Evaluation of Intercropping System of Wheat and Egyptian Clover in the Nile Delta of Egypt

Aki KUBOTA1,2,5, Sayed Ahmed SAFINA3,4, Saad Mohamed SHEBR3,5, Alaa El-Din Hassan MOHAMED4,5, Naoto ISHIKAWA1,5, Katsuyoshi SHIMIZU1,5, Korany Abdel-GAWAD2,5, and Sachio MARUYAMA1,5

1 Faculty of Life and Environmental Sciences, University of Tsukuba, Ibaraki 305-8572, Japan
2 Faculty of Agriculture, Cairo University, Giza 12613, Egypt
3 Rice Research and Training Center, Agricultural Research Center, Kafr El-Sheikh 33713, Egypt
4 Animal Production Research Institute, Agricultural Research Center, El-Dokki, Cairo, Egypt
5 JICA/JST SATREPS

Abstract Using limited water resources efficiently and increasing crop production for population growth are critical issues in Egypt. A field experiment was conducted to evaluate wheat-Egyptian clover intercropping system with the purpose of maximizing the land use and minimizing evaporation from soil surface in the Egyptian Nile Delta. An additional treatment was cutting at 15 cm from the land level at 69 days after sowing to provide animal feeds in winter. Leaf area index (LAI) and ratio vegetation index (RVI) of the canopies, SPAD values of leaves, biological yields, grain yield, land equivalent ratio (LER), and water use efficiency (WUE) were measured. LAI and RVI of monocultured wheat were improved by intercropping with Egyptian clover, which can contribute to reduction in water evaporation from the soil surface. Wheat grain yield was not affected by intercropping but significantly reduced by cutting during the vegetative stage. Egyptian clover production was notably reduced by both intercropping and cutting treatment. WUE based on the yield was 6% higher for intercrops than for wheat monocrop. The total LER of intercropping system was 1.17. The results suggested that the cultivation of wheat intercropped with Egyptian clover has possibility to provide food both for human and animal of small-scale farmers with limited land and water resources, and also to reduce evaporation from soil surface in the Egyptian Nile Delta. The cutting treatment at early stage of crops was not effective of production of neither wheat nor clover.

Key words: Biological yield, Canopy development, Cutting treatment, Land equivalent ratio, Water use efficiency

Introduction

In Egypt, agriculture is entirely depending on the water from River Nile, and the water usage for agricultural activities is about 90% of total water use in the country (Koth et al., 2000). The climate of Egypt is classified as arid and semi-arid to desert with very hot dry summer. Mean annual rainfall is usually less than 80 mm, except the coastal area, and evaporation rate is very high (El-Nahrawy, 2011). The arable land for crop production is only 2.6% of whole country, and arable land per capita is 0.04 ha which is very low for agriculture dependent country (FAO, 2012). Thus, efficient management of limited water and land is a key for increasing demand of crop production.

Wheat (Triticum aestivum L.) is one of the most important winter crops in Egypt. The country imported 9.8 million tons in 2012 while the total domestic production was 8.8 million tons (FAOSTAT, 2012). One of the reasons for the low self-sufficiency of wheat of approximately 47% is that most farmers keep animals in the farms, and prefer producing Egyptian clover (Trifolium alexandrinum L.) to wheat for feeding their animals.

Water use of winter wheat is very low in early growing season because of cool temperature. The daily water use is often lower than 2.0 mm (Asseng, 2012). Until the canopy enlarges during tillering and stem elongation periods, soil surface evaporation stays high as a proportion of evapotranspiration (ET) in wheat field for some months. Therefore, an approach to reduce ET of wheat field, especially in early stage, is to minimize the soil surface evaporation. Intercropping wheat with Egyptian clover can be the solution of the early soil surface evaporation issue.

Intercropping systems for winter cultivation have been studied by many researchers. The advantages of intercropping cereal crops with legumes are N fixation of legumes (Lithourgidis et al., 2007), higher quality of forage (Ross et al., 2004; Vasilakoglou and Dhima, 2008; El-Karamany et al., 2012), and higher biomass production (Ghaffarzadeh, 2013). Disadvantage of intercropping can be the extra labor work for preparation, limited choices of herbicide which can be used for both
monocots and dicots, and lower yield of individual crops under intercropping system.

