

Full Length Research Paper

## Gas exchange and morpho-physiological response of soybean to straw mulching under drought conditions

Lan-lan Xue<sup>1</sup>, Long-chang Wang<sup>1\*</sup>, Shakeel Ahmad Anjum<sup>1</sup>, Muhammad Farrukh Saleem<sup>2</sup>,  
Ming-chen Bao<sup>1</sup>, Asif Saeed<sup>3</sup> and Muhammad Faisal Bilal<sup>2</sup>

<sup>1</sup>College of Agronomy and Biotechnology, Southwest University, Engineering Research Center of South Upland Agriculture, Ministry of Education, P. R. China.

<sup>2</sup>Department of Agronomy, University of Agriculture, Faisalabad, Pakistan.

<sup>3</sup>Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad, Pakistan.

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A pot experiment was conducted to investigate the morphological, physiological and biochemical straw mulch-induced response of soybean under water-deficit conditions. Soybean (*Glycine max* L. Merrill) variety "Xidou 7" was treated with varying quantity of wheat straw mulch viz: (control (no straw mulch), 3750, 7500, 11000 and 14750 kg/ha) under water-deficit conditions. The experimental results indicate that the varying quantity of straw mulch significantly improved the plant growth in terms of plant height, leaf area, number of leaves/plant and stem diameter. Mulch treatment also significantly enhanced the photosynthesis ( $P_N$ ), intercellular  $CO_2$  concentration (Ci), transpiration rate (E) and stomatal conductance (gs) over the control. The gas exchange parameters were improved depending on the quantity of wheat straw mulch; significantly high  $P_N$  and E was observed in the treatment where wheat straw was applied at the rate of 11000 kg/ha. Wheat straw mulch treatments led to noticeable reduction in malondialdehyde (MDA) contents, which protected the drought stressed soybean plants from membrane damage. Furthermore, the free proline contents linearly increased with increase in straw mulch quantity. It is evident that wheat straw mulch can considerably modulate growth, photosynthetic and physio-biochemical attributes of soybean under drought. The research will effectively solve seasonal drought problem and can provide technical assistance for sustainable agriculture development.

**Key words:** Soybean, growth, water-deficit, wheat straw mulch.

### INTRODUCTION

With the change in global climate, the soil moisture balance and available moisture in soil is going to change and the frequency of regional drought will increase (Fuhrer, 2003). Soybean (*Glycine max* L. Merrill.) is considered as a miracle crop due to its extraordinary qualities. It contains 40 to 42% good quality protein and 18 to 22% oil, so it is highly desirable in human diet. As the best source of

protein, it truly deserves the title "the meat that grows on plant" (Arshad et al., 2006). It is considered highly sensitive to drought stress, especially during critical growth stages resulting in yield reduction. Water stress as a key abiotic limiting factor for soybean production can cause soybean yield reduction up to 40% or even more (Pathan et al., 2007). Most soybean production can cause soybean

yield reduction up to 40% or even more (Pathan et al., 2007). Most studies indicate that surface mulch application presents opportunities for utilizing a range of organic matter that may benefit crop, soil and water relations (Movahedi and Cook, 2000).

Straw mulch leads to increase in yield by improving soil physical conditions and stability in the topsoil (De Silva and Cook, 2003). Straw mulch can lower soil temperature, reduce evaporation of soil water and thus conserve soil moisture (Jalota et al., 2001). Straw mulch reduced soil moisture loss and soil temperature through shading (Kar and Singh, 2004). Researchers have studied the utilization of available organic materials as mulch in orchards (avocado, citrus and other fruit trees) and landscaping (Claassen, 2000).

