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Tolerance *vs*. avoidance: two strategies of soybean (*Glycine max*) seedlings in response to shade in intercropping

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Abstract

Intercropping is a sustainable agricultural practice used worldwide for highly efficient utilization of resources. However, short crops often grow under the shade of the canopy of tall crops in intercropping systems. Plants evolved two main strategies to deal with shade: avoidance and tolerance. Soybean (*Glycine max*), a legume crop, is often planted in intercropping. But little is known about a strategy that soybean may employ to deal with shade at seedling stage. Therefore, we determined morphological and physiological traits related to shade tolerance and shade avoidance in seedlings of two varieties. Generally, both varieties showed similar shade tolerance traits, such as increased specific leaf area and chlorophyll (Chl) content, and reduced photosynthetic capacity and the Chl *a*/*b* ratio. The light-limiting environment eliminated the benefits of shade tolerance traits for the carbon gain, which led to similar real-time photosynthesis and biomass in intercropping. By contrast, two varieties expressed different changes in shade avoidance traits. The variety Guixia 3 exhibited clear preference of shade avoidance that resulted in a high main stem, hypocotyl elongation, and biomass allocation towards the stem. The variety Gongxuan 1 showed those traits less. We suggested that the genetic variation occurs within soybean, thus the shade avoidance related traits might be important for variety selection for intercropping. Hence, the evaluation of performance should focus on shade avoidance in soybean genotypes in future experiments.

Additional key words: biomass partitioning; gas exchange; leaf structure; monocropping; palisade mesophyll; stem elongation.

Introduction

Intercropping is a sustainable agricultural practice widely used in many developed and developing countries to enhance food security and to use natural resources more efficiently. In intercropping, two or more crops are grown simultaneously in the same field during a growing season (Willey 1979, Francis 1989). During the simultaneous growth of the mixed crops, light is frequently the most important factor related to crop yields (Francis 1989), and to overyielding by crop mixtures that exhibit temporal complementarity and high efficiency (Willey 1979, Malézieux *et al.* 2009). Due to the morphological and physiological differences among intercropped crops, light can be partitioned into different components of crop canopies. Most studies have focused on the total light utilization of intercropping pattern at seasonal and spatial scales (Keating and Carberry 1993, Mushagalusa *et al.* 2008, Zhang *et al.* 2008, Gao *et al.* 2009, Bedoussac and Justes 2010, Ghanbari *et al.* 2010, Shili-Touzi *et al.* 2010, Knörzer *et al.* 2011). Unfortunately, as the partitioned incident light by different canopies can induce above-ground competition on light between the component crops, light is frequently the limiting resource in intercropping

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Abbreviations: Chl – chlorophyll; $\mathrm{Chl}_{\mathrm{area}}$ – chlorophyll content per unit of area; $\mathrm{Chl}_{\mathrm{mass}}$ – chlorophyll content per unit of dry mass; C_i – intercellular CO_2 concentration; f_L – fractional investment of biomass in leaves; f_P – fractional investment of biomass in petiole; f_S – fractional investment of biomass in stem; g_s – stomatal conductance; INT – relay strip intercropping; LA – leaf area; LAR – leaf area ratio; LCP – light compensation point; LSP – light saturation point; MON – monocropping; N_{area} – nitrogen content per unit of area; N_{mass} – nitrogen content per unit of dry mass; $P_{N\max}$ – light-saturated photosynthetic rate; P_N – net photosynthetic rate; R_D – dark respiration; SLA – specific leaf area; Φ_{PSII} – effective quantum yield of PSII photochemistry.

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systems. Therefore, the effects of shade on understory crops should be considered when we attempt to increase the productivity of an intercropping system (Keating and Carberry 1993, Wallace *et al.* 1996, Malézieux *et al.* 2009, Lithourgidis *et al.* 2011).

Light has profound effects on plants. Either the presence of neighboring plants or self-shading within the canopy could reduce the availability of PAR and alter light quality for each plant. Hence, shade is ubiquitous in nature and all plants are shaded to some degree during their lifetime (Valladares and Niinemets 2008). To acclimate to shade, plants have evolved two opposing strategies in response to competition for light: shade tolerance and shade avoidance. Shade avoidance includes a set of traits to reach for the light, such as elongation of stem and petioles, hyponasty, and reduced branching (Ballaré 1999, Smith 2000, Franklin and Whitelam 2005, Vandenbussche *et al.* 2005, Casal 2012). Contrary, shade tolerance is a set of traits that typically optimize the carbon gain under low light conditions, such as an increased Chl content, specific leaf area (SLA), and the PSII/PSI ratio, together with the reduced Chl *a*/*b* ratio (Givnish 1988, Valladares and Niinemets 2008, Niinemets 2010).

Genetic differences within species determine an adaptation ability to cope with shade. For example, *Impatiens capensis* grown in shade were taller and possessed more elongated internodes than those grown in full sun, and notably the open-habitat population were more responsive to shade than the shade-habitat population (Dudley and Schmitt 1995). The shade-induced production of taller, thinner internodes was stronger in grass genotypes than in forest genotypes of *I. capensis* (Anten *et al.* 2009). Therefore, genetic variation is likely to occur in different crop varieties.