Most of the studies of winter intercropping were focused on forage production. They are intercrops of wheat, barley or oat - Egyptian or berseem clover (El-Karamany et al., 2012; Ross et al., 2004; Vasilakoglou and Dhima, 2008), and wheat or barley-vetch (Roberts et al., 1989; Lithourgidis et al., 2007). Studies on evaluating intercrops by grain yield of cereal crops are yet very limited.

The objectives of this study are to evaluate the intercropping system of winter wheat and Egyptian clover by biological yield, grain yield of wheat, and water use efficiency. The land equivalent ratio (LER) is also discussed as it is an useful single index for expressing the yield advantage of intercropping system (Willey, 1979; Mead and Willey, 1980; Willey and Rao, 1980), and expresses the relative land area required as sole crops to produce the same yield as intercropping.

We additionally tried cutting the wheat plants during their vegetative growth stage. In Egypt there often are not enough feed sources for farm animals in winter. Trials on clipping barley during the vegetative growth for feeding animals are reported in some studies (Yau, 2003; El-Shatnawi, et al., 1999), but the information on wheat is very limited. If the cutting treatment have no negative effect on the final wheat grain yield, this can encourage farmers to grow wheat not only for the grain yield but also for animal feed in winter. It would be also possible for farmers to allow animals to graze wheat leaves together with Egyptian clover in the intercropping field, for better quantity and quality of feeds, if no negative effects.

**Materials and Methods**

**Experimental site**

The field experiment was conducted during the period from December, 2012 to May, 2013 in the experimental field of Sakha Research Station (31°05’58”N and 30°55’19” E), Agricultural Research Center in the Nile Delta of Arab Republic of Egypt. The soil was classified as Vertisols by Soil Taxonomy. Some characteristics of the soil was reported by Kubota, et al. (2015), and it had a bulk density ranged from 1.54 to 1.73 g cm⁻³ in 0 to 80 cm depth from the soil surface. The climate of the area belonged to the arid climate in Köppen’s Mediterranean climate zone with very hot dry summer and some rainfall in winter. Mean monthly temperature and precipitation recorded near the experimental field during the experiment are given in Fig. 1. The total precipitation received was 106 mm during the experimental period.

**Treatments and field layout**

The field experiment had two factors, i.e. cropping system and cutting treatment. For the cropping system, monocultured wheat (*Triticum aestivum* L.) and Egyptian clover (*Trifolium alexandrinum* L.), and intercropping of the two crops were compared. The cutting treatment was to cut the both crops at 15 cm height from the soil surface during the vegetative growth stage of wheat. In each plot of cropping system, crops in the half portion were cut on the 69th day after sowing (DAS), Feb. 12, 2013, and the rest of the half was grown without cutting until harvest (control for cutting treatment).

The wheat seeds were sown in lines, and seeds of Egyptian clover were broadcasted. The seeding rate of Egyptian clover in intercropping plots were half of that in the monocultured plots.

The experiment was conducted as a split-plot design, with intercropping treatment as a main factor and cutting treatment as a sub factor. The size of each plot was 6.0 m × 5.0 m and repeated 3 times. The total area was 540 m².

**Crop management**

Nitrogen (N), phosphorus (P₂O₅) and potassium (K₂O) were applied to the field at the rate of 178.6, 35.7, and 59.5 kg ha⁻¹, using urea, superphosphate and potassium sulfate, respectively. These fertilizer application rates are conventional among wheat producing farmers in the area.

Varieties used in this study were Giza 168 for wheat and Sakha 4 for Egyptian clover. Both seeds were sown on December 5, 2012. Wheat seeds were sown by hands in line with a distance of 20 cm and seeding rate was
95 kg ha\(^{-1}\) for both monocropping and intercropping plots. Seeds of Egyptian clover were broadcasted at the seeding rates of 86 kg ha\(^{-1}\) for monocrop and 43 kg ha\(^{-1}\) (a half the amount for the monocrop) for intercropping plot.

Irrigation was applied 5 times during the experiment on Dec. 6, Jan. 15, Feb. 24, Mar. 13, and Apr. 3 by surface basin irrigation. The total amount of irrigation water was 831 mm in all plots.

