

ASIAN CROP SCIENCE ASSOCIATION

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**PROCEEDINGS OF 8th ASIAN CROP SCIENCE
ASSOCIATION CONFERENCE**

Ha Noi, 23-25 September, 2014

AGRICULTURAL UNIVERSITY PRESS - 2015

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Foreword

We are now living in a world which is affected by global challenges such as population growth, climate change, food un-security and environmental degradation. The recent financial and food crises remind us that the world is changing quickly and dramatically. While world food production has increased dramatically, 820 million people worldwide are still undernourished. It is important to increase not only crop productivity to meet the world's food demand but also the value in crop production chains for farmers. Therefore, providing a growing population with food, water, shelter, and livelihoods, without further degradation of the environment and sustaining crop production under global climate changes present great challenges worldwide.

From 23-25 September 2014, the 8th Asian Crop Science Association Conference (ACSAC8-2014) on “*Sustainable Crop Production in Response to Global Climate Change and Food Security*” was held at Viet Nam National University of Agriculture, Ha Noi, Viet Nam.

The aims of ACSAC8-2014 are to provide a platform for leading academic scientists, researchers and scholars to share and exchange the most current advances in environmental and crop science; as well as to explore and discuss approaches, technologies and strategies to sustain and increase crop productivity under the pressure of global climate changes.

In particular, the ACSAC8-2014 includes General Session with invited speakers and five specific sessions as follows:

Session 1: Climate change and its impacts on crop production and society

Session 2: Molecular approaches for improving crop adaptation to climate change

Session 3: Approaches of crop morphology, physiology and ecology to cope with climate change

Session 4: Innovative cultivation technology for enhancing crop production under climate change

Session 5: Plant physiology-based approaches to climate changes; impact of low to high temperatures and other disaster on crop productivity (Special session in cooperation with the Crop Science Society of Japan and the Korean Crop Science Society)

We would like to express our sincere thanks to all participants. The acknowledgement is also addressed to our sponsors for financial support of the conference.

On behalf of the Organizing committee

Assoc. Prof. Pham Van Cuong

Kernel Quality and Ripening Ability in *Japonica* and *Indica* Type Rice Cultivars Grown under Different Temperature Conditions

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ABSTRACT

The effect of high temperature during ripening on the occurrence of white immature kernels and ripening ability was compared among rice cultivars, and the percentages of white immature kernels and grain filling were then analyzed in relation to yield characters. Five *indica* and nine *japonica* cultivars in 2011, and eight *indica* and eight *japonica* cultivars in 2013 from China (collected from Hunan, Jiangsu and Tianjin), with six Japanese *japonica* cultivars in each year, were cultivated in a paddy field and transplanted to pots (one plant/pot) at the flag leaf emergence stage. From full heading stage to the mature stage, plants were grown under low (23°C daytime/19°C at night), medium (28°C/23°C) and high (34°C/26°C) temperature conditions. The results were as follows: (1) the percentage of white immature kernels in Chinese cultivars was significantly higher than that in Japanese cultivars, because of the higher percentage of milky white kernels. In *japonica*, the Chinese cultivars had a wide and thick kernel compared with Japanese cultivars. (2) With increasing temperature during ripening, the percentage of white immature kernels significantly increased because of the increase in milky white kernels. High temperature decreased the grain filling percentage and kernel weight with a decrease in kernel width and thickness. (3) The percentage of white immature kernels in middle temperature conditions was significantly related to kernel thickness in *japonica*, while in *indica* it was significantly related to kernel width. These results indicated that cultivars with a wide or thick kernel had a high percentage of white immature kernels. However, these relationships were not significant under high temperature conditions.

Keywords: Chinese cultivar, Grain filling percentage, High temperature, White immature kernel.

1. INTRODUCTION

A rapid temperature increase in the summer seasons (about 1.34°C) has been observed in Kochi Prefecture, Southeast Japan during the last three decades (Japan Meteorological Agency, 2011). Severe high temperatures in the summer seasons (37-40°C) have also been reported often in Hunan Province, China, where a temperature increase of 1.02°C in July and August has been observed during these three decades (Matsui, 2009; Miyazaki et al., 2011). High temperatures in summer have degraded the quality of rice kernels in various regions in Japan, and the percentage of first-grade rice kernels declined to only 14 % in 2010 in Kochi Prefecture, Japan (Morita, 2008; Sakata et al., 2011). This is caused by a decrease in perfect kernels with an increase in white immature kernels. The percentage of white immature kernels shows large variation among rice cultivars (Takata et al., 2010; Sakata et al., 2011), which is clear under high-temperature conditions (Sonoda et al., 2012).

High temperature during the ripening stage decreases the kernel weight, beginning at an average temperature of 25°C and decreasing significantly at more than 30°C (Matsushima and Manaka, 1957). The decrease in kernel weight is due to a decrease in the kernel size, causing a decrease in grain filling below a certain threshold.

Recent Chinese cultivars have high-yield with high grain filling, a large number of spikelets per panicle, and heavy kernels compared with Japanese cultivars, resulting from a high capacity to supply starch (Yao et al., 2000a, 2000b; Gendua et al., 2009b). By comparing Chinese cultivars including both *japonica* and *indica* cultivars with Japanese cultivars, the effect of high temperature on white immature kernels and grain filling was elucidated in this experiment. In addition, the relationships between these characters and yield-related characters were examined under different temperature conditions to identify the crop characters affecting cultivar differences.

2. MATERIALS AND METHODS

2.1. Plant materials and cultural conditions

Five *indica* and nine *japonica* cultivars in 2011, and eight *indica* and eight *japonica* cultivars in 2013 collected from China (Hunan, Jiangsu, and Tianjin), with six Japanese *japonica* cultivars in each year, were used.

Experimental plants were firstly cultivated in a paddy field of Kochi University, Japan. One seedling/hill was transplanted at 22.2 hill/m² on 24 May, 2011 and 27 May, 2013. Basal dressing was applied with slow-release fertilizer at a rate of 12 g/m² each of N, P₂O₅, and K₂O.

Plants with an average number of panicles were transplanted from the paddy field to pots (one plant/pot) at the flag leaf emergence stage. From the full heading stage to the mature stage, plants were grown under low- (23°C daytime/19°C at night), medium- (28°C/23°C) and high- (34°C/26°C) temperature conditions using temperature-controlled rooms with natural sunlight.

2.2. Measurements

Three pots were sampled at the root base in the full heading and mature stages to determine the panicle number and dry matter weight after drying at 95°C for 2 h and 65°C for 2 days. The panicles were dried at 40°C for 2 days to measure the dry weight and yield components in the mature stage. After selection with salt solution, hulled kernels were used to measure kernel shapes and classify white immature kernels with a grain quality analyzer (model RGQI10A, Satake Corporation, Hiroshima, Japan) for *japonica* cultivars. The white immature kernels of *indica* cultivars were classified by visual inspection because the *indica* cultivar kernels did not fit into the analyzer.

The increase in dry matter weight after heading (ΔW) was calculated by the difference in aboveground dry matter weight between the mature and heading stages. The amount of carbohydrate translocated from leaves and stems to panicles (ΔT) was estimated by the decrease in dry matter weight of leaves and stems after heading (Gendua et al., 2009a; Kokubo et al., 2013).

3. RESULTS

Similar results were obtained in both 2011 and 2013, and the common results are shown below.

3.1. Differences among production areas

The percentage of white immature kernels in Chinese *japonica* cultivars was significantly higher than that in Japanese *japonica* cultivars, because of a higher percentage of milky white kernels (Table 1). The percentage of white immature kernels in *indica* cultivars classified by visual inspection was quite high; milky white kernels were predominant (Table 2). The kernel thickness in Chinese *japonica* cultivars was greater than that in Japanese *japonica* cultivars (Table 3). Compared

with *japonica* cultivars, *indica* cultivars had a large number of spikelets per panicle, a high percentage of grain filling, and long and narrow kernels (Table 3, 4). A lower number of panicles, larger number of spikelets per panicle, and heavier 1000-kernel weight were found in Tianjin *japonica*, and Hunan and Jiangsu *indica* cultivars than Japanese *japonica* cultivars.

Table 1 Effects of production area and temperature treatment on the percentage of white immature kernel in *japonica* cultivar (2013).

Production area	Subspecies	White immature (%)	Milky white (%)	White-based (%)	White belly and back (%)
Japan	<i>japonica</i>	40.0 c	19.4 c	9.7 a	10.9 b
Tianjin	<i>japonica</i>	51.0 b	34.5 b	11.6 a	4.8 c
Jiangsu	<i>japonica</i>	77.0 a	47.9 a	10.9 a	18.2 a
Low temp		37.4 c	15.3 b	10.7 b	11.4 b
Medium temp		50.1 b	16.4 b	17.7 a	16.0 a
High temp		73.7 a	64.0 a	3.4 c	6.3 c
Area		***	***	ns	***
Temp		***	***	***	***
Area × Temp		**	***	***	***

Classified by the inspection analyzer.

Means followed by the same letter in the same column do not differ significantly at the 5% level among production areas and among temperature treatments.

** and ***, significant at the 1 and 0.1% levels, respectively; ns, not significant at the 5% level.

Table 2 Effects of production area and temperature treatment on the percentage of white immature kernel in *indica* cultivar (2013).

Production area	Subspecies	White immature (%)	Milky white (%)	White-based (%)	White belly and back (%)
Jiangsu	<i>indica</i>	95.7 a	85.6 b	0.0 a	10.0 a
Hunan	<i>indica</i>	96.7 a	93.8 a	0.0 a	3.0 b
Low temp		91.4 b	77.1 b	0.0 a	14.2 a
Medium temp		97.2 a	92.8 a	0.0 a	4.5 b
High temp		99.9 a	99.1 a	0.0 a	0.8 b
Area		ns	**	ns	**
Temp		*	***	ns	***
Area × Temp		ns	**	ns	**

Classified by the visual inspection.

Means followed by the same letter in the same column do not differ significantly at the 5% level among production areas and among temperature treatments.

*, ** and ***, significant at the 5, 1 and 0.1% levels, respectively; ns, not significant at the 5% level.

3.2. Differences among temperature conditions

The percentage of white immature kernels in both *japonica* and *indica* cultivars increased with an increase in temperature, mainly because of an increase in milky white kernels (Table 1, 2). The kernel width and thickness significantly decreased (Table 3). The grain filling percentage and 1000-kernel weight in high-temperature conditions were significantly lower than in medium- and low-temperature conditions, resulting in a lower yield (Table 4).

Table 3 Effects of production area and temperature treatment on the kernel shape in *japonica* and *indica* cultivars (2013).

Production area	Subspecies	Length (mm)	Width (mm)	Thickness (mm)
Japan	<i>japonica</i>	5.03 b	2.57 b	1.99 b
Tianjin	<i>japonica</i>	5.10 b	2.69 a	2.01 b
Jiangsu	<i>japonica</i>	4.91 b	2.64 ab	2.08 a
Jiangsu	<i>indica</i>	6.26 a	2.26 c	1.99 b
Hunan	<i>indica</i>	6.54 a	2.21 c	1.90 c
Low temp		5.53 a	2.54 a	2.01 a
Medium temp		5.51 a	2.50 a	2.01 a
High temp		5.53 a	2.40 b	1.97 b
Area		***	***	***
Temp		ns	***	**
Area × Temp		ns	ns	ns

Means followed by the same letter in the same column do not differ significantly at the 5% level among production areas and among temperature treatments.

** and ***, significant at the 1 and 0.1% levels, respectively; ns, not significant at the 5% level.

Table 4 Effects of production area and temperature treatment on the yield components in *japonica* and *indica* cultivars (2013).

Production area	Subspecies	Panicle No. (/hill)	Spikelet No. (/panicle)	Spikelet No. (/hill)	Grain filling (%)	1000 kernel weight (g)	Brown rice yield (g/hill)
Japan	<i>japonica</i>	15.3 a	74.4 c	1124 b	52.2 b	20.2 c	12.7 c
Tianjin	<i>japonica</i>	12.8 b	110.2 b	1396 a	53.2 b	21.6 b	16.9 b
Jiangsu	<i>japonica</i>	15.3 a	72.4 c	1104 b	52.3 b	20.8 bc	12.5 c
Jiangsu	<i>indica</i>	9.4 c	145.5 a	1367 a	60.1 ab	23.9 a	20.7 a
Hunan	<i>indica</i>	12.0 b	121.4 b	1422 a	63.3 a	21.8 b	20.1 a
Low temp		13.1 a	99.8 a	1246 a	72.1 a	23.3 a	20.8 a
Medium temp		13.1 a	103.7 a	1275 a	70.5 a	22.9 a	20.5 a
High temp		13.2 a	102.7 a	1283 a	25.0 b	21.6 b	7.4 b
Area		***	***	***	**	***	***
Temp		ns	ns	ns	***	***	***
Area × Temp		ns	ns	ns	***	ns	***

Means followed by the same letter in the same column do not differ significantly at the 5% level among production areas and among temperature treatments.

** and ***, significant at the 1 and 0.1% levels, respectively; ns, not significant at the 5% level.

3.3. Relationship between the percentage of white immature kernels and yield-related characters

The percentage of white immature kernels in *japonica* cultivars was significantly related to kernel thickness in medium-temperature conditions ($r=0.86$, $p<0.01$), suggesting that the white immature kernel percentage was low in cultivars with thin kernels (Table 5). However, the percentage of white immature kernels in *indica* cultivars was significantly related to kernel width in medium-temperature conditions ($r=0.75$, $p<0.05$); in addition, the kernel width was negatively related to kernel length ($r=-0.85$, $p<0.01$). These results suggested that the percentage of white immature kernels was low in *indica* cultivars with long slender kernels. However, these relationships were not significant under high-temperature conditions, in which other factors may have more influence on the occurrence of white immature kernels. Similar relationships between

kernel shapes and white immature kernels were reported in the experiments using different cultivars (Takata et al., 2008; Nagaoka et al., 2012).

Table 5 Correlation coefficients between the percentage of white immature kernel and the yield-related characters in *japonica* and *indica* cultivars (2013).

	<i>Japonica</i>			<i>Indica</i>		
	Low temp	Medium temp	High temp	Low temp	Medium temp	High temp
Panicle No.	-0.07	-0.08	-0.05	-0.83 *	0.39	0.23
Spikelet No. (/panicle)	<u>-0.18</u>	0.03	-0.03	0.71	-0.16	-0.11
Spikelet No. (/hill)	-0.36	-0.23	-0.03	-0.18	0.40	0.19
Grain filling (%)	-0.08	-0.37	0.08	0.04	0.64	0.15
1000 kernel weight	0.49	0.39	0.07	-0.22	-0.79 *	<u>-0.26</u>
Brown rice yield	-0.15	-0.35	0.16	-0.22	0.51	0.12
Kernel length	-0.02	-0.12	-0.14	-0.20	<u>-0.56</u>	-0.21
Kernel width	0.32	0.32	0.21	0.16	0.75 *	0.08
Kernel thickness	<u>0.28</u>	<u>0.86</u> **	0.42	-0.03	0.08	0.48
ΔW (/spikelet)	0.40	-0.01	<u>0.00</u>	-0.69	-0.23	0.13
ΔT (/spikelet)	-0.37	0.02	-0.12	0.52	0.03	-0.11
$\Delta W + \Delta T$ (/spikelet)	0.07	-0.02	-0.37	-0.26	-0.44	-0.02

* and **, significant at the 5 and 1% levels in 2013, respectively.

Correlation coefficients with the underline, significant in 2011.

ΔW , dry matter increase after heading; ΔT , dry matter decrease in leaves and stems after heading;

$\Delta W + \Delta T$, dry matter increase in panicles after heading.

3.4. Relationship between the of grain filling and dry matter production

The percentage of grain filling of *japonica* cultivars was significantly related to $\Delta W + \Delta T$ under low temperature and ΔT under high temperature, consistently in both years (Table 6). This result suggested that ripening ability at high temperature might be related to the dry matter decrease in leaves and stems. The reduction in ripening ability depends on reduced starch accumulation resulting from the deterioration of source capacity (Nagata et al., 2001; Gendua et al., 2009a). This is partly explained by the shortage of carbohydrate translocated from leaves and stems to panicles (Hirai et al., 2003). Therefore, the kernel weight is enhanced by an increase in carbohydrate stored in leaves and stems (Wada, 1969). However, in *indica* cultivars, consistent results between both years were not obtained.

Table 6 Correlation coefficients between the percentage of grain filling and the ΔW , ΔT , $\Delta W + \Delta T$ in *japonica* and *indica* cultivars (2013).

	<i>Japonica</i>			<i>Indica</i>		
	Low temp	Medium temp	High temp	Low temp	Medium temp	High temp
ΔW (/spikelet)	0.07	0.34	<u>-0.46</u>	-0.63	-0.47	-0.39
ΔT (/spikelet)	0.23	0.16	<u>0.56</u> *	0.78 *	0.46	0.66
$\Delta W + \Delta T$ (/spikelet)	<u>0.82</u> **	0.78 **	0.28	0.57	0.19	0.89 **

* and **, significant at the 5 and 1% levels in 2013, respectively.

Correlation coefficients with the underline, significant in 2011.

4. CONCLUSIONS

The percentage of white immature kernels in medium-temperature conditions was significantly related to kernel thickness in *japonica*, while in *indica* it was significantly related to kernel width. These results indicated that cultivars with a wide or thick kernel had a high percentage of white immature kernels. However, these relationships were not significant in high-temperature conditions. On the other hand, the percentage of grain filling in *japonica* cultivars was significantly related to the dry matter decrease in leaves and stems after heading under high-temperature conditions. This result suggested that the cultivar difference in ripening ability under high-temperature conditions might be affected by the amount of assimilation supply.

ACKNOWLEDGEMENT

We thank Mr. Yasuo Takemura and Mr. Nobuyuki Kira for their technical support on field experiments. This study was supported by Grant-in Aid for Scientific Research (No. 25450023) from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

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Relationships among Global Climate Indices, Rainfall and Rain-fed Crop Yields in the Southern Highlands of Java: A Case Study in Gunungkidul, Yogyakarta, Indonesia

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ABSTRACT

Although there has been a high interest to investigate the relationships among global climate indices, rainfall and rain-fed crop yields in Indonesia, little evidence is available for crops in highland agriculture of the country. This study evaluates the relationships among global climate indices (Southern Oscillation Index, SOI; sea surface temperature, SST), rainfall and rain-fed crop yields in Gunungkidul district, highland areas of southern Indonesia, an important area of rain-fed crop production. Rainfall data were collected during the rainy season (October-March) from 1981 to 2009, and crop yields data were collected from 1990-2009 from Gunungkidul district. Global climate indices (SOI and SST) were collected from the Japan Meteorological Agency (JMA). Rainfall was highest in the southern and western mountainous areas, especially near the coast, and SOI and SST Niño.West values were highly correlated with rainfall in those areas. Soybean and dryland paddy yields were highly correlated with the amount of rainfall in January, and the average SOI during June-September was highly correlated with maize yield during January-April.

Keywords: correlations, rain-fed agriculture, rainfall variability, SOI-SST, yields

1. INTRODUCTION

In Indonesia, variable rainfall during the growing season, mostly due to El Niño and La Niña events, critically affects rain-fed agriculture. El Niño events affect delaying the beginning of the rainy season (typically, October-March) and thereby negatively affecting agricultural production (Hamada et al., 2002). During the past 20 years, several El Niño events (McPhaden, 1999) delayed the paddy harvest in Indonesia and threatened the stability of food security (Harger 1995; Amien et al., 1996). In 1997/1998 and 1982/1983, decrease of harvest area for wetland paddy was about 670 thousand hectares in 1982/1983 and 700 thousand hectares in 1997/1998. In percentage terms the decrease was 4.5% and 3.8% in 1982 and 1983, respectively, and in 1997 and 1998 these values were 4.0% and 3.0%, respectively. Harvest area decrease for all food crops was two million hectares for both 1982/1983 and 1997/1998 (Irawan, 2002). The total loss to the Indonesian economy due to the decreased agricultural production in 1997/1998 and 1982/1983 was about US\$2.75 billion out of total losses to the national economy that were close to US\$9 billion (Kirono and Tapper, 1999; BAPPENAS, 1999). Especially in Java, the rain-fed paddy yield in some subdistricts of Java dramatically decreased, by 58% in wetland areas and by 52% in dryland areas, mostly in southern of Java highland areas (Irawan, 2002) due to the delayed start of the rainy season. The delayed start of the rainy season due to El Niño events has a greater effect on crop yields in highland areas, where agriculture is rain-fed (Irawan, 2002; Kirono and Tapper, 1999). Therefore, detailed investigations of the relationship between rainfall variability in the rainy season and rain-fed crop yields in highland areas are needed.

Many studies have investigated the relationship between rainfall variability and topography in Indonesia. Hamada et al. (2008) showed that rainfall and wind speed are greater on the windward side than on leeward side of the mountains of western Sumatera and suggested that surface

topography plays a role in determining the rainfall distribution not only in Sumatera but also in and around the entire Indonesian archipelago.

Rainfall variability in Indonesia also correlates with global climate indices, such as the Southern Oscillation Index (SOI) and sea surface temperature (SST) (Saji et al., 1999). SST fluctuations are most pronounced around Indonesia and in the nearby tropical Pacific (Trenberth and Shea, 1987; Trenberth and Hoar, 1996). Nicholls (1981, 1984) showed that surface pressure at Darwin, northern Australia and SST around Indonesia could be used to predict Indonesian climate variations and Indonesian rainfall. Kirono and Tapper (1999) found that rainfall from June to November was positively correlated with the SOI from 1951 to 1997, and that the relationship was particularly strong in southern Java, including Yogyakarta Special Province. Thus, rain-fed crop production in highland areas of Indonesia is simultaneously influenced by rainfall variability, global climate as reflected by the SOI and SST indices, and the highland topography.

Therefore, the aim of this study was to clarify the relationships among global climate indices (SOI and SST), rainfall and rain-fed crop yields during the rainy season from 1981 to 2009.

2. MATERIALS AND METHODS

2.1. Study Area

Our study area was Gunungkidul district ($7^{\circ}46' - 7^{\circ}09'S$, $110^{\circ}21' - 110^{\circ}50'E$), a rain-fed agricultural area in the highlands of southern Java bordering the Indian Ocean. This area of 1485.36 km² is divided into 18 subdistricts and includes 144 villages (www.gunungkidulkab.go.id; accessed 5 December 2011). The district can be divided into three areas according to elevation (Agricultural Service for Food Crops and Horticulture, ASFCH): the northern part ranges between 200 and 700 m above sea level (a.s.l.); the central lowland area is at 150-200 m a.s.l.; and the southern area is hilly with elevations from 0 to 300 m a.s.l. (Fig. 1).

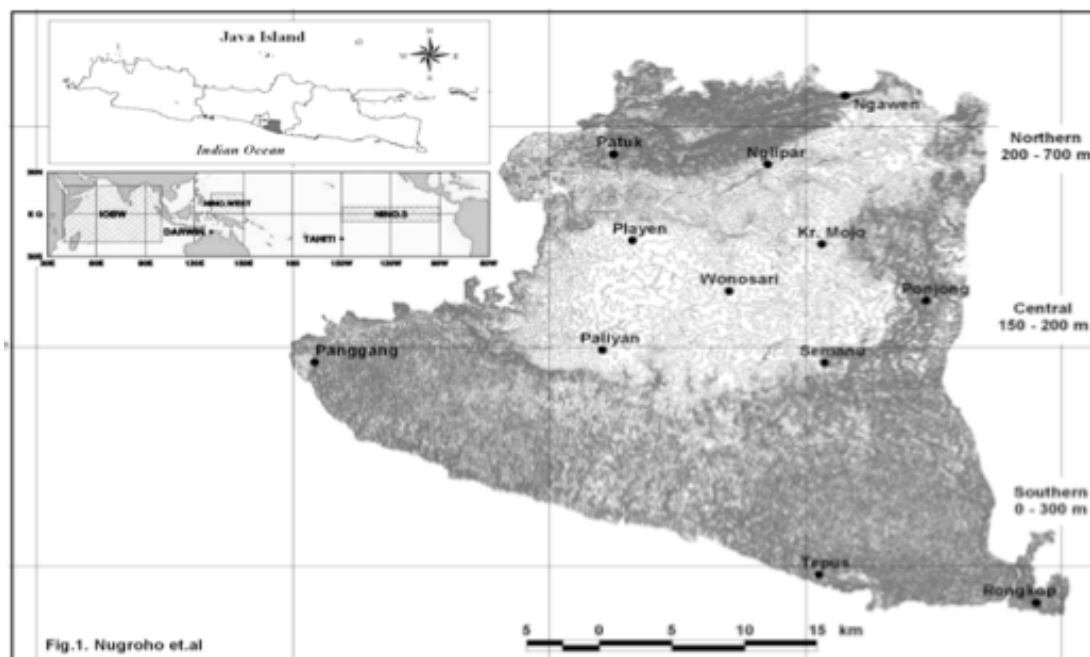


Fig. 1. Topography of Gunungkidul district

Of the total agricultural land area, rain-fed agriculture is practiced in 61,989 ha, with October precipitation being especially important (ASFCH, 2006), and the remaining 7804 ha are irrigated. The mean annual rainfall in the district during 1989-1998 was 2041 mm. On average, the rainy and dry periods each last for 4 to 6 months (ASFCH, 2006). The main crops are dryland paddy and other dryland food crops. Two main cropping patterns are used: monoculture and multiple cropping. Monoculture is the repetitive growing of the same crop on the same piece of land and usually planted in wetland or irrigated areas. Whereas multiple cropping is the intensification of cropping in time and space dimensions, growing two or more crops on the same field in a calendar crop (Huang, 2003; Beets, 1975), and usually planted in dryland or rain-fed agricultural areas (Sarjiman and Mulyadi, 2005).

In the rain-fed highlands of Gunungkidul district, multiple cropping is used as the main cropping pattern. Crop production is dominated by cassava (62%), and other crops include dryland paddy (11.4%), maize (9.6%), peanut (8%), soybean (5.5%), and wetland paddy rice (3.3%) (ASFCH, 2006). Cassava is the dominant crop in multiple cropping system of the district because it can be grown year-round and is resistant to drought (ASFCH, 2006).

2.2. Data and Methods

2.2.1. Crop yields and rainfall data

Crop yields from 1990 to 2009 and rainfall data from 1981 to 2009 were collected at 12 stations (see Fig. 1) (ASFCH, 2009). We used data from 12 of the 18 rainfall observation stations from which adequate data were available. Rainfall data in Indonesia, especially for large areas, are often incomplete or unreliable. Because the records are updated by hand, both the human error and the time required for records to be updated are considerations. Data for all of Indonesia are particularly hard to obtain. There are 6000 rainfall stations in 39 regions of Indonesia, and although some data are collected and recorded at Jakarta, other data are kept only at each provincial office (Hamada et al., 2002). In this study, to minimize errors in the rainfall data, we limited the size of the study area and obtained rainfall data directly from the rainfall observation station in each sub district.

2.2.2. Global climate indices

We used SOI and SST data from the Japan Meteorological Agency (JMA) (<http://www.data.jma.go.jp/gmd/cpd/db/elnino/index/dattab.html>; accessed 20 November 2011) averaged over the Niño.3 (5°N-5°S, 150°W-90°W), Niño.West (15°N-0°, 130°E-150°E), and the Indian Ocean Basin-wide (IOBW; 20°N-20°S, 40°E-100°E) regions (Fig. 1). Munawar et al. (2000) reported that anomalies in the Niño.3 area influence precipitation in Indonesia more strongly than those in the Niño.4 area. We used JR-25 re-analysis data (Onogi et al., 2007) to analyze normal (*i.e.*, the 26-year mean from 1979 to 2004) specific humidity (kg/kg), wind direction, and wind speed in January at 850 hPa at Java Island. Spatial data for Gunungkidul district (scale, 1:25,000) are from the National Coordinating Agency for Survey and Mapping (Bakosurtanal) of Indonesia (2007).