Most studies revealed that straw mulch under water stress led to increased rate of photosynthesis and free proline accumulation (Chaum and Kirdmanee, 2009). The improved photosynthesis has direct positive impact on the yield of soybean. Rice straw mulch was reported to increase the grain yield of soybean from 0.95 to 1.25 t ha<sup>-1</sup> (Adisarwanto, 1985). Under moisture-stress, net photosynthetic rate and the flow of water of soybean leaves was also decreased (Liu et al., 2004). There are many defense mechanisms in plants which induce the stress tolerance against water-stress, such as osmoregulation, ion homeostasis, antioxidant and hormonal systems (Sairam and Tyagi, 2004; Mahajan and Tuteja, 2005). Proline is generally regarded as a plant osmoregulator, whose accumulation is altered when plants face stressful environments. Yamada et al. (2005) reported proline to be one of the osmotic protections in plant under drought. Enhanced proline contents are considered to be correlated with drought tolerance. Malondialdehyde (MDA), a product of lipid peroxidation, exhibited greater accumulation in plants under stress condition (Diego et al., 2003). This study aimed to investigate the effects of straw mulch on growth, yield and physiological aspects of soybean and showed the optimum quantity of straw mulch required for optimum growth and yield under water limiting conditions.

## MATERIALS AND METHODS

The experiment was carried out in plastic pots during summer 2008 in artificial rain-protected wire-house of Southwest University, Chongqing, China. The soil was typical purple soil having total nitrogen of 1.214 g/kg, total phosphorus of 3.031 g/kg, total potassium of 10.331 g/kg, available phosphorus of 16.842 mg/kg and available potassium of 56.472 mg/kg. The seeds of soybean variety "Xidou 7" were sown in plastic pots in unified model with diameter 31 cm, depth 24 cm, inside bottom diameter of 21 cm and pots were filled with 8 kg soil per pot. Five seeds of soybean were sown per pot and thinning was carried out at 3<sup>rd</sup> leaf stage to keep three plants per pot. Wheat straw was harvested and cut into small pieces of 4 to 6 cm in order to ease application into pots after 15

days of planting. Compound fertilizer was applied at the rate of 20 g per pot. The total fertilization contents were higher than 45%, and the rate of N:P:K was 13:23:9. During the course of the experiment, the highest day temperature was 42°C and the daily average temperature was 35°C. The experiment comprised of five levels of wheat straw mulch viz: control (T<sub>1</sub>), 3750 kg/ha (T<sub>2</sub>), 7500 kg/ha (T<sub>3</sub>), 11000 kg/ha (T<sub>4</sub>) and 14750 kg/ha (T<sub>5</sub>). The treatments were set by keeping in view the previous reports regarding straw mulch. The pots were arranged in completely randomized design (CRD) with three replicates, and 20 pots were used for each treatment. Drought stress was applied at the start of blooming stage by withholding water and sampling for proline and MDA was carried out on 5, 10 and 15 days after withholding water at blooming stage. All other agronomic practices were kept normal and uniform.

Plant height was measured at flowering stage of soybean. Leaf area was measured with LI-3100 leaf area meter (Li-Cor, Lincoln, NE) CI-203 (CID, Inc., USA). The photosynthetic rate, intercellular CO<sub>2</sub> transpiration rate and stomatal conductance of the top fully expanded leaves was determined at reproductive growth stage after withholding water. 20 leaves were used for each treatment selected randomly. Number of leaves/plant and the stem diameter of soybean were measured 62 days after planting. Sampling was done for fully expanded third leaf from the top to assess free proline and MDA contents with 5, 10 and 15 days interval after withholding water.

New and fully expanded leaves (the third leaf from the top) were selected for the leaf measurements. A gas exchange analyzer (Li-COR 6400 portable photosynthesis measurement system, Li-Cor, Lincoln, NE, USA) was used for these measurements during 9:00 to 11:00 am when photosynthetic active radiation above the canopy was 1000 to 1100 μmol m<sup>-2</sup> s<sup>-1</sup>. 20 leaves were selected for each treatment with the following adjustments: molar flow of air per unit leaf area of 388.33 mmol l<sup>-1</sup> m<sup>-2</sup> s<sup>-1</sup>, water vapour pressure into leaf chamber of 3.56 mbar, PAR at leaf surface of up to 1199 mol m<sup>-2</sup> s<sup>-1</sup>, temperature of leaf ranged from 36.67 to 42.59°C, ambient temperature of 36.39 to 40.98°C, ambient CO<sub>2</sub> concentration of 362.3 mol mol<sup>-1</sup> and relative humidity (RH) of 55.12%.