**Parameter investigated**

Leaf area index (LAI) was measured in the field by Plant Canopy Analyzer (LAI-2200, LI-COR, Inc.) at 3 spots in each plot on Jan. 23 (49 DAS), and ratio vegetation indices (RVI) were measured at approximately 30 cm above the canopy (about 80 cm above the ground level) by portable spectroradiometer (MS-720, EKO Instruments) at 3 spots in each plot on Jan. 25 (51 DAS) to estimate the crop coverage ratio of the field. SPAD value of the youngest expanded leaves of wheat and Egyptian clover were measured by Chlorophyll Meter (SPAD-502 Plus, Konica Minolta, Inc.) at 5 plants in each plot on Jan. 25 to evaluate chlorophyll contents.

On Feb.12 (69 DAS), both crops in 6 m \(\times\) 5 m of each plot were cut at 15 cm high from the soil surface, and above-ground parts were sampled from 1 m \(\times\) 1 m as the 1st cut. In intercropping plots, completed crops (both wheat and Egyptian clover) were sampled and weighed together after being dried at 70 °C for 72 hr for dry weight measurement.

Yield survey was conducted on May 11 (157 DAS). All above-ground parts were sampled from 1 m \(\times\) 1 m in each plot as the final cut. The wheat samples were separated for grain yield and yield components, and dried as same as samples from the cutting at 69 DAS. Grain yield, grain weight per spike and 1000-grain weight of wheat were adjusted at 15 % moisture content. The significance of the treatment effects were analyzed by Tukey method using software of Statistix 9 (Analytical Software, Co.).

Using the data sets of yield survey, land equivalent ratio (LER) was calculated as follows (Willey, 1979);

\[
\text{LER} = L_1 + L_2 - \frac{Y_I}{YM_I} + \frac{Y_I}{YM_2}
\]

where \(L_1\) and \(L_2\) are the LERs for individual crops, \(Y_I\) and \(Y_M\) are the yields of individual crops when intercropped, and \(YM_I\) and \(YM_2\) are their yields when monocultured.

Water use efficiency (WUE) was also calculated as the biological yield (WUE\(_{\text{bio}}\)) and the economic yield (WUE\(_{\text{eco}}\)) divided by the sum of irrigation water and precipitation. The biological yield is the dry matter produ-

**Results**

**LAI, RVI, and SPAD values of canopy**

Figures 2, 3 and 4 show LAI, RVI and SPAD values measured on Jan. 23 (49 DAS) and Jan. 25 (51 DAS). The LAI values show the trend of Egyptian clover monoculture (CM) > intercropping (IC) > wheat monoculture (WM), though there were no significant differences (Fig. 2). The LAI of CM plot tended to be higher than that of IC plots most probably due to the higher seeding rate of Egyptian clover in CM than in IC plot. The IC plot gave the highest RVI value among the treatments (Fig. 3). The RVI was identical in WM and CM plots. In WM plot, RVI had a large deviation within the plots which was also observed in LAI values.

The SPAD values of both wheat and Egyptian clo-

![Fig. 2. Leaf area index measured during January 23, 2013 (49 DAS). The error bars indicate SE (n = 3).](image)

![Fig. 3. Ratio vegetation index measured during January 25, 2013 (51 DAS). The error bars indicate SE (n = 3).](image)
ver were not affected by intercropping method (Fig. 4). Wheat leaves showed higher SPAD values than Egyptian clover.

**Biological yield**

Figure 5 shows biological yield which is the sum of 1st cut in February and the final cut at the harvest in May. The result presents that the biological yield at the 1st cut was very low, and the cutting treatment affected the final biological yield in all the plots. The final biological yield of wheat was more affected and decreased by the cutting treatment when intercropped with Egyptian clover, whereas the final biological yield of Egyptian clover was less affected by cutting treatment in intercropping.

Intercropping reduced the biological yield of both crops (Fig. 5). In case of wheat it was reduced by 47% (18.9 t to 11.3 t ha⁻¹). In case of Egyptian clover it was reduced by 71%. This reduction rate in biological yield of Egyptian clover was more than the reduction in seeding rates (50%) between mono (86 kg ha⁻¹) and intercropping (43 kg ha⁻¹).

**Yield, yield components, WUE and LER**

Plant height at harvest, yield and yield components of wheat, and yield of Egyptian clover are presented in Table 1. The final plant height of wheat suggests that wheat could re-grow after cutting treatment but not as the same height as wheat without cutting.