Soybean is the fourth most widely cultivated crop worldwide, and also one of the major crops often planted in intercropping patterns (Ghosh *et al.* 2009, Gao *et al.* 2010, Echarte *et al.* 2011). Previous studies have demonstrated the acclimation mechanisms of soybean at a leaf level in responses to light environments. Total Chl content and Chl *a*/*b* of soybean leaves between the top and the side of the canopy were significantly different when analyzed across genotypes (Fritschi and Ray 2007), and soybean leaves were thinner as shade increased under crop/tree intercropping system (Manceur *et al.* 2009). However, our understanding is limited on how crops respond to shading in intercropping. Maize (*Zea mays*)/ soybean relay-intercropping pattern is one of the widely spread relay-intercropping. In this pattern, soybean is sowed into skip-strips of standing maize, thus, soybean grows under shade conditions. To know more about the soybean acclimation responses to shade in relay-intercropping, morphological, physiological, and anatomical traits of soybean associated with shade avoidance and shade tolerance were investigated in two soybean varieties. In addition, crop varieties were compared under unshaded, sole-cropping conditions; therefore results might be unsuitable for intercropping conditions, where shading alters the phenotypes of crop varieties. Thus, the objective of these comparisons was to determine the preference on either shade avoidance, or shade tolerance strategy, the soybean may take under shade in maize/soybean relay strip intercropping. Then, the knowledge of preferential strategy to deal with shade may be beneficial for future genotype selection to perform better in a relay-intercropping pattern.

Materials and methods

Field site and experimental design: The experiment was carried out in the maize/soybean relay strip intercropping system during the growing season in 2011 at the Teaching and Experimental Farm of Sichuan Agricultural University, Ya'an (29°59'N, 103°00'E), located on the western border of the Sichuan Basin. The soil of the experimental field is a purple clay loam (pH 7.5), and at the beginning of experiment, total N, P, K, available N, P, K, and organic matter were 2.79 g kg^{-1}, 0.383 g kg^{-1}, 12.89 g kg^{-1}, 168.6 mg kg^{-1}, 81.3 mg kg^{-1}, 140.1 mg kg^{-1}, and 4.32%, respectively. Field experiment was done in a two-factor, completely randomized design with three replications, totally containing 12 plots. Each plot was 6 m in length by 4 m in width. All crop rows were oriented north-south. Irrigation, weeding, fertilizers, and other field practices were kept the same in all treatments. Two soybean varieties, Gongxuan 1 (Go1) and Guixia 3 (Gu3), were used in this experiment. Go1 is a traditional variety, while Gu3 is a new bred variety. Soybean was planted under either in relay strip intercropping (INT) or as monocropping (MON) system. The PAR above soybean canopy were monitored by a light sensor (*LiCor SA190, LiCor Inc*., USA) at 5 min intervals, and R:FR ratio was measured every 1–2 h using a spectrometer (*AvaSpec-2048, Avantes Inc*., Netherland). The overall daily light irradiance in INT was around 40% of that in the MON treatment, and the R:FR ratio was reduced significantly (Fig. 1S; *supplementary material available online*). For INT, soybean and maize were planted in alternating strips; every soybean strip was relay-intercropped between maize strips. Each soybean and maize strip consisted of two soybean and two maize rows, respectively. All the strip spacing (distance between maize and soybean rows), soybean row spacing and maize row spacing were 0.5 m. Maize was sown in a seed bed on March 28 and transplanted into field on April 9 within 0.4 m intra-row spacing per hole and two plants per hole. Soybean was drilled on June 11 within 0.35 m intra-row spacing per hole and two plants per hole. For MON, soybean was planted in solid rows with 0.5 m row spacing, thus, plants have the same growing space for the individual plant as in INT. Intra-row spacing per hole of soybean in two planting patterns were 0.35 m within two plants per hole. Maize was harvested on August 9, 2011, and shading environment lasted nearly for two month after soybean plantation. The overall daily light irradiance in INT before maize harvesting was around 30% of that in the MON treatment, and the light irradiance level above soybean canopy recovered to MON treatment after maize harvesting. Sampling and field measurements were performed from July 29 to August 2 (during the co-existing period of maize and soybean) to analyze shade responses of soybean seedling.

Growth characteristics: Aboveground parts of five plants per plot were sampled to measure biomass, leaf area (LA), and specific leaf area (SLA). After the sampling, plants were divided into leaf, petiole, and stem. Main stem length, hypocotyl length and diameter, and the first internode length and diameter were investigated. After the leaf, petiole, and stem were oven-dried to constant mass, the total aboveground biomass and the fractional investment of biomass in leaf (f_L), petiole (f_L), and stem (f_S) were calculated by dividing leaf, petiole, and stem mass by aboveground biomass, respectively.

Leaf morphological and anatomical features: After the sampling, the latest fully expanded leaves (the 3rd, 4th, and 5th leaves from top, only leaf laminae) were scanned by flatbed scanner (*CanoScan LiDE 200*, *Canon Inc*., Japan) and their area was measured using *ImageJ 1.45s*. Then, the leaves were placed in an envelope for drying. SLA was calculated by dividing the measured area by its dry mass (DM), total leaf area (LA) was calculated by multiplying SLA by total leaf DM, and leaf area ratio (LAR) was calculated by dividing LA by total aboveground biomass.