For determination of free proline, fresh leaf samples (0.5 g) were homogenized in 5 ml 3% (w/v) sulphosalicylic acid, heated for 10 min, cooled down at room temperature (25°C) and centrifuged at 4000 g for 10 min. 2 ml of filtrate was mixed into 15 ml centrifuge tube; the mixture was heated at 98°C for 30 min in water bath and then allowed to cool down at room temperature. The mixture was extracted with 5 ml toluene and the absorbance of fraction with toluene aspirated from liquid phase was read at 520 nm. Free proline concentration was determined using calibration curve and expressed as μ mol free proline g<sup>-1</sup> FW.

MDA content was measured by following the method of the Zhang et al. (1990) with some modifications. Leaf samples (0.5 g) were homogenized with a pestle in a mortar using liquid nitrogen. The homogenate powder was mixed with 5 ml 5% trichloroacetic acid. The homogenate was centrifuged at 4000 × g for 10 min at 25°C and then 2 ml liquid gotten was put into centrifuge tube of 3 ml 2-thiobarbituric acid. The mixture was heated at 98°C for 10 min in water bath, and centrifuged at 4000 × g for 10 min again. Liquid phase was read at 532 and 600 nm. Data was presented as the mean ± SE for each treatment (n = 5), were tested with analysis of variance (ANOVA) and Newman-Keuls test, and were marked by letters, where the values sharing the same letters were not significantly different at 5% level, using SPSS 16.0.

## RESULTS

Wheat straw mulch considerably improved the growth

**Table 1.** Effect of wheat straw mulch on growth parameters of soybean under drought.

Treatment	Plant height (cm)	Number of compound leaves/plant	Leaf area (cm <sup>2</sup> )	Stem diameter (mm)
T <sub>1</sub>	28.19±0.48 <sup>c</sup>	4.90±0.12 <sup>d</sup>	14.86±0.51 <sup>b</sup>	2.83±0.02 <sup>d</sup>
T <sub>2</sub>	28.81±0.47 <sup>c</sup>	8.74±0.24 <sup>c</sup>	18.65±0.56 <sup>b</sup>	2.98±0.03 <sup>c</sup>
T <sub>3</sub>	31.64±0.44 <sup>b</sup>	10.53±0.29 <sup>b</sup>	20.20±0.54 <sup>a</sup>	3.18±0.03 <sup>b</sup>
T <sub>4</sub>	32.78±0.42 <sup>b</sup>	11.35±0.24 <sup>a</sup>	18.58±0.63 <sup>a</sup>	3.74±0.07 <sup>a</sup>
T <sub>5</sub>	35.94±0.61 <sup>a</sup>	11.57±0.25 <sup>a</sup>	16.04±0.50 <sup>a</sup>	3.87±0.06 <sup>a</sup>

T<sub>1</sub> = Control (no straw mulch); T<sub>2</sub> = 3750 kg/ha straw mulch; T<sub>3</sub> = 7500 kg/ha straw mulch; T<sub>4</sub> = 11000 kg/ha straw mulch; T<sub>5</sub> = 14750 kg/ha straw mulch. Values are mean ± SE (n = 3). Values followed by the same letter within columns are not significantly different according to Newman-Keuls test (P < 0.05).

