation and degradation into cellulose in the soybean stem. Less content of cellulose in soybean stem at the seedling stage resulted in lodging in the intercropping system. Compared with shade susceptible varieties, Nandou 12 had higher cellulose accumulation and related enzyme activities in the stem, and thus the stem breaking strength and lodging resistance of the basal stem increased. The higher activities of sucrose phosphate synthase and sucrose synthetase in the stem were associated with shade tolerance and lodging resistance. Thus, we concluded that the high physical strength and high cellulose contents of the stem of shade tolerant soybean reduced the rate of lodging in the maize–soybean intercropping system.

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1. Introduction

Soybean [*Glycine max* (L.) Merr.] originated from China and has been cultivated for more than 5000 years (Fukuda, 1933; Zhao and Gai, 2004). Soybean is ranked in the world at fourth position regarding production and at second position with respect to its processing and consumption. China is the largest soybean importing country (Xu, 2013). There is a large gap between soybean production and the fast growing demand in China, leading to a gradual decline in the self-supply rate of domestic soybean production. In addition, in northeast China, which is the main region for soybean cultivation, the soybean area is declining and is being replaced by maize and rice (Yang et al., 2008).

Maize–soybean relay intercropping system has contributed greatly to soybean production. During the past 13 years, intercropping has been practiced on three million hectares in southwest China, and 4.8 million tonnes of soybean has been produced. The Government of China has recommended greatly expanding this system to be a major agricultural technology in southwest and southern China and the Huang-Huai-Hai Plain (Liu et al., 2015).

However, in the maize–soybean relay intercropping system, lodging of soybean seedlings is a serious problem because of over shading by maize during the co-growth stage (Zou et al., 2015). Many studies have indicated that morphological, physiological and biomechanical characteristics of stems are closely related to lodging resistance. Above all, main stem strength is a key factor in improving the lodging resistance of soybean (Jian et al., 2006; Luo et al., 2007; Liang and Guo, 2008; Zhou, 2009). A strong fibrillar network structure of plant cell walls can provide mechanical support for cells and the entire plant. As the main component of the cell wall, cellulose has an obvious effect on maintaining stem mechanical strength (Xiang et al., 2010). Some research has shown that with a decrease in cellulose content, the soybean stem loses mechanical strength and easily lodges (Wu et al., 2007). Studies of wheat (Jian

Abbreviations: AI, acid invertase; ALR, actual lodging rate; NI, neutral invertase; SPS, sucrose phosphate synthase; SS, sucrose synthetase.

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et al., 2006), rice (Yang et al., 2009) and maize (Xia et al., 2013) also found that varieties with high cellulose content in the stem were more lodging resistant than those of low cellulose content.

Previous studies concerning soybean lodging were based on monoculture conditions, focused on regulating fertilization and using lodging resistant varieties (Board, 2001; Liu et al., 2014; Yamaguchi et al., 2014). In the maize–soybean intercropping system, some studies have shown differences in lodging resistance of different soybean genotypes, and varieties with shade tolerance and lodging resistance have been chosen from the germplasm (Liu et al., 2015; Luo et al., 2015). However, little is known about the effect of different planting patterns on the physical strength of the soybean main stem, especially regarding how cellulose biosynthesis and accumulation in the stem is affected in different varieties. The objective of present study was to investigate the effects of intercropping on lodging behavior and cellulose accumulation and activity of related enzymes in the main stem of different soybean varieties. This information should provide a theoretical basis for breeding soybean varieties with high physical strength of the stem to prevent lodging in maize–soybean intercropping.

2. Materials and methods

2.1. Plant material and experimental design

From 30 soybean varieties (genotypes) identified with difference degree of shade tolerance in 2010 and 2012 (Liu et al., 2015), three varieties were selected: B1 (Nandou 032-4, shade susceptible, breeding material of Nanchong Academy of Agricultural Sciences in Sichuan Province); B2 (Jiuyuehuang, moderately shade tolerant, landrace at Langzhong City, Sichuan Province); and B3 (Nandou 12, shade tolerant, main variety in southwest China, bred by Nanchong Academy of Agricultural Sciences). The variety of maize (*Zea mays* L.) was Zhenghong 505 (semi-compact maize), which is the main variety used in maize–soybean intercropping.

Field experiments were conducted at the Experimental Farm, Sichuan Agricultural University, Ya'an (29°59'N, 103°00'E), Sichuan Province, China in 2013 and 2014 on a clayey textured soil.