All the yield components of wheat, except grain numbers, were affected by cutting treatment (Table 1). The spike number per unit area was greatly increased by cutting treatment in both cropping methods. While spike length, grain number per spike, grain weight per spike, and 1000-grain weight decreased in wheat with the cutting treatment. As a result, grain yield was lower with cutting treatment than that without cutting in both monocultured and intercropping plots. The most affected yield component was grain weight per spike (54-67% reduction).

In contrary, intercropping with Egyptian clover did not significantly affect the yield components of wheat. However, the grain yield and yield components of wheat had a consistent tendency to be suppressed by intercropping.

In case of Egyptian clover, the length was not clearly influenced by treatments. However, the biological yield, which is the final economic yield for the clover, was greatly reduced by intercropping, and also by cutting treatment in monocultured plot.

Table 2 presents summary of the results without the cutting treatment. The ‘economic yield’ in the table is grain yield in case of wheat (adjusted for 15% moisture content), and equal to biological yield in case of Egyptian clover (dry weight). It is already discussed above but it is clear in Table 2 that intercropping affected yields of Egyptian clover more extensively than those of wheat, and the reduction rate in the yield was more than the reduction of seeding rate. Water use efficiency based on biological yields (WUEbi) did not have significant differences among the plots. However, total WUEey of intercropping was same level as that of monocultured wheat (2.02 kg m⁻³), and lower than that of monocultured Egyptian clover (3.27 kg m⁻³). Water use efficiency based on economic yields (WUEey) was the lowest for monocultured wheat in an order of monocultured Egyptian clover > intercropping sum > monocultured wheat with statistical significance (P < 0.01). Land equivalent ratio (LER) of wheat and Egyptian clover in intercropping plot
Table 1. Growth and yield components of wheat and Egyptian clover (May 11, 2013).

<table>
<thead>
<tr>
<th>Cropping method</th>
<th>Cutting treatment</th>
<th>Plant height (cm)</th>
<th>Biological yield (t ha⁻¹)</th>
<th>Spike length (cm)</th>
<th>Spike No (m⁻²)</th>
<th>Grain no./spike</th>
<th>Grain wt/spike (g)</th>
<th>Grain No (m⁻²)</th>
<th>1000-grain wt (g)</th>
<th>Grain yield (t ha⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monocultured</td>
<td>Control</td>
<td>94.7</td>
<td>18.9</td>
<td>9.9</td>
<td>225</td>
<td>43.6</td>
<td>2.42</td>
<td>9370</td>
<td>54.4</td>
<td>5.0</td>
</tr>
<tr>
<td></td>
<td>Cut</td>
<td>75.6 (79.8) *</td>
<td>11.6 (61.4)</td>
<td>8.3 (83.8)</td>
<td>464 (206.2)</td>
<td>22.5 (51.6)</td>
<td>0.79 (32.6)</td>
<td>10359 (110.6)</td>
<td>35.2 (64.7)</td>
<td>3.6 (72.0)</td>
</tr>
<tr>
<td>Intercropped</td>
<td>Control</td>
<td>97.3</td>
<td>10.1</td>
<td>9.2</td>
<td>246</td>
<td>36.4</td>
<td>1.83</td>
<td>8797</td>
<td>50.3</td>
<td>4.4</td>
</tr>
<tr>
<td></td>
<td>Cut</td>
<td>64.7 (66.5)</td>
<td>4.8 (47.5)</td>
<td>8.2 (89.1)</td>
<td>345 (140.2)</td>
<td>25.2 (69.2)</td>
<td>0.85 (46.4)</td>
<td>8711 (99.0)</td>
<td>33.7 (67.0)</td>
<td>2.9 (65.9)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cropping method</th>
<th>Cutting treatment</th>
<th>Plant length (cm)</th>
<th>Biological yield (t ha⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*: Grain weight and grain yield were adjusted for 15% moisture content.

**: ** indicates significant effect at P < 0.01, * at P < 0.05, and n.s. indicates no significant difference at P < 0.05 by Tukey's test.

*: Figures in the parenthesis present percentage of the control.
was 0.88 and 0.29, respectively (Table 2). The total LER of intercropping was, therefore, higher than 1.0 (1.17).

