Effects of Shading on Growth and Photosynthetic Potential of Greengram (Vigna radiata (L.) Wilczek) Cultivars

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The effects of shading on growth and photosynthetic potential was analyzed with 8 cultivars with greengram (Vigna radiata (L.) Wilczek). Plants grown in 5 L pots were subjected to 2-week shading treatment and after 2-week recovery by removing a black-cheese cloth. Photosynthetic potential was evaluated by electron transport rate sealed on the both leaf surfaces by vaseline, which is close relationship to gross photosynthetic rate because CO2 released by the reaction of photosynthesis is recycled by stroma in chloroplast. Shading treatment reduced total dry weight of all cultivars. In particular, significant reduction in total dry weight was found in Nyangoo, Magwe and VC. Leaf specific area under shading treatment showed increment tendency and remarkable increase in this parameter was found in Nyangoo and VC, which cultivars decreased total dry weight under shading, indicating that shading treatment lead to thin of the leaf thickness. The ratio of plant height to diameter of stem was significantly decreased in R-288-8, Kanti and Yezin-5 by shading, indicating the restriction of succulent growth under shading condition and these cultivars are superior in lodging resistance to other cultivars. Photosynthetic rate as evaluated by electron transport rate sealed by vaseline was decreased by shading treatment. The extent of decrease in photosynthetic potential was found cultivaral difference. According to these results, Kanti is capable of applicable to the highest in resistance to shading and its recovery.

Keywords: electron transport rate, greengram, gross photosynthetic rate, photochemical system II, shading

INTRODUCTION

Light is an indispensable resource for plant growth because the light energy supplied from the sun is the basic factor that regulates growth rate, organ development or structure, function and behavior. The adaptation and acclimation to light environment is also critical to the plant survival and production efficiency in any ecosystem.

In particular, photosynthetic active radiation is the major factor regulating photosynthesis and other physiological processes in plants, and hence the dry matter production and yield depend on photosynthetic active radiation to a great extent (Rao and Mitra, 1988). In general, plants grown in high light intensity and adapted to this condition have been known to reduce the photosynthetic rate under the shading condition. But if we can select a cultivar that performs a stable photosynthesis under different light intensities, it will be a greater advantage to get high and stable productivity under the natural environments.

Islam (1993) reported that Vigna radiata was greatly affected by shading, and the total dry weight per plant decreased with an increase in shading ratio, and this trend continued till the maturity stage. The low economic yield of greengram in intercropping conditions is largely attributed to the less solar energy supply by shading during the entire life cycle. The cultivation of greengram characterized by having an early maturity is allowed to be successfully grown before and after rice cropping, or intercropping with sugarcane (Pookpakdi, 1978). However, in the summer season, the irradiance fluctuation frequently occurs, and it may cause the yield instability for greengram species (Karim et al., 2003). The resistance to low light intensity or shading is important factor for greengram cultivars grown under the agro-forestry cropping system and intercropped with such a tall plant as sugarcane plants.

Although numerous studies have been carried out on photosynthetic light responses in many crops, yet the effects of irradiance intensities on the photosynthetic apparatus of carbon assimilation and photochemical systems in a leaf have not been sufficiently studied, especially in tropical grain legumes (Karim et al., 2003). The accumulation of knowledge on the shading response or adaptation, and mechanisms of shading resistance is important as the foundation of improving and stabilizing the yield of leguminous crops grown in Myanmar.

Many of the smallholders farming system in Myanmar were so far characterized by variable seasonal rainfall and poor soil fertility with minimal external inputs of nitrogen and phosphorous. This results in reducing crop yields to extremely low levels. However, recently, the efforts to improve soil fertility have been begun introducing leguminous species into the farming systems of many rural communities. The nitrogen benefit from legumes in intercropping...
systems is depending on the symbiotic activity. The importance of legume variety in nitrogen nutrition in intercropping systems in Myanmar not yet been sufficiently documented.

In the intercropping combination with tall and short species, the amount of light that is received by short species such as greengram is greatly influenced by the plant population density of the taller partner crops. Under such circumstances, shade-resistant legumes become valuable to get higher yield. Of the legume crops grown in Myanmar, greengram is a promising grain legume that given much attention from farmers for its high price and short growth duration. Thus, the successful introduction of this legume species is expected to bring a greater advantage from the intercropping system.

In order to raise and stabilize the yield of greengram in Myanmar, various technological improvements are necessary for the intercropping and cultivar breeding. However, very few reports are available on the effect of shading on growth and photosynthetic activity in greengram. It is an important factor to search greengram cultivars that have the ability to tolerate partial shading for intercropping with sugarcane and pigeon pea. However, refined techniques for evaluating shade tolerance have not yet developed in Myanmar. By identifying genotypic differences for resistance to shading condition, to obtain new information which is indispensable for the improvement of cropping system unique in Myanmar will be expected.