Rainfall data are the averages of cumulative rainfall during the rainy season (October-March) from 1981 to 2009 at the twelve stations in Gunungkidul district (Fig. 2). We analyzed the data as follows. First, we mapped the rainfall distribution during the entire rainy season (6 months) from 1981 to 2009, and then we separately mapped the distribution during the first three months (October-December; OND) and the last three months (January-March; JFM) of the season. We chose October as the first month because it is also the beginning of the growing season (Sarjiman and Mulyadi, 2005). Second, we compared the distribution of rainfall in Gunungkidul district and the average SOI and SST values to identify global climatic influences on rainfall in the district by

conducting a regression analysis of the data for the entire rainy season and the data for OND and JFM against the climatic indices. Third, we performed correlation analyses, comparing the average rainfall during the rainy season and crop yields and average values of the global climate indices during the rainy season and crop yields.

3. RESULTS

3.1. Correlations of rainfall in southern Java with global climate indices

During the El Niño years (low SOI years, Fig. 2) of 1982/1983, 1997/1998 and 2004/2005, Gunungkidul district received less rainfall during the rainy season. The correlation between rainy season rainfall and SOI variations during 1981-2009 was significant and positive ($r = 0.48, p < 0.01$; Fig. 2a). Rainfall variations also showed a significant, positive correlation with SST Niño.West variations ($r = 0.39, p < 0.05$; Fig. 2b), and a significant negative correlation with SST Niño.3 ($r = -0.49, p < 0.01$; Fig. 2b) and SST IOBW variations ($r = -0.50, p < 0.01$; Fig. 2b). Therefore, SOI and SST can be used to predict variations in precipitation in rain-fed agricultural areas of the southern Java highlands.

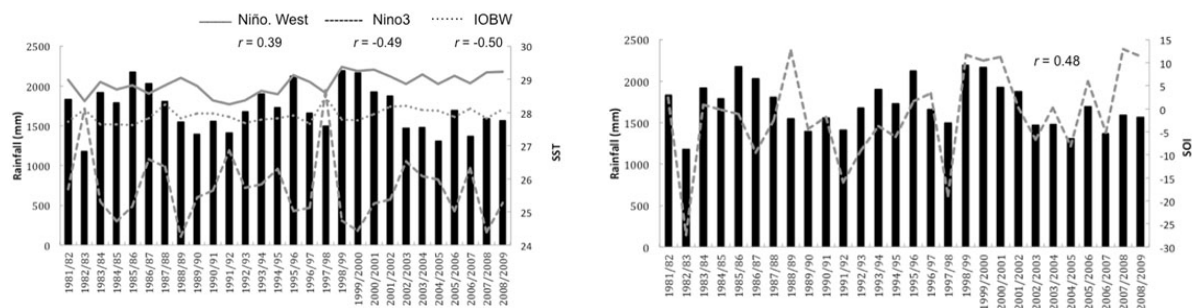


Fig. 2. Average precipitation (bars) and (a) average SOI (dashed line) and (b) average SST Niño.West (solid line), Niño.3 (dashed line), and IOBW (dotted line) during the rainy season (October-March) from 1981 to 2009

3.1.1. Rainy season rainfall distribution from 1981 to 2009

We analyzed the rainfall distribution from 1981 to 2009 using data for the entire 6 months of the rainy season and separately for OND and JFM (Fig. 3). Overall during the rainy season, rainfall ranged from 1800 to 2200 mm in the southern coastal and western inland areas, and around 1500-1600 mm of rain fell in the central lowland and northern areas (Fig. 3a). During the early rainy season (OND), rainfall was 750-900 mm in the southern coastal areas and 500-600 mm in the central lowland area (Fig. 3b), and the highest amounts of rainfall occurred during the late rainy season (JFM, Fig. 3c). During the rainy season, especially at its peak in January, westerly winds bring moisture from the Indian Ocean to Java (Fig. 4), and this moisture carried by the winds produces dense clouds and orographic precipitation (Roe, 2005) along the mountain ranges of southern and western Java. As a result, the windward side of the mountains in Gunungkidul district receives much more precipitation than the leeward side. Orographic precipitation occurs particularly in the mountainous northern Gunungkidul district (Patuk, Nglipar, and Ngawen). Moreover, rainfall is higher in Patuk sub district (in the northwest) than in Ngawen sub district (northeast), even though both are mountainous, because the Patuk mountain block the southwesterly winds.

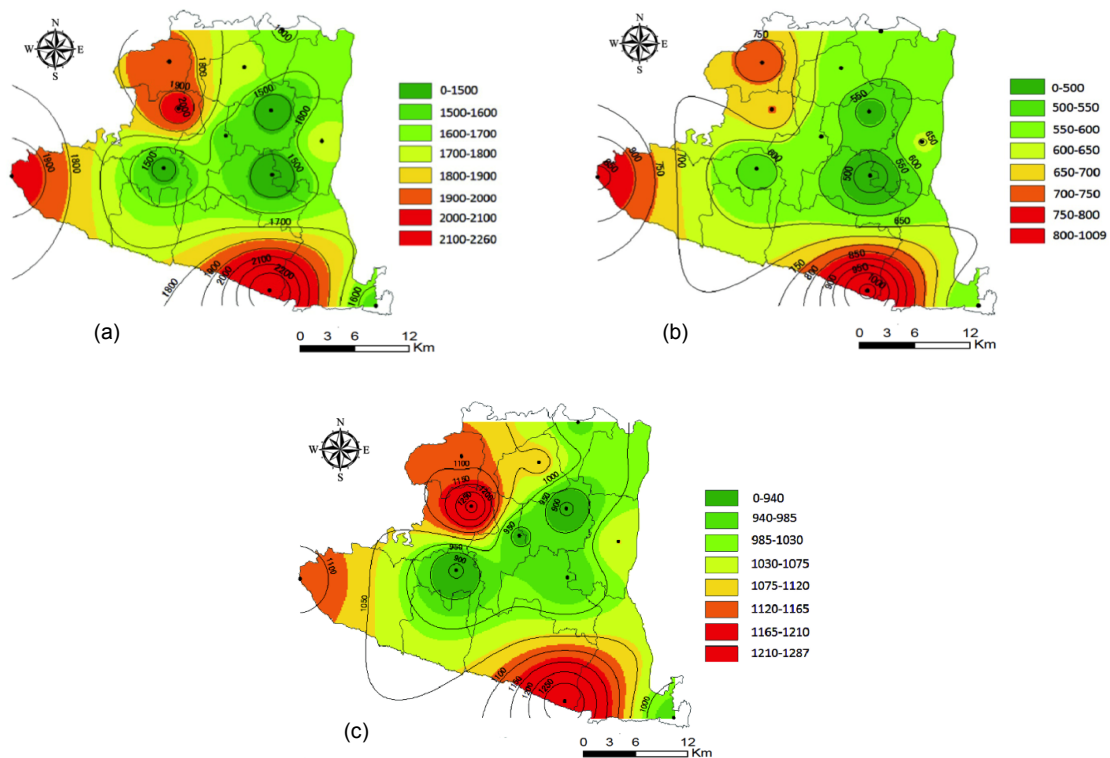


Fig. 3. Rainfall distribution in Gunungkidul district: (a) rainy season (October-March), (b) October-December (OND), and (c) January-March (JFM)

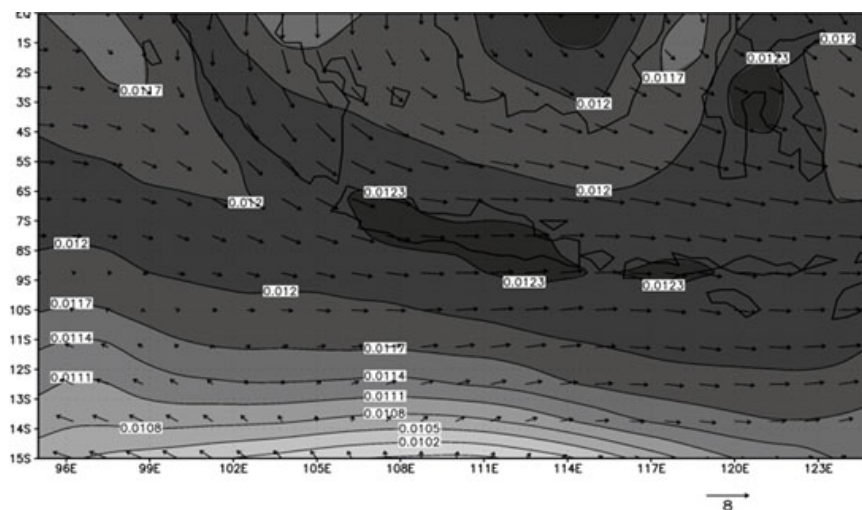


Fig. 4. Humidity (contours) and wind direction and wind speed (vectors) at 850 hPa over Java Island in January from 1979 to 2004

3.1.2. Relationships between rainfall and climate indices during 1981-2009 by sub district

We compared the average rainfall with average values of the global climate indices during the rainy seasons from 1981 to 2009 in each subdistrict, and found significant correlations between the rainfall variation in the subdistricts and SOI and SST values. SOI and SST Niño.West were both correlated with rainfall in only three subdistricts (Figs. 5 and 6): Panggang (SOI, $r = 0.58$; SST Niño.West, $r = 0.55$),

Playen (SOI, $r=0.44$; SST Niño.West, $r = 0.52$), and Rongkop (SOI, $r = 0.49$; SST Niño.West, $r = 0.38$) in $p < 0.01$ for all correlations. Rainfall variation was also correlated with SST Niño.3 in Panggang ($r = -0.57$), Playen ($r = -0.38$), and Rongkop ($r = -0.50$) in $p < 0.01$ for all correlations, and with SST IOBW in Panggang ($r = -0.44$) and Rongkop ($r = -0.54$) in $p < 0.01$ for all correlations. Low significant correlation between rainfall and SST IOBW and Niño.3 is influenced by location of both SST. Also we focused on SST Niño.West in our subsequent analysis because Niño.West is closest to our study area.

In Panggang subdistrict, rainfall ranged from 700 to 4000 mm, SOI from -25 to +14, and SST Niño.West from 28.2 to 29.4. Most years, the average rainfall was between 1200 and 2500 mm, and it was below 1000 mm in only one year. In Playen subdistrict, the average rainy season rainfall varied greatly from year to year, from 1000 to 3700 mm, and SOI varied from -27 to +14 and SST Niño.West from -27 to +14. The average rainfall was usually between 1200 and 3500 mm and always above 1000 mm. The correlations between rainfall and SST Niño.West were similar in these two adjacent subdistricts. In Rongkop subdistrict, rainfall variation was highly correlated with the indices: average rainfall varied from 900 to 2400 mm, SOI from -27 to +14, and SST Niño.West from -28.20 to 29.40. In Rongkop, although the highest rainfall amount was lower than the highest amounts in Panggang and Playen, the average rainfall was generally above 1500 mm.

Panggang, Tepus and Rongkop are the southernmost subdistricts in Gunungkidul district and border directly on the Indian Ocean, and the SOI and SST Niño.West averages were Panggang and Rongkop strongly correlated with the average rainfall in these subdistricts. In contrast, rainfall in Playen subdistrict, in western-central areas of Gunungkidul district, is little influenced by wind direction from the west. Thus, rainfall in areas along the coast and west area shows stronger correlations with SOI and SST Niño.West than central areas and northern areas, and positive/negative SOI/SST Niño.West values influence the amount of rainfall in coastal areas.

The average rainfall in the other nine subdistricts of Gunungkidul district was not significantly correlated with either SOI or SST Niño.West averages, which we attribute to the effect of the mountainous terrain on precipitation.

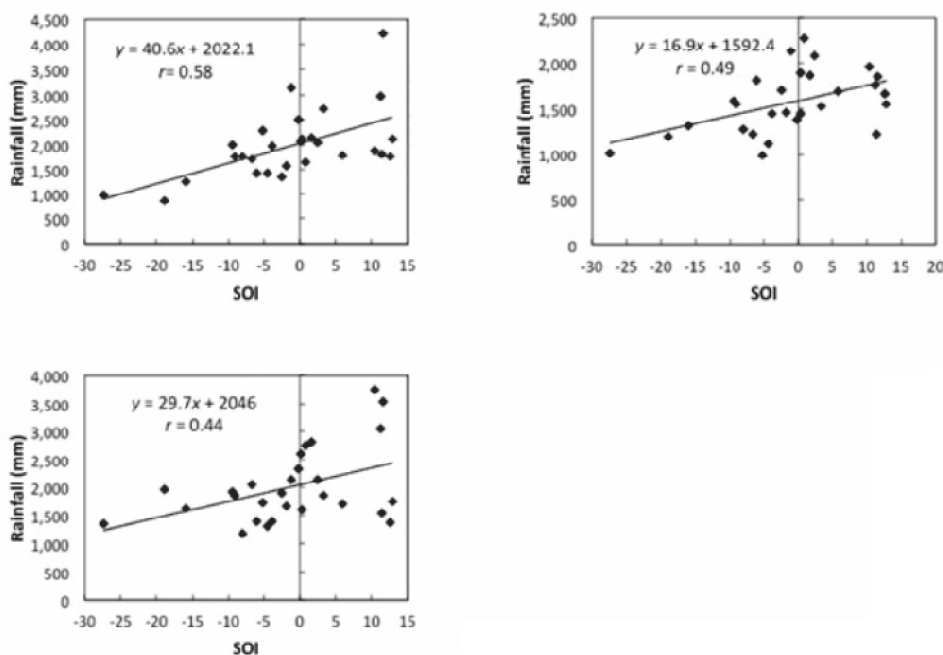


Fig. 5. Relationships between average rainfall and the average SOI in the rainy season during 1981-2009 for (a) Panggang, (b) Playen, and (c) Rongkop

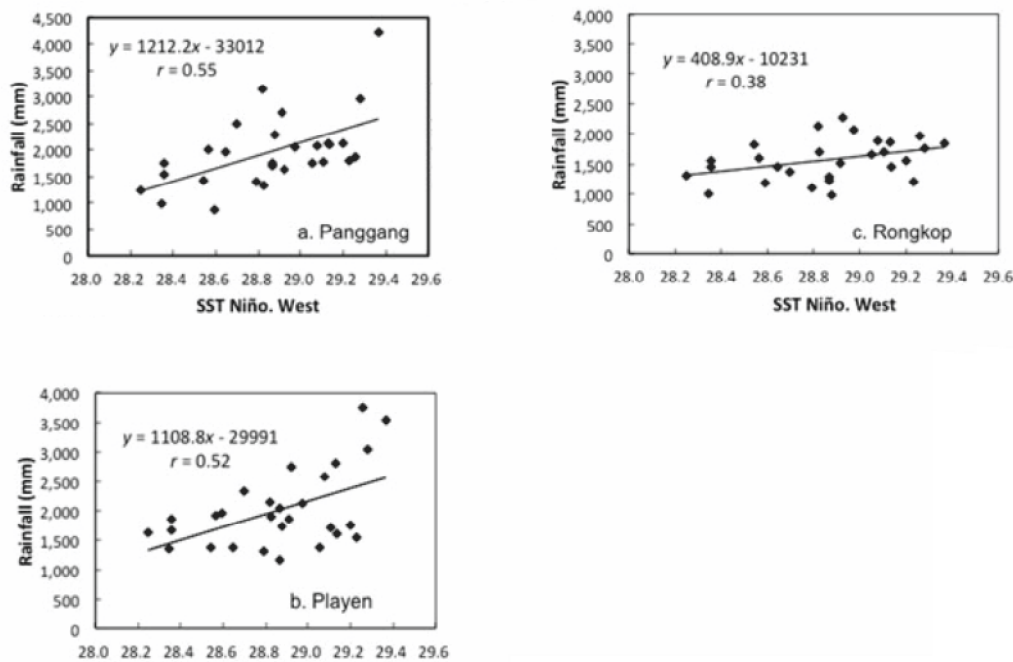


Fig. 6. Relationships between average rainfall and the average SST Niño.West in the rainy season during 1981-2009 for (a) Panggang, (b) Playen, and (c) Rongkop

3.2. Relationships with crop yield

3.2.1. Relationships between rainfall and crop yield

We determined correlations between average rainfall and crop yields during 1990-2009. During January-April (JFMA), both soybean ($r = 0.62$, $P < 0.01$) and dryland paddy ($r = -0.54$, $P < 0.05$) yields were significantly correlated with the amount of rainfall in January (Fig. 7), but whereas January rainfall was positively correlated with soybean yields, it was inversely correlated with dryland paddy yields. This can be explained as follows: traditional and economic factors determine the planting system in Gunungkidul district. Sarjiman and Mulyadi (2005) divided the growing season in dryland areas of the district into three stages, beginning in October, February, and May. During the early growing season (October-December or January), farmers prefer to plant dryland paddy for their own consumption (Sarjiman and Mulyadi, 2005). In contrast, in the late growing season (January or February-March or April), farmers prefer to plant soybeans over dryland paddy because they can get a higher price for soybeans than for dryland paddy in the market. The average price of late-season soybeans is IDR3.000 (per hectare per kg), compared with around IDR1.000 (per hectare per kg)¹ for late-season dryland paddy (ASFCH, 2006). If precipitation is low in January, however, farmers do not plant soybeans because the quality of the soybean crop would be poor. Instead, they plant dryland paddy for their own consumption. Therefore, soybean yields correlate positively, and dryland paddy yields correlate negatively, with January rainfall, and the amount of rainfall in January can be used to predict soybean and dryland paddy yields during the late growing season (JFMA).

¹IDR: Indonesian rupiah.

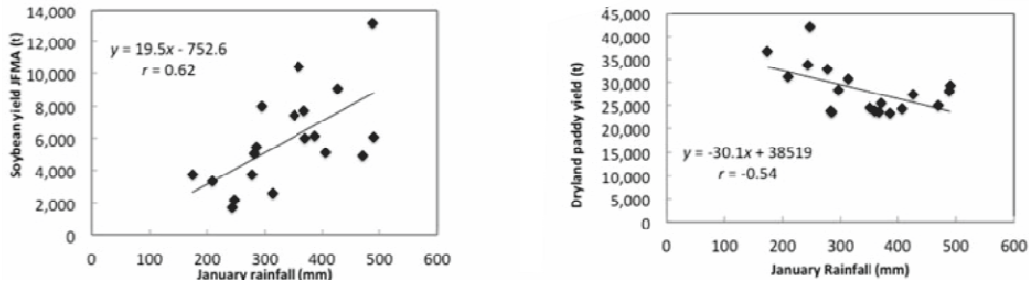


Fig. 7. Relationships between the amount of rainfall in January and (a) soybean yield and (b) dryland paddy yield during January-April (JFMA)

3.2.2. Relationships between SOI and crop yield

The average SOI during June-September (JJAS) (x) was correlated with the maize yield during JFMA (y) ($r = 0.50$, $P < 0.05$; Fig. 8), whereas we found no correlation between SST Niño.West and any crop yield.

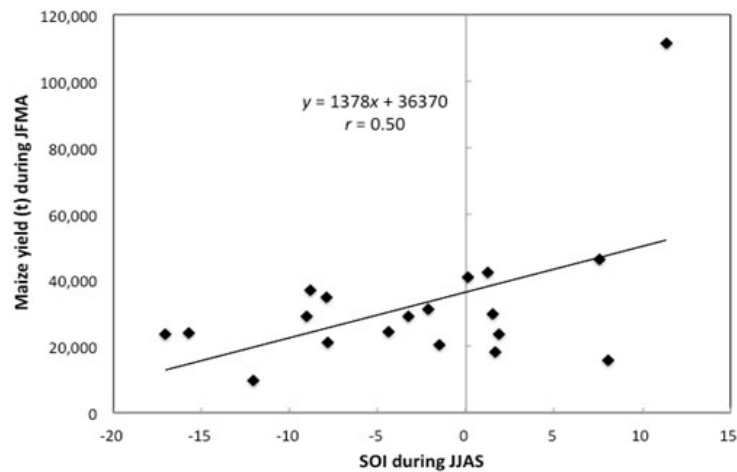


Fig. 8. Relationship between the average SOI during June-September (JJAS) and maize yield during JFMA

4. DISCUSSION

Some sub-districts in the coastal and western parts of the study area, the variations in rainfall during the rainy season were also significantly correlated with the SOI and with SST Niño.West, Niño.3, and IOBW. Although we found SST Niño.West to be significantly correlated with rainfall in Gunungkidul district, Surmaini and Susanti (2009) reported that SST Niño 3.4 (5°N - 5°S , 120° - 170°E) is significantly correlated with rainfall in Indonesia generally. They contend that SST in the Niño 3.4 zone in May and June can be used to predict rainfall in Indonesia during August-November. The discrepancy between their results and ours reflects the difference in the scope of the study area and seasonal factors. Our microscale analysis focused on rainfall in highland areas during the rainy season only (October-March), whereas the mesoscale analysis of Surmaini and Susanti (2009) included the entire Indonesian archipelago during both the rainy and dry seasons.

Average values of the indices SOI and SST Niño.West were correlated with rainfall in the southern and western mountainous areas of the district; coastal areas in particular received more rainfall than inland areas. Similarly, annual rainfall is generally higher in the coastal regions of West Sumatera Province, Indonesia, than in the inland regions (Hamada et al., 2008). This pattern of distribution can be attributed to the prevailing westerly winds being blocked by the mountains in the southern and western areas (Hamada et al., 2008). Thus, in the future, the relationships between rainfall variations and global climate indices in not only southern Java but also all of Indonesia need to be examined.

By relating global climate indices to rainfall patterns, the suitability of crops can be determined, which can help farmers make decisions about which crops to plant, especially in rain-fed agricultural areas in the highlands. Kirono and Khakhim (1998) proposed that if the weather can be predicted well in advance, and then so can crop production. We found that the amount of rainfall in January corresponded well to soybean and dryland paddy yields during JFM: In Gunungkidul district, increased rainfall during January increases the soybean yield and decreases the dryland paddy yield. Rice is the primary staple food for most Indonesian peoples, but we showed that during the peak of the rainy season (JFMA), most farmers in Gunungkidul district planted soybeans instead of dryland paddy rice. In the future, the correlation between soybean yield quality and rainfall variation in rain-fed agricultural areas should be deeply investigated.

The average SOI in JJAS corresponded well to maize yields during JFMA. According to Naylor et al. (2001), rice production in Java strongly depends on SST anomalies: Indonesia's paddy rice production varies on average by 1.4 million t for every 1°C change in the August SST anomaly. Furthermore, the SOI also corresponds to maize production (Naylor et al., 2002).

Overall, our results show that global climate indices and rainfall can be used as predictors of crop yields in rain-fed agricultural areas in the highlands of southern Java. Therefore, policymakers and farmers can anticipate those areas that will have less rainfall during the next growing season. The result of our study provides information to policymakers in the agricultural sector that will be useful in the future. Further studies should investigate crop yields in rain-fed croplands in both the highlands and the lowlands in relation to both rainfall and climate indices.

5. CONCLUSIONS

The rainfall distribution pattern shows that most rainfall during the rainy season from 1981 to 2009 fell in the southern and western mountainous areas, especially near the coast, and less rain fell in the central lowland area. In the central lowlands, which have hilly topography, the prevailing wind direction and orographic effects cause the amount of rainfall to differ among sub-districts. The SOI and SST Niño.West were correlated with the average rainfall during the rainy season in the coastal southern and western areas. Rainfall in January correlated well with soybean ($r = 0.62$) and dryland paddy rice yields ($r = -0.54$) in JFMA. The average SOI in JJAS correlated well with the maize yield in JFMA ($r = 0.50$). Thus, the amount of precipitation in January can be used to predict soybean and dryland paddy yields, and the SOI during JJAS can be used to predict the maize yield in JFMA.

Because precipitation in southern Java is significantly related to the global climate indices (SOI and SST), rainfall can be predicted from variation in these indices, and precipitation and the SOI can be used to predict crop yields in rain-fed agricultural areas of the southern Java highlands.

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Growth Response of Three Rice Cultivars to Nitrogen Foliar Application under Submerged Condition

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ABSTRACT

Growth characteristics of three Indonesian rice cultivars (Inpara 5, IR 64, and Si Kuning) were evaluated under 7 and 14 days submergence condition. Nitrogen foliar treatment of 2300 ppm was applied to rice plants and split into two application times: before (N1) and after (N2) submergence. Growth analysis then was calculated separately for both under submerged condition and recovery period. Results showed that tiller number was identified as an important character determining plant survival under submergence and growth after de-submergence. Greater tiller number after de-submergence would lead to an improved leaf area ratio (LAR) and finally will affect relative growth rate (RGR) and the accumulation of total dry weight (TDW). Therefore, N2 treatment was assumed as an effective effort to enhance tiller number and maintain plant growth after de-submergence. Nevertheless, under longer submergence duration, the application of N1 appeared to give better performance.

Keywords: Growth analysis, nitrogen, recovery period, submergence, tiller number.

1. INTRODUCTION

Rice is a well-adapted species of plant to flooded condition. However, excessive flooding may result in plant stress leading to decline of productivity. Flooding is a common problem occurred in lowland area across South and Southeast Asia (Ella and Ismail, 2006; Das et al., 2009), including swamp areas in Indonesia. Swamp rice cultivation in Indonesia has suffered from an adverse impact from high flooding which occurs in various stages of plant cycle. The risk of flooding to rice plant is not only during early growth but also on the later stage or even during reproductive stage causing the farmers suffer from massive harvest loss (Ito et al., 1999).

Poorly drained fresh-water swamp is the most prone area to flash-flooding that generally will last less than a few weeks and is caused by heavy rain but the depth is not very deep (Catling 1992; Hattori et al., 2009). Flash floods that result in stagnant flooding or complete submergence (Singh et al., 2011) of paddy fields can cause yield losses from 10% to 100% depending on water depth, duration of the flooding event, turbidity, temperature and the developmental stage of the plant (Das et al., 2009).

The increase in the frequency and intensity of flash floods due to changing global weather patterns (Singh et al., 2011) makes the development of more flood tolerant rice varieties critical (Sasidharan and Voesenek, 2013). Nowadays, breeders are making efforts to develop and release new rice cultivars that have submergence tolerance character (Septiningsih et al., 2015). However, this effort will take longer time due to the complexity of floodwater characteristics among different environments (Das et al., 2009). Furthermore, existing submergence cultivars in Indonesia, such as INPARA cultivars, are not preferable for both farmers and consumers due to the unfavorable taste. Farmers still cultivate popular commercial cultivars which are less adaptive to flooding injury. Consequently, agronomists should consider cultivation technique to cope with this submergence problem.

Proper nutrient adjustment then is considered as one of the subjects that can be modified to adapt to this situation. Nitrogen (N) is one of the essential macronutrients for plant growth and yield (De Datta, 1986; Ye et al., 2007). Many studies have reported the importance of N in plant body to show better performance either in plant elongation, dry matter production or tillering (Ahmed et al., 1998; Chaturvedi, 2005; Duan et al., 2006; Irmawati et al., 2015). Ehara et al (1993) mentioned that application of 2300 ppm N would give the best effect on rice growth. The application of N fertilizer to improve plant tolerance under submerged condition has also been investigated. As reported by Rao et al. (1985), basal fertilization with N promoted early vigor allowing plants to tolerate submergence at later stages of growth. Sharma (1995) also stated that top dressing of N after recession of flood water improved recovery of plants and partially compensated for the mortality due to flooding.

Furthermore, the combination of N application through plant foliage along with soil application is believed more efficient compared to application only through soil (Mosluh et al., 1978; Khemira et al., 1998; Saleem et al., 2013). However, very limited references of the effect of foliar N application to growth of rice under submergence were found. Thus, the study was carried out to examine the effect of foliar application of high N concentration on growth parameters of rice under submerged condition.