**Discussion**

**Canopy development and SPAD**

LAI of IC tended to be higher than that of WM plots (Fig. 2). They were 3.4 and 2.8 in IC and WM plots, respectively. The denser cover with higher LAI of the canopy in IC plots can be the advantage for suppressing water evaporation from the soil surface (Ritchie, 1972; Walker and Ogindo, 2003). Ritchie (1972) used LAI values to predict the soil surface evaporation with incomplete canopy cover. The model was developed with a threshold LAI of 2.7 which is an apparent ‘full cover’ canopy (Ritchie and Burnett, 1971). After the threshold LAI is obtained, the soil evaporation becomes insignificant. In our study, since the LAI value of WM was just close to the ‘full cover’, intercropping wheat with Egyptian clover is effective to control soil surface evaporation in early growth stage before 50 DAS. The RVI value, which has a linear relationship with above-ground biomass (Li et al., 2010), was higher in IC than in WM and CM as well (Fig. 3). Ghanbari et al. (2010) also concluded in a study under arid environment that evaporation of the soil surface decreased due to shading in maize-cowpea intercropping and increased the water amount potentially available for transpiration and growth of crops.

Intercropping with Egyptian clover did not improve the chlorophyll contents of wheat leaves, as measured as SPAD value (Fig. 4). Several studies reported on N-transfer between legumes and non-legumes in intercrops (Singh et al., 1986; Giller et al., 1991; Sawatsky and Soper; 1991, Jensen, 1996a; 1996b), and results varied. Singh, et al. (1986) summarized that some legumes, such as soybean and blackgram, increase NO$_3^-$ and NH$_4^+$ concentration in soil. In contrast, Jensen (1996b) concluded that there was no evidence for N fixed by pea being transferred to barley in a 4-year field experiment on pea-barley intercropping.

In the same field site of this study, an advantage of maize-cowpea and maize-soybean intercrops was reported with higher SPAD values of maize leaves by Kubota et al. (2015). The difference of these responses in effect of intercropping with legumes may be due to the difference between N demand of maize and wheat, in other words maize plants may require higher amount of N than wheat. To confirm the advantage of N-transfer in wheat-Egyptian clover intercropping, we have to test the effect under a limited application rate of N fertilizer.

**Effects of cutting**

The biological yield by the 1st cut at 69 DAS was very low (1.9 – 2.3 t ha$^{-1}$) (Fig. 5). This cutting treatment was tried in order to obtain feeds for farm animals in winter because the feeds are not enough during winter in Egypt. However, the cutting crops in February did not show any advantages in this study since it was not productive and gave a negative effect on growth of both crops in terms of biological yield (Fig. 5 and Table 1). The cutting reduced 27% of total biological yield of wheat, 39% of Egyptian clover in monocrops, and 18% of sum of 2 crops in intercropping plot (Fig. 5). The cutting cannot be conducted in later stage of wheat because wheat would not regrow well when cereal crops are cut in reproductive phase (Aase and Siddoway, 1975; El- Shatnawi et al., 1999). Aase and Siddoway (1975) tested effects of clipping the plants at 9 different times during wheat growth, and concluded that clipping after tillering stage results in a definite and progressive reduction in grain production. El-Shatnawi et al. (1999) reported that clipping barley at tillering stage did not affect the final
production but clipping to 5 and 10 cm above soil surface at booting stage reduced 52% and 38% of the final shoot weight, respectively.

Similarly, the final grain yield of wheat was affected by cutting treatment (Table 1). The reduction rates were 28% in monocultured and 34% in intercropping plots. Most of other yield parameters, except the grain numbers per unit area, were significantly affected by cutting treatment (Table 1). The spike numbers per unit area increased by cutting, but grain number per spike and grain weight per spike were notably reduced. Reduced 1000-grain weight and grain number per spike lead to the great reduction in grain weight per spike (54-67% reduction), and then in total grain yield. Our hypothesis is that the plants increased tillering after the cut in February and produced more spikes than plants without cutting, but the growth could not catch up due to the cool temperature. The minimum and maximum temperatures were lower than 10 °C and 25 °C, respectively, until the end of February (Fig. 1). The final biological yield was much lower in plants being cut than those without cutting, which led to the smaller grains.