Two middle segments (5 × 8 mm) without midrib from each middle leaflet of three latest fully expanded leaves of each plot were sampled and fixed in a formaldehyde (FAA) solution (water/ethanol/acetic acid/formaldehyde, 27:63:5:5, v/v). Leaf segments were dehydrated and cleared through a graded ethanol series and graded ethanol/xylene series, then embedded in paraffin, and cut by rotary microtome (*RM2235*, *Leica Microsystems Ltd*., Germany) at thickness of 5 µm. Sections were stained with Strafine O/Fast green and observed under light microscope (*Eclipse 50i*, *Nikon Instruments Inc*., Japan). Micrographic images were captured by digital camera (*Digital Sight DS–U1*, *Nikon Instruments Inc.*, Japan). Total leaf thickness, palisade and spongy mesophyll thickness, and adaxial and abaxial epidermis thickness were quantified by using *ImageJ 1.45s*. At least five fields of view from each section were stored and measured at least in three different positions per each image.

Leaf photosynthesis: Light response curves were measured from July 29 to August 1. All these measurements were conducted from 9:00 to 12:00 h. Net photosynthetic rate (P_N)-PAR response curve was estimated by measuring P_N at 11 stepwise levels of PAR (2,000; 1,500; 1,200; 1,000; 800, 500, 200, 100, 50, 20, and 0 µmol m^{-2} s^{-1}) under CO_2 concentration of 380 µmol mol^{-1}. Measurements were conducted on the third leaves from top; and two to three leaves from each plot were used for measurements. Light-saturated net photosynthetic rate (P_{Nmax}), dark respiration (R_D), light compensation point (LCP), light saturation point (LSP), and apparent quantum yield (α) were then estimated by the method of Ye (2007).

Real-time diurnal courses of P_N, stomatal conductance (g_s), intercellular CO_2 concentration (C_i), and effective quantum yield of PSII photochemistry (Φ_{PSII}) were measured on a clear sunny day, August 2. One day before the measurements, three middle leaflets of the third leaves from top on three different plants in each plot were tagged for tracking measurement. Photosynthesis was measured with the portable photosynthesis system (*LI-6400XT*, *Li-Cor Inc*., USA) equipped with 2 × 3 cm clear chamber (*6400-02*, *Li-Cor Inc*., USA). PAR, P_N, g_s, C_i, and Φ_{PSII} values were recorded at intervals of 2 h from 08:00 to 20:00 h (between sunrise and sunset) to show a diurnal variation. To assess real-time photosynthesis, measured leaves were kept at their natural angle of posture exposing to direct irradiance outside leaf chamber (Murchie *et al.* 1999). Air flow rate was maintained around 500 ml min^{-1}; CO_2 concentration, temperature, and relative humidity were kept at ambient conditions. Diurnal chlorophyll (Chl) fluorescence variations were measured on the same leaves used to measure P_N with *Mini-PAM* (*Walz*, Germany) under the same diurnal courses. The Φ_{PSII} was then calculated as described by Baker (2008).

Chl and nitrogen contents: Fresh leaves of the latest fully expanded leaves from each plot were sampled and quickly brought to laboratory; four leaf discs were punched and extracted in 80% aqueous acetone solvent to determine total Chl and Chl *a*/*b* by spectrophotometric analysis as described by Lichtenthaler (1987). The dry leaf fragment after determining SLA was used for determining nitrogen content. After the dry fragments were ground into fine powder and digested, N content was determined using the segmented flow analysis (*FUTURA II*, *Alliance Instrument*, France). Pigment and nitrogen contents were expressed both per DM (Chl_{mass}, N_{mass}) and per area (Chl_{area}, N_{area}).

Statistical analysis: Data analysis was performed by analysis of variance (*ANOVA*) of *SPSS 19.0* for *Windows* (*SPSS*, Chicago, USA). All measured and calculated mean values per individual plot were analyzed as dependent variables; variety and cropping pattern were analyzed as fixed factors. Significant differences were examined by using the *F*-test at $p<0.05$.

Results

Growth characteristics and biomass partition: Both varieties showed stronger stem elongation response under INT (Table 1). Longer, but slimmer main stem were observed in shade-grown soybean. Under MON, main stem length of Go1 and Gu3 was 53.9 and 58.6 cm, respectively. Under INT, main stem length of both varieties increased significantly to 72.5 and 111.1 cm, representing 34.5 and 89.6% of main stem elongation, respectively. Meanwhile, hypocotyl length and the first internode length increased significantly on both varieties under INT, roughly two fold elongations were observed. However, the diameters of hypocotyl and the first internode of both varieties became slimmer; they declined by 40–50% than those under MON.

The elongating degree of the main stem length of Go1 was smaller than that of Gu3 under INT conditions. More detailed analysis showed that both varieties exhibited similar hypocotyl elongation degree of 82.3 and 78.5% respectively, although Go1 had lower elongation degree on the first internode lengths than Gu3. Combined with the main stem length elongation, the shorter main stem of Go1 than that of Gu3 under INT resulted from the lesser elongation of the late-emerged internode.

Biomass in INT was significantly lower than that in MON for both soybean varieties (Table 2). Under MON condition, Gu3 showed significantly higher biomass compared with Go1. But under INT, both varieties had similar biomass. Thus, the advantage of Gu3 did not persist under INT. Both varieties showed similar f_L, f_P, and f_S under MON. However, under INT, both varieties showed higher f_S with reduced f_L and f_P. Interestingly, Gu3 showed higher f_S than that of Go1. Hence, the biomass partition differed between both varieties under INT conditions.