**Table 2.** Effect of wheat straw mulch on gas exchange attributes of soybean under drought.

Treatment	Net photosynthesis (P <sub>N</sub> ) (μmol CO <sub>2</sub> m <sup>-2</sup> s <sup>-1</sup> )	Intercellular CO <sub>2</sub> (C <sub>i</sub> ) (μmol mol <sup>-1</sup> )	Stomatal conductance (g <sub>s</sub> ) (μmol H <sub>2</sub> O m <sup>-2</sup> s <sup>-1</sup> )	Transpiration rate (E) (mmol m <sup>-2</sup> s <sup>-1</sup> )
T <sub>1</sub>	15.37±0.61 <sup>b</sup>	243.25±6.76 <sup>ab</sup>	0.281±0.03 <sup>a</sup>	6.81±0.33 <sup>b</sup>
T <sub>2</sub>	16.39±0.82 <sup>ab</sup>	241.88±5.74 <sup>ab</sup>	0.288±0.02 <sup>a</sup>	7.46±0.38 <sup>ab</sup>
T <sub>3</sub>	16.99±0.49 <sup>ab</sup>	234.59±4.31 <sup>ab</sup>	0.285±0.02 <sup>a</sup>	7.68±0.26 <sup>ab</sup>
T <sub>4</sub>	17.78±0.47 <sup>a</sup>	230.70±5.44 <sup>b</sup>	0.296±0.01 <sup>a</sup>	8.30±0.32 <sup>a</sup>
T <sub>5</sub>	17.29±0.55 <sup>ab</sup>	252.51±4.28 <sup>a</sup>	0.335±0.12 <sup>a</sup>	8.14±0.28 <sup>a</sup>

T<sub>1</sub> = Control (no straw mulch); T<sub>2</sub> = 3750 kg/ha straw mulch; T<sub>3</sub> = 7500 kg/ha straw mulch; T<sub>4</sub> = 11000 kg/ha straw mulch; T<sub>5</sub> = 14750 kg/ha straw mulch. Values are mean ± SE (n = 3). Values followed by the same letter within columns are not significantly different according to Newman-Keuls test (P < 0.05).

attributes of moisture stressed soybean. Plant height was increased by the application of varying rates of mulch when compared with the control. Statistically highest plant height (35.94 cm) was recorded in treatment where wheat straw mulch was applied at the rate of 14750 kg/ha which increased the plant height by 27.5% over control and where no wheat straw mulch was applied. Number of leaves per plant followed increasing trend with increase in mulch quantity. Statistically, the lowest number of leaves/plant was noted in the control treatment. Application of mulch at the rate of 14750 kg/ha enhanced the number of leaves by 136% against control which was statistically at par with 11000 kg mulch ha<sup>-1</sup>. Leaf area of soybean was increased with the increase in quantity of wheat straw mulch and then started to decline. Minimum leaf area (14.86 cm<sup>2</sup>) was recorded in the control treatment and highest (20.20 cm<sup>2</sup>) was noted in treatment where wheat straw mulch was applied at the rate of 7500 kg/ha. Stem diameter also followed the linearly increasing trend with increase in straw mulch quantity. Consequently, minimum stem diameter (2.83 mm) was observed in the control treatment and the highest (3.87 mm) was found in the treatment where maximum quantity of straw mulch was applied (Table 1).

Leaf net photosynthesis rate was enhanced with increase in straw mulch quantity when compared with the control. Minimum photosynthesis (15.37 μmol CO<sub>2</sub>m<sup>-2</sup>s<sup>-1</sup>) was observed in the control treatment whereas, the statistically highest photosynthesis (17.78 μmol CO<sub>2</sub>m<sup>-2</sup>s<sup>-1</sup>) was found in treatment where straw mulch was applied at the rate of 11000 kg/ha and then started to decline and was found to be 17.29 μmol CO<sub>2</sub>m<sup>-2</sup>s<sup>-1</sup> in 14750 kg/ha mulch treatment. Stomatal conductance (g<sub>s</sub>) was improved with the increase of straw mulch quantity. Statistically, the lowest photosynthesis was recorded in the control and the highest was found where 14750 kg/ha of straw mulch was applied, thus improved by 19.2% over the control. The C<sub>i</sub> of all treatments decreased, except 14750 kg/ha. The transpiration rate (E) substantially increased with addition of wheat straw mulch. Highest transpiration rate was recorded in the treatment where straw mulch was applied at the rate of 11000 kg/ha. Application of wheat straw mulch at the rate of 14750 kg/ha led to maximum intercellular CO<sub>2</sub> and stomatal conductance (Table 2). Statistically minimum proline contents were observed in the control and maximum in the 14750 kg/ha straw mulch (Table 3). The MDA contents of soybean plants treated with different straw lower than the control as recorded