2. MATERIALS AND METHODS

2.1. Plant materials, treatments and growth conditions

The experiment was conducted from September to December 2013 in Faculty of Agriculture, Sriwijaya University, Indonesia. Three rice cultivars were evaluated in this research: IR 64 (common commercial cultivar), Inpara 5 (IR 64 sub-1 cultivar), and Si Kuning (local fresh water swamp cultivar in South Sumatra). Germinated seeds were first sown in a plastic tray, then 28-d-old seedlings were transplanted into plastic pots with 1 seedling per pot. Submergence treatments were started at 25 days after transplanting (DAT) by shifting the pots into $4 \times 1.5 \times 0.8$ m concrete water tank filled with tap water until whole plants were fully submerged. Two submergence treatments were applied to the plants based on the duration: 7 days and 14 days. Water level and plant condition was monitored to ensure submergence condition.

N treatment was applied to the plants by spraying 2300 ppm of N solution to the leaves. Tween 20 500 ppm was mixed in the N solution to confirm that foliar N will attach firmly on the leaflet. Urea was used as N source considering its characteristic that can easily be absorbed by plants. The application of N foliar treatment was divided into two treatments: before (N1) and after (N2) submergence and added with one non N treatment (N0) with three replicates. N treatment before submergence was applied 7 days before submergence treatment (at same time for both 7 and 14 days submergence), while after submergence treatment was applied one day after de-submergence for each 7 days and 14 days submergence treatment.

2.2. Growth analysis

Plant samplings were conducted four times. First was one day before submergence treatment (t_0 : 25 DAT), second and third sampling were carried out at the end of submergence treatment [t_1 : end of 7 days submergence (32 DAT); t_2 : end of 14 days submergence (39 DAT)] and final sampling (t_3 : 49 DAT) was conducted 10 days after recovery period of 14 days submergence treatment. Plant growth characters were measured at each sampling time, while plant length was measured on 25, 32, 35, 39, 42, and 49 DAT. During samplings, the treated plants were taken out and washed thoroughly in distilled water. The plants were separated into three parts: roots, stems and leaves. Leaf area and dry weight were also measured. Growth analysis then was calculated separately for both under submerged condition according to the following equations,

$$\text{Relative Growth Rate (RGR)} = \frac{\ln(W_2) - \ln(W_1)}{t_2 - t_1} \quad (1)$$

$$\text{Net Assimilation Rate (NAR)} = \frac{W_2 - W_1}{A_2 - A_1} \times \frac{\ln(A_2) - \ln(A_1)}{t_2 - t_1} \quad (2)$$

$$\text{Leaf Area Ratio (LAR)} = \frac{A_2 - A_1}{\ln(A_2) - \ln(A_1)} \times \frac{\ln(W_2) - \ln(W_1)}{W_2 - W_1} \quad (3)$$

$$\text{Relative Tillering Rate (RTR)} = \frac{\ln(T_2) - \ln(T_1)}{t_2 - t_1} \quad (4)$$

Where, W is total dry matter weight, t is time of treatment, A is leaf area and T is tiller number, respectively.

2.3. Statistical analysis

A statistical analysis was performed using two-way analysis of variance to determine the differences on growth parameters among cultivars and N treatments under both 7 days and 14 days submergence. Differences among mean values of N treatments were evaluated by using 0.05 level of LSD test. Pearson product moment correlation was also calculated for certain pairs of growth analysis parameters.

3. RESULTS

3.1. Plant growth

N treatment had insignificant impact to plant length for all cultivars in both 7 days and 14 days submergence either under submergence or after recovery period condition ($P > 0.05$). Fig. 1 showed the change of plant length increment of three rice cultivars under both submergence durations and foliar N treatments. From Fig. 1, it was found that rapid increase occurred during submergence treatment (25-32 DAT for 7 days submergence treatment, and 25-39 DAT for 14 days submergence treatment) for all cultivars. At the end of experiment, plants which had been submerged under 7 days duration reduced their lengths due to N treatment, especially in Inpara 5 and IR 64 where the leaves suffered either from rapid senescence or detached from the stems. While most plants (especially from N0 and N2 plot) could not survive under 14 days submergence treatment so that the effect of N treatment could barely be evaluated. Si Kuning could not survive under 14 days submergence treatment.

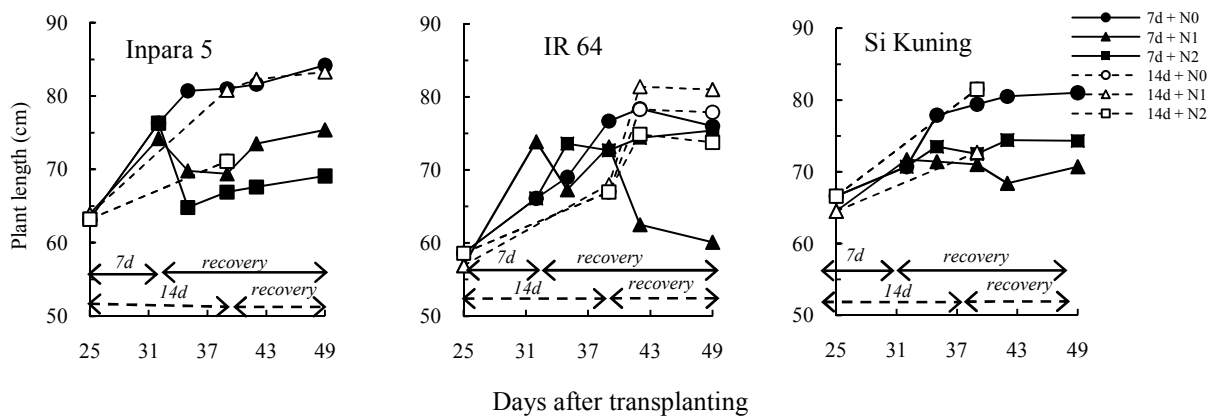


Fig. 1. Plant length increment of three rice cultivars under 7 days and 14 days submergence and after de-submergence condition

Tiller numbers of rice plants at the end of experiment (49 DAT) were measured as given in Fig. 2. Increase in tiller number was found in all cultivars due to N treatment, except for N2 plot of

IR64 under 14 days submerged duration. The increase caused by both N treatments was more apparent in IR 64, while Si Kuning had a greater increase of tiller number due to N2 treatment in 7 days submergence duration.

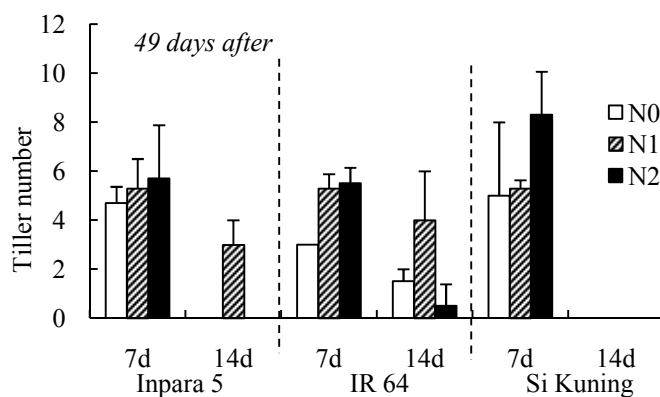


Fig. 2. Tiller number of three rice cultivars measured at the end of experiment (49 DAT). Error bars indicated standard error (n = 3)

3.2. Dry matter weight

Under submergence condition for both 7 days and 14 days, N1 treatment had significantly ($P < 0.01$) increased total dry matter weight (TDW) of rice plants. The highest increase was obtained in Inpara 5 and IR 64 in 7 days submergence with 2.39 and 2.33 g respectively, while in 14 days submergence the highest increase was Inpara 5 with 1.93 g as given in Fig. 3. However, TDW did not significantly change after recovery period as it measured at the end of experiment ($P > 0.05$). N1 treatment showed opposite response from the previous result (Fig. 3) by having decreased TDW in 7 days submergence in all cultivars (Fig. 4). In contrast, N2 treatment showed various results among cultivars. TDW was decreased in Inpara 5, increased greatly in IR 64 and only slightly increased in Si Kuning. In 14 days submergence treatment, the application of N1 had improved TDW for Inpara 5 and IR 64.

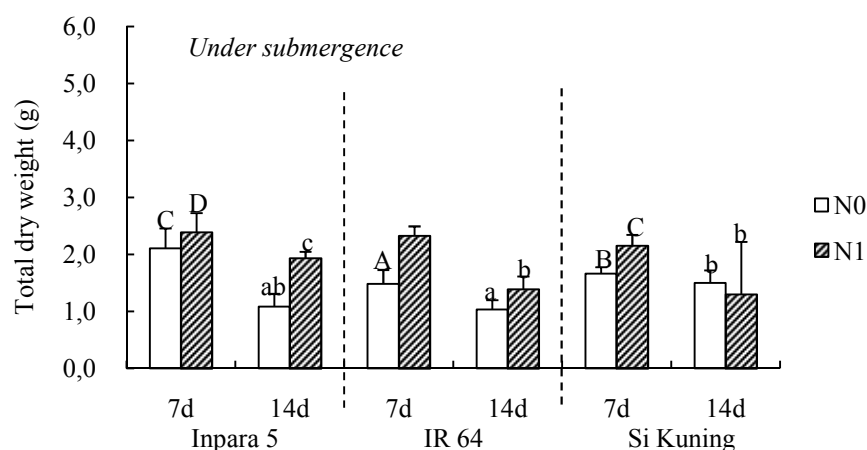


Fig. 3. Total dry weight of three rice cultivars under submergence treatment, measured on 32 DAT for 7 days submergence, and on 39 DAT for 14 days submergence treatment. Error bars indicated standard error (n = 3)

Note: Values with the same letters are not significantly different at the 0.05 level by LSD test. Capital letter indicates comparison among TDW of rice plants under 7 days submergence treatment. Lowercase letter indicates comparison among TDW of rice plants under 14 days submergence treatment.

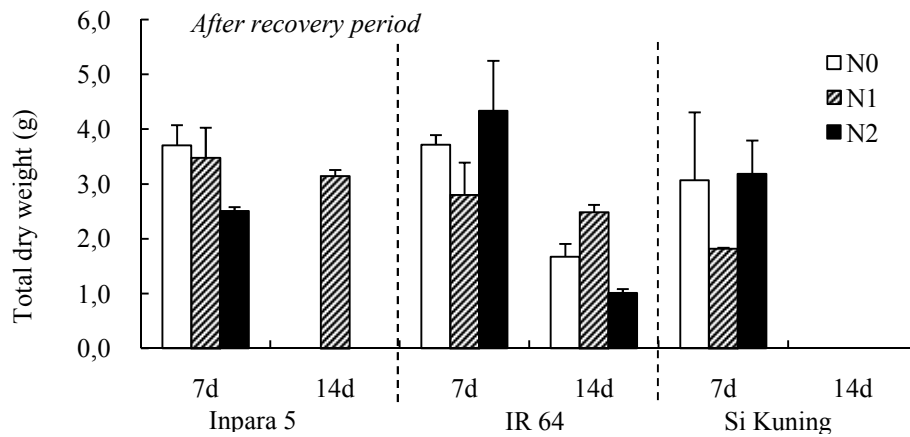


Fig. 4. Total dry weight of three rice cultivars, measured after recovery period or at the end of experiment (49 DAT). Error bars indicated standard error (n = 3)

3.3. Growth analysis

Growth analysis was calculated to evaluate growth of rice plant both under submergence condition and after de-submergence. The results then were divided as following.

(1) Under submergence

Results showed that N1 treatment had generally improved RGR of three cultivars under both 7 days and 14 days submergence, except for Si Kuning under 14 days submergence (Fig. 5). Fig. 6 illustrated the effect of N treatments in NAR and it was found that NAR was escalated due to N1 treatment in Inpara 5 and IR 64 and conversely declined in Si Kuning for both submergence durations. LAR change among cultivars was given in Fig. 7. The results showed that under 7 days submergence, N1 treatment had caused a decrease in LAR value in Inpara 5 and IR 64, and only a slight change in Si Kuning. Different results were obtained under 14 days submergence where IR 64 and Si Kuning had a decreasing LAR though the result was more apparent in Si Kuning, while Inpara 5 was opposite due to N1 treatment. N1 treatment also gave a positive impact to RTR as seen in Fig. 8 where all cultivars had an increasing RTR compared to N0 for both submergence durations.

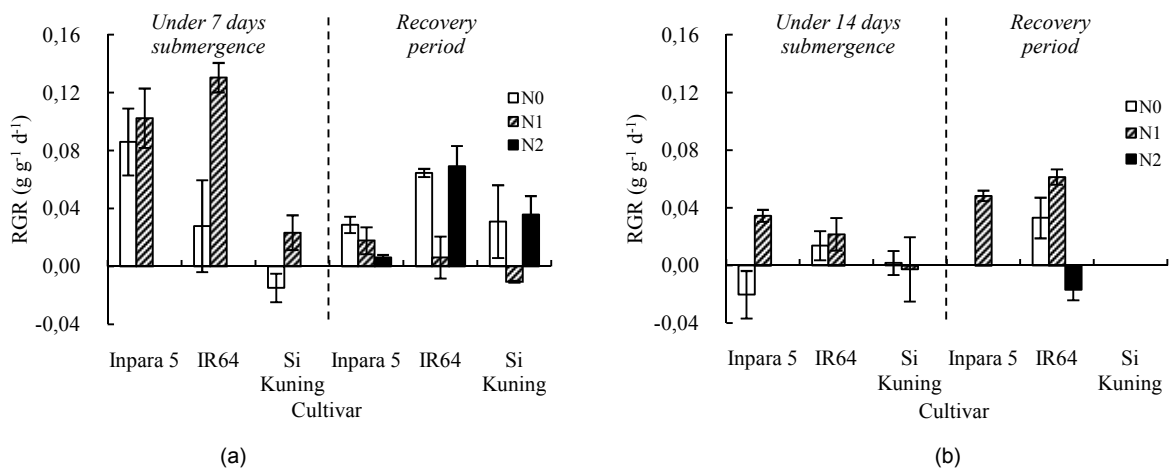


Fig. 5. Effect of N treatment to RGR of three rice cultivars under 7 days submergence (a) and 14 days submergence (b). Error bars indicated standard error (n = 3)

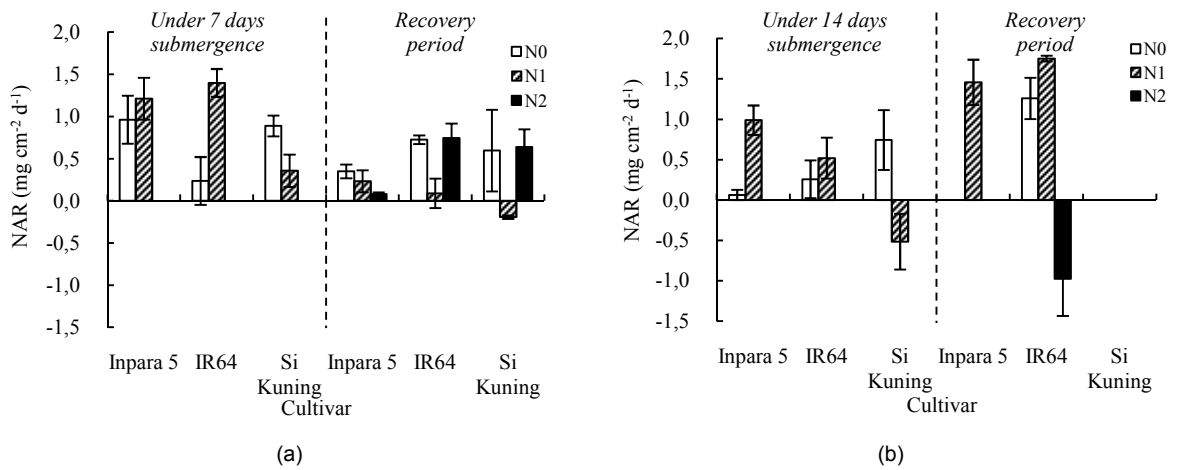


Fig. 6. Effect of N treatment to NAR of three rice cultivars under 7 days submergence (a) and 14 days submergence (b). Error bars indicated standard error (n = 3)

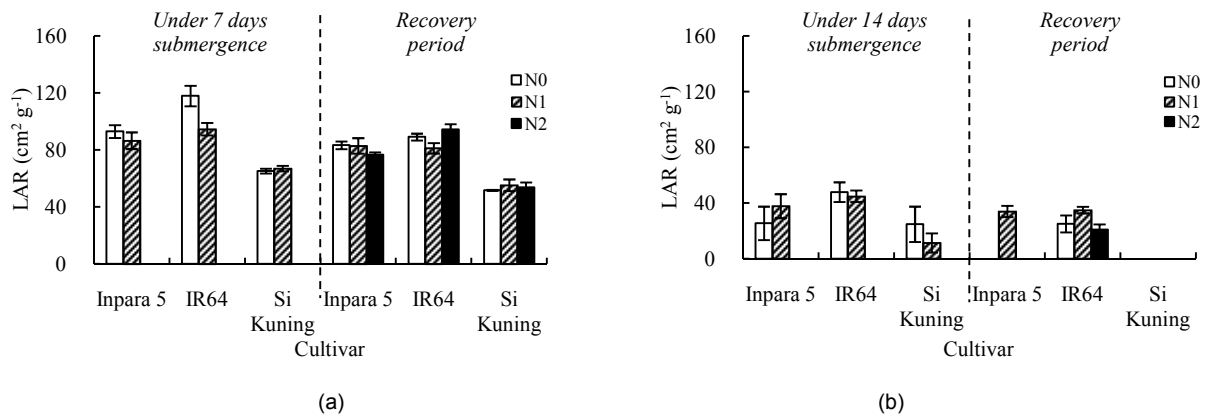


Fig. 7. Effect of N treatment to LAR of three rice cultivars under 7 days submergence (a) and 14 days submergence (b). Error bars indicated standard error (n = 3)

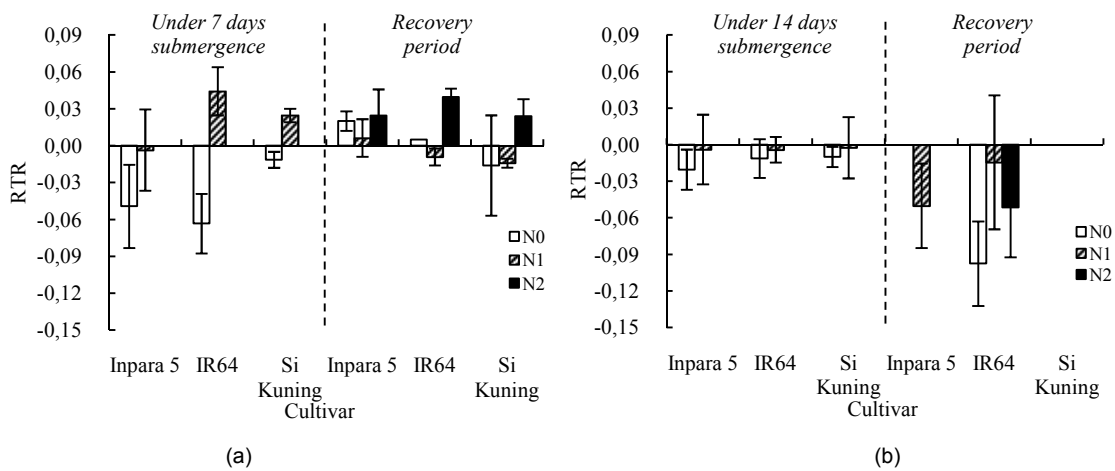


Fig. 8. Effect of N treatment to RTR of three rice cultivars under 7 days submergence (a) and 14 days submergence (b). Error bars indicated standard error (n = 3)

(2) Recovery period

After de-submergence, N2 treatment then was applied to rice plants. During recovery period, RGR of plants applied with N1 treatment after submerged for 7 days was significantly decreased. There was only a slight increase of RGR by the application of N2 in IR 64 and Si Kuning compared to N0 (Fig. 5). However, different response was shown by Inpara 5 by having a decreasing trend of RGR due to N treatment. NAR showed a similar response to RGR due to N treatment in both submergence durations (Fig. 6). LAR was only slightly changed in all rice cultivars during recovery period among N treatments in 7 days submergence (Fig. 7). RTR of IR 64 and Si Kuning were apparently improved by the application of N2 treatment in 7 days submergence, while N1 gave better results in all the growth analysis parameters in 14 days submergence treatment (Fig. 8).

4. DISCUSSION

In this experiment, the evaluation of growth parameters could be mostly performed only for 7 days submergence duration since most of rice plants under 14 days submergence could not survive until the end of experiment. Therefore, the evaluation of N2 treatment was also very limited. Some studies reported that survival during submergence was closely related to plant elongation growth that led to plant avoidance mechanism under submerged condition (Setter et al., 1997; Visser et al., 2003; Kotera and Nawata, 2007; Nishiuchi et al., 2012). As seen in Fig. 1, plant length increment was not so apparent in 7 days submergence among N treatments, except for N1 in IR64 which later resulted in drastic decrease of plant length during recovery period. Generally, plant length increase was slower or relatively constant, and even declined due to detached leaf or enhanced leaf senescence occurred after de-submergence. Nevertheless, enhanced stem elongation was actually an unfavorable character for submergence rice. Kawano et al. (2009) reported that tolerance was greater in cultivars where acceleration of elongation caused by submergence was minimal. Elongation growth competed with maintenance processes for energy and hence reduced survival during submergence (Setter and Laureles, 1996). The report was also validated by result of this study as shown by IR 64 which had the best survival under 14 days submergence since it had minimal plant length under submergence, while Inpara 5 which was basically a sub-1 rice cultivar and anticipated to have better survival performed an unexpected result. The application of N1 treatment improved survival of Inpara 5 by enhancing stem elongation under submergence.

As described above, N1 treatment had significantly affected TDW under submergence conditions. However, the results were varied at the end of experiment. The relationship between TDW and RGR then was investigated as given in Fig. 9 and showed significant positive correlations for both under submergence and during recovery conditions. As RGR was a product of leaf area ratio (LAR) and net assimilation rate (NAR) (Yamauchi et al., 1988; Poorter and Werf, 1998), observing the trend of both traits would be beneficial in differentiating the roles of morphological and physiological response of rice plant under submergence condition. By comparing the tendency in NAR results, it was understandable that RGR change was mostly attributed by the alteration in NAR (Fig. 5, Fig. 6). Under both 7 and 14 days submergence, NAR was increased by the application of N1 as seen in Inpara 5 and IR64 and this trend was similar to RGR as aforementioned. Hence, the opposite result was shown by Si Kuning by having smaller NAR compared to N0 indicating that RGR change of Si Kuning was not determined by the change in NAR and more subjected by the change in LAR as given in Fig. 7, though the increase was quite small. Increase in LAR due to N1 treatment in Si Kuning defined plant investment in leaves and larger LAR was indicative of plant emphasizing on leaf production (Azarpour et al., 2014). Inpara 5 actually also had increased its LAR under 14 days submergence. However, greater increase was initiated by NAR attributing to a superior impact to RGR change.

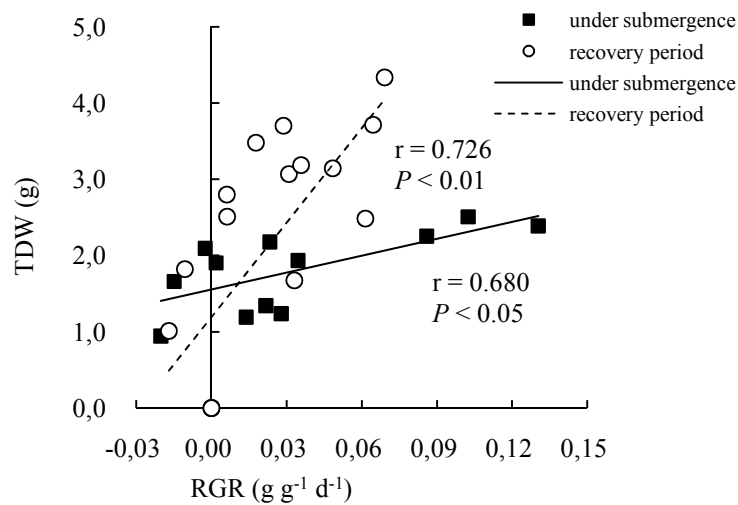


Fig. 9. Correlation between TDW and RGR for both under submergence and recovery period

From the results, it was also noted that there was a negative relationship between NAR and LAR caused by N1 treatment under submerged condition regarded as a compensatory response between these two variables. Some studies also reported the occurrence of compensatory effect in rice. Yan and Wang (2009) reported the compensatory effect in rice seedling induced by water deficit. Compensatory effect on yield was also found in rice planted at different seedling densities (Ehara et al., 1998). Contributions from both NAR and LAR to RGR then were further investigated (Fig. 10 and Fig. 11) and found that both variables affected RGR though the impact was not similar. From the results, it was also considered that during recovery period, NAR gave a greater impact to RGR rather than LAR.

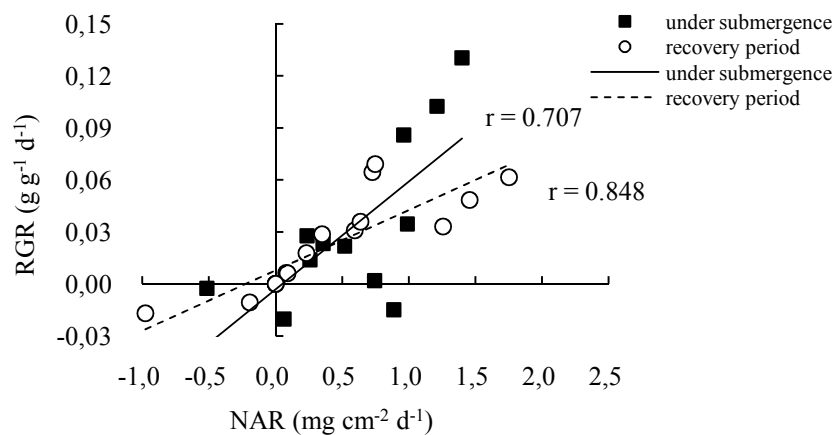


Fig. 10. Correlation between RGR and NAR for both under submergence and recovery period

Gautam et al. (2014) reported that a quick re-growth following submergence was a desirable quality as it could ensure production of sufficient biomass for plant productivity indicating the importance of plant growth during recovery period. Thus, growth characters in this period were

beneficial. As NAR during recovery showed the great impact to RGR as mentioned, NAR related traits then were identified. Some studies reported that NAR of rice was affected by leaf morphogenesis such as thinning of the leaf blade when nutrients were supplied sufficiently (Ehara et al., 1990; Ehara, 1993). Therefore, the relationship between NAR and SLA was also evaluated. However, the result showed that the correlation coefficient between NAR and SLA was significant ($r = 0.530$, $P = 0.076$) only under submergence and insignificant during recovery period (data not shown).