Leaf morphological and anatomical structures: Comparisons of SLA, LAR, and LA are presented in Table 3. Significantly higher SLA and LAR under INT was observed in both varieties, and no significant difference in SLA was observed between Go1 and Gu3. By contrast, absolute LA per plant showed significantly lower value under INT. Variety difference was observed in LA; larger decrease in LA was observed in Gu3 than in Go1 under INT.

Leaves became thinner; the total leaf thickness declined under INT in both varieties. However, microscopic observations of transverse sections revealed different

Table 1. Main stem length, hypocotyl length and diameter, first internode length and diameter of Gongxuan 1 and Guixia 3 grown under monocropping (MON) and intercropping (INT). Data represent mean ± SE of three replicate plots ($n = 3$). * represents significant difference at 0.05 probability level in *ANOVA*.

Variety	Pattern	Main stem length [cm]	Hypocotyl length [cm]	Hypocotyl diameter [mm]	First internode length [cm]	First internode diameter [mm]
Gongxuan 1	MON	53.9 ± 8.2	4.40 ± 0.75	7.99 ± 0.06	4.68 ± 0.08	7.06 ± 0.11
	INT	72.5 ± 6.7	8.02 ± 0.89	4.77 ± 0.20	8.81 ± 0.60	3.78 ± 0.10
Guixia 3	MON	58.6 ± 6.1	3.68 ± 0.76	7.66 ± 0.25	4.45 ± 0.14	6.74 ± 0.15
	INT	111.1 ± 5.0	6.57 ± 0.57	4.38 ± 0.15	10.24 ± 0.19	3.37 ± 0.18
Analysis of variance						
Variety		0.011^{*}	0.186	0.082	0.097	0.028^{*}
Pattern		0.001^{*}	0.003^{*}	$< 0.001^{*}$	$< 0.001^{*}$	$< 0.001^{*}$
Variety × pattern		0.033^{*}	0.639	0.848	0.033^{*}	0.754

Table 2. Aboveground biomass and its partition among leaf, petiole, and stem of Gongxuan 1 and Guixia 3 grown under monocropping (MON) and intercropping (INT). f_L, f_P, and f_S represents fractional investment of biomass in leaf, petiole, and stem, respectively. Data represent mean ± SE of three replicate plots ($n = 3$). * represents significant difference at 0.05 probability level in *ANOVA*.

Variety	Pattern	Biomass per plant [g]	Leaf mass per plant [g]	f_L [%]	Petiole mass per plant [g]	f_P [%]	Stem mass per plant [g]	f_S [%]
Gongxuan 1	MON	10.36 ± 0.25	5.17 ± 0.20	49.9 ± 1.3	1.67 ± 0.12	16.1 ± 0.9	3.52 ± 0.04	33.9 ± 1.1
	INT	4.06 ± 0.29	1.87 ± 0.13	46.2 ± 1.6	0.50 ±0.05	12.2 ± 0.7	1.70 ± 0.12	41.6 ± 1.0
Guixia 3	MON	14.87 ± 1.19	7.50 ± 0.60	50.4 ± 0.3	2.28 ±0.22	15.4 ± 0.4	5.08 ± 0.37	34.3 ± 0.2
	INT	4.19 ± 0.63	1.77 ± 0.27	42.1 ± 1.0	0.43 ±0.06	10.2 ± 0.2	2.00 ± 0.31	47.7 ± 1.1
Analysis of variance								
Variety		0.010^{*}	0.012^{*}	0.162	0.076	0.050^{*}	0.006^{*}	0.009^{*}
Pattern		$< 0.001^{*}$	$< 0.001^{*}$	0.001^{*}	$< 0.001^{*}$	$< 0.001^{*}$	$< 0.001^{*}$	$< 0.001^{*}$
Variety × pattern		0.014^{*}	0.008^{*}	0.094	0.033	0.314	0.036^{*}	0.015^{*}

anatomical structures in Go1 and Gu3 (Table 4). Under MON, both varieties showed that the predominant tissue was palisade, while under INT, both varieties were predominantly composed of spongy tissue. Go1 showed thicker leaf because of thicker palisade layer under MON and there was no significant reduction in spongy mesophyll when grown under INT. Contrary, in Gu3, palisade and spongy mesophyll was reduced dramatically and the second layer of palisade became ill-defined when grown under INT.

Leaf gas exchange, Chl and nitrogen content: Go1 and Gu3 expressed similar changes in light response curves under INT (Table 5, Fig. 2S; *supplementary material available online*). All estimated P_{Nmax}, R_D, LCP, and LSP were significantly lower under INT than those under MON. Apparent quantum yield was significantly different between both varieties, Gu3 showed higher one than that of Go1. For interaction of planting pattern and variety, only estimated P_{Nmax} was significant. Gu3 exhibited the highest value of P_{Nmax} in MON, but it showed the lowest value in INT. However, Go1 showed lesser difference in P_{Nmax} under INT and MON.