**Table 3.** Effect of wheat straw mulch on free proline and malondialdehyde (MDA) contents of soybean under drought.

Treatment	Proline ( $\mu\text{g/g FW}$ )			Malondialdehyde MDA ( $\mu\text{mol/g FW}$ )		
	5 days	10 days	15 days	5 days	10 days	15 days
T <sub>1</sub>	33.23 $\pm$ 0.92 <sup>b</sup>	51.13 $\pm$ 3.47 <sup>b</sup>	158.09 $\pm$ 5.39 <sup>b</sup>	26.51 $\pm$ 0.20 <sup>a</sup>	36.40 $\pm$ 1.74 <sup>a</sup>	35.37 $\pm$ 0.22 <sup>a</sup>
T <sub>2</sub>	33.45 $\pm$ 0.81 <sup>b</sup>	53.70 $\pm$ 4.08 <sup>b</sup>	158.66 $\pm$ 7.04 <sup>b</sup>	23.96 $\pm$ 0.23 <sup>bc</sup>	34.12 $\pm$ 1.82 <sup>ab</sup>	26.17 $\pm$ 1.16 <sup>c</sup>
T <sub>3</sub>	34.87 $\pm$ 1.15 <sup>b</sup>	54.64 $\pm$ 3.02 <sup>b</sup>	204.67 $\pm$ 8.30 <sup>a</sup>	25.01 $\pm$ 0.72 <sup>b</sup>	35.84 $\pm$ 1.89 <sup>a</sup>	31.11 $\pm$ 0.92 <sup>b</sup>
T <sub>4</sub>	35.80 $\pm$ 1.33 <sup>b</sup>	53.34 $\pm$ 1.41 <sup>b</sup>	173.81 $\pm$ 13.98 <sup>ab</sup>	20.45 $\pm$ 0.65 <sup>d</sup>	34.26 $\pm$ 0.97 <sup>ab</sup>	32.32 $\pm$ 1.58 <sup>a</sup>
T <sub>5</sub>	37.41 $\pm$ 1.40 <sup>a</sup>	84.75 $\pm$ 4.09 <sup>a</sup>	204.99 $\pm$ 13.35 <sup>a</sup>	23.27 $\pm$ 0.41 <sup>c</sup>	31.03 $\pm$ 0.42 <sup>b</sup>	28.47 $\pm$ 0.35 <sup>bc</sup>

T<sub>1</sub> = Control (no straw mulch); T<sub>2</sub> = 3750 kg/ha straw mulch; T<sub>3</sub> = 7500 kg/ha straw mulch; T<sub>4</sub> = 11000 kg/ha straw mulch; T<sub>5</sub> = 14750 kg/ha straw mulch. Values are mean  $\pm$  SE (n = 3). Values followed by the same letter within columns are not significantly different according to Newman-Keuls test (P < 0.05).

after 5, 10 and 15 days of water stress imposition (Table 3).

## DISCUSSION

Straw mulch potentially increased the soil moisture content which in turn led to improved growth. These results are in agreement with that of Ahmed et al. (2007) who reported that the increase in mulch rate from 1000 to 4000 kg ha<sup>-1</sup> when compared with control progressively increased the plant height (10 to 37 %). Rahman et al. (2006) also reported increase in plant height of tomato mulched with rice straw, while lowest height was observed in the control. The increase in plant height could be attributed to moisture conservation and weed suppression due to the application of mulches (Ullah et al., 1998).

The increase in number of leaves per plant by mulching is in conformity with the results of Kayum et al. (2008) which reported that there was 33% increase in number of leaves/plant by application of straw mulch as compared to the control in tomato.