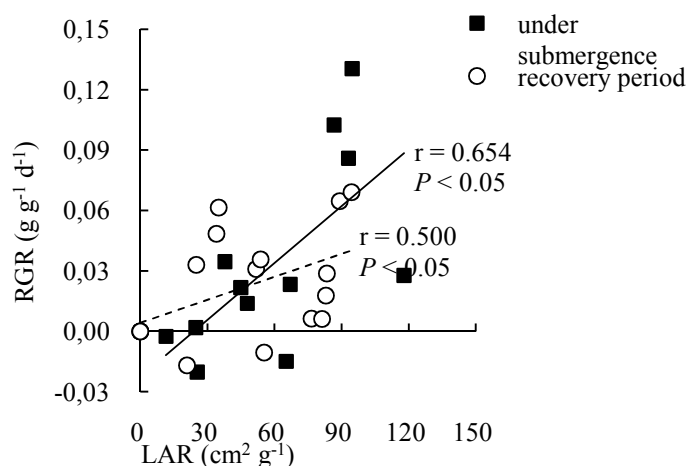


Fig. 11. Correlation between RGR and LAR for both under submergence and recovery period

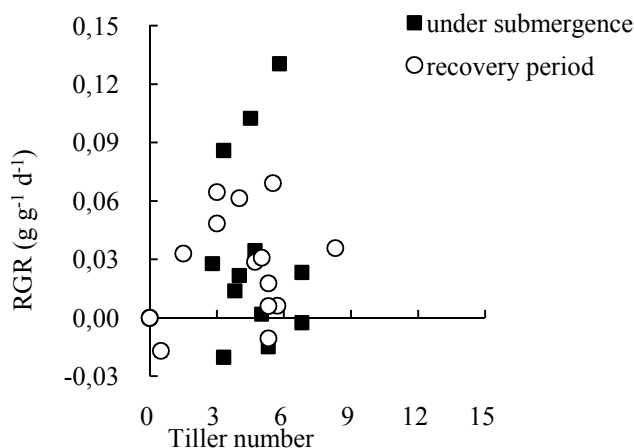


Fig. 12. Correlation between RGR and tiller number for both under submergence and recovery period

The present study also attempted to investigate the relationship between RTR and RGR as they both showed a similar tendency as previously given in Fig. 5 and Fig. 8. The relation, however, was insignificant (data not shown). Similar insignificant result was shown between RGR and tiller number ($r = 0.424$, $P = 0.080$) as given in Fig. 12. Hence, when tiller number was associated with LAR, the correlation coefficient showed significant result ($r = 0.815$, $P < 0.01$) (Fig. 13). Furthermore, the role of tiller number was more apparent to TDW for both under submergence and during recovery period with $r = 0.577$, $P < 0.05$ and $r = 0.828$, $P < 0.01$ respectively (Fig. 14).

These findings indicated that tiller number was an important character determining plant adaptation under submergence and survival after de-submerged condition. Corresponding to this result, Fageria et al. (1997) clarified that greater tiller number would increase the leaf area index (LAI) (or LAR in this case) and consequently the radiation interception capacity resulting in high biomass production. Similar results were also reported by some studies indicating the contribution of tiller number to dry matter production (Wu et al., 1998; Ntanos and Koutroubas, 2002). Moreover, tiller number was also considered as a substantial trait affecting yield (Counce et al., 1996; Wang et al., 2007). The former study in submergence-prone swamp area in South Sumatra also discovered that fewer tillers led to less panicle number per hill resulting in a lower yield due to the absence of N fertilizer (Irmawati et al., 2015).

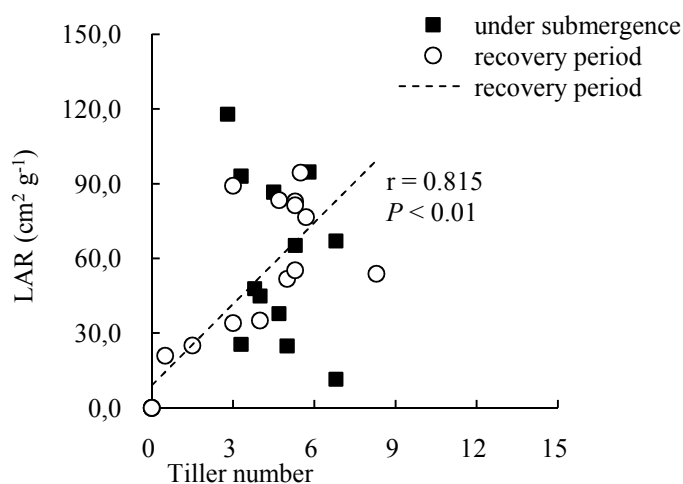


Fig. 13. Correlation between LAR and tiller number for both under submergence and recovery period

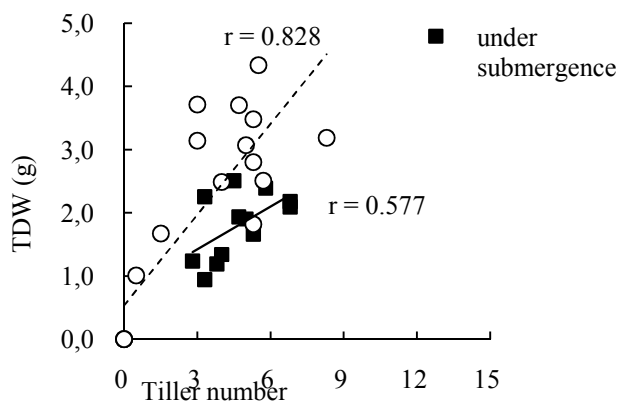


Fig. 14. Correlation between TDW and tiller number for both under submergence and recovery period

5. CONCLUSIONS

In conclusion, three cultivar tested showed different response to N treatment under submergence stress. IR 64 was the cultivar with highest dry weight increase in 7 days submergence, Inpara 5 was more apparent in 14 days submergence treatment, and Si Kuning increased slightly

during both submergence treatments. Tiller number was regarded as one of beneficial characters determining plant adaptation under submerged condition. Greater tiller number after de-submergence would lead to an improved LAR and finally will affect RGR and the accumulation of TDW. Considering these findings, the application of N after submergence (N2) was considered more effective to enhance tiller number after de-submerged condition. However, this result was limited only for relatively short duration submergence, while under longer duration, the application of N before submergence (N1) appeared to give better performance. Further study would be beneficial to ensure the effect of N application for either short or longer submergence durations.

ACKNOWLEDGEMENT

This work was supported by JSPS KAKENHI Grant Number 24405021. We are also indebted to Faculty of Agriculture, Sriwijaya University for granting of favorable treatment to conduct the experiment.

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Evaluation of Rice Productivity with Plant Canopy Analyzer in Farmers' Fields in Lao PDR

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ABSTRACT

Rice is the most important crop in Lao People's Democratic Republic (Lao PDR). In crop monitoring for precision farming and yield forecasting, the timely observation of leaf area index (LAI) is critical. The present study analyzed the relationship between rice production and LAI growth rate measured by plant canopy analyzer, and discussed suitability of these measurements to estimate grain yield in farmers' fields in Lao PDR. 66 farmers' paddy fields from 33 places were selected in this area for surveying throughout the growth period. Because the LAI in the farmers' fields increased almost linearly, a straight-line regression was used for the analysis. The LAI growth rate, the slope of the straight-line, was significantly correlated with the total dry weight (TDW) and the grain yield. However, compared to TDW, correlation coefficient between the LAI growth rate and the grain yield was lower. The result might derive from larger variance of harvest index (HI). This large variance of HI was considered to be mainly caused by the difference of cultivar and fertilizer and water management. Combining the measurements by plant canopy analyzer with the simulation models to evaluate cultivar or crop management may help to estimate rice growth and then rice grain yield in a regional scale.

Keywords: Lao PDR, Leaf area index (LAI), rice, plant canopy analyzer, yield.

1. INTRODUCTION

Rice is by far the most important crop in the Lao People's Democratic Republic (Lao PDR), and here, approximately 70% of the total calorie supply in diets comes from rice (Maclean et al., 2002). The wet season lowland is the main rice-producing environment in Lao PDR (Schiller, 2006).

Rice productivity is strongly affected by water, temperature, and solar radiation (Inoue et al., 2014). Therefore, in crop monitoring for precision farming and yield forecasting, the timely observation of plant biophysical and ecophysiological status is critical (Doraiswamy et al., 2004; Inoue, 2003). The leaf area index (LAI) is an important biophysical variable that is related to canopy photosynthetic rate and dry matter production during growth periods (Vaesen et al., 2001). Indeed, Hirooka et al. (2015b) reported that rice productivity in Lao PDR is affected by LAI growth.

This study used a plant canopy analyzer, LAI-2200, to measure the LAI. The analyzer also reduces the laboriousness of the destructive measurements for LAI and makes frequent measurements easier. Accordingly, the most advantageous aspect of this analyzer may be the quantification of the LAI dynamics based on frequent measurements (Hirooka et al., 2013).

The present study analyzed the relationship between rice production and LAI growth rate measured by plant canopy analyzer, and discussed suitability of these measurements to estimate grain yield in farmers' fields in Lao PDR.

2. MATERIALS AND METHODS

2.1. Sites

This study was conducted in 2013 in farmers' fields in Vientiane province, Lao PDR (18°01' - 18°30'N, 102°24' - 103°02'E, 168 - 178 m asl.). 66 farmers' paddy fields from 33 places were selected in this area for surveying throughout the growth period. The mean air temperature for the measuring period (from 22 July to 16 September) was 28.0°C. The longitude and latitude of the study sites were recorded by the Global Positioning System (GPSMAP 62SJ, GARMIN). The rice plants of the investigated farmers' fields varied in cultivation methods (direct seeding/transplanting, fertilizer, planting density and cultivar). Preliminary interviews with farmers suggested that transplanting, non-fertilization, 25 hill m⁻² of planting density and traditional cultivation were the majority.

2.2. Measurements

The leaf area index (LAI) was measured using a plant canopy analyzer (LAI-2200, LI-COR) with a single sensor mode in a sequence of two above and four below canopy at each field (Hirooka et al., 2015b). In order to reduce the influence of the adjacent fields and the operator, a 90° view-cap was applied to the optical sensor. The measurement was conducted 4 times before the heading period (from 22 to 25 July, from 10 to 12 Aug, from 30 Aug to 1 Sep and from 16 to 18 Sep). The LAI for each measurement was referred to as LAI_{1st}, LAI_{2nd}, LAI_{3rd} and LAI_{4th}. Because the peak of the heading in these study sites was on 22 Sep, LAI_{4th} contained almost all of the maximum LAI values.

Nine rice plant samples were harvested, and the plant density was measured in 56 of the 66 farmers' fields to determine the grain yield (g m⁻²) and total dry weight (TDW; g m⁻²) at the maturing stage (from 22 to 25 Oct). The other 10 fields were excluded from the sampling because the rice plants during these periods were too premature to harvest or had already been harvested. The grain yield and TDW were determined after oven-drying at 70°C for more than 2 days.

2.3. Data analysis

The air temperature (maximum, minimum and average temperature) was measured by thermometer/hygrometer (DCA, Decagon). The data was recorded using a field monitoring system (Mizoguchi, 2012) in Vientiane.

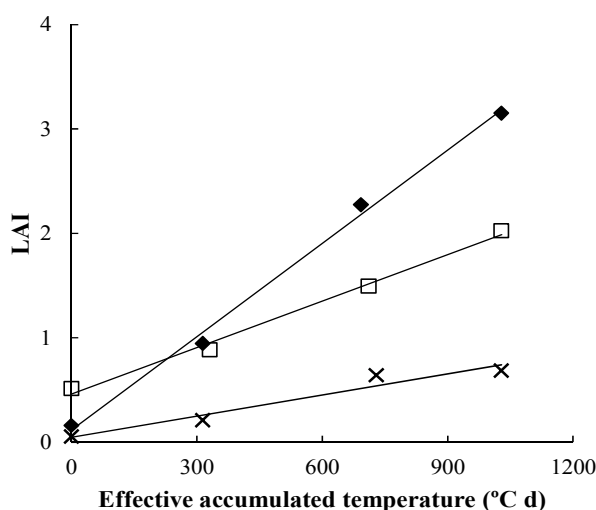


Fig. 1. Changes with the effective accumulated temperature (°C d; base temperature of 10°C) of the LAI. The effective accumulated temperature was calculated from 22 July

Because the measurements were conducted only 4 times in this study, linear growth was assumed (Hirooka et al., 2015b) (Fig. 1). LAI growth rate was calculated using the following linear function.

$$\text{LAI} = a T + b$$

where a and b are the regression coefficients, and T is the effective accumulated temperature ($^{\circ}\text{C}$ d; base temperature of 10°C) from 22 July. The coefficient a represents LAI growth rate, and b represents approximate LAI at the transplanting date.

3. RESULTS AND DISCUSSION

The grain yield ranged from 63.8 g m^{-2} to 411.8 g m^{-2} (235.8 g m^{-2} on average), and the total dry weight at maturing stage (TDW) ranged from 248.4 g m^{-2} to 1089.0 g m^{-2} (660.0 g m^{-2} on average) (Fig. 2). The TDW was correlated with the grain yield (Fig. 2). The grain yield and the TDW of Nipponbare (normal japonica rice cultivar) grown in Kyoto, Japan in 2013 was 563.4 and 1256.5 g m^{-2} , respectively (Hirooka et al., 2014). These two values were much higher than those in this study area (Fig. 2). This may be resulted in the poor soil fertility and cultivation method (less or no fertilizer) in this area (Hirooka et al., 2015a).

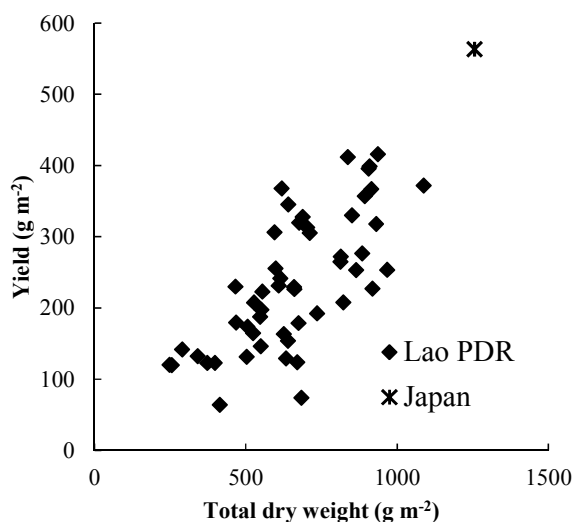


Fig. 2. Relationship between the grain yield (g m^{-2}) and the total dry weight at the maturing stage (g m^{-2}). Japan; Nipponbare cultivated in Kyoto in Japanese typical cultivation method

The LAI growth rate in the 66 fields varied widely from 0.56×10^{-3} to $3.63 \times 10^{-3} \text{ m}^2 \text{ m}^{-2} \text{ }^{\circ}\text{C}^{-1}$ ($1.94 \times 10^{-3} \text{ m}^2 \text{ m}^{-2} \text{ }^{\circ}\text{C}^{-1}$ on average) (Fig. 3). The LAI growth rate was significantly correlated with the TDW and the grain yield (Fig. 3). However, compared to TDW, correlation coefficient (r) between the LAI growth rate and the grain yield was lower (TDW, $r = 0.597$; grain yield, $r = 0.415$). The result might derive from larger variance of harvest index (HI) from 0.11 to 0.54 (0.36 on average) (Fig. 4). This large variance of HI was considered to be mainly caused by the difference of cultivar and fertilizer and water management.

Because the LAI in this study area increased almost linearly against effective accumulated temperature (Hirooka et al., 2015b), LAI growth rate theoretically can be determined by at least two measurements. Hirooka et al. (2015b) also reported that LAI growth rate in the study area was

closely associated with soil C and N levels, which is a major indicator of soil fertility and consequent rice production. Consequently, LAI growth rate is thought to be useful indicator to evaluate rice productivity such as in the study area, which would make it possible for remote sensing to evaluate regional scale evaluations even when it is measured in wider area by remote sensing.

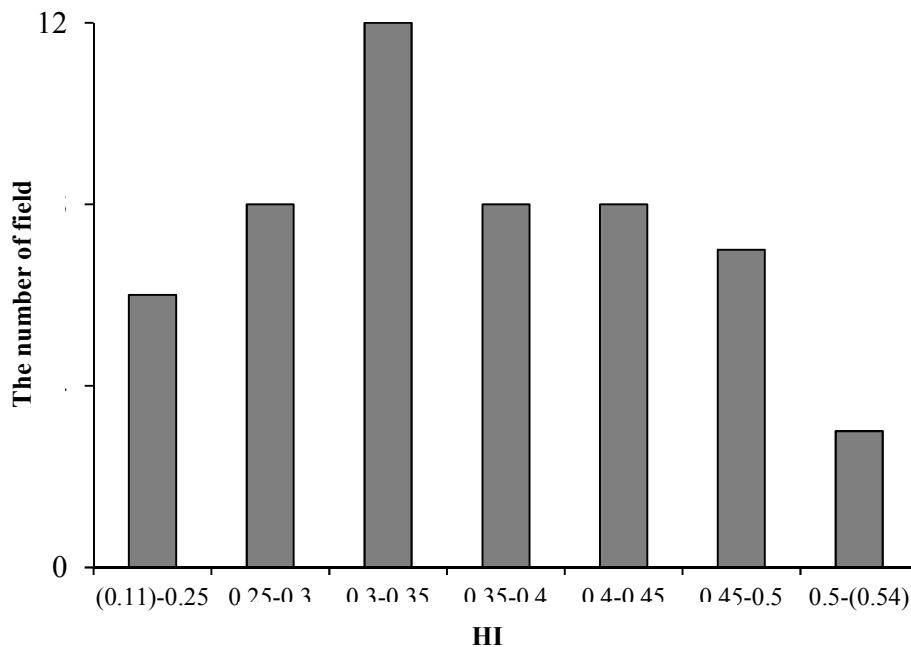


Fig. 3. The difference of harvest index (HI) in farmers’ fields in Lao PDR

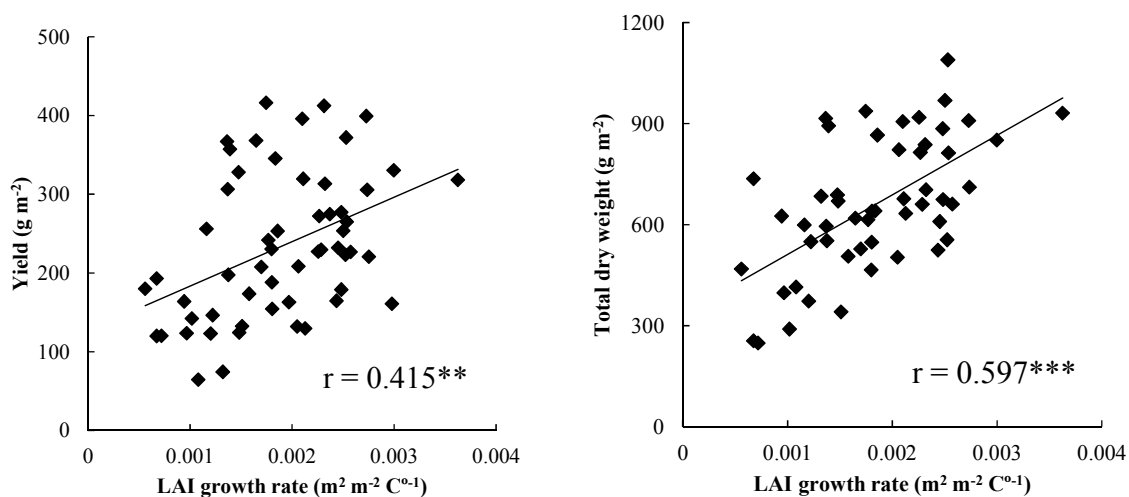


Fig. 4. Relationships of LAI growth rate ($m^2 m^{-2} C^{-1}$) with (a) total dry weight at the maturing stage ($g m^{-2}$), and with (b) grain yield ($g m^{-2}$)

Hirooka et al. (2015b) showed that LAI growth rate could be estimated by satellite based remote sensing of synthetic aperture radar (SAR) (Fig. 5). The remote sensing of SAR also provides estimation of transplanting date (Miyaoaka et al., 2015). Hashimoto et al. (2009) developed new index (TIPS: Time-series change Index of Plant Structure) to express seasonal change of plant canopy

structure by remote sensing for spectral reflectance. Rice cultivars would be distinguished by using TIPS and NDVI (Kambayashi et al., 2014). Maki et al. (2015) and Tsujimoto et al. (2013) developed the new simulation models to evaluate rice production combined with remote-sensing and hydrology, respectively. These newly developed techniques may help to estimate rice growth and then rice grain yield in a regional scale.

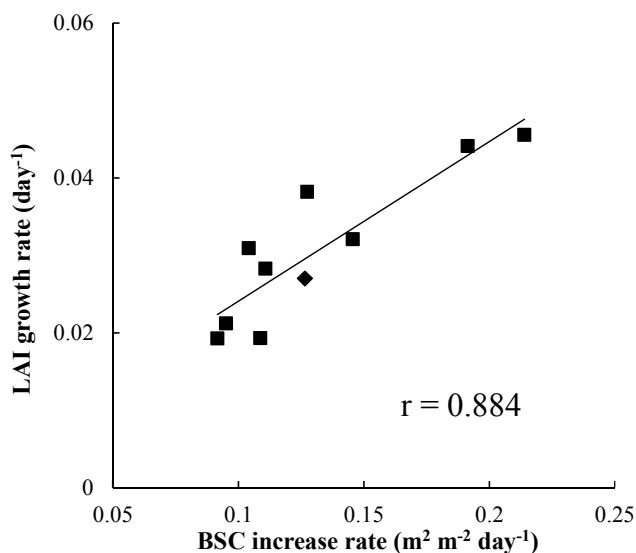


Fig. 5. Relationships between LAI growth rate (day⁻¹) and BSC increase rate (m² m⁻² day⁻¹) (Hirooka et al., 2015b)

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Greenhouse Gas Emission From on-Field Straw Burning in The Mekong Delta of Viet Nam

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ABSTRACT

Greenhouse gas emission from burning rice straw on field in the Mekong delta of Viet Nam was investigated by prepared questionnaires and field surveys in An Giang, Dong Thap, Kien Giang and Can Tho provinces. The results showed that the most common method to remove rice straw is field burning while other methods such as burying, cultivating mushroom, selling, raising cattle, giving away and leaving on fields accounted for the small proportion. Removing rice straw methods vary depending on the rice crop seasons. The estimated quantity of rice straw in the Mekong Delta region is approximate 28.98 million tons in 2014, in which 27.68 million tons is burned directly on the fields. The survey indicates most rice farmers tend to continue burning straw as the most popular method in the next few years. This practice releases into the atmosphere with about 32.42 million tons of CO₂, 768.40 thousand tons of CO and 68.65 thousand tons of NO_x. The amount of CO₂ accounting for more than 97% of the total greenhouse gas released from field burning. Studying on the methods to utilize the straw after harvests is highly recommended in order to limit the straw burning activities which waste a biomass resource and causes environmental pollution.

Keywords: Greenhouse gas, on-field burning, rice straw, the Mekong Delta.

1. INTRODUCTION

The Mekong Delta (MD) of Viet Nam is the most important rice producing area where rice production makes up approximately 51.5% of the national one and contributes to more than 90% of the national rice export. The rice cultivating area in the MD keeps increasing over the years, reaching 4.3 million ha in 2013 with the production nearly 25 million tons (GSO, 2014). Given that big amount of cultivating area and rice production, there is a huge amount of rice straw which is either discarded or burned every year in the MD (MONRE, 2010). At present, alternative of rice straw uses is very limited and the most common rice straw practice is on-field burning which causes air pollution, health impacts and nutrient loss. From Truc and Ni (2009), burning one tone rice straw lost 91.3% carbon which equivalent to 291.2 kg C and may release 1,067.6 kg CO₂ and 12.6 kg NO.

Concerning to health impacts, a former study of Kumar and Kumar (2010) showed that rice straw burning leads to air pollution and several other problems. Irritation in eyes and congestion in the chest were the two major problems faced by the majority of their survey household members. Respiratory allergy, asthma and bronchial problems were the other smoke related diseases which affected household members in the survey villages. Almost 50% of the survey households indicated that their health related problems get aggravated during or shortly after harvest when rice straw burning is in full swing period. In the peak season, affected families had to consult doctor or use some home medicine to get relief from irritation/itching in eyes, breathing problem and similar other smoke related problems.

Therefore, studying on rice straw utilization is needed to understand gas emission from on-field rice straw burning in the MD which could help establish rice straw management strategy to reduce the health effects at local communities and to mitigate greenhouse gas emission.

2. METHODOLOGIES

2.1. Household interview selection

To define the rice straw useage, data from various provinces in MD where rice is grown on large hectarage, is needed. Based on the statistical rice production data, four districts were chosen for surveying, including Chau Thanh district in An Giang province, Thap Muoi district in Dong Thap province, Giong Rieng district in Kien Giang province and Thoi Lai district in Can Tho city. After selecting the surveying districts, a list of all households including those who were agricultural cultivators was worked out. At each district, 100 farmers were selected using random sampling method. Thus, total 400 farmers were selected from four districts.

2.2. Questionnaire design

The questionnaire used for the household survey had four sections seeking detailed information on various aspects of inputs - outputs used by the farmers for agricultural practices, and disposal of crop residues. Section 1 of the questionnaire provides information on individual household members' profile in terms of their age, education, sex, occupation, land area, farm activities such as number of rice crops per year, harvest forms, rice varieties used, and rice yield, etc. Section 2 deals with the information on end use of straw in each crop of the year. Sub-sections also ask information on rice straw utilization such as on-field burning, burying, cultivating mushroom, selling, feeing cattle, giving away and leaving on fields. Section 3 recorded farmers' opinion on rice straw utilization, rice straw treatment, etc. In the last section, interviewers recorded any comments on farmers' situations.

To complete the questionnaire, 5 farmers were interviewed as testing phase. The questionnaire was then revised, for officially interviews.

2.3. Survey for residue rice straw

At each selected district, five households who cultivate the popular rice variety at that area were chosen to collect rice straw. At these five selected rice field, we used 1 square meter frame to fix five sampling areas. The rice harvested following farmer's procedure either by hand or by machine, was separated to grain and residue, then all the left rice straw was cut. The fresh weight of each part was determined onsite. Then grain, rice residue and rice straw were put into plastic bags and brought back to the laboratory for testing with dry weight.

The dry weight of grain was recorded when grain moisture reached 14% (good for storage and sell). The dry weight of rice residue and rice straw was determined using oven drying at 70°C until constant weight.

According to Gadde et al. (2009), quantity of straw burnt on the fields is estimated based on the following formula.

$$Q_{st} = Q_p \times R \times k$$

where

Q_{st} : amount of straw burned on the fields (ton)

Q_p : rice production (ton)

R: proportion of rice straw to rice production

k: proportion of straw burned on the fields to amount of straw

The amount of greenhouse gas emitted from burning was estimated based on the formula (Gadde et al., 2009):

$$E_i = Q_{st} \times E_{Fi} \times F_{co}$$

where

E_i : amount of gas_i emitted into the environment due to burning straw on the fields (ton)

E_{Fi} : coefficient of gas_i emission from burning of straw on the fields (g/kg)

$E_{CO_2} = 1464E_{CO} = 34.7E_{NO_x} = 3.1$

$F_{co} = 0.8$: the conversion proportion for gas emission when burning straw

3. RESULTS AND DISCUSSION

3.1. Rice straw utilization in the MD

The results from the interviews involving 400 households revealed seven methods to deal with rice straw by local people, namely burning, burying, cultivating mushroom, selling, feeding cattle, giving away and leaving on fields. These straw treatment methods were different between crops and between the farmer's habits at each region (Table 1).

- In Winter-Spring crop, there were 4 ways to deal with straw, i.e. burning, using for mushroom cultivation, selling and giving away. The surveys show that 98.23% of households burned their straw after harvest, 0.99% used for mushroom cultivation, 0.73% sold the straw and 0.06% gave away.

- In Summer-Autumn crop the interview results show that there were two more methods than those used in Winter-Spring crop including burying the straw and using the straw to feed cattle. Eventhough burning straw was the most popular treatment among farmer households in the Summer-Autumn crop, but the number of farmers burning rice straw only 87.9%, less than the rice straw burning in Winter-Spring crop.