Diurnal course of PAR reaching soybean leaves in INT was drastically reduced by maize (Fig. 1). But the shading level was not stable over the diurnal course. In the morning (08:00–11:00 h) and afternoon (15:00–19:00 h), the PAR was generally lower than 500 μmol m^{-2} s^{-1} (Fig. 1), however, at the midday from 11:00–15:00 h, the PAR exceeded 1,000 μmol m^{-2} s^{-1}. Diurnal courses of P_N under MON increased rapidly after sunrise, and reached its maximum during 10:00–12:00 h, while P_N was drastically constrained under INT condition. Significantly lower values of P_N under INT were observed at all recording times from 8:00–18:00 h in both varieties (Fig. 2*A*,*B*). Simultaneously, g_s under MON and INT showed unimodal patterns as well, and reached its maximum around 12:00–14:00 h (Fig. 2*C*,*D*). Significantly higher values of g_s were observed under MON than those under INT at 08:00–12:00 and 16:00–20:00 h. C_i showed opposite alteration course compared to P_N and g_s (Fig. 2*E*,*F*). C_i declined in the morning, reaching minimum values in the midday, but it increased in the afternoon. Overall, C_i was higher under shade conditions in both varieties. Φ_{PSII} of both varieties under MON and INT followed similar changing trends. Φ_{PSII} decreased drastically as the PAR rose after 10:00 h, and recovered after 16:00 h with PAR decline (Fig. 2*G*,*H*). Leaves under INT condition exhibited higher Φ_{PSII} values than those under MON, in the morning and afternoon, indicating the higher efficiency of light utilization. However, no distinguishable difference between both varieties was observed under INT in the afternoon.

Total Chl content, Chl *a*/*b*, and nitrogen content are

Table 3. Specific leaf area (SLA), leaf area ratio (LAR), and total leaf area (LA) per plant of Gongxuan 1 and Guixia 3 grown under monocropping (MON) and intercropping (INT). Data represent mean ± SE of three replicate plots ($n = 3$). * represents significant difference at 0.05 probability level in *ANOVA*.

Variety	Pattern	SLA [m^2 kg^{-1}]	LAR [cm^2 g^{-1}]	LA per plant [cm^2]
Gongxuan 1	MON	32.44 ± 1.43	161.7 ± 7.7	1678.2 ± 119.5
	INT	49.60 ± 0.49	228.4 ± 6.1	927.0 ± 70.6
Guixia 3	MON	34.55 ± 1.42	174.3 ± 6.4	2586.0 ± 182.7
	INT	52.12 ± 1.01	219.8 ± 9.5	919.5 ± 141.2
Analysis of variance				
Variety		0.080	0.792	0.010*
Pattern		< 0.001*	< 0.001*	< 0.001*
Variety × pattern		0.867	0.197	0.009*

Table 4. Anatomical structures of Gongxuan 1 and Guixia 3 leaves grown under monocropping (MON) and intercropping (INT). Data represent mean ± SE of three replicate plots ($n = 3$). * represents significant difference at 0.05 probability level in *ANOVA*.

Variety	Pattern	Total leaf [μm]	Adaxial epidermis [μm]	Abaxial epidermis [μm]	Palisade tissue [μm]	Spongy tissue [μm]
Gongxuan 1	MON	133.6 ± 5.0	7.9 ± 0.2	7.8 ± 0.3	64.6 ± 2.2	53.2 ± 2.9
	INT	102.7 ± 2.2	7.7 ± 0.2	7.1 ± 0.3	35.3 ± 1.3	52.6 ± 2.4
Guixia 3	MON	128.0 ± 1.0	7.0 ± 0.1	7.1 ± 0.1	62.2 ± 1.1	51.6 ± 1.9
	INT	84.0 ± 1.9	6.8 ± 0.4	6.2 ± 0.2	30.6 ± 1.4	40.4 ± 1.7
Analysis of variance						
Variety		0.003*	0.005*	0.012*	0.052	0.016*
Pattern		< 0.001*	0.395	0.009*	< 0.001*	0.032*
Variety × pattern		0.057	0.935	0.796	0.473	0.048*

Table 5. Light-saturated net photosynthetic rate (P_{Nmax}), dark respiration rate (R_D), light compensation point (LCP), light saturation point (LSP), and apparent quantum yield (α) of the latest fully expanded leaf (third leaf from top) of Gongxuan 1 and Guixia 3 grown under monocropping (MON) and intercropping (INT). Data represent mean ± SE of three replicate plots ($n = 3$). * represents significant difference at 0.05 probability level in *ANOVA*.

Variety	Pattern	P_{Nmax} [μmol(CO$_2$) m^{-2} s^{-1}]	R_D [μmol(CO$_2$) m^{-2} s^{-1}]	LCP [μmol(photon) m^{-2} s^{-1}]	LSP [μmol(photon) m^{-2} s^{-1}]	α [mol mol^{-1}]
Gongxuan 1	MON	20.20 ± 0.02	3.00 ± 0.70	44.86 ± 7.90	1679.39 ± 26.92	0.072 ± 0.009
	INT	17.63 ± 1.38	1.45 ± 0.33	20.38 ± 3.47	1405.94 ± 69.30	0.073 ± 0.006
Guixia 3	MON	23.17 ± 0.41	3.25 ± 0.50	46.24 ± 6.91	1702.99 ± 58.18	0.077 ± 0.007
	INT	16.67 ± 0.96	2.06 ± 0.15	22.54 ± 2.58	1481.16 ± 39.31	0.101 ± 0.006
Analysis of variance						
Variety		0.278	0.384	0.763	0.362	0.046*
Pattern		0.001*	0.019*	0.003*	0.001*	0.093
Variety × pattern		0.049*	0.710	0.947	0.627	0.143

shown in Table 6. Chl$_{mass}$ under INT was significantly higher than those under MON. But no significant difference between Go1 and Gu3 was observed. There was no difference in total Chl$_{area}$ between both varieties and between planting patterns. For Chl *a*/*b*, significantly lower values were observed under INT in both Go1 and Gu3, while no difference was found between both varieties. Nitrogen content per unit of DM differed in both varieties. Go1 showed increase of nitrogen content under INT, while in Gu3 no alteration occurred. N$_{area}$ showed significant difference between MON and INT; MON promoted higher nitrogen content per unit of area than that under INT in both varieties.