Our data indicated that cutting wheat plant or Egyptian clover, even at 15 cm above the soil surface, is not profitable for both animal feed production and grain production.

Effects of intercropping

The total and the final biological yield of both crops were lowered by intercropping (Fig. 5 and Table 1). Wheat decreased its final biological yield by approximately half (47%), and clover by 71% (Table 1). The reduction rate in the biological yield of Egyptian clover was more than 50% which was the reduction in seeding rate. The biological yield of individual crop in winter cereal-legume intercrops also decreased in some studies (Agegnehu, et al. 2006; Lithourgidis et al., 2007; El-Karamany et al., 2012). Lithourgidis et al. (2007) confirmed that the growth rate of common vetch, wheat and barley was lower when they were intercropped than in sole cropping because of competition.

The total biological yield produced by intercrops (19.1 t ha⁻¹) was similar with that of monocultured wheat (18.9 t ha⁻¹) (Table 2), but less than that of monocultured Egyptian clover (30.7 t ha⁻¹) in monocultured plot (Fig. 5 and Table 2). However, it is still an advantage to intercrop wheat with Egyptian clover because intercropping cereals with legumes enhances protein production and then quality of forage (Vasilakoglou and Dhima, 2008).

The grain yield and yield components of wheat tended to be lower when intercropped with Egyptian clover (Table 1). Both grain number and grain size (1000-grain weight) influenced the grain yield of wheat, and the grain yield decreased by 12% in intercropping plots (Table 1). This disadvantage of intercropping for growth and grain yield of wheat in our study can be probably improved by modifying sowing time of the 2 crops to minimize the risk of above and below-ground competition (Mariotti, et al., 2009).

Although WUEᵥₑᵥ did not differ among the cropping system (Table 2), monocultured Egyptian clover showed the highest WUEᵥₑᵥ, but intercropping plot had the similar value as monocultured wheat plot. WUEᵥₑᵥ based on the final yield of both crops showed the clear differences. The WUEᵥₑᵥ of wheat was notably improved by intercropping with Egyptian clover from 0.53 kg m⁻³ to 1.42 kg m⁻³. This agrees with a review study of Morris and Garrity (1993) that WUE of intercrops greatly exceeds WUE by sole crops, often by more than 18%. In our study, WUEᵥₑᵥ of intercropping plot was 63% higher than WUEᵥₑᵥ of wheat monocrop (Table 2). The advantage of water use in intercropping is a part of ‘resource conversion efficiency’ (Willey, 1990) where water evaporation from the soil surface can be converted to transpiration for crops. However, WUEᵥₑᵥ of monocultured Egyptian clover was the highest (3.27 kg m⁻³) (Table 2).

The LER value of 1.17 in intercropping system indicated that this wheat-Egyptian clover intercropping system was more productive per unit area than monoculture of individual crop. Interestingly, this LER value was identical to LER (1.16) obtained in the study of maize-soybean intercropping conducted in the same experimental field (Kubota et al., 2015). Our result with LER higher than 1.0 agrees with a study on barley-faba bean with LER of 1.05 to 1.23 (Agegnehu et al., 2006), on maize-cowpea in all systems with different planting ratios (Ghanbari et al., 2010), and a study of a simulation model of cereal-legume intercrops in all scenarios, using different levels of soil water, planting date, and planting densities (Tsubo et al., 2005), but differs from a study of berseem clover-barley intercrops of Vasilakoglou and Dhima (2008) where LER was lower than 1.

Our results suggest that the intercropping system of wheat-Egyptian clover has possibility to provide food both for human and animal of small-scale farmers with limited land and water resources although the intercropping system reduced the yield of individual crop. The denser canopy of intercrops also has an advantage of reducing evaporation of soil in the Egyptian Nile Delta. The cutting treatment at early stage of crops was not
effective of production of neither wheat nor the clover.

Acknowledgements

This study was a part of JST/JICA project “Sustainable Systems for Food and Bio-energy Production with Water-saving Irrigation in the Egyptian Nile Basin”, SATREPS. We acknowledge Mr. Hussein Nashaat, Mr. Hassan Mohamed, Mr. Sayed El Behlak and Ms. Momoko Higuchi for their technical supports. We would like to express gratitude to Dr. Masayoshi Satoh for supporting the study.

References