- In Autumn-Winter crop, burning straw still accounted for a large percentage yet it has much decreased (54.1%) compared with the Winter-Spring and Summer-Autumn crops. The percentage of burying is higher than that in the Summer-Autumn crop (26.1%).

Table 1. The popular rice straw utilization practices in various crops

Utilization practice	Autumn-Winter	Winter-Spring	Summer-Autumn
Selling (%)	2.92	0.73	1.27
Burning (%)	54.10	98.23	89.67
Cultivating mushroom (%)	8.14	0.99	1.26
Burying (%)	26.10	0.00	6.65
Giving away (%)	1.65	0.06	1.13
Raising cattle (%)	0.36	0.00	0.02
Leaving on fields (%)	6.74	0.00	0.00

Among three crops, the amount of straw burnt was highest in the Winter-Spring crop. The reason is that there are favorable weather conditions in the harvest time of the Winter-Spring crop. The hot weather helps burn rice straws more quickly. In addition, collecting large amount of straw may be costly. In addition, burning can improve the field sanitation condition to prepare for the next crop, and the ash from the burning can be utilized as fertilizer.

In the Summer-Autumn and Autumn-Winter crops, due to the less favorable weather conditions than the Winter-Spring crop such as heavy rainfall, the straw burning decreases. In the recent years, the harvest combines have been widely used in the MD; but by mechanized harvesting the straw spreads all over the fields which makes costly for collection work. Therefore, local people often let the straw on the fields, and then burn it if it is sunny or bury it in unfavorable conditions such as rainy days or the fields are inundated.

In contrast, the Summer-Autumn and Autumn-Winter crops produce good quality straw for mushroom cultivation, thus, so there were more straw buyers. This helps explain the high percentage of dry straw in some regions from the surveys. However, for the fields harvested manually, due to high rainfall and difficult in transportation (especially for farm households locating far from their fields), many households can neither sell the straw nor transport the straw home, and they therefore leave the straw on the fields for natural decomposition. The survey results also shows that the majority of farmers (> 95%) tended to keep burning straw practices in the following years.

3.2. Trend of straw utilization by local people in the MD

The trend of rice straw utilization depends largely on the number of paddy rice crops per year, the weather and farming conditions of individual households. In the selected areas, burning is the most popular method used by local people due to their long time habits (Fig. 1).

Besides burning straw directly on the fields, burying straw in the soil profile is a popular method in the MD due to specific condition. The Winter-Spring crop has the most favorable conditions for rice production in the year, and its harvest time is in hot sunny days which are ideal straw burning practices on the fields. In the Summer-Autumn and Autumn-Winter crops, straw burning is not suitable due to high rainfall. Therefore, farming households usually bury the straw directly under the soil layer on the fields. Other methods to deal with straw are less popular and depend on the farming conditions.

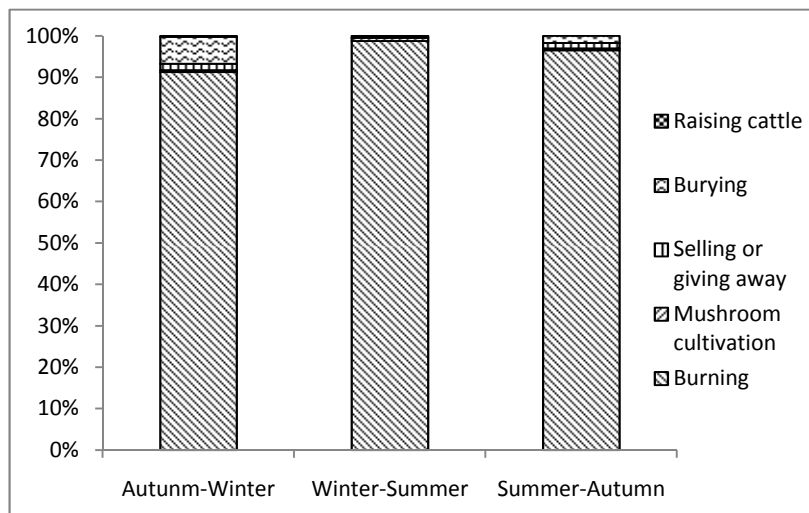


Fig. 1. Trends of straw utilization in the following years

The trends in straw utilizations also reveal low perception in straw burning among local people. Burning straw across large areas in the MD will have great impact on the environment of soil and air, human health conditions, and the increase of climate change (Gadde et al., 2009). Burning straw also wastes a great biomass resource. Therefore, identifying the trends in dealing with straw in the

following seasons among local farming households is essential in coming up with recommendations and solutions to restrict burning straw practices, and at the same time reuse this resource in a most logical and efficient way.

3.3. Estimating the quantity of rice straw after harvest

3.3.1. The ratio of straw and grain

The ratio of straw to grain fluctuated between the lowest rate at Thoi Lai in the Winter-Spring crop and the highest rate at Thap Muoi in the Autumn-Winter crop. This ratio is closely related to the rice varieties and rice productivity in each crop (Table 2).

Table 2. The straw to grain ratio in different crops and locations

Locations	Autumn-Winter	Winter-Spring	Summer-Autumn ¹
Giong Rieng	0.92 - 1.10	0.85 - 1.10	0.88 - 1.10
Thap Muoi	1.27 - 1.39	1.19 - 1.42	1.23 - 1.41
Thoi Lai	1.01 - 1.14	0.80 - 1.07	0.91 - 1.11
Chau Thanh	1.12 - 1.34	1.21 - 1.39	1.17 - 1.37
The MD ²	1.08 - 1.24	1.01 - 1.25	1.05 - 1.25

Note: ¹: excluding surveyed result, presented as average value of Autumn-Winter and Water-Spring crops; ²: expressed as average value of surveyed data

The research results show that the ratio is usually lower in the Winter-Spring crop than in the Autumn-Winter crop. This can be explained by the fact that the rice productivity in the Winter-Spring crop is the highest of the year whereas the rice productivity in the Autumn-Winter crop is usually low due to rainy weather and low sunshine.

3.3.2. Estimating the amount of rice straw in the MD

The amount of rice straw in the MD is estimated based on the provincial rice outputs (which referenced from GSO, 2014) and the ratio of straw to grain (based on this survey results). The average values of the ratio of straw to grain in selected areas are used to estimate the amount of rice straw for whole MD. The estimated results show that the straw produced in Winter-Spring crop is higher than it in the Autumn-Winter crop because the total cultivating areas and rice yields in the Winter-Spring crop are higher than those in the Autumn-Winter crop (Table 3).

Table 3. Estimation of annual quantity of rice straw (million tons)

Locations	Autumn-Winter	Winter-Spring	Summer-Autumn ¹
Kien Giang	0.26 - 0.31	1.77 - 2.29	1.87 - 2.33
Dong Thap	-	1.75 - 2.09	2.28 - 2.61
Can Tho	-	0.51 - 0.69	0.66 - 0.81
An Giang	0.03 - 0.04	2.12 - 2.43	2.61 - 3.01
The MD	2.03 - 2.33	10.97 - 13.58	12.86 - 15.31

According to the estimation, the total amount of straw in the whole MD in 2013 was 28.55 million tons in which An Giang accounted for the most and the lowest amount came from Can Tho.

The differences in the amount of straw among the selected areas came mainly from differences in the land areas for cultivation in 2013 among these regions. The total land area for rice cultivation in An Giang was 641.3 thousand ha, while that in Can Tho was 236.6 thousand ha (GSO, 2014).

3.4. Estimating the greenhouse gas emission from straw burning

The research results for the proportion of local farming households which adopt straw burning on the fields after harvest are presented in Table 1. The results show that the majority of households choose straw burning method in the Winter-Spring and Summer-Autumn crops. The Autumn-Winter crop showed a lower proportion of straw burning. In Giong Rieng district, the proportion for straw burning is lower than those in other districts due to specific low terrain features. Besides burning straw, local people also bury straw under the soil.

The amount of straw burned on the field is estimated based on the straw burning proportion and the amount of straw. The average proportion is considered for the estimation of straw burning in the whole MD. Most regions had low straw burning proportion in the Autumn-Winter crop and high one in the Winter-Spring crop. The estimated results reveal that the total amount of straw burned in the Winter-Spring crop accounted for more than 50% while the Autumn-Winter crop had the lowest amount. Among the regions in the research, the highest amount of burning was in An Giang and the lowest one was in Can Tho (Fig. 2).

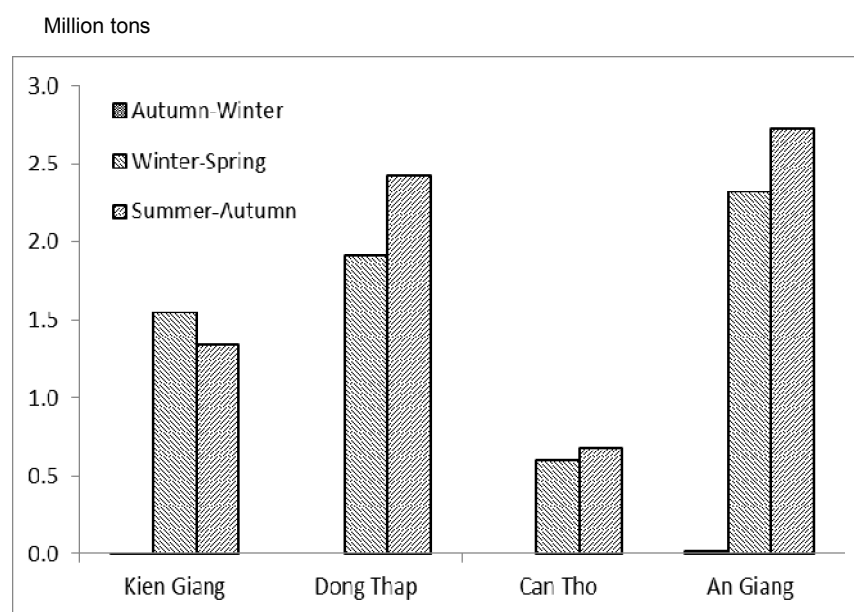


Fig. 2. Average quantity of straw burned on the fields at different locations (million tons)

The amount of greenhouse gas emitted from burning straw practices is in linear proportional to the amount of straw burned. Among the selected areas, An Giang had the highest amount of greenhouse gas emission, especially in the Winter-Spring crop when there was big amount of straw and high proportion of households adopting straw burning method (which releases 2,717 thousand tons of CO₂). At the same crop, Dong Thap and Kien Giang had nearly similar amounts of greenhouse gas emission while Can Tho had the lowest emission (703 thousand tons of CO₂). These greenhouse gases volumes could increase according to the development of rice cultivation area (Table 4).

In the composition of greenhouse gas emitted from straw burning, CO₂ is of the biggest amount. It is estimated that the amount of CO₂ generated in the MD in 2014 was 32.419 thousand tons, accounting for more than 97% of the total gas released. CO and NO_x had also contributed the total amount of gas released (Table 5).

Table 4. The amount of greenhouse gas (thousand tons) emitted from burning rice straw in 2013

Locations	Autumn-Winter			Winter-Spring			Summer-Autumn		
	CO ₂	CO	NO _x	CO ₂	CO	NO _x	CO ₂	CO	NO _x
Kien Giang	23.42	0.56	0.05	1815.4	43.03	3.84	1569.4	37.20	3.32
Dong Thap	-	-	-	2248.7	53.30	4.76	2834.3	67.18	6.00
Can Tho	-	-	-	702.7	16.66	1.49	808.1	19.15	1.71
An Giang	30.45	0.72	0.06	2717.2	64.40	5.75	3197.4	75.78	6.77

Table 5. The amount of greenhouse gas emitted from burning rice straw in the MD

Year	Grain (mil. tons)	Residue straw (mil. tons)	Burning straw (mil. tons)	Greenhouse gases (thousand tons)		
				CO ₂	CO	NO _x
2010	21.6	24.84	23.72	27783.44	658.53	58.83
2011	23.3	26.80	25.59	29970.10	710.36	63.46
2012	24.3	27.95	26.69	31256.37	740.84	66.19
2013	25.0	28.75	27.46	32156.76	762.19	68.09
2014	25.2	28.98	27.68	32418.82	768.40	68.65

4. CONCLUSIONS AND RECOMMENDATIONS

In the MD, onsite burning is currently the most popular method to treat straw from rice production, from which the highest straw burning rate takes place in the Winter-Spring crop, followed by the Summer-Autumn and Autumn-Winter crops. Other straw treatment methods including mushroom cultivation, cattle raising, selling and giving shared a small proportion. Most of the farmers tend to repeat the straw burning practices in the following years.

The annual amount of straw from rice production in the MD is very large, but most of this material is burned off. This practice wastes an abundant biomass resource from agricultural activities and releases a large amount of CO₂, CO, NO_x into the atmosphere. There should be research studies on the methods to utilize the straw after harvests, like using straw to produce gases as a renewable energy to limit the straw burning activities which waste a biomass resource and causes environmental pollution.

ACKNOWLEDGEMENT

The authors acknowledge financial support of the project “Sustainable Biogas Production from Waste Rice Straw” (DFC 11-016AU) by the Danish International Development Agency.

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Alleles for Good Grain Filling in Rice Extra-Heavy Panicle Types and Their Distribution among Rice Cultivars

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ABSTRACT

Rice cultivars with numerous spikelets in a panicle (extra-heavy panicle type, EHPT) have been developed to attain higher yield, but could not realize this goal, mainly due to their low grain filling. EHPT with relatively better grain filling, however, were also found and they showed a higher activity of ADPglucose pyrophosphorylase (AGPase) in developing endosperm. We then explored the single nucleotide polymorphisms (SNPs) among six cultivars including EHPT for the loci of AGPase small subunit (*APS2*) and large subunit (*APL2*), as well as a locus of sucrose transporter (*SUT1*). More than 30 SNPs in each locus were detected. Notably, in these six cultivars, one of the alleles in every locus, designated as *APS2-2*, *APL2-2* and *SUT1-2* at *APS2*, *APL2* and *SUT1*, respectively, was shared in common by the EHPT with good grain filling. Cultivars with the genotypes of *APS2-2 APL2-2 SUT1-2* showed higher AGPase activity and better grain filling in field experiments. These findings strongly suggests that these alleles could contribute to good grain filling in EHPT. Cleaved amplified polymorphic sequences markers and others were developed at several available SNPs to search for alleles among 179 rice cultivars. Results showed that there were at least four, five and four major alleles at *APS2*, *APL2* and *SUT1*, respectively. Of these, the plausible alleles for good grain filling, *APS2-2*, *APL2-2* and *SUT1-2*, existed most frequently in *indica*-type cultivars but not in *japonica*-types. These alleles could contribute to the improvement of yield sink strength, grain filling, and finally yield in EHPT cultivars.

Keywords: ADPglucose pyrophosphorylase, grain filling, molecular marker, nucleotide polymorphism, rice, sucrose transporter.

1. INTRODUCTION

Increasing of grain yield is an ultimate goal for all research in crop science in order to supply sufficient food for the growing population worldwide. In rice (*Oryza sativa* L.), many cultivars with numerous spikelets in a panicle (heavy panicle type or extra-heavy panicle type [EHPT]) have been developed in the course of breeding programs to attain higher yields, such as Hybrid Rice, New Plant Type, Super Rice, etc. (Khush, 1996; Peng et al., 1999; Yang and Zhang, 2010). These EHPT cultivars increased extensively the number of spikelets in a panicle, particularly on secondary branches, and obtained higher yield sink potential to accumulate photoassimilates. The spikelets on secondary branches, however, generally showed poor grain filling compared with those on primary branches. Consequently, EHPT cultivars often failed to realize their potential and to produce higher actual yield (Kato and Takeda, 1996; Yang et al., 2007; Yang and Zhang, 2010). This grain-filling problem has been pointed out in the first generation of New Plant Type, for example (Peng et al., 1999). Therefore, genetic improvement of poor grain filling is an important step to attain higher yields consistently in EHPT cultivars.

On the other hand, there exists clear genetic variation in the degree of grain filling among EHPT cultivars, which should contribute to the genetic improvement of their grain filling. Venkateswarlu et al. (1986) showed that the proportion of high-density rice grains, which were grains with a specific gravity of more than 1.20, varied including among EHPT cultivars. Peng et al. (1999) also demonstrated the variation in grain filling among New Plant Type cultivars. Kato (2010)

showed an apparent difference in the proportion of high-density grains, as well as the rate of grain filling, among EHPT cultivars. Therefore, some internal physiological factors underlying the genetic variation in grain filling among EHPT cultivars should be explored and utilized.

We have to consider the transportation of sucrose, the form of photoassimilates transported from source to sink, in this context. In rice, and many other cereal and pulse crops, most of the photoassimilates to be accumulated in the sink and harvested were generated in source organs, not in the sink. Patrick and Offler (2001) demonstrated that a steeper gradient of sucrose concentration between source and sink termini along the network of sieve tubes (source > sink) results in distinct differences in osmotic pressure, leading to the flow of water from higher pressure terminus (source) to the lower pressure terminus (sink). This water flow consequently drives sucrose transport from source to sink along sieve tubes, resulting in better grain filling. In other words, sucrose is not transported if the sucrose concentrations are the same in both the source and sink termini, resulting in poorer grain filling. This situation may be the case in the spikelets on secondary branches in EHPT cultivars.

One of the factors to generate this gradient is to keep higher sucrose concentration in source terminus, by supplying enough photoassimilates from higher photosynthetic activity in the source organs. Another factor to be considered is to keep a low sucrose concentration in sink terminus. This should be achieved by active unloading of sucrose from the sink terminus to the adjacent apoplast, and by metabolizing sucrose into starch, which is compartmented in amyloplasts. Kato et al. (2007) examined the activities of two key enzymes of the metabolism of sucrose to starch in developing rice endosperm after anthesis, namely sucrose synthase (EC 2.4.1.13) and ADPglucose pyrophosphorylase (EC 2.7.7.27, AGPase), using several EHPT cultivars with different degrees of grain filling. Results clearly showed that EHPT cultivars with higher AGPase activity exhibited higher degrees of grain filling, as well as higher rates of grain growth. It was strongly suggested that AGPase plays an important role in determining the activity of sink organs to decrease sucrose concentration in the sink terminus. Liang et al. (2001) also demonstrated the importance of AGPase activity, as well as sucrose synthase activity, to determine grain filling in hybrid rice.

In higher plants, AGPase is a hetero-tetramer enzyme consisting of two large and two small subunits (Tetlow et al., 2004; Lee et al., 2007). For each subunit, several isoforms, i.e., several corresponding loci, have been identified. They function in an organ-specific manner: the loci of *OsAGPS2* (*APS2*, Os08g0345800) and *OsAGPL2* (*APL2*, Os01g0633100) express the small and large subunits, respectively, in the developing endosperm of rice (Ohdan et al., 2004). Thus, Kato et al. (2010) explored nucleotide sequence polymorphisms in these two loci using six cultivars including EHPT cultivars with different degrees of grain filling. The locus of sucrose transport (*OsSUT1*, *SUT1*, Os03g0170900) (Aoki et al., 2003), which functions in unloading sucrose from the sink terminus of sieve tubes into the apoplast, was also examined. Their results identified 28, 30 and 37 single nucleotide polymorphisms (SNPs) from translation start to translation stop codon compared with a reference cultivar, Nipponbare, for *APS2*, *APL2* and *SUT1*, respectively (Kato et al., 2010; Fig. 1). These SNPs did not alter any amino acid sequences within these three loci. Kato et al. (2010) demonstrated that EHPT cultivars with better grain filling commonly shared the same haplotypes or alleles of *APS2-2* and *SUT1-2*, and those with poorer grain filling shared *APS2-1* and *SUT1-1*, at *APS2* and *SUT1* loci, respectively. The former alleles, *APS2-2* and *SUT1-2*, were strongly suggested to be alleles for good grain filling in EHPT cultivars.

We recently re-examined the nucleotide sequences of the *APL2* locus, resulting that one more novel plausible allele for good grain filling, *APL2-2*, was identified at *APL2* (Kato, unpublished data). The present study examined the effects of *APL2-2*, as well as *APS2-2* and *SUT1-2*, on grain filling and AGPase activity in developing endosperm. Kato and Horibata (2012) revealed a non-random distribution of alleles at *APS2* and *SUT* among more than 300 cultivars, and detected a tendency that *indica*-type cultivars have alleles for good grain filling in combination. This study further explored the allelic variations in *APS2*, *APL2* and *SUT1* loci, for a wide range of rice

cultivars, and using several cleaved amplified polymorphic sequences (CAPS) and insertion/deletion (Indel) markers available to detect SNPs. The distributions of the alleles including newly detected ones at *APS2*, *APL2* and *SUT1* were evaluated again among 179 cultivars. The objectives of this study were to characterize the function of these three alleles of good grain filling and their distribution among rice cultivars, in order to facilitate the utilization of these alleles in the improvement of grain filling in EHPT cultivars.

2. MATERIALS AND METHODS

2.1. Materials

Seven rice EHPT cultivars; Akenohoshi (the genotype for *APS2*, *APL2* and *SUT1* is *APS2-1 APL2-1 SUT1-1*, hereafter *111*.), Momiroman (*111*), Hokuriku 193 (*121*), IR65598-112-2 (*121*), Takanari (*121*), Milyang 23 (*222*) and Nanjing 11 (*222*), were used in field experiment to evaluate the degree of grain filling and AGPase activity in developing endosperm. For the molecular marker study, 179 rice cultivars including 83 *japonica*-type and 96 *indica*-type were used to survey SNPs and the distribution of alleles at *APS2*, *APL2* and *SUT1*. These 179 cultivars were included in those used in Kato and Horibata (2012).

2.2. Methods

In the field experiment, the seven cultivars were sown in nursery boxes on 14 May, 2014, and grown in a greenhouse. On 11 June, these seedlings were transplanted into a paddy field at the Faculty of Biology-Oriented Science and Technology, Kinki University, Kinokawa, Wakayama, Japan, with a single plant per hill and at inter-hill and inter-row densities of 15 cm and 30 cm, respectively. Each cultivar was arranged in the field in three rows and 12 hills per row. Fertilizers were applied at the rate of 12:10:10 g m⁻² for N:P₂O₅:K₂O in total. Standard cultivation practices were conducted throughout the growing season.

After recording heading dates of about 10 panicles individually, panicles were sampled at 15 days after heading (DAH), frozen with liquid nitrogen, and their spikelets on secondary branches collected. These spikelets were stored at -80°C until use. Extraction of enzyme fractions from the endosperm and assays of AGPase activity were performed in accordance with Kato et al. (2007). The extractions and assays were replicated four times for each cultivar. The results were expressed as specific activity (mU mg protein⁻¹), in which protein content was determined as described in Bradford (1976).

From 45 to 50 DAH, 5 panicles were sampled from every 6 hills at the center of each cultivar plot, air dried and the spikelets on secondary branches collected. These spikelets were classified as those showing specific gravities >1.00 and those >1.15. The former and latter spikelets were defined as fine grains and well-filled grains, respectively. The proportions of fine grains and well-filled grains to total grains were calculated and used as an index of grain filling in the earlier filling stage and that in the later stage, respectively. Longest culm length, panicle length on the longest culm, spikelet number per panicle on the longest culm, panicle number and spikelet number per panicle were also recorded for every 6 hills.

For molecular marker studies, DNA was extracted from seedlings of all of the 179 cultivars by CTAB method (Doyle and Doyle 1987). From the SNPs detected in six cultivars (Koshihikari, Nakatashinsenbon, Akenohoshi, Takanari, Milyang 23 and Nanjing 11) (Kato et al., 2010; Kato, unpublished data), 6, 5 and 5 SNPs for *APS2*, *APL2* and *SUT1* loci, respectively, from transcription start to translation stop codon could be converted to CAPS or Indel markers (Fig. 1, Table 1). A total of 179 cultivars were genotyped using these 16 markers. Table 1 also shows the primers for PCR and restriction enzymes used for these markers. From the SNPs data, alleles for each of the three loci were identified in every cultivar.

Table 1. Cleaved amplified polymorphic sequences (CAPS) and insertion/deletion (Indel) markers available to detect single nucleotide polymorphisms (SNPs) from transcriptional start to stop codons in *APS2*, *APL2* and *SUT1* of rice cultivars

Position ¹⁾	Forward primer (5' - 3')	Reverse primer (5' - 3')	Annealing temp. (°C)	Restriction enzyme
<i>APS2</i>				
-1	TGTAATTCTTTAATTTGTTGCAACC	GATTGAAACATCATGCATGGAGAA	61	(Indel) ²⁾
	TGTAATTCTTTAATTTGTTGCAAA	GATTGAAACATCATGCATGGAGAA	57	
549	AGGGGTTTTTGTCTGCATTG	TTCTCCATGCATGATGTTTCA	59	CviRI
2750	CAGATAATCCTAACTGGTTTCAGGT	GTCAAGAAAATTCCCAATGAAATAA	58	CviRI
2837	CAGCATCTGCAGTACCCTGA	TGCAGAGTGTGCTTGGAAATC	59	EcoRV
4461	GTCTGCTCCAATTTATACACAACCT	TTGCAGCATGATAAATTACTTTGAA	58	TasI
5274	CGCAGAAAGCAGTTTTACGTT	TCCCCATTGTTCTTGAATG	58	Hin1II
<i>APL2</i>				
-3680	ACCTGACAGGCCTAATTCCTGA	GACGGGTCCATCACCATCAG	61	Eco47III
-2549	TTGGCAGAGAAACAAATCCTGA	TAGGAGTTTACAAGGCAATTCTG	60	Hin1II
-455	ATTGACTTGTTTTGGGTTTTGAC	ATAGAGCTGAACTACTGCTCCTGCT	59	(Indel)
	GAATGGTTTGTAATTTTGCTTGTT	TCTATGGCACTCAAGATAGCAC	58	
1137	TCATTTGGTTATTTTCGACTTGC	CATTTGTGTAGCAGCCAATACC	58	NdeI
1281	TCATTTGGTTATTTTCGACTTGC	CATTTGTGTAGCAGCCAATACC	58	MunI
<i>SUT1</i>				
1467	TTCCATTTTACCTCAGATGCTGT	AATCGCTCCACTTGCTCTGT	58	SspI
1908	AACTGCCAAACGATGTGTTTC	CAGGGGTGATGAACTGCATA	58	HhaI
4743	CCTACTGGGATGCCTTCTGT	AGGATGAATGGGAACCAAGA	58	TasI
4751	CCTACTGGGATGCCTTCTGT	AGGATGAATGGGAACCAAGA	58	TaqI
5064	CCTACTGGGATGCCTTCTGT	CTGCAGTGATGGCTTTCTGA	58	CviRI

Note: ¹⁾ Number of nucleotides from the start of translation (minus values mean upstream positions from the start nucleotide); ²⁾ In the case of Indel markers, two pairs of primers were individually used in separate PCR amplifications.

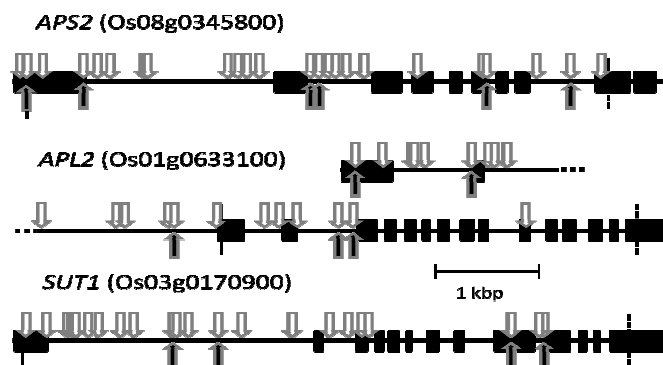


Fig. 1. Structure of rice *APS2*, *APL2* and *SUT1* loci, the sites of single nucleotide polymorphisms (SNPs) and the positions of molecular markers available to detect SNPs

Note: Filled rectangles indicate exons. Straight and dotted vertical lines indicate the positions of translation start and stop codons, respectively. Open downward arrows and filled upward arrows indicate the sites of SNPs and the positions of molecular markers, respectively.