Discussion

Shade avoidance and tolerance strategies: Shade avoidance and shade tolerance are two strategies that plants have evolved in response to shading. For shade avoidance, plants often increase stem and hypocotyl elongation rates at the expense of leaf growth and the elongated hypocotyl is often seen as an indicator of shade avoidance (Ballaré 1999, Smith 2000, Franklin and Whitelam 2005, Vandenbussche *et al.* 2005, Casal 2012). In this study, the more elongated main stem, hypocotyl and the first internode length confirmed that soybean dealt with shading in relay-intercropping *via* shade avoidance. It is interesting that two soybean varieties showed different elongation responsiveness under shade: Go1 exhibited less extensive main stem, hypocotyl, and the first internode elongations than Gu3 (Table 1). Thus, we could infer that Go1 expressed relatively weaker ability for shade avoidance than Gu3. In addition, increased biomass partition to the stem suggested that soybean invested more resource in the stem growth at the expense of the leaf expansion; this might be used for searching light under deep shade (Valladares *et al.* 2011). These features suggested soybean, as a classic sun-habitat crop, expressed typical shade avoidance symptom, such as searching for light and growing towards light under shading in relay-intercropping system.

Besides shade avoidance, plants usually optimize light capture and utilization under shading, which is needed for their higher efficiency of carbon gain. Many features, such as increased SLA and α, reduced Chl *a*/*b*, and R_D, are components of the carbon gain hypothesis for shade

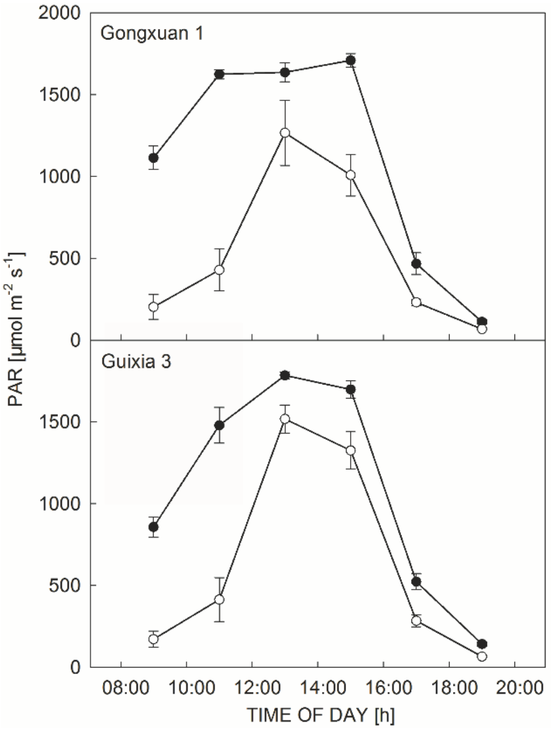

Fig. 1. Diurnal course of actual PAR reaching the latest fully expanded leaf (third leaf from top) of Gongxuan 1 and Guixia 3 grown under monocropping (MON) and intercropping (INT). *Closed circles* and *open circles* represent MON and INT condition, respectively. Each circle represents mean ± SE of three replicate plots ($n = 3$).