This enhancement of leaf area by mulching is in accordance with the report of Tolk et al. (1999) which reported that leaf area was greater in the mulched treatments when compared with the bare soil treatments. Furthermore, Qin et al. (2006) also reported the increased leaf area per plant by application of rice straw mulch. It is noteworthy, that leaf area increased with the increase in straw mulch quantity at low mulch quantity but declined at high straw mulch quantity in order to reduce transpiration and to alleviate the effects of drought stress.

Straw mulch validly promoted the crop growth in terms of plant height, number of leaves, leaf area and stem diameter over the control. The improvement in growth with straw mulch may be ascribed to the lowering of daytime soil temperature and increase in moisture supply to the crop. The considerable enhancement in soybean growth was the result of soil water being used for crop growth and yield rather than evaporation of soil water. The greater

soil profile moisture under mulch has important implications in the utilization of water by crop and in soil reactions that control the availability of nutrients and biological nitrogen fixation (Surya et al., 2000).

Thakur et al. (2000) reported increased photosynthesis in chillies with application of mulch. Mulch is reported to retain soil moisture for longer duration providing additional amount of nutrition which help to enhance the rate of photosynthesis as a result, improving plant health (Pongsa-Anutin et al., 2007). Enhancement of transpiration rate by straw mulch is in accordance with Zhang et al. (2009) report. Wheat straw mulch provides the ground with cover to prevent evaporation; the higher evapotranspiration under water limiting conditions improved photosynthesis and ultimately increased crop growth and yield.

The Ci of 11000 kg/ha was sharply lower than the decreased CK (5.2%). Early researches concluded that the reduction of soil dehydrates led to swelling pressure that made the stomata to close (Schulze, 1993). In our study, photosynthesis (P<sub>N</sub>), transpiration rate (E) and stomatal conductance (gs) were considerably improved by different rates of wheat straw mulch as compared to the control. Higher stomatal conductance observed in the present study might have increased CO<sub>2</sub> diffusion into the leaf and favored higher photosynthetic rates.

This improvement in gas exchange parameters could be attributed to two main reasons: firstly, straw mulch increased the medium between the atmosphere and soil layer, which acted as effective barrier to the part of the solar radiation, thus reducing the soil evaporation; secondly, the larger quantity of straw mulch caused less bare surface which led to more reduced evaporation, and ultimately more water used for photosynthesis was available.

Free proline accumulation is one of the key features of protein metabolism under drought stress, and also the first response of plants exposed to water-deficit stress in order to reduce injury to cells (Ashraf and Foolad, 2007). Free proline accumulation in plant under water stress can reduce water loss of plants. During the period of withholding

water at 5, 10 and 15 days, the free proline contents linearly increased with the increase in straw mulch quantity. This enhancement of free proline contents could be attributed to straw mulch which blocked soil water evaporation and the effect increased with the quantity of wheat straw dynamically.

The contents of malondialdehyde (MDA) reflect the degree of membrane lipid peroxidation. A decrease in membrane stability reflects the extent of lipid peroxidation. When the crop plants are under stress, membrane lipid peroxidation often occurs. The protective action of wheat straw mulch was demonstrated by the decreased MDA contents, which protected the drought stressed soybean plants from membrane damage.

## Conclusions

Wheat straw mulch applied during the growing season of soybean substantially improved the growth and gas-exchange attributes. Furthermore, wheat straw mulch considerably modulated the proline and malondialdehyde contents. Plant growth in terms of plant height, number of leaves/plant and stem diameter were highest with 14750 kg/ha; however, leaf area was observed to be highest with 7500 kg/ha wheat straw mulch. The gas exchange parameters like  $P_N$ ,  $E$  and  $g_s$  were also improved by wheat straw mulch as compared to the control;  $P_N$  and  $E$  were highest with 11000 kg/ha, while  $C_i$  and  $g_s$  were higher with 14750 kg/ha wheat straw mulch. Straw mulching significantly reduced the MDA contents which averted the membrane damage, while, proline contents improved with increase in straw mulch quantity.

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