In many studies, photosynthetic rate measured with a leaf of full open stomata is evaluated as a potential standard of photosynthetic activity of the leaf. The measurement of photosynthetic is a time taking operation because it is necessary to keep the stomata at a sufficient openness, and hence, measurements of many leaves are difficult (Kubota et al., 1991). Thus, photosynthetic measurements under stomatal resistance free conditions may indicate the potentiality of crop for photosynthesis. In this study, the photosynthetic activities of greengram cultivars were accurately and quickly estimated from ETR of sealed leaves according to the method described by Haimeirong et al. (2002). The objective of this study is to compare the cultivar features in growth responses and electron transport rate under shading treatment for developing the shade-resistant genotype.

MATERIALS AND METHODS

Plant materials and treatment

The experiment was conducted during August to September, 2006 at Kyushu University, Japan, using eight greengram cultivars: two landraces ( cvs. Magwe, Nyaungoo) are from China, six Myanmar promising cultivars ( cvs. Yezin-4, Daumo, R288-8, Kanti, Yezin-5 and VC 1973A) are released from Central Agricultural Research Institute in Myanmar.

The pre-germinated seeds were sown in a 5 L pot filled with sandy loam soil. Then, the young shoots were thinned to one plant per pot for each cultivar after germination. The plants were grown outdoors under natural light conditions for 30 days after sowing. The experiments were designed with four replications. The pots were divided into two groups. One group was continuously grown under the natural light condition, control, and the other group was subjected to the low light condition, shading. The shading treatment was conducted at 30 days after sowing and was divided into two stages, 2-week shading and 2-week recovery. Shading was carried out by placing pots under the frames covered with a black cheese-cloth to get 60% of full sunlight. During the recovery stage, the black cheese-cloth was removed and then the pots were returned back under natural light for two weeks.

Measurements of growth parameters and electron transport rate

Four plants for each treatment and cultivars were harvested before and after 2 weeks shading, and were measured leaf area per plant with an automatic area meter (Model AAM 8, Hayashi Denko, Japan). The plants harvested were dried at 80°C for 3 days and were measured dry matter weight per plant. Chlorophyll degree of a leaf is an index determined as SPAD value by the chlorophyll meter (SPAD-502, Konica-Minolta, Japan), leaf area per plant, specific leaf area (SLA), plant height and diameter ratio under the control and shaded conditions were measured before and after the shading.

To observe the relationship between gross photosynthetic rate under 2% O2 level (Pg 2%) and electron transport rate sealed on both leaf surfaces with vaseline (ETRvase), the measurement of photosynthetic rate was conducted at 390 μmol mol−1 of CO2 concentration, 2% O2 level and photosynthetic photon flux density (PPFD) range from 200 to 700 μmol m−2 s−1. The temperature, relative humidity and air flow rate were similar as described in Araki et al. (2014). The calculation equations for ETR and Pg were the same as those described in Araki et al. (2012).

In this experiment, the uppermost fully expanded leaves were used for the determination of ETR of sealed leaf and measured at two weeks after the shade and the recovery treatments. According to the method described by Haimeirong et al. (2002), both surfaces of a leaf were sealed with vaseline to stop the gas exchange between the leaf and the atmosphere; hence the functions of both photosynthetic assimilation (CO2 uptake) and photorespiration (CO2 release) are restricted within the leaf. After both functional rates became equally balanced, electron transport rate of PSII in sealing leaves was monitored within 15 min with a fluorescence probe (PAM-2000, Walz, Germany) at different PPFD levels (200, 300, 400, 500 and 600 μmol m−2 s−1).

RESULTS AND DISCUSSION

The growth parameters, leaf, stem, root and total dry weights under the control and shading conditions are shown in Table 1. The accumulation of dry matter in crops frequently has a close relationship with LA. As a result of reduction in leaf dry weight under shading, total dry weight was declined especially in cvs. Nyaungoo, Magwe and VC. The reduction in stem and root dry weight was also
attributed to the reduction in total dry weight in these cultivars. Particularly, remarkable reduction in root dry weight was found in these cultivars, which corresponded to 42 to 45% as compared to that under control. Dry weight of individual organs was not significantly different between the control and shaded plants in cvs. Yezin-4, R288-8, Kanti and Yezin-5 and their total dry weight ranged 92 to 98% of the control and shaded plants in cvs. Yezin-4, R288-8, Kanti and Yezin-5 and their total dry weight ranged 92 to 98% of the control. An additional feature of these cultivars was the increase in leaf area under the shaded condition (Table 1).