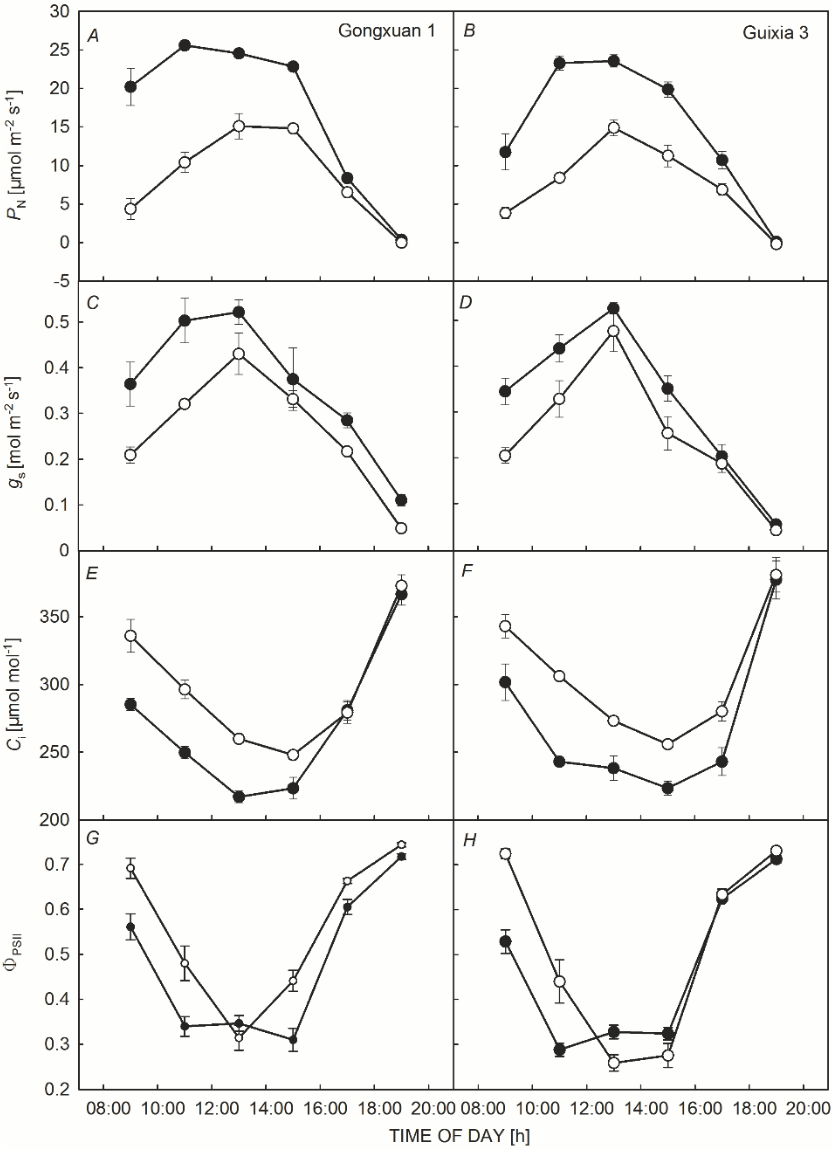

Fig. 2. Diurnal courses of net photosynthetic rate (P_N) (*A*,*B*), stomatal conductance (g_s) (*C*,*D*), intercellular CO_2 concentration (C_i) (*E*,*F*), and effective quantum yield of PSII photochemistry (Φ_{PSII}) (*G*,*H*) of the latest fully expanded leaf (third leaf from top) of Gongxuan 1 and Guixia 3 grown under monocropping (MON) and intercropping (INT). *Closed circles* and *open circles* represent MON and INT condition, respectively. Each circle represents mean ± SE of 3 replicate plots ($n = 3$).

Table 6. Total chlorophyll content (Chl_{mass} and Chl_{area}), chlorophyll *a*:*b* ratio (Chl *a*/*b*), and nitrogen content (N_{mass} and N_{area}) of Gongxuan 1 and Guixia 3 grown under monocropping (MON) and intercropping (INT). Data represent mean ± SE of three replicate plots ($n = 3$). * represents significant difference at 0.05 probability level in *ANOVA*.

Variety	Pattern	Chl_{mass} [g kg^{-1}]	Chl_{area} [mg m^{-2}]	Chl *a*/*b*	N_{mass} [g kg^{-1}]	N_{area} [g m^{-2}]
Gongxuan 1	MON	7.60 ± 0.07	237.03 ± 8.73	3.73 ± 0.08	50.94 ± 0.97	1.59 ± 0.08
	INT	11.31 ± 0.10	227.95 ± 3.10	3.42 ± 0.12	57.79 ± 0.33	1.17 ± 0.02
Guixia 3	MON	7.56 ± 0.11	217.64 ± 8.34	3.78 ± 0.08	58.86 ± 1.34	1.69 ± 0.07
	INT	12.04 ± 0.43	231.05 ± 7.95	3.50 ± 0.06	57.66 ± 0.98	1.11 ± 0.03
Analysis of variance						
Variety		0.172	0.302	0.511	0.004*	0.696
Pattern		< 0.001*	0.777	0.009*	0.020*	< 0.001*
Variety × pattern		0.133	0.167	0.878	0.003*	0.191

tolerance (Givnish 1988, Valladares and Niinemets 2008).

For soybean in INT, higher Chl content per leaf mass in leaves (Table 6) suggested more pigment-binding proteins. Besides total Chl content, Chl *a*/*b* serves as another characteristic for sun and shade leaves (Boardman 1977, Anderson 1986), since the light-harvesting complex of PSII contains majority of Chl *b* (Evans 1989); thus, accumulation of LHCII causes decline of the Chl *a*/*b* ratio under shade condition. Therefore, the reduced Chl *a*/*b* ratio in both varieties in this study coincided with previous studies (Table 6). Larger SLA under low light is seen as an acclimatory trait, because larger SLA results in higher opportunity to receive light per unit of leaf mass (Terashima *et al.* 2001, 2006, 2011). However, larger SLA indicates negative consequences for CO_2 carboxylation due to the reduced thickness of palisade mesophyll. Carboxylation reactions of photosynthesis take place in chloroplasts, and most chloroplasts are located in palisade tissue; thus, thinner layer of palisade leads to smaller surface area of mesophyll, which constrains CO_2 diffusion from intercellular cavities into chloroplast and it ultimately reduces the P_{Nmax} in shade leaves (Terashima *et al.* 2006, 2011). Increased α is beneficial for higher light-use efficiency under light-limited conditions, and reduced R_D suggested lower cost of growth in shade. The larger SLA, the thinner leaf from a view of anatomical structures, and reduced R_D indicated the lower photosynthetic capacity and lower CO_2 losses of soybean grown in INT in our experiment.

P_{Nmax} is closely related to N_{area} (Niinemets 1999, Makino 2011). The increase in N_{area} with increasing light availability is generally mediated through light-dependent changes in SLA (Niinemets 2007). In this study, P_{Nmax} was positively correlated with N_{area} in both varieties and light treatments and coincided with this relationship. Nitrogen in leaf can be allocated to Rubisco or light harvesting complex. Under low light conditions, the greater nitrogen allocation to light harvesting complex and increased SLA give rise to increased Chl_{mass}, rather than Chl_{area} (Niinemets 2010). Our results confirmed SLA was an important determinant of soybean leaf photosynthesis and nitrogen content in INT conditions. In addition, the inconsistence of N_{area} and Chl_{area} across all treatments in this study suggested the nitrogen allocation to Rubisco and light harvesting complex was different in both soybean varieties.