2.3. Data analysis

Analysis of variance by one-way classification was applied to the data of the proportions of fine grains and of well-filled grains after arcsine transformation, as well as to the data of AGPase activity, to obtain the respective least significant differences at a probability level of 0.05. Cluster analysis was conducted to express the relationships among alleles at each of *APS2*, *APL2* and *SUT1* using an adin software of Excel (Mulcel) (Yanai 2005).

3. RESULTS

Table 2 shows the results of agronomic traits of the seven cultivars used. The number of spikelets ranged from about 180 to 220 among these seven cultivars, indicating that all of them could be regarded as EHPT cultivars. Three genotypes, 111, 121 and 222, were compared for the proportion of fine grains and well-filled grains in spikelets on secondary branches (Fig. 2). Although significant variations were found within each genotype, the genotype of 222 apparently showed significantly higher proportions of fine grains and also of well-filled grains than other genotypes ($P<0.05$). Genotype 121 also showed significantly higher proportions than genotype 111 for well-filled grains ($P<0.05$). Fig. 3 shows the results of AGPase activity in the developing endosperm of these three genotypes. Similar to the results for the degree of grain filling, genotype 222 exhibited significantly higher activity than genotypes 111 and 121 ($P<0.05$).

Table 2. Agronomic traits of seven rice cultivars examined in the present field experiment. Mean \pm S.D.

Cultivar	Genotype ¹⁾	Days to heading	Panicles hill ⁻¹	Culm length (cm)	Panicle length (cm)	Spikelets panicle ⁻¹
Akenohoshi	111	102.3 \pm 1.2	9.2 \pm 3.1	81.7 \pm 5.5	20.5 \pm 0.4	221.0 \pm 27.7
Momiroman	111	105.5 \pm 0.5	8.7 \pm 2.1	80.8 \pm 6.0	20.6 \pm 1.3	223.2 \pm 35.6
Hokuriku 193	121	103.8 \pm 2.3	9.4 \pm 3.7	81.0 \pm 4.5	20.7 \pm 1.3	186.2 \pm 29.6
IR65598-112-2	121	97.8 \pm 1.3	9.3 \pm 0.5	80.0 \pm 2.2	20.8 \pm 0.8	195.5 \pm 37.4
Takanari	121	98.8 \pm 0.8	8.7 \pm 2.1	77.3 \pm 3.1	20.7 \pm 2.1	194.7 \pm 22.6
Milyang 23	222	98.7 \pm 1.8	8.0 \pm 2.5	76.0 \pm 6.3	21.3 \pm 0.8	210.3 \pm 19.7
Nanjing 11	222	92.0 \pm 2.9	10.3 \pm 1.6	75.0 \pm 3.8	22.0 \pm 2.5	180.8 \pm 22.9

Note: ¹⁾ 111, 121 and 222 indicate *APS2-1 APL2-1 SUT1-1*, *APS2-1 APL2-2 SUT1-1* and *APS2-2 APL2-2 SUT1-2*, respectively.

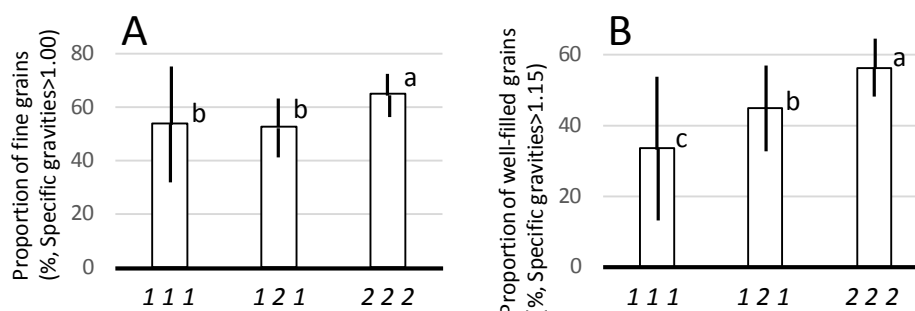


Fig. 2. Degrees of grain filling of the three genotypes of *APS2*, *APL2* and *SUT1* for the spikelets on secondary branches. A - proportion of fine grains (specific gravity >1.00); B - proportion of well-filled grains (specific gravity >1.15).

Note: Vertical bars on each mean indicate S.D. Means with different letters differed significantly at a probability level of 0.05. 111, 121 and 222 indicate *APS2-1 APL2-1 SUT1-1*, *APS2-1 APL2-2 SUT1-1* and *APS2-2 APL2-2 SUT1-2*, respectively.

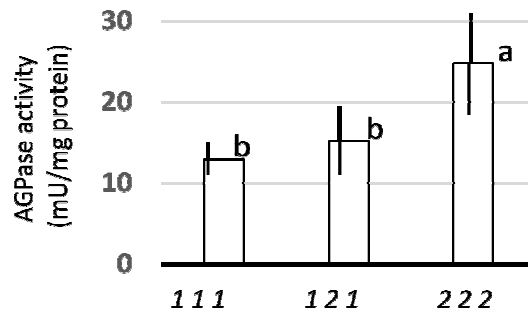


Fig. 3. Activity of ADPglucose pyrophosphorylase (AGPase) in developing endosperm at 15 days after heading of the three genotypes of *APS2*, *APL2* and *SUT1* for spikelets on secondary branches

Note: Vertical bars on each mean indicate S.D. Means with different letters differed significantly at a probability level of 0.05. 111, 121 and 222 indicate *APS2-1 APL2-1 SUT1-1*, *APS2-1 APL2-2 SUT1-1* and *APS2-2 APL2-2 SUT1-2*, respectively.

Table 3 shows SNPs detected for *APS2*, *APL2* and *SUT1* in 179 rice cultivars, resulting in 4, 5 and 4 alleles for *APS2*, *APL2* and *SUT1*, respectively, except for rare alleles with the frequencies of less than 5%. Fig. 4 shows dendrograms of these alleles for each locus, derived from a cluster analysis based on dissimilarity index using the furthest neighborhood procedure. These dendrograms demonstrate that *APS2-1* and *APS2-3*, *APL2-2* and *APL2-4*, *APL2-3* and *APL2-5*, *SUT1-1* and *SUT1-3*, and *SUT1-2* and *SUT1-4*, showed similar nucleotide sequences to each other.

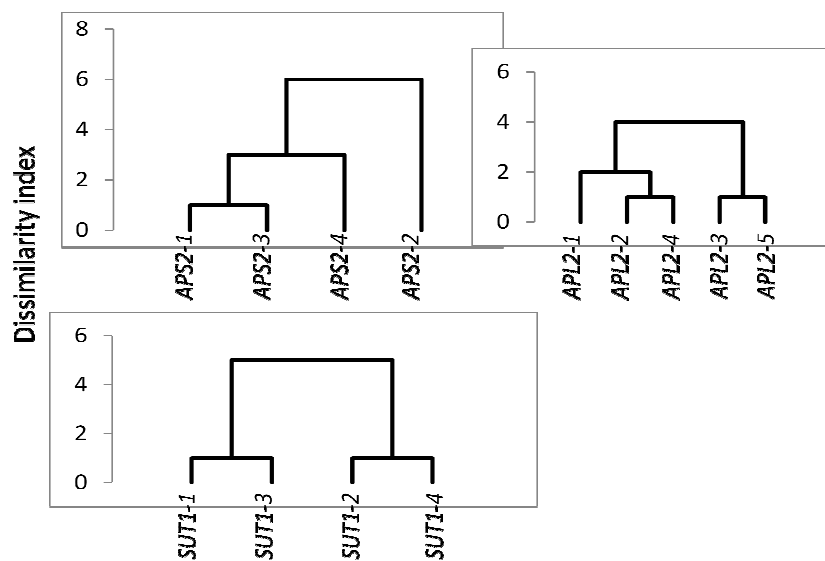


Fig. 4. Dendrograms representing dissimilarities of alleles at *APS2*, *APL2* and *SUT1*, constructed using the furthest neighborhood procedure

Response to reviewer's comments

P1, L17: loci => locus

P2, L11: higher rates of grain filling => higher rates of grain growth

P2, L30: I deleted "which".

P3, L16: I added "on the longest culm".

Distributions and associations of these alleles were evaluated separately in *japonica*-type and *indica*-type cultivar groups, as shown in Table 4. These tables clearly demonstrate that *APS2-2*, *APL2-2* and *SUT1-2* were found most frequently in *indica*-type cultivars (68.2%, 58.1% and 51.8%, respectively), whereas they appeared at very low frequencies in *japonica*-type cultivars (6.5%, 5.2% and 5.2%, respectively). Moreover, these three alleles tended to be shared in an *indica*-type cultivar, although they are located on different chromosomes. Tests of independency from 2×2 contingency tables between the major allele and other alleles resulted in significant χ^2 values in *indica*-type cultivars: $\chi^2=29.733$ ($P<0.001$) for *APS2* vs. *APL2*, $\chi^2=21.636$ ($P<0.001$) for *APS2* vs. *SUT1* and $\chi^2=8.497$ ($P=0.004$) for *APL2* vs. *SUT1*. In *japonica*-type cultivars, *APS2-3* (44.2%) and related *APS2-1* (32.5%), and *APL2-4* (40.3%) and *APL2-1* (31.2%) were major alleles at *APS2* and *APL2*, respectively. *SUT1-1* existed exclusively at the *SUT1* locus at a frequency of 90.9%. The same tests of independency in *japonica*-type cultivars demonstrated no significant association among the three loci: $\chi^2=0.104$ ($P=0.747$) for *APS2* vs. *APL2*, $\chi^2=0.005$ ($P=0.942$) for *APS2* vs. *SUT1* and $\chi^2=2.160$ ($P=0.142$).

Table 3. Single nucleotide polymorphisms at the sites where molecular markers were available and detected alleles at *APS2*, *APL2* and *SUT1* in 179 rice cultivars

Position ¹⁾	Allele ²⁾			
	<i>APS2-1</i>	<i>APS2-2</i>	<i>APS2-3</i>	<i>APS2-4</i>
-1	CC	del	CC	CC
549	T	G	G	G
2750	A	G	A	A
2837	G	A	G	A
4461	AT	del	AT	AT
5274	T	C	T	C

Position ¹⁾	Allele ²⁾				
	<i>APL2-1</i>	<i>APL2-2</i>	<i>APL2-3</i>	<i>APL2-4</i>	<i>APL2-5</i>
-3680	G	C	C	C	C
-2549	C	C	T	C	T
-455	AC	GT	AC	AC	AC
1137	A	A	G	A	G
1281	C	C	T	C	C

Position ¹⁾	Allele ²⁾			
	<i>SUT1-1</i>	<i>SUT1-2</i>	<i>SUT1-3</i>	<i>SUT1-4</i>
1467	A	T	A	T
1908	G	C	C	C
4743	T	A	T	A
4751	A	C	A	A
5064	C	T	C	T

Note: ¹⁾ Number of nucleotides from the start of translation (minus values mean upstream positions from the start nucleotide);
²⁾ Except for rare alleles.

Table 4. Frequencies of the alleles at *APS2*, *APL2* and *SUT1*, and their association among *japonica*-type and *indica*-type rice cultivars

Japonica-type cultivar

	<i>APL2-1</i>	<i>APL2-2</i>	<i>APL2-4</i>	<i>APL2-3</i>	<i>APL2-5</i>	Total	Freq. (%)
<i>APS2-1</i>	9	0	7	9	0	25	32.5
<i>APS2-3</i>	15	0	13	5	1	34	44.2
<i>APS2-4</i>	0	0	10	3	0	13	16.9
<i>APS2-2</i>	0	4	1	0	0	5	6.5
Total	24	4	31	17	1	77	100.0
Freq. (%)	31.2	5.2	40.3	22.1	1.3	100.0	

	<i>APL2-1</i>	<i>APL2-2</i>	<i>APL2-4</i>	<i>APL2-3</i>	<i>APL2-5</i>	Total	Freq. (%)
<i>SUT1-1</i>	24	0	30	16	0	70	90.9
<i>SUT1-3</i>	0	0	1	1	1	3	3.9
<i>SUT1-2</i>	0	4	0	0	0	4	5.2
<i>SUT1-4</i>	0	0	0	0	0	0	0.0
Total	24	4	31	17	1	77	100.0

	<i>APS2-1</i>	<i>APS2-3</i>	<i>APS2-4</i>	<i>APS2-2</i>	Total
<i>SUT1-1</i>	25	31	13	1	70
<i>SUT1-3</i>	0	3	0	0	3
<i>SUT1-2</i>	0	0	0	4	4
<i>SUT1-4</i>	0	0	0	0	0
Total	25	34	13	5	77

Indica-type cultivar

	<i>APL2-1</i>	<i>APL2-2</i>	<i>APL2-4</i>	<i>APL2-3</i>	<i>APL2-5</i>	Total	Freq. (%)
<i>APS2-1</i>	0	1	0	2	0	3	3.5
<i>APS2-3</i>	4	1	2	0	1	8	9.4
<i>APS2-4</i>	2	2	8	4	0	16	18.8
<i>APS2-2</i>	0	45	9	0	4	58	68.2
Total	6	49	19	6	5	85	100.0
Freq. (%)	7.0	58.1	22.1	7.0	5.8	100.0	

	<i>APL2-1</i>	<i>APL2-2</i>	<i>APL2-4</i>	<i>APL2-3</i>	<i>APL2-5</i>	Total	Freq. (%)
<i>SUT1-1</i>	6	3	2	6	0	17	20.0
<i>SUT1-3</i>	0	2	6	0	1	9	10.6
<i>SUT1-2</i>	0	32	10	0	2	44	51.8
<i>SUT1-4</i>	0	12	1	0	2	15	17.6
Total	6	49	19	6	5	85	100.0

	<i>APS2-1</i>	<i>APS2-3</i>	<i>APS2-4</i>	<i>APS2-2</i>	Total
<i>SUT1-1</i>	3	7	6	1	17
<i>SUT1-3</i>	0	1	5	3	9
<i>SUT1-2</i>	0	0	4	40	44
<i>SUT1-4</i>	0	0	1	14	15
Total	3	8	16	58	85

4. DISCUSSION

Kato et al. (2010) demonstrated that EHPT cultivars with the genotype *APS2-2 SUT1-2* exhibited a higher degree of grain filling and also a higher AGPase activity than *APS2-1 SUT1-1*, leading to the conclusion that *APS2-2* and *SUT1-2* could be alleles for good grain filling. The same tendencies could also be detected in the present results for alleles at the *APS2* and *SUT1* loci. Additionally, a novel allele of *APL2-2* also revealed an enhancing effect on grain filling, together with *APS2-2* and *SUT1-2*, compared with *APL2-1*. The genotype *APS2-2 APL2-2 SUT1-2* showed a significant increase in the proportion of well-filled grains compared with *APS2-1 APL2-2 SUT1-1*, indicating that the alleles *APS2-2*, *APL2-2* and *SUT1-2* could function in an additive manner in enhancing grain filling. The present conclusion should be further elucidated using near-isogenic lines with all combinations of these three alleles.

The present results may propose a plausible mechanism for the enhancing effect of these three alleles on grain filling, i.e., these alleles strengthen the sink activity of developing endosperm of EHPT cultivars through an increase in AGPase activity and sucrose transporter properties. This strengthened sink activity maintained the concentration gradient of sucrose from the source terminus to the sink along the phloem network, and allowed sustainable transportation of photoassimilates from the source organs. The fact that any SNPs detected in the present study do not have altered amino acid sequences suggests that the three alleles should act quantitatively, not qualitatively. These three alleles might, therefore, function sufficiently to enhance grain filling not in individual forms but in combined genotypes.

AGPase is well-known as a key enzyme in starch biosynthesis not only in sink organs but also in source organs. Molecular manipulation of this enzyme in rice endosperm resulted in very high AGPase activity and higher grain weight (Smidansky et al., 2003; Sakulsingharoj et al., 2004). Mo et al. (2014) also reported several SNPs in *APS2* and *APL2* in Korean *japonica* rice cultivars, which affected several grain quality traits, especially in different temperature conditions. These reports suggested that the allelic variation in *APS2* and *APL2*, and also in *SUT1*, could affect various traits related to rice grain growth including grain filling.

The present survey for SNPs among 179 rice cultivars in *APS2*, *APL2* and *SUT1*, detected several additional novel alleles to those reported in Kato et al. (2010) and Kato and Horibata (2012). The number of alleles in each locus will be the minimum estimates, because this study could not examine polymorphic nucleotide positions that cannot be designed as CAPS or other markers. Actually, reported SNPs in several databases corresponds to those in this study in many cases, but do not match in several cases (for example in OryzaSNP@MSU, <http://oryzasnp.plantbiology.msu.edu/index.html>).

The most pronounced results for the distribution of alleles at the three loci were that the alleles were not distributed randomly among rice cultivars, particularly between *japonica*-type and *indica*-type cultivars. More than half of *indica*-type cultivars had *APS2-2*, *APL2-2* and *SUT1-2*, all of which are suggested to be for good grain filling as above, and tended to be associated to each other, resulting that the genotype *APS2-2 APL2-2 SUT1-2* was much more frequent than expected from independent segregation in *indica*-type cultivars. On the other hand, in *japonica*-type cultivars, none of the *APS2-2*, *APL2-2* and *SUT1-2* were frequently found. In addition, no association among these three alleles was detected. For the *SUT1* locus, the *SUT1-1* allele was mostly found in *japonica*-type cultivars at a frequency of more than 90%. Kato and Horibata (2012) reported this tendency for the prevalence and association of *APS2-2* and *SUT1-2* in *indica*-type cultivars. The present study clearly demonstrated that the tendency obtained from these two alleles was also revealed when one more plausible allele for good grain filling, *APL2-2*, was newly involved. The association of these three

alleles might reflect natural and/or unconscious artificial selection toward obtaining good grain filling genotypes. These three alleles should be utilized consciously to resolve grain filling problems in EHPT cultivars in future breeding programs.

5. CONCLUSIONS

This study emphasized that the *APL2-2* at the *APL2* locus could be an allele for good grain filling in EHPT cultivars, together with two known alleles of *APS2-2* and *SUT1-2* at other loci, compared with *APS2-1*, *APL2-1* and *SUT1-1*. *APS2* and *APL2* encode AGPase small and large subunits, respectively, and the alleles of *APS2-2* and *APL2-2* could enhance the activity of AGPase in developing endosperm, resulting in accelerating sucrose to starch metabolism, and finally in a decrease in sucrose concentration in the sink terminus of phloem network. The allele of *SUT1-2* could also contribute to the decrease in sucrose concentration through enhanced unloading of sucrose from the sink terminus to the endosperm apoplast. This decrease in sucrose concentration in the sink terminus may provide a steeper downward gradient of sucrose concentration from the source terminus to the sink, leading to sustainable transportation of photoassimilates, and consequently contribute to better grain filling. These plausible alleles for good grain filling, *APS2-2*, *APL2-2* and *SUT1-2*, are frequently found in *indica*-type cultivars, and not so much in *japonica*-type cultivars. In addition, these three alleles tended to result in the genotype of *APS2-2 APL2-2 SUT1-2* in *indica*-type cultivars. These three alleles or this genotype should be introduced and utilized in breeding for improving grain filling in EHPT cultivars.

ACKNOWLEDGEMENT

The authors wish to express sincere thanks to M. Fukawa, M. Ishida, H. Kitano, Y. Endo and N. Sakamoto, Faculty of Biology-Oriented Science and Technology, Kinki University, for technical assistance. This study was in part supported by the grants from the JSPS KAKENHI Grant No. 22580020, and Wakayama Prefecture Collaboration of Regional Entities for the Advancement of Technological Excellence, JST.

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Residue Management Effects on Survival Rate, Growth and Yield of Rice Cultivar IR64-Sub1 Subjected to Submergence at Young Seedling Stage in Pots

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ABSTRACT

Flooding is one of the factors that causes serious damage to agricultural production, in paddy rice areas. In the Mekong river delta of Viet Nam, intensive rice production systems consist of 2 to 3 consecutive crops per year with short intervals between each crop. This causes straws and stubbles to remain un-decomposed under field water, potentially change physical, chemical and biological characteristics of soil that affect rice growth and yield. The study investigated the effects of straw and stubble incorporation equivalent to 5 tons per ha under artificial flooding on growth and yield of IR64-Sub1 rice variety grown in pots. IR64-Sub1 seedlings' survival decreased consequently with prolonged submergence, around 10% after being flooded continuously for 20 days. Organic residual incorporation under 60 cm depth of water continuously in 15 days at 3-day-seedlings altered pH, EC, and dissolved oxygen of the water and affected first internode elongation and delayed harvest time up to 20 days in comparison to that of the control. The incorporation of burnt straws and stubbles had a tendency to improve rice yield regardless to submergence but flooded condition reduced seed discoloration and enhanced spikelet filling significantly.

Keywords: Anaerobic germination, Mekong river delta, organic matter, rice seedlings, submergence, yield

1. INTRODUCTION

In many parts of the Mekong Delta (MD) of Viet Nam, farmers grow 3 crops of rice annually. Typically, Winter - Spring crop (WS) is from December to March, Summer - Autumn (SA) crop from April - August, and Autumn - Winter (AW) crop from September to November. Among the three, only WS is harvested in the dry season. With the average rice (*Oryza sativa* L.) yield of about 5 tons/ha and with harvest index of the typical high yield rice cultivars grown in the MD, the amount of rice residue (straw and stubble) after harvesting can be as high as 5 - 7 tons/ha. Managing a large amount of residue is challenging and the solution is a trade-off between labour, decomposition rate, environmental regulation, and the cropping calendar. After the dry season harvests, most farmers in MD burn the rice straw and stubble before establishing the next crop. Residue burning may contribute to quick nutrient recycling (Gadde et al., 2009) and eliminate pests (Dobermann and Fairhurst, 2002). But this practice contributes to the reduction in soil organic matter leading to production issues and regional air pollution. In the rainy season crops, particularly in AW crop, residue burning is not practical due to rains and increasing water level on the field from annual flooding of the Mekong River. Farmers often leave the residues in the inundated fields and incorporated them into the soil during land preparation for the next crop. Under these anaerobic conditions, organic matter from rice residual can undergo fermentation and may lead to the toxic concentrations of in soil solution (e.g. Fe²⁺, S²⁻, PO₄; Ponnaperuma, 1984) and immobilisation of soil nitrogen. This can cause mortality of rice seedlings and reduce yield of the subsequent crop. These processes have not been fully investigated for highly intensive cropping

systems in the MD. Rice straw consisted about 5.4, 1.4, and 13.0 kg of N, P, and K per ton, respectively. Analysis from IRRI also showed that it contained up to 0.03, 0.8, 6.5, 2.0, 0.3, 0.45, 0.003, and 0.01 kg of Zn, S, Si, Mg, Ca, Fe, Mn, Cu, and B, respectively per one ton of rice straw. Incorporating residues may increase nutrient status of the soil. Ruensuk et al. (2010) showed that incorporation of less than 5 tons/ha of rice straw in Thailand did not impact on plant density; but higher levels of incorporation decreased number plants per m² after 15 days after crop establishment and rice yield. Another long-term comparison reported that straw incorporation improved microbial biomass, nitrogen and carbon in the soil than those of burnt straw (Bird et al., 2001). Long term research on straw management on the field at Cuu Long Delta Rice Research Institute, Viet Nam reported that incorporation or burnt rice straw resulted in higher yield than residual removal, improved nitrogen and phosphorous level but caused no change in Ca, Mg, Na, Zn and Cu in the soil (Tuyen and Tan, 2001). Most of previously researched residue incorporation reported was not carried out under submerged conditions.

Rice in the MD is mostly established by direct sowing of pre-germinated seeds onto wet or saturated soil. Sowing time of AW crop (September) coincides with period of high rainfall and annual flood of the Mekong river. Fields may become inundated after crop establishment, drowning the young seedlings. In these cases, farmers have to re-sow the seeds. The availability of cultivars with high tolerance to submergence at young seedling stage is highly desirable. Submergence tolerance gene in rice, Sub1, was discovered by Xu et al. (2006) and afterwards transferred to other high yielding rice cultivars, e.g. IR64-Sub1 (Septiningsih et al., 2009) but studies on this variety in practice are still limited in Mekong Delta. The tolerance to submergence at vegetative stage of Sub1-cultivars has been well documented (Xu et al., 2006), but there is little information on performance of IR64-Sub1 subjected to submergence during young seedling, especially when the bio-chemistry of the flood water may be governed by anaerobic decomposition of the residue.

This research aimed to investigate the effects of rice residue anaerobic decomposition on survival rate, growth and yield of IR64-Sub1 rice cultivar subjected to submergence at young seedling stage in pots.

2. MATERIALS AND METHODS

The study was carried out at green house of College of Agriculture and Applied Biology, Can Tho University from May to September 2013. It comprised of two experiments, the first one formed the basis for designing the second. Both experiments used breeder's seed of IR64-Sub1, obtained from Cuu Long Rice Research Institute.

2.1. Experimental lay out and treatments

Experiment 1

Experiment 1 aimed at investigating the survival rate of IR64-Sub1 seedlings subjected to different durations of submergence, starting from 3 days after emergence (DAE). There were 5 submergence duration treatments: control (without submergence), 5, 10, 15, and 20 days. Rice was grown in pots of 23 cm high and 30-cm inner diameter. Experiment pots were arranged in randomized complete blocks, with 4 replications. In each pot, 100 germinated seeds were sown onto a layer of saturated soil. After 3 days, rice seedlings anchored their roots firmly into the soil layer. Pots were lowered into 1-m height by 0.75-m diameter plastic container and were flooded to

60 cm depth with domestic tap water, except for the control treatment, which was covered by a layer of water 1 cm above the soil surface. All the containers were covered by black plastic sheet. Rice seedlings thus grew in darkness, to mimic conditions of submergence under high turbidity flood, typical of the MD. At the end of the submergence treatment, water was gradually drained from the containers. Pots were exposed to natural light for 2 days and the survival rate was calculated from number of turned-green seedlings.

Experiment 2

Experiment 2 investigated effects of residue managements under submerged and non-submerged conditions on field water chemistry and growth and yield of IR64-Sub1. The experiment consisted of two factors:

(i) Submergence was main factor (S): Seedlings were subjected to submergence for duration of 15 days (S1); or without submergence (S0, control). The exposure to submergence treatment was similar to that of Experiment 1. The submergence duration in S1 was limited to 15 days - based on results of Experiment 1 - to ensure adequate survived seedlings to carry out the experiment.

(ii) Residue management was sub-factor (R): With three levels, namely R0: without residue incorporation; R1: Incorporation of burnt residue; and R2: Incorporation of fresh residue. The residue was rice straw and stubble collected from surrounding rice fields. The amount of residue in each pot of treatments R1 and R2 were equivalent to 5 tons/ha. The experimental pots were arranged in split plot design with 4 replications.

2.2. Monitored parameters

In Experiment 2, electrical conductivity (EC), pH (using Toledo meter), and dissolved oxygen (Oxi330 meter) of water during the submergence period were monitored daily around 9 am.

At the end of 15-day submergence period, 3 seedlings were left in each pot to grow with water depth of 2 cm, until harvest. Inorganic fertilizer supplement was applied equivalent to 100 - 60 - 30 kg of nitrogen, P₂O₅, and K₂O per ha, respectively.

The length of first, second, third and fourth 4 internodes from randomly selected tiller of each plant in each pot were measured at the harvest. Yield components were also examined at harvest, yield recorded. Seed discoloration was evaluated following IRRI method (IRRI, 1998).