The different soybean varieties were grown using two cropping systems: maize–soybean relay intercropping and soybean monoculture. The trials were designed with split-plot arrangements, with three replications. The main area was two cropping systems, and secondary area was three soybean varieties (genotypes). The planting methods of the relay intercropping system were the same as those described in the technical regulations for maize–soybean intercropping issued by the Ministry of Agriculture of the People's Republic of China in 2014 (Yang et al., 2015). In the intercropping system, two rows of soybean between two rows of maize were planted in alternating 200-cm-wide strips including a 40-cm-wide maize strip (two maize rows with 40 cm inter-row distance) and a 40-cm-wide soybean strip (two soybean rows with 40 cm inter-row distance). There was 60 cm between maize strips and soybean strips. The plant spacing within rows of maize and soybean was 20 and 10 cm, respectively (Fig. 1). In the soybean monoculture system, the row and hill spacings of soybean were 55 and 10 cm, respectively. The row length was 600 cm. Maize was sown into a flat wet seedbed on 28 March and transplanted into the field on 5 April (7 days after sowing). Soybean seeds were sown on 13 June. Maize was harvested on 5 August, and soybean on 23 October. The co-growth time of maize and soybean was 53 d. Fertilization and other management measures in this study were the same as used in field production.

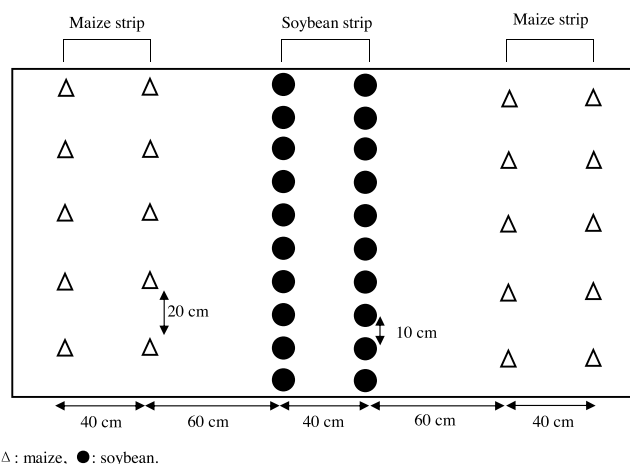


Fig. 1. Diagram showing the arrangement of the rows of maize and soybean in the maize–soybean relay intercropping system.

2.2. Stem morphological characteristics

At 30 d after seedling emergence, 20 soybean plants of each treatment were sampled every 7 d until the end of the co-growth period (i.e. maize harvested). The first internodes (basal internode) were used to measure the stem breaking strength according to the procedure described by Liu et al. (2015), using a digital plant lodging tester (YYD-1, Zhejiang Top Instrument Co. Ltd., Hangzhou, China). At the same time, the actual lodging rate (ALR) of soybean was determined according to the following standard: when the angle between the main stem of soybean and the ground was $\leq 30^\circ$, it was considered as lodged.

2.3. Light transmittance of maize

At 30 d after soybean seedling emergence, the light transmittance of the maize canopy was measured from 11:00–12:00 am at 7-d intervals on sunny days. The sensors of the light intensity instrument (LI-191SA quantum sensors, LI-COR Inc., Lincoln, NE, USA) were placed above the canopy of the soybean row. The 20 horizontal points with 10-cm spacing in each plot of three replications were measured every time. The light transmittance of the maize canopy was averaged.

2.4. Carbohydrate determination

The first internodes of the soybean stem were dried to a constant weight at 70 °C after denaturing enzymes at 105 °C for 0.5 h. The stems were crushed and passed through a 60-mesh screen, and then used for measuring the sucrose and cellulose contents by resorcinol method (Bajracharya, 1999) and semi-automatic fiber analyzer (Fibertec 1020, Foss Scino (Suzhou) Co. Ltd, Suzhou, China), respectively.

2.5. Enzyme assays

The first internodes of the fresh soybean stem were frozen in liquid nitrogen for 10 min and stored at -80°C . The extractions of neutral invertase (NI) and acid invertase (AI) were conducted as previously described (Lowell et al., 1958; Komatsu et al., 1999). Samples were weighed and extraction buffer added [200 mM potassium phosphate buffer, 5 mM magnesium chloride (MgCl_2), 0.1% β -mercaptoethanol, 0.05% Triton-X 100, 0.05% bovine serum albumin (BSA) and 2% polyvinylpyrrolidone (PVPP) at pH 7.5]

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