Combination of leaf morphological and physiological traits with irradiance explained the lower diurnal real-time P_N and Chl fluorescence in INT than that in MON, which consequently gave rise to reduced aboveground biomass of soybean. Photosynthesis is determined by stomatal or nonstomatal limitations, and only when the C_i and g_s decrease simultaneously, it is supposed that the decline in P_N was mainly caused by stomatal limitation (Farquhar and Sharkey 1982). As shown in Fig. 2, the change of C_i was nearly opposite to that of g_s under both INT and MON at midday, when C_i increased, while g_s decreased. The directions of changes in C_i and g_s caused by INT treatment were opposite. Therefore, the diurnal courses obtained in this study suggested that the decrease in P_N under INT condition was caused by nonstomatal rather than stomatal limitations. Combined with the diurnal courses of PAR (Fig. 1) and the light response curve (Fig. 2S), we could infer that photosynthesis of soybean leaf in INT was light-limited in the morning and afternoon, when the PAR was generally lower than 500 μmol m^{-2} s^{-1} (Fig. 1). At the midday from 11:00–15:00 h, although the PAR exceeded 1,000 μmol m^{-2} s^{-1}, no significant difference in P_N was observed between Go1 and Gu3 due to their similar light-saturated net photosynthetic rates estimated from the light curves (Table 5). In general, no matter what photosynthetic capacity at the leaf level the soybean variety exhibited under MON, the real-time photosynthesis was not significantly different in both soybean varieties grown under INT. Similar whole plant traits related to light interception, such as LA and LAR, in both varieties (Table 3) suggested that both varieties intercepted similar light under the light-limited INT condition. Hence, we suppose that the limited light diminished the benefits of soybean leaf gained from morphological and physiological traits in intercropping, even if soybean expressed shade tolerance traits for higher light-use efficiency.

Varietal difference on avoidance and tolerance strategies: The new bred variety, Gu3, showed higher P_{Nmax} and biomass than the traditional variety, Go1, under MON conditions. This result demonstrated the advantage of Gu3. However, Gu3 did not show any advantage under the INT condition. These results suggested that the shading diminished the advantage of Gu3 in the intercropping system. Gu3 showed the stronger capacity to shade avoidance than Go1; it suggests that soybean genotypes possess larger variability in shade avoidance aiming to reach light than that in shade tolerance for increasing the carbon gain.

Shade-intolerant species usually possess higher overall phenotypic plasticity than shade-tolerant species (Dudley and Schmitt 1995, Valladares *et al.* 2000, Sánchez-Gómez *et al.* 2006, Portsmuth and Niinemets 2007). In this study, Gu3 also showed higher overall variation than Go1 between MON and INT treatment, indicating that Gu3 is the relatively shade-intolerant variety. The measured traits in this study showed that Gu3 owned the overall higher plasticity than Go1. More specifically, the plasticity of shade tolerance traits, such as SLA and Chl content, was lesser than that of shade avoidance traits, such as stem length. Therefore, shade avoidance traits might be better indicators to evaluate genotype responses to shade in relay-intercropping.

Although modern 'Green Revolution' crops are semi-dwarfs, they still compete for light in dense communities and show shade avoidance responses. Since these traits might exacerbate crop lodging and reduce harvest index, hence, shade avoidance is not favored in crop production. In intercropping, shade avoidance strategy is not also an optimal option for understory crops, because it is impossible to outgrow the tall neighboring plants. Hence, the shade avoidance strategy to acquire light irradiance is not the best strategy for soybean grown in intercropping. In addition, we observed severe lodging of Gu3 in our field experiment, which coincided with the main stem elongation.

In contrast, the lesser elongation of main stem and less lodging might be the reason why Go1 is the traditional soybean variety used in relay-intercropping. In plant natural communities, many plants, such as grasses and herbs, can adapt their phenotypes by the shade tolerance strategy to cope with permanent shade in their life cycle. These plants usually exhibit larger SLA, the higher Chl content, and the lower Chl *a*/*b* ratio, which can increase the carbon gain in shading environment. Furthermore, the typical shade tolerant species suppress shade avoidance traits, showing little plasticity in photosynthetic traits and reduced, or absent, elongation responses in stems and petioles compared to open habitat counterparts (Gommers *et al.* 2013). These plants prove that it is possible to promote plant capacity for shade tolerance and inhibit sensitivity for shade avoidance. Therefore, selection of soybean genotypes displaying small preference for shade avoidance might be a possible way to improve productivity in intercropping. However, we must admit that only two soybean varieties were used in this study. As the important crop around the world, a large amount of genotypes had been bred and reserved by researchers. Therefore further evaluation of other varieties must be carried out to compare the relationship between shade tolerance and shade avoidance in soybean.

In summary, this study provided the analysis of the shade avoidance and tolerance strategies in two soybean varieties grown in relay-intercropping system. Both varieties, Guixia 3 and Gongxuan 1, showed similar shade tolerance traits for carbon gain. Nevertheless, the light-limiting condition reduced the benefits of physiological changes. Gu3 expressed the stronger response in elongation of the main stem and invested more into stem biomass, which indicated its preference for searching light *via* the shade avoidance strategy. Hence, future experiments should focus on plant traits of shade avoidance.

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