2.3. Data analysis

Microsoft Excel and SPSS 16.0 were employed for data comparison between treatments for each experiment. For Experiment 2, analysis of variance, effects of submergence and residue management treatments, and their interaction were carried out by the mentioned statistic software.

3. RESULTS AND DISCUSSION

3.1. Experiment 1 - Survival rate of IR64-Sub1 subjected to submergence at young seedling stage

Fig. 1 shows that the survival rate decreased sharply as the duration of submergence increased. Indeed, the survival rate was significantly negatively correlated with the submergence duration. Despite carrying Sub1 gene, less than 10% of the 3-days old IR64-Sub1 seedlings survived after 20 days of continuous submergence.

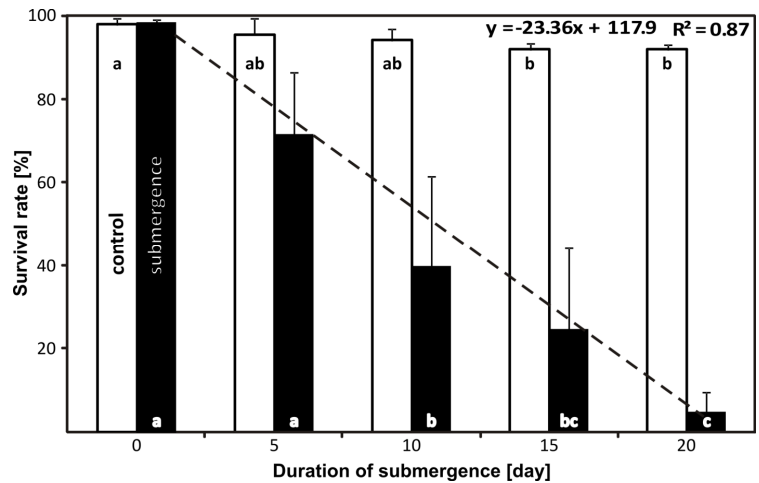


Fig. 1. Survival rate of IR64-Sub1 seedlings submerged in pots for different duration. In “control”, seedlings were not submerged

Note: Columns with the same letters (a, b,c) inside are not significantlt different by Duncan’s Multiple Range Test at the 5% level. Vertical and capped bars are standard deviations of the means of 4 replications. The dashed line shows correlation between survival rate and submergence duration by relation equation showed on the top right corner.

3.2. Experiment 2 - Residue management and IR64-Sub1 performance

3.2.1. EC and pH

A few days at the beginning of submerged duration, EC values of non-submerged treatments were clearly higher than the submerged ones. Low EC of the submerged treatments could be attributed to the dilution effects of added water to increase the flood depth in the containers. Among the S0, EC of treatments with burnt residue had a tendency to be higher than those with fresh residue, this in-turn higher than those without residues. This implies that materials from burnt residues could dissolve more quickly than those of fresh ones. Due to large variations of the EC values, however, the difference among the residue treatments were not significant. At the end of the submergence period, the water EC of all treatments were similar (Fig. 2).

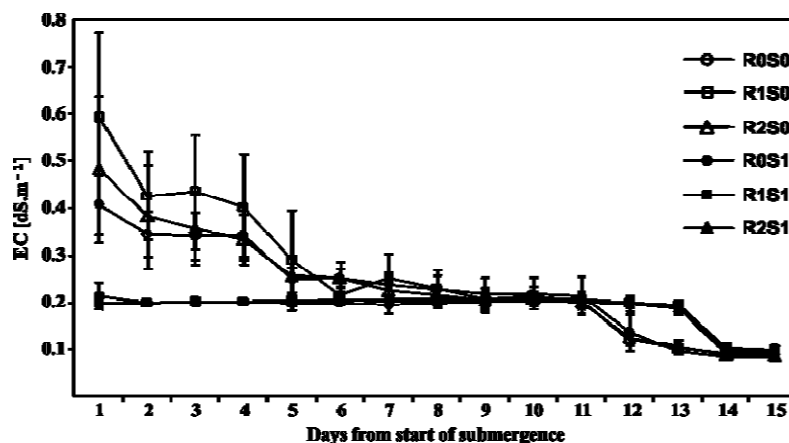


Fig. 2. Effects of submergence (S0 = control, no submergence, S1 = 15-day submergence) and residue treatments (R0 = without residue incorporation; R1 = incorporation of burnt residue; R2= incorporation of fresh residue) on water EC

Note: Vertical and capped bars are standard deviations of the means of 4 replications

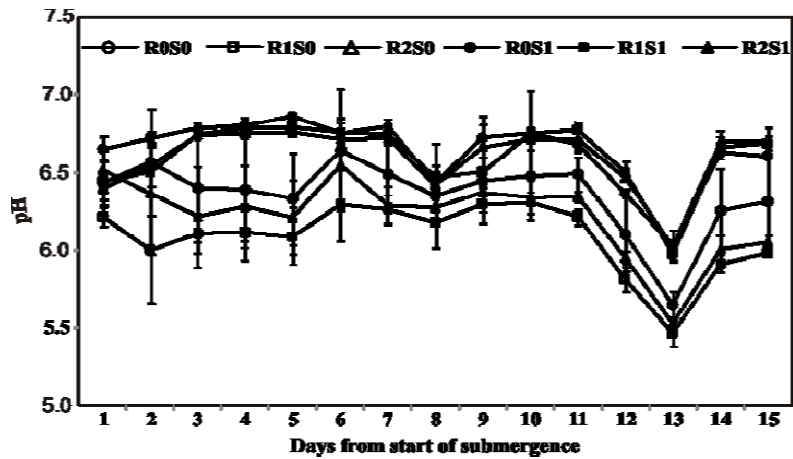


Fig. 3. Effects of submergence (S0 = control, no submergence, S1 = 15-day submergence) and residue treatments (R0 = without residue incorporation; R1 = incorporation of burnt residue; R2= incorporation of fresh residue) on water pH

Note: Vertical and capped bars are standard deviations of the means of 4 replications.

3.2.2. Dissolved Oxygen

For the first 11 days, the non-submerged treatments had significantly higher level of dissolved oxygen in solution than those in submerged ones (Fig. 4). The last 5 days of submerged duration, there were no difference in soluble oxygen in water recognized.

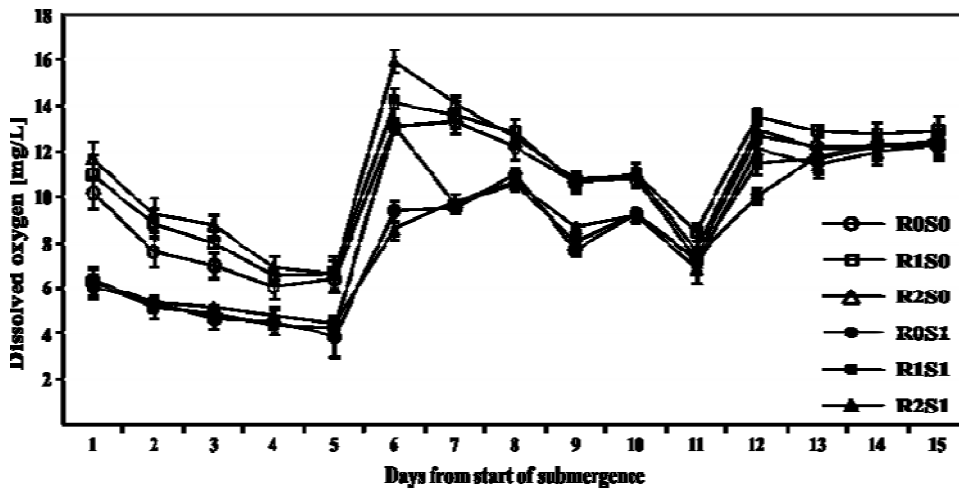


Fig. 4. Effects of submergence (S0 = control, no submergence, S1 = 15-day submergence) and residue treatments (R0 = without residue incorporation; R1 = incorporation of burnt residue; R2= incorporation of fresh residue) on dissolved oxygen in water

Note: Vertical and capped bars are standard deviations of the means of 4 replications.

3.2.3. Effects of submergence and residual treatment on survival, growth and yield of IR64-Sub1 rice

The survival rate of submerged treatments from Experiment 2 was less (<20%) than Experiment 1 (>20%, compare Fig. 1 and Fig. 5). Similar to Experiment 1 submergence of the seedlings

significantly reduced the seedling survival rate compared with the non-submerged treatment. This may be related to the reduced dissolved oxygen in submerged treatments during the first 11 days of treatments (Fig. 4). Straw incorporation regardless fresh or burnt enhanced the survival rate of IR64-Sub1 seedlings under submergence, though the difference was small.

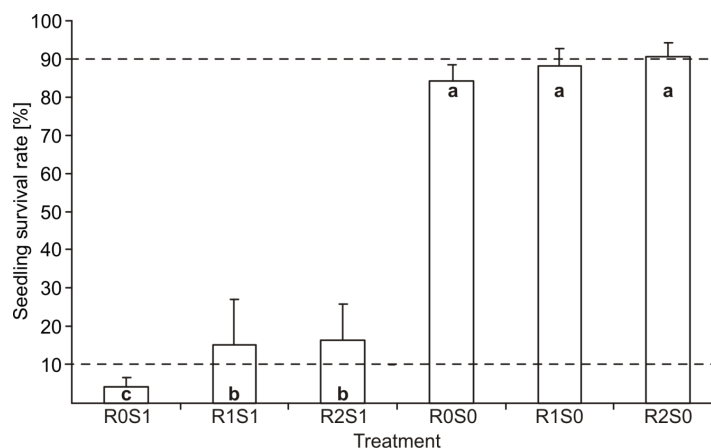


Fig. 5. Effects of submergence (S0 = control, no submergence, S1 = 15-day submergence) and residue treatments (R0 = without residue incorporation; R1 = incorporation of burnt residue; R2= incorporation of fresh residue) on survival of IR64-Sub1 rice seedlings

Note: Vertical and capped bars are standard deviations of the means of 4 replications. Columns with the same letter are not significant difference by Duncan Multiple Range Test at the 5% level.

Submerged treatments contributed to improve the number of filled spikelet per panicle but no difference in yield was found. Submerged plants produced more spikelets per panicle and resulted in lighter weight of 1000-grains. The incorporation of burnt residual in both water treatments increased rice yield significantly in comparison to other residue treatments. Among them, fresh residue incorporation caused negatively effect on yield in contrast with free-residual treatment. In overall, submergence showed effectively impact on growth and yield components of IR64-Sub1 rather than residual incorporation. The interaction between submergence and residual incorporation was rarely found except for the final rice yield (Table 1).

Table 1. Effects of submergence and residue treatments on yield and yield components of IR64-Sub1 rice

Factor	Unfilled spikelets/panicle	Filled spikelets/panicle	1000-grain weight	Yield (g/pot)
Residue management				
No residue	24.6 ± 4.6	63.4 ± 17.3 b	23.7 ± 1.3	52.1 ± 17.7 b
Burnt residue	23.4 ± 5.2	73.9 ± 22.4 a	24.3 ± 2.1	72.5 ± 12.5 a
Fresh residue	25.6 ± 6.2	67.4 ± 15.8 ab	24.4 ± 1.4	40.0 ± 6.4 c
Submergence				
Non-submergence	22.0 ± 4.2	51.8 ± 5.7	25.3 ± 1.1	59.1 ± 19.9
Submergence	27.1 ± 5.1	84.6 ± 9.6	23.0 ± 1.0	50.7 ± 16.8
F (R)	ns	*	ns	**
F (S)	*	**	**	ns
F (R x S)	ns	ns	ns	**

Note: In the same column, numbers followed by the same letter (a, b,c) are not significant different by Duncan Multiple Range Test; ns = non-significance; * = significant difference at 5% level; ** = significant difference at 1% level.

The submerged plants were higher than non-flooded plants and tended to lodge. Submergence enhanced elongation of the first internode from top of plant significantly but the length of following internodes among treatments were found no apparent difference (Fig. 6). In overall, the plant height from submerged treatments was significantly higher than those of non-submerged ones.

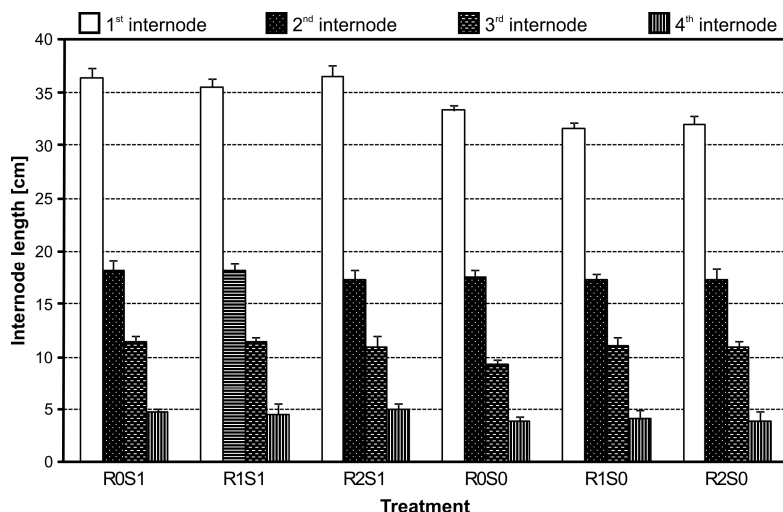


Fig. 6. Effects of submergence (S0 = control, no submergence, S1 = 15-day submergence) and residue treatments (R0 = without residue incorporation; R1 = incorporation of burnt residue; R2= incorporation of fresh residue) on the length of 4 internodes from top of IR64-Sub1 rice at harvest

Note: Vertical and capped bars are standard deviations of the means of 4 replications.

During the second experiment was that submergence treated plants appeared greener and less disease contaminated than those of non-submergence ones. There was also a significant in seed discoloration from the submerged plants than that in the non-submerged treatments (Fig. 7).

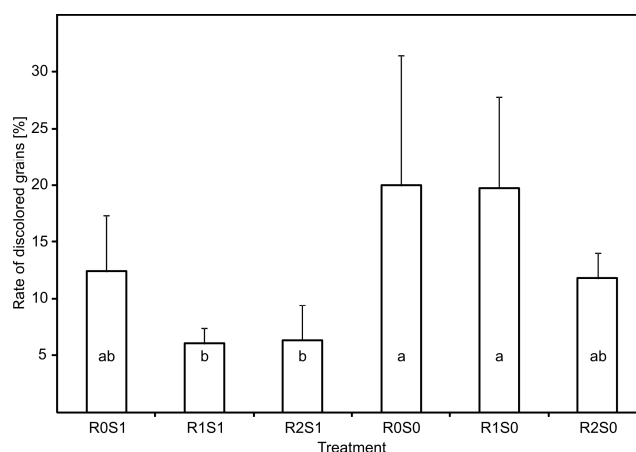


Fig. 7. Effects of submergence (S0 = control, no submergence, S1 = 15-day submergence) and residue treatments (R0 = without residue incorporation; R1 = incorporation of burnt residue; R2= incorporation of fresh residue) on occurrence of seed discoloration of IR64-Sub1 rice at harvest

Note: Vertical and capped bars are standard deviations of the means of 4 replications. Columns with the same letter(s) inside are not significant difference by Duncan Multiple Range Test at 5% level.

4. GENERAL DISCUSSION AND CONCLUSIONS

Survival under completely submerged conditions of 3-day seedlings IR64-Sub1 rice might be not longer than 3 weeks. In the dark conditions, imposed to mimic the natural condition of water turbidity, there was a low probability of survival after 15 days. This is similar to the findings of (Das et al., 2009, Singh et al., 2009, Bailey-Serres et al., 2010) for older plants >21 days after emergence. In the field there may be better survival because plants would receive scattered natural sunlight. Submergence delayed harvest by twenty days and affect yield relative to the control similar to other studies (Das et al., 2009, Singh et al., 2009, Bailey-Serres et al., 2010). The results indicate that a 15 day flood on starting 3 DAE on IR64-Sub 1 would still significantly reduce yield due to seedling mortalities of 70-80% (Fig. 1 and 5). Further testing of the survival of high yielding rice cultivars under field condition is required.

Submerged rice plants elongated more in contrast to those of non-submergence, this is surprising because IR64-Sub1, like other high yielding rice cultivars, uses the quiescence strategy for survival under completely flooded conditions (Voesenek and Bailey-Serres, 2009; Nishiuchi et al., 2012). The presence of Sub1 should limit the elongation response (Bailey-Serres et al., 2010). It is also possible that early flooded rice seedlings might result in more frequency of lodging at harvest.

The incorporation of the rice straw stubble and rice straw ash improved the level of rice seedlings survival (Fig. 5). This was unexpected because fresh rice straw incorporation typically causes seedling mortality. It is not clear why rice straw stubble or ash improved survival rates. The residue treatments did not affect the EC or pH of the water, rather the depth of water was more important probably through dilution. The ash had a significant effect relative to the other treatments on yield. Rao et al. (1976) found that the incorporation of fresh straw cause the immobilization of mineral N and subsequently effected yield. This could be overcome if the planting was delayed by 15 days to all the straw to begin decomposing and subsequent mineralization of the organic nitrogen pool.

In conclusion, IR64-Sub1 can survive a 3 week-duration of complete submergence in darkness beginning 3 days after emergence, but there is significant mortality. This indicates that early season flooding could still have a significant effect on yield if growers are unable to replant. The incorporation of rice straw or ash increased the survival rate relative to the control. At harvest the ash had the greatest effect on yield when the rice under both flooding treats. The management of rice straw is an important factor for intensive rice production systems whilst incorporation of fresh residue boosted survival it also had a negative impact on yield.

ACKNOWLEDGEMENT

This research was undertaken under project “Climate change affecting land use in the Mekong delta: adaptation of rice-based cropping system” (CLUES), funded by Australian Federal Government Australian Center for International Agricultural Research.

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Effects of Alternate Wetting and Drying Irrigation and Phosphorous Fertilizer Rates on the Growth and Yield of Rice in Double Rice Cropping System at An Giang Province

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ABSTRACT

Alternate wetting and drying (AWD) irrigation is a water saving irrigation technique in rice production and can be an important adaptation strategy under climate change where water scarcity may become more prevailing. The suitability of AWD in acid sulphate soils (ASS) has not been tested. There is a possibility that the drying cycles of AWD may enhance oxidation and acidification. The drying cycles may also reduce the phosphorous availability to the plants. Rice irrigated with AWD may require more P fertilizer than under continuous flooded condition. In highly intensive cropping areas of the Mekong river delta of Viet Nam, such as An Giang province, farmers have applied high doses of P fertilizer to compensate for the P-binding of toxicities associated with ASS such as aluminum and iron. Excess of P, on the other hand can lead to phosphorous accumulation over time. This may reduce the requirement for P fertilizer input, even under AWD irrigation. This study investigated the interactive effects of AWD irrigation and phosphorous rates on water use, soil properties, rice growth and yield in double rice system grown on ASS in Ta Danh, An Giang, for 3 consecutive crops from March 2012 to June 2013. AWD application reduced about 50% of irrigated water quantity and increased EC value of field water in contrast with continuous flooded of farmers' practice. Difference in water management strategies and phosphorous levels showed no impact on pH values of field water, rice growth, biomass accumulation, and rice yield in 3 continuous cropping seasons. Without or reduced phosphorous fertilization varied the availability of phosphorous in the soil for at least 2 crops that could waste farmers' resource and create environmental issues. Obtained data promote the application of AWD irrigation and less phosphorous use for rice production in acid sulphate soil to improve resource management and local farmers' adaptation under impact of climate change.

Keywords: Acid sulphate soil, climate change adaptation, Mekong river delta, phosphorous, rice yield, water saving.

1. INTRODUCTION

Rice is one of among majority crops to be grown to feed billion of people around the world (Grierson et al., 2011). About half of the world population depends on rice as staple food (Timmer, 2010) and rice is the most important crop in Viet Nam, particularly in the Mekong river delta (MRD) where it accounts for more than 80 percents of rice export of the country (De, 2008).

Phosphorous (P) is one of three macronutrients and among 17 essential elements required for plant growth (Raghothama, 1999). P involves in many metabolic processes of plants such as energy generation, nucleic acid synthesis, photo-synthesis, glycolysis, respiration, membrane synthesis and stability, enzyme activation/inactivation, redox reactions, signaling, carbohydrate metabolism, and nitrogen fixation. On dry weight basis, P level in plant materials ranges from 0.05 to 0.50% (Vance et al., 2003) which exceeds the P concentration in the soil solution about 2000 folds (Schachtman et al., 1998). In arable land around the world, about 30 - 40% area is limited by P availability (von Uexküll and Mutert, 1995). Application of P-containing fertilizers is usually the recommended treatment for enhancing soil P availability and stimulating crop yields (Vance et al., 2003). P limiting condition

can lead to alterations in root architecture, carbon metabolism, exudation of organic acids, gene expression, etc. (Vance et al., 2003).

In many soil types, P is abundant but presents in unavailable forms for plant and P is usually considered as limiting element for plant growth and development, particularly in acid soils with high presence of aluminum and iron (Vance et al., 2003). Unfortunately, more than 50% of the MRD is overlaid with acid sulphate soil (ASS) with low pH, high concentration of soluble aluminum and ferrous ions (Foy et al., 1978), which are highly toxic to plants including rice (Becker and Asch, 2005; Panda et al., 2009). P availability is enhanced by phosphate solubilizing bacteria via lowering soil pH, producing organic acids, and mineralizing organic P (Awasthi et al., 2011). In addition, mycorrhiza also plays an important role in plant P nutrition (Smith et al., 2011) and using microorganisms to improve plant acquisition of phosphorous appears to be a potential prospect (Richardson, 2001). Recently, high yield rice under P deficiency with Pup1 gene has been developed (Chin et al., 2011).

Fertilizer recommendation for the MRD was developed about 3 decades ago, mostly for single crop of rice. Since then, farmers in many parts of the MRD have increased the cropping intensity to 2 or 3 crops/year. In ASS, farmers tend to use high rates of P, from 70 to 90 kg P₂O₅ ha⁻¹, to reduce the negative effects of ASS-toxicities. With most of residues either burned in the fields or ploughed into the soil, apart from the amount of P in the grains, which are removed from the fields, the major part of phosphorous fertilizer is returned to the soil. Through the years, there might be substantial accumulation of P in the soil. In other words, excessive phosphorus fertilization has occurred. We hypothesized that farmers in ASS areas in the MRD can reduce P fertilizer rates without compromising rice growth and yield.

The normal practice of rice production in MRD is keeping the field continuously flooded. This practice requires high volume of water and actually supplies more water than the requirement of rice plants and the field need (Guera et al., 1998). In many parts of the MRD, farmers experience water scarcity for irrigation the dry season rice crops. This water scarcity may become more prominent with the eminent climate change. Alternate wetting and drying irrigation (AWD) has been shown a viable irrigation technique to help farmers cope with water scarcity and has been widely practiced in “normal” soil and water conditions. In ASS, there are some concerns that the drying cycles of AWD may exposed the soil to oxidation, which may lead to increased acidification. There has not been systematic investigation on the use of AWD in ASS.

AWD has been proven a robust technology. It did not require any adjustment in N fertilizer management compared with continuous flooded conditions (Cabangon et al., 2004; Cabangon et al., 2011). The effects of AWD on P fertilizer management has, however, not investigated. AWD exposes the soil to temporary aerobic conditions, which may limit the bioavailability of phosphorus, especially if soil is affected by salinity or acidity. Plants under AWD may need more P than those under continuous flooding. Optimum P fertilization under AWD and continuously flooded conditions on ASS need to be identified.

The aim of this study was to examine the effects of AWD irrigation in combination with phosphorous fertilizer rates on water use, chemical properties of soil and field water and yields of rice grown on ASS in a double rice cropped areas of the MRD.

2. MATERIALS AND METHODS

2.1. Study site

Experiment was done at Ta Danh village, Tri Ton district, An Giang province, from March 2012 to June 2013. The experimental site is a typical annually flooded area of An Giang province. Local

farmers usually culture double rice crops annually during (Winter-Spring and Summer-Autumn; see Fig. 1) because their fields are located outside the flood protection area where triple rice cropping systems are grown. During the wet season (AW) fields outside the flood protection area are left fallow and inundated until the on-set of the dry season (WS). The weather data were collected at the An Giang Provincial Station located 20 km away from the study site. Farmers always burn rice residue after harvest and the average phosphorous application at study site is around 40 kg P₂O₅ per ha.

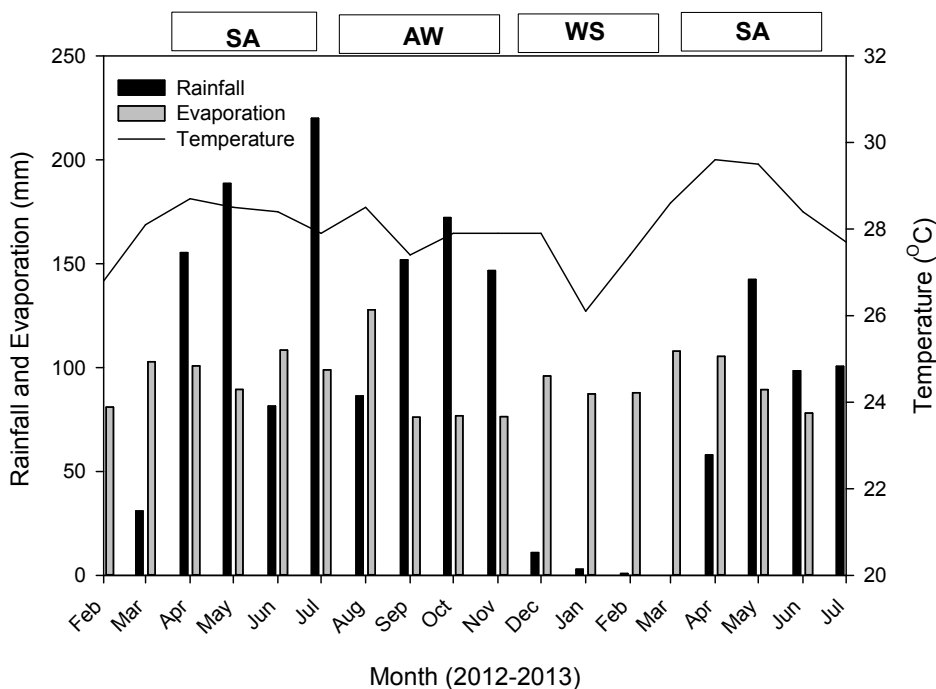


Fig. 1. Cropping calendar, temperature, rainfall and evaporation

The soil is classified as Typic Sulfaquept (USDA) or Epi-Orthi-Thionic Fluvisol (IUSS WRB 2006) and is commonly known as an acid sulphate soil. The top soil is mixed with decomposed organic matter and has a field pH <5.5; soil is less developed, ripe to nearly ripe down to 500 mm depth. The sulphuric horizon with jarosite mottles occurs from 250-800 mm depth with a pH <4.5.

2.2. Treatments and lay out

The experiment was arranged in split-plot design with 3 replications. Main plot factor was water management (W) and the sub-plot factor was P rates.

The main plot factor W had 3 levels:

W_{CF} = Farmer Practice- continuously flooded irrigation water management. The field is always flooded, irrigate when water level in the field is about 1 cm

W_{AWD5} = AWD irrigation water management. Irrigate when the water level in the field is 5 cm below the soil surface.

W_{AWD15} = AWD irrigation water management. Irrigate when the water level in the field is 15 cm below the soil surface.

In the AWD plots, a water tube (Tuong et al., 2009) water depth measuring device in form of a PVC tube approximately 15 cm diameter x 25 cm depth is installed to a depth of 15 cm in the field

at 20 days after direct sowing. The buried parts of these tubes were holed by 5 cm diameter with a distance at 5 cm from each other allowing water to percolate whenever field water increases or decreases.