Chlorophyll plays a key role in determining the light absorption efficiency within a leaf. The chlorophyll degree was influenced by shading. In all the cultivars except cultivar VC and Nyaungoo, SPAD values were increased during the shading treatment. In particular, the value of R288-8 under shading was significantly higher than those under control. Many previous studies have been reported that chlorophyll per unit leaf area increased under low PPFD, which allowed increasing the absorption of photosynthetic active radiation and enhancing the assimilation efficiency (Nilsen and Orcutt, 1996; Pearcy, 1998; Evans and Pooter, 2001).

Kubota et al. (1992) stated that SLA was increased with reduction in light intensity in both Vigna radiata and Vigna mungo. The similar response was found in this experiment and SLA of all the cultivars except cv. Yezin-4, was increased under shaded condition. According to the report of Björkman (1981), the shaded plant generally had a higher SLA, because the leaves were characterized by having larger layer of palisade cells. This may cause the increase in the number of chlorophylls, by which the photosynthetic rate per unit leaf area increased at low light intensity.

Greengram plants were elongated in height by shading, and this may reduce the physical strength of the plant, and cause to increase lodgingss (Kubota and Abdul, 1992).

The ratio of plant height to stem diameter (H/D) is used as an indicator for lodging resistance. In this study, H/D ratio differed between the cultivars, and the smallest values were found in Yezin-5 (Table 1). This may suggest that this cultivar have an increased lodging resistance because of keeping a larger stem diameter under the shaded condition.

Net photosynthetic rate measured in the normal atmospheric air containing 21% O₂ did not show a linear relationship with ETR in C3 leaves, because the functional strength of photorespiration in leaves greatly affects the photosynthetic electron distribution (Krall et al., 1991). But photorespiration restricted in the air of low O₂ concentration, the sink for electrons transported from the photosystem is limited to the CO₂ assimilation function, and hence a close relationship appears between the rate of CO₂ assimilation without photorespiration and ETR (Krall and Edwards, 1992).

Light responses in Pg2%, ETR2% and ETRvase in a young active leaf were determined at a leaf temperature of 30°C in the PPFD range from 200 to 700 μmol m⁻² s⁻¹. Pg2% increased with an increase in PPFD, having a turning point at about 600 μmol m⁻² s⁻¹ (Fig. 1A). Both ETR2% and ETRvase had an almost the same response pattern to light intensity (Fig. 1B).

The relationship between Pg2% and ETRvase for three greengram cultivars, Kanti, Yezin-4 and VC grown under the control and shading are shown in Fig. 2. Under each measurement condition, ETRvase showed a close positive

### Table 1 Cultivaral difference in dry matter weight, leaf area, leaf specific area (SLA), the ratio of plant height to stem diameter (H/D) and SPAD values of greengram cultivars under the control and shading conditions.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Treatment</th>
<th>Leaf (g plant⁻¹)</th>
<th>Stem (g plant⁻¹)</th>
<th>Root (g plant⁻¹)</th>
<th>Total (g plant⁻¹)</th>
<th>Leaf area (m² plant⁻¹)</th>
<th>SLA (cm² g⁻¹)</th>
<th>H/D value</th>
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<td>Nyangoo</td>
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<td>2.1</td>
<td>1.1</td>
<td>7.1</td>
<td>16.1</td>
<td>423</td>
<td>3.9</td>
</tr>
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<td>1.8ns</td>
<td>0.5**</td>
<td>4.8*</td>
<td>14.2ns</td>
<td>561*</td>
<td>6.2**</td>
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<td></td>
<td></td>
<td>(67)</td>
<td>(86)</td>
<td>(44)</td>
<td>(68)</td>
<td>(88)</td>
<td>(133)</td>
<td>(155)</td>
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<tr>
<td>Magwe</td>
<td>Control</td>
<td>4.2</td>
<td>2.6</td>
<td>1.4</td>
<td>8.3</td>
<td>15.0</td>
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<td>2.2</td>
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<td>2.5ns</td>
<td>0.6***</td>
<td>6.4*</td>
<td>12.5ns</td>
<td>391ns</td>
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<td></td>
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<td>(96)</td>
<td>(45)</td>
<td>(77)</td>
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<td>Yezin-4</td>
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<td>2.7</td>
<td>1.1</td>
<td>6.6</td>
<td>14.6</td>
<td>533</td>
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<td>6.0ns</td>
<td>13.6ns</td>
<td>446ns</td>
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<td>Daumo</td>
<td>Control</td>
<td>3.3</td>
<td>2.1</td>
<td>0.9</td>
<td>6.3</td>
<td>12.1</td>
<td>365</td>
<td>3.5</td>
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<td>2.0</td>
<td>1.0</td>
<td>6.0</td>
<td>12.3</td>
<td>396</td>
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<td>VC</td>
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<td>343</td>
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<td>1.9ns</td>
<td>0.5***</td>
<td>4.9**</td>
<td>13.6ns</td>
<td>540**</td>
<td>5.7**</td>
</tr>
<tr>
<td></td>
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<td>(69)</td>
<td>(105)</td>
<td>(157)</td>
<td>(227)</td>
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<td>Kanti</td>
<td>Control</td>
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<td>2.7</td>
<td>1.1</td>
<td>6.6</td>
<td>15.9</td>
<td>580</td>
<td>4.9</td>
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<td>2.6ns</td>
<td>0.8ns</td>
<td>6.4ns</td>
<td>19.8**</td>
<td>650ns</td>
<td>5.5ns</td>
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<td></td>
<td></td>
<td>(110)</td>
<td>(95)</td>
<td>(73)</td>
<td>(98)</td>
<td>(125)</td>
<td>(112)</td>
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<td>Yezin-5</td>
<td>Control</td>
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<td>2.1</td>
<td>0.9</td>
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<td>600*</td>
<td>4.4ns</td>
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<td>(90)</td>
<td>(93)</td>
<td>(119)</td>
<td>(127)</td>
<td>(112)</td>
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</tbody>
</table>

Values in the parentheses are percent of shading treatment to control. *** , ** , * and ns represent significant varietal differences and not significant at 0.1%, 1%, 5% level, respectively.
relationship with $P_g2\%$. This means that $P_g2\%$ is accurately estimated from $ETR_{vase}$, which can be used as an indicator to estimate the photosynthetic activity of greengram cultivars in different PPFD. However, the accuracy of estimated values might gradually decrease as the PPFD increases beyond 600 μmol m$^{-2}$ s$^{-1}$, because energy dissipation mechanisms such as the Mehler reaction may become more activated (Oberhuber and Edwards, 1993).

By using $ETR_{vase}$, the photosynthetic potential of different cultivars was estimated within the PPFD range of 200–600 μmol m$^{-2}$ s$^{-1}$ (Fig. 3).

$ETR_{vase}$ was determined under the control, shading and recovery conditions to identify the varietal difference. Four cultivars measured $ETR_{vase}$ in Fig. 3 were selected based on the results of the changes in total dry matter weight to shading; cultivars of shading sensitive were Nyangoo and Magwe, and those of resistance were Yezin-4 and Kanti. The varietal differences in $ETR_{vase}$ response were observed in both shaded and recovery conditions. Among the eight cultivars, $ETR_{vase}$ of a leaf was the highest in cvs. R288-8, Kanti and Yezin-5 and kept a stable level in both shading and recovery conditions (data not shown). However, $ETR_{vase}$ of cvs. Nyaungoo, Magwe, Yezin-4, Daumo and VC may be greatly reduced under shading and are very sensitive to light intensity fluctuation.

In cv. Magwe, a large reduction in $ETR_{vase}$ was observed in shaded plant but it recovery level was higher than control plant after two weeks. On the other hand, a
significant reduction of total dry weight was also found in this cultivar and it may be suggested that this cultivar may sensitive to changing light environment. The reduction degree of ETRvase under shading condition showed same level as that of total dry weight matter, which suggests that the measurement of ETR sealed on both leaf surfaces with vaseline is available to efficient screening for shading resistant cultivars.

Cultivars used in this study may be divided into some groups according to the results. Firstly, we could divide the response in total dry weight to shading into two groups, that is, shading sensitive cultivars were Nyangoo, Magwe and VC, and shading resistant ones were Yezin-4, Daumo, R288-8, Kanti and Yezin-5. The former three cultivars (shading sensitive) possessed common feature of shading response, decrease in root dry weight and maintenance of leaf area. However, this group was further divided into two groups by the results of ETRvase. Magwe was sensitive in ETRvase to shading, while the others, Nyangoo and VC were non-sensitive. Latter five cultivars were further also divided some groups based on growth and photosynthetic features. Yezin-4 and Daumo showed the increment in H/D with the maintenance of total dry weight matter under shading condition, which feature could lead to lodging. The residual three cultivars, R288-8, Kanti and Yezin-5 maintained H/D and photosynthetic feature. It seems that the classification of cultivars based on dry matter production and photosynthetic features under shading conditions may be available for application of genetic resources such as breeding.

The results of the present study agree with the finding of Haimerrong et al. (2002) and they were reported that ETR of sealed leaf is able to be used as one of the photosynthetic indicators for leaves of C3 crops. Varietal difference of ETRvase response to shading was significantly different in greengram cultivars and it seems to be possible to improve shade-resistant cultivars for intercropping. However, there is a little information about sealed leaf method and further study is necessary to improve the resistant cultivars. The order of shading resistance from high to low is as follow; cvs. Kanti, Yezin-5, R288-8, Yezin-4, Daumo, VC, Magwe and Nyangoo.

REFERENCES


REFERENCES


Araki, T., Oo, T. T., Kubota, F. 2014. Effects of flooding...