The sub-plot factor P had 3 levels

P_0 = No P fertilizer

P_{20} = 20 kg P_2O_5 per ha

P_{40} = 40 kg P_2O_5 per ha

There were 27 subplots (5 x 8m). Small bunds (20 cm height and 30 cm width) were built between subplots. For the two main plot of AWD the bunds (30 cm height and 40 cm width) were built with the insertion of plastic film in the middle to a depth of 30 cm from soil surface to minimize lateral water movement.

2.3. Management

The residue from previous crop was burnt and ash and remaining residue was incorporated using a rotary hoe. After tillage was completed, the field was flooded until the soil was saturated. The field surface was then leveled off. Rice cultivars were OM4218 and OM2517 (certified seed) for SA and WS, respectively and drum seeding was used to apply the seed at the density of 100 kg per ha. Pre-germination herbicide, Sofit was used to control weeds.

Nitrogen and potassium were the fixed amounts as farmer practice (120 kg N and 60 kg K_2O per ha, respectively). One third of total nitrogen was applied at 10, 20 and 35 days after crop establishment, and 30 kg K_2O ha^{-1} was applied at 20 and 35 days..

Water in the fields was controlled according to water treatments. Whenever ground water level reached the critical depth of soil surface at 5 and 15 cm, water from nearby canal was pumped mechanically into the respective treatments up to about 5 cm above the soil surface. The volume of irrigation water that was applied to each treatment was measured by a 90 mm flow meter.

2.4. Parameters monitored

2.4.1. Water

Water level in each plot was measured every three days inside AWD tools using a tape measure and the height was referenced to the ground surface. The water inside the AWD water tube was sampled every 6 days for pH and EC measurement, using a hand held meter (FG2 and FG3 Fieldkit, Mettler Toledo, Switzerland). At each measurement, water inside the water tube was purged by hand bailing prior to sampling. During the 2013 WS, the concentration of aluminum and ferrous ions in the ground water was determined in the field using test strips (Al 1.10015.0001 and Fe 1.10004.0001 MERCK, Germany).

2.4.2. Soil

Available phosphorous was analysed after harvesting. Soil samples were collected by a using a soil core of 50 mm diameter and 200 cm depth at the centre of each plot. The soils were stored in plastic bags and transported bag to the laboratory for analysis. On receipt the soils were oven-dried at 60 degrees Celsius for 24 hours. Each soil sample was crushed, ground and sieved and the fraction > 2 mm was discarded. The soils at the site are acid sulfate soils and characteristically acidic and contain iron and aluminum mineral. In soils such as these the Bray-Krutz method (Bray and Krutz, 1945) is the most suitable for the determination phosphorus. The input of nutrients from flood sediments was determined after the 2012 annual wet season. Three plastic sheets (4 x 5 m) sheets

were placed in the field before the start of the flooding season. Immediately after the flood water receded, the sediment was collected, weighed and samples for dried weight at 60°C in an incubator. Total and available phosphorous was determined.

2.4.3. Plant

Plant biomass at flowering and physiological maturity was done by collection of 0.5 m² at each stage, made sub-sample and dried until constant weight at 60°C. For yield component: harvested 0.5 m² at physiological maturity (about 5 days before harvesting) for counting the number of panicles/m², filled and unfilled spikelets, 1000 grains weight, and grain moisture content. Grain yield was measured from 5 m² at harvest, converted to tons per ha at 14% of moisture content.

2.5. Data analysis

Microsoft Excel and SPSS 16.0 were employed for data comparison between treatments, factors and their interaction for each crop season.

3. RESULTS

3.1. Water quantity

During the crop systems when the ground water reach trigger values of -5 and -15cm the treatments where irrigated (Fig. 2). Figure 1 shows that the use of water was not uniform across the field, treatment W_{AWD15} lost water through seepage and evapo-transpiration at a greater rate than W_{CF} and W_{AWD5} (Fig. 2). Three consecutive crops were cultured but only two of them were recorded for volume of pumped water quantity. Difference in water quantity between water management treatments is presented in Table 1.

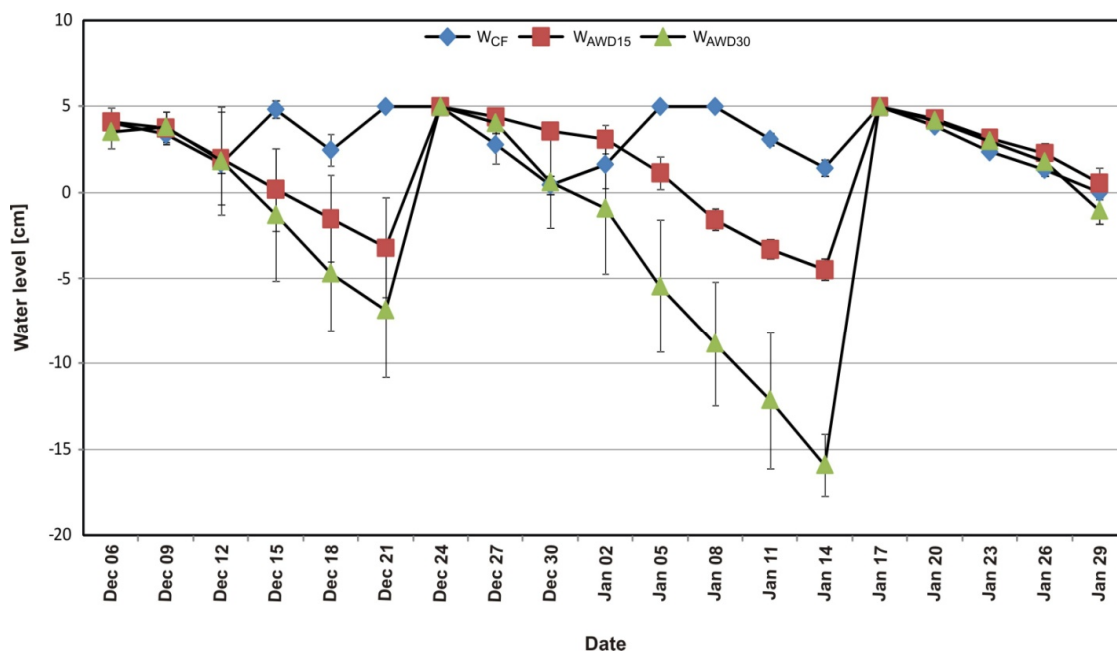


Fig. 2. Dynamics of field water in different water management treatment in 2013 WS crop, from November 2012 to February 2013 at Ta Danh, Tri Ton, An Giang

Note: Vertical and capped bars indicate means of 3 measurements

Table 1. Effects of water management on the volume of pumped water. Total water volume equates to the rainfall and the irrigated water inputs

Water management	Irrigation water volume (m ³ /ha)		Total water volume (m ³ /ha)	
	2013 WS	2013 SA	2013 WS	2013 SA
W _{CF}	2,519a	2,061 a	4135	5052
W _{AWD15}	1,183b	1,433 b	2799	4424
W _{AWD30}	1,167b	1,292 b	2783	4283
F	41.9**	65.4**		

Note: In a column, means followed by the same letter (a, b) are not significantly different at the 1% level by Duncan's multiple range test.

AWD treatments saved more than 50% of irrigated water for both cropping seasons compared with continuous flooding treatment. The difference between the two AWD regimes was not significant. This result also reveals that field water management as farmers' practice waste fresh water and might contribute to increase the risk of seawater intrusion for the downstream areas in MD. During the 2013 WS crop rain contributed additional 1600 m³ water and in the 2013 SA crop 2991 m³.

3.2. pH and EC value of field water

Experimented factors caused very little change in pH of field water in 3 continuous crops but data from 2013 WS chosen for presentation (Fig. 3). At no time did the pH drop below 5.5 and the concentration of Fe²⁺ and Al³⁺ was below the detection limit of each test (<3 and <10 mg L⁻¹ respectively). These outcomes also demonstrated that if there were not much change in water chemistry characteristics of the field water, AWD technique is a promising application even on acid sulphate soil areas as in MD to save natural fresh water resource in rice production.

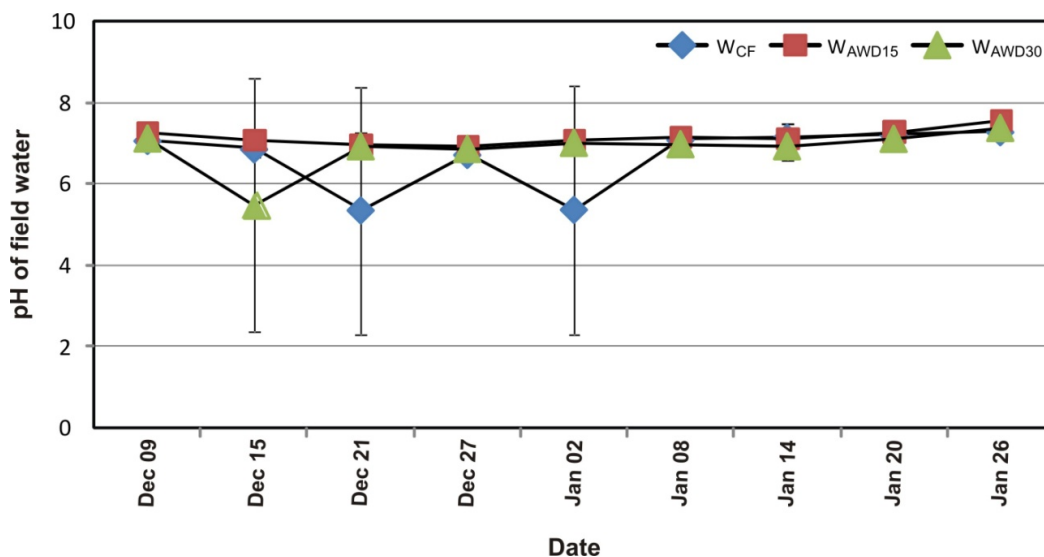


Fig. 3. Effect of water management on pH of field water in 2013 WS from November 2012 to February 2013 at Ta Danh, Tri Ton, An Giang

The EC of the field water during the WS season increased during the cropping seasons and the EC of W_{AWD5} and W_{AWD15} was greater than the W_{CF} (Fig. 4). Variation in phosphorous application showed no effect on pH and EC values of field water which were not presented here.

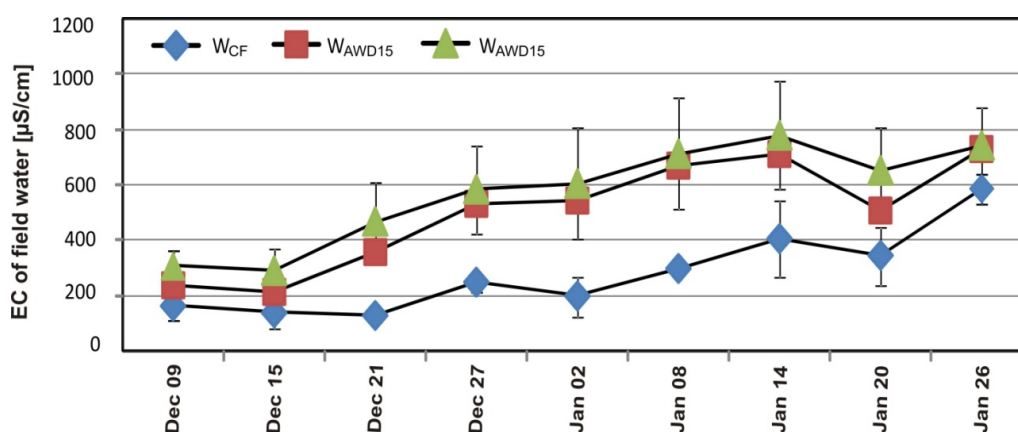


Fig. 4. Effect of water management on EC of field water in 2013 WS from November 2012 to February 2013 at Ta Danh, Tri Ton, An Giang

These outcomes also demonstrated that if there were not much change in water chemistry characteristics of the field water (see below), AWD technique is a promising application even on acid sulphate soil areas as in MD to save natural fresh water resource in rice production.

3.3. Above ground biomass and rice yield

In 3 consecutive crops, 2 experimented factors caused no significant difference in biomass partitioning among leaves, culm, and panicles between treatments.. The water management or phosphorous levels did not contributed the observe differences in the above ground biomass (Table 2). It was also recognized that biomass accumulation was higher in WS than in SA.

Table 2. Effects of water management and phosphorous levels on above ground biomass at physiological maturity

Factor	Total biomass (tons/ha)		
	2012 SA	2013 WS	2013 SA
Water management (W)			
W _{CF}	13.5	15.3	12.6
W _{AWD15}	13.8	15.6	12.9
W _{AWD30}	13.5	15.4	12.0
Phosphorous level (P)			
P ₀	14.0	16.0	12.6
P ₂₀	14.1	15.1	12.1
P ₄₀	12.7	15.1	12.8
F (W)	0.17ns	0.09ns	0.79ns
F (P)	2.77ns	0.97ns	1.61ns
F (WxP)	0.79ns	1.38ns	1.14ns

Note: ns: non-significance

Local farmers always chose suitable rice cultivars for their cropping seasons which explain un-uni-formed cultivars among crops at the same experimental location leading to difficulty in elucidation of rice yield difference between crops. However, water management or phosphorous

levels showed no impact on rice yield in 3 consequent crops (Table 3). The combination of less water and phosphorous use for rice production in double rice areas can be approaching strategy for improvement of local farmers' income.

Table 3. Effects of water management and phosphorous levels on rice yield

Factor	Yield (tons/ha)		
	2012 SA	2013 WS	2013 SA
Water management (W)			
W _{CF}	6.3	7.4	6.7
W _{AWD5}	6.6	7.7	6.5
W _{AWD15}	6.2	7.3	6.5
Phosphorous level (P)			
P ₀	6.4	7.6	6.0
P ₂₀	6.3	7.5	6.9
P ₄₀	6.5	7.4	6.7
F(W)	0.50ns	0.53ns	0.29 ns
F(P)	1.77ns	0.26ns	3.02 ns
F(WxP)	1.26ns	0.58ns	0.75 ns

Note: ns: non-significance

3.4. Availability of phosphorous in the soil

Normally, less phosphorous application to the soil would lead to its phosphorous reduction but the analysis of available phosphorous in soil in 2 crops at the same experimental site shown no difference for both factors, water management or phosphorous applied levels, even for the null phosphorous fertilized treatment (Table 4).

Before the first crop establishment, the total phosphorous and its availability in the soil were $0.13 \pm 0.01\%$ and 6.80 ± 1.79 mg P/kg of dried soil, respectively. After harvesting, the available phosphorous in the soil was comparable to its value at starting point (2012 SA). At the highest dose, 40kg of P₂O₅ per ha did not contribute to build up the availability of phosphorous in the soil after consequent cropping seasons (Table 4).

Table 4. Effects of water management and phosphorous levels on available phosphorous in the soil after harvesting each crop

Factor	Available P in soil (mg P/kg dried soil)	
	2012 SA	2013 WS
Water management (W)		
W _{CF}	8.61	11.32
W _{AWD5}	8.58	12.96
W _{AWD15}	9.73	11.89
Phosphorous level (P)		
P ₀	9.46	12.59
P ₂₀	8.69	9.83
P ₄₀	8.78	13.76
F (W)	0.46ns	0.19ns
F (P)	0.19ns	1.13ns
F (WxP)	0.43ns	0.33ns

Note: ns: non-significance

During the wet season flood a significant amount of nutrients are supplied to the soils (Table 5) It is not clear how much of the supplied P will be mineralized during the cropping season and converted to plant available P.

Table 5. Nutrients supplied to the non-flood protected areas during the wet season floods

Nutrients in dried sediment	Replicate			Average
	1	2	3	
Dry sediment (kg/ha)	9,511	12,694	10,648	10,951
Total nitrogen (kg N/ha)	30.4	40.6	36.2	35.7
Total phosphorous (kg P ₂ O ₅ /ha)	11.4	15.2	11.7	12.8
Total potassium (kg K ₂ O/ha)	82.7	93.9	56.4	77.7
NH ₄ ⁺ (kg N/ha)	0.885	1.273	0.769	0.976
NO ₃ ⁻ (kg N/ha)	0.027	0.096	0.063	0.062
Available P (kg/P ₂ O ₅)	0.129	0.188	0.179	0.165
Available K (kg K/ha)	0.167	0.233	0.174	0.191

4. DISCUSSION

In the double rice area the application of AWD to 15 cm could reduce the application of irrigation water by ~50% in the WS and SA seasons. This represents a significant saving in both labour and capital for the farmer because the pumping time would be reduced by 50%. The application of AWD in the acid sulfate soil areas of An Giang did not affect the paddy water pH, and Al³⁺ or Fe²⁺ concentrations. This indicates that the AWD did not promote further profile oxidation and acidity generation. But under drier conditions, predicted to occur under future climate change scenarios, when the ground water level is allowed drain to ~30 cm then profile oxidation and acidification may become a significant issue. If AWD is applied broadly in acid sulphate soil double rice production areas then it is recommended that farmers and extension offices monitor and evaluate the potential profile oxidation. Further, the application of lime may be warranted to increase the buffering capacity of the surface soil.

The phosphorous trial results where no fertilisation in 3 consecutive crops had no impact on rice yield in free-dyke area of Ta Danh. This indicates that there has been soil phosphorous accumulation from the application of phosphorous fertilizer and the yearly inputs of P with the flood sediments. In double rice cropping areas farmers apply 40 kg P₂O₅ ha⁻¹ which is barely enough fertilizer to replace the P that is removed in the grain. This suggests that the flood sediment supplied P augments the applied fertilizer. Changes to the supply of flood sediments caused by climate change adaptations such as dyke and dam constructions would cause farmers to increase synthetic P applications.

The increased in available P after the end of the WS season is due to the increased EC of the paddy water. During the dry season the irrigation source canal solute concentration increases through evap-concentration and inputs of ground water. This water would contain sulphate which is subsequently reduced in the paddy soil releasing P. A similar process has been found to regenerate salt marsh PO₄²⁻ through mineralization due to SO₄²⁻ metabolism (Portnoy and Giblin., 1997a; Portnoy and Giblin, 1997b; Williams et al., 2014).

5. CONCLUSIONS

The combination of AWD irrigation and maintenance of phosphorous fertilization thru flooding is perspective adaptation for farmers in double rice area in An Giang. Farmers could make significant water savings, which would improve farm profitably and hence climate change resilience. The P balance at the site is dependent on the delivery of P within the flood water sediments. If climate change adaptations or future developments change the supply of P then farmers will need to increase the application of synthetic P to the crop.

Overall, there is significant potential for farmers in the double rice cropping areas in An Giang and possibly other provinces to apply AWD and apply 40 kg P₂O₅ ha⁻¹ P without yield penalty. Farmers will need to monitor and adjust AWD and P management if acidification and P deficiencies become an issue.

ACKNOWLEDGEMENT

The work was done under scope of “Climate change affecting land use in the Mekong delta: adaptation of of rice-based cropping system” (CLUES), funded by ACIAR.

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Developing A Mathematical Model to Measure The Spreading of Soil Moisture under Drip Irrigation

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ABSTRACT

Water plays a crucial role to crops. Therefore, determining the suitable time for irrigation and the quantity of water needed is very important in the whole irrigating system and is a key factor to the crop growth and production. In alluvial soils of the Red River delta, the field capacity (β_{dr}) is 32.41%; if the average moisture of soils before irrigation (β_0) 28.42%, the standard irrigation was calculated as $M = 221.84 \text{ m}^3 \text{ ha}^{-1}$. Based on experiments, the function of moisture before irrigation was found to be $g(y) = 25.28203(y-1.5)^{0.057362}$, with the coefficient of determination $R^2 = 0.933298$; while the function of moisture at the end of drip irrigation was $F(x;y) = 8.9918 \cdot 10^{-5}(25 - x)^{1.240306}(25 - y)^{2.311185} + 25.28203(y-1.5)^{0.057362}$, with the coefficient of determination $R^2 = 0.946491$. One day after finishing the drip irrigation, the function of moisture became $F(x;y) = 0.0003325(25 - x)^{1.269}(25 - y)^{1.83194} + 25.28203(y-1.5)^{0.057362}$ with $R^2 = 0.9256$. The function can be used to simulate soil moisture as model validation resulting in small errors (5-10%).

Keywords: Soil moisture, the function of moisture content.

1. INTRODUCTION

Water plays a very important role for agricultural production. Crop needs a lot of water; however, each type of crop (at different growth stages) requires a different amount of water for growth, and irrigation is needed in order to compensate the lack of water. Therefore, determining the exact moment of irrigation and the amount of water needed is significant and important for crop growth and yield (Nguyen, 2008).

In addition, the horizontal permeability and vertical permeability processes during irrigation depend on the irrigation discharge (Shrivastava et al., 2011). For drip irrigation, these processes are affected by the distance between sprinklers (Abdallah et al., 2011).

There have been several studies based on the Richard's equation which represents one-dimensional vertical flow to predict the movement of water in the unsaturated soil. Nguyen (1994) has developed a general model of moisture transfer in the unsaturated soil area for all dryland crops in the condition of changing upper and lower bounds. However, this model is one-dimensional moisture equation in which the irrigation method, the root characteristics and crop density were not taken into consideration. For one-dimensional moisture model studies of the unsaturated soil due to the drip irrigation, several models were developed. For analytic methods, the simplified model in the hemispherical form for the stable unsaturated permeable form due to the drip irrigation in the hose line/point style was used based on the assumptions of permeable in the hemispherical style and linear solution. This model was developed by Bresler et al. (1971), Philip (1971), and Warrick (1974). Nevertheless, some limitations exist in this model such as its assumption that the flow is stable, and that the water pressure and permeability have an exponential relationship.

Healy and Warrick (1988) have successfully developed a model of unsaturated permeability in the soil due to drip irrigation by applying the finite difference method for Richard dimensionless

variant equations, but still, the method is complex because its output is humidity without units. The group in Aristotle University of Thessaloniki (2001) has applied the finite difference method in the heterogeneous soil but the method is limited to one-dimensional mathematical problem.

In this paper, we introduce a mathematical model to build a function that determines the moisture in the soil under drip irrigation. The aim is to find the coefficients of soil moisture spreading vertically and horizontally based on soil types, discharge, and density of crop to recommend an irrigation regime to obtain the high efficiency.

2. MATERIALS AND METHODS

2.1. Drip irrigation

Drip irrigation was carried out in the experimental room using 3 soil (Red river alluvial soil) blocks each of of 40 x 40 x 40 cm in size. The soil blocks are covered around by the plastic to avoid evaporation and seepage. One drip nozzle was positioned at the center of each soil block (Fig. 1). For the drip system, PE main irrigation pipe with 25 mm in diameter and smaller branch pipe of 15 mm were used. The nozzle discharge was 0.43 L hr⁻¹.



Fig. 1. Drip irrigation in the soil block in the experiment room

2.2. Determination of physical and chemical characteristics of the soil

The physical and chemical characteristics of the soil were determined: pH_{KCl} by pH meter, OC% (Walkley and Black), P₂O₅ (Oniani), K₂O by the photometer fire, N (Tiurin and Kononova). The soil texture was measured by the Robinson suction method and the soil density measured by the cylinder method.

Soil moisture and field capacity were determined according to the percentage of the dry weight.

2.3. Determination of standard irrigation

The irrigation standards for each soil block were determined according to the following formula:

$$M = 10^4 \cdot h \cdot d \cdot (\beta_{dr} - \beta_0) \cdot (\text{m}^3 \text{ha}^{-1})$$

Where:

h: depth of the soil to be moistened (m)

d: soil density (g cm⁻³)

β_{dr} : field capacity (% dry weight)

β_0 : moisture before irrigation (% dry weight)

2.4. Monitoring criteria

To predict the distribution process of soil moisture before drip irrigation, immediately after the irrigation and one day, two days and three days after the irrigation, five positions with a horizontal spacing of 5 cm and at the depths of 0-5 cm, 10-15 cm, 15-20 cm, and 20-25 cm were fixed. Soil sampling was taken by hand drilling. Soil moisture was determined by the dried soil using the weight method.

2.5. Construction of mathematical model

The soil moisture function $F(x,y)$ at position $M(x,y)$ was formulated. The coefficients k , α , β , a and b of soil moisture function were determined by monitored data. Mathematical model of the spread of the moisture in the soil using was constructed using regression function in the Microsoft Excel.

3. RESULTS AND DISCUSSION

3.1. Physical and chemical characteristics of the soil and the standard irrigation

The soil used for experiment is clay soil, pH_{KCl} : 7; OC: 1.92%; P_2O_5 : 332 mg kg^{-1} soil; K_2O : 55.3 mg kg^{-1} soil; N: 80.5 mg kg^{-1} soil

- The components of the grain: clay 5.4%; silt 40.3%; sand 54%
- The soil density $d = 1.39 \text{ g cm}^{-3}$
- The field capacity $\beta_{dr} = 32.41\%$
- The average moisture before irrigation $\beta_0 = 28.42\%$
- Irrigation standard $M = 221.84 \text{ m}^3 \text{ ha}^{-1}$

3.2. Construction of a function to determine soil moisture

Soil moisture depends on many elements such as soil profile, the amount of irrigation water, temperature, radiation, and specific time, etc. Based on the results of our experiment, we introduce the following function to determine soil moisture:

$$F(x,y) = f(x,y) + g(y)$$

Where: y is the depth of soil layer,

x is the distance from the position where moisture is to be calculated to the irrigation position on the vertical axis,

$g(y)$: the function that determines the moisture before irrigation at $M(x,y)$,

$f(x,y)$: the function that determines the additional moisture at $M(x,y)$ after the irrigation time (at the measured time),

$F(x,y)$: the function that determines the moisture at $M(x,y)$ after the irrigation time (at the measured time),

Experimental data shows that the function $g(y)$ has a linear form as follows:

$$g(y) = a(y-1.5)^b$$

Where 1.5 is the factor identifying natural moisture at the midpoint of the 0-5 cm layer (at the midpoint of the 0-5 cm layer, $y=2.5$ then $g(y) = a$)

The function $f(x,y)$ decreases when x increases, y increases and $f(x,y) = 0$ when $x \geq x_0$ or $y \geq y_0$

Where: x_0 , y_0 are the maximum spreading of moisture in horizontal and vertical directions respectively.

Therefore, the function of $f(x,y)$ is as follows:

$$f(x, y) = k (x_0-x)^\alpha (y_0-y)^\beta \text{ when } 0 \leq x \leq x_0; 0 \leq y \leq y_0$$

$$f(x, y) = 0 \text{ when } x > x_0; y > y_0$$

Where: k, α, β are positive constants depending on the physical and chemical properties of the soil.

The function $f(x,y)$, as previously determined, is reasonable because when x increases, y increases leading to the decrease of the function and when one of two parameter increases to the critical levels, it will be cancelled, at that time the soil moisture at the position $M(x,y)$ equals the natural soil moisture before irrigation.

The function of $F(x,y)$ in the range $0 \leq x \leq x_0; 0 \leq y \leq y_0$ has the following form:

$$F(x,y) = k(x_0-x)^\alpha(y_0-y)^\beta + a(y-1.5)^b$$

To define the function of $F(x,y)$ we have to determine the coefficients k, α, β, a, b based on the experimental data.

3.3. Defining the moisture function before irrigation $g(y)$

The function of $g(y)$ has the form: $g(y) = a(y-1.5)^b$

$a; b$ are defined based on the observations of the moisture before irrigation

Table 1. The moisture before irrigation

Distance Depth	Irrigation point	x = 5 cm	x = 10 cm	x = 15 cm	x = 20 cm
0 - 5 cm	24.98	24.98	24.90	25.00	24.96
5 - 10 cm	28.87	28.89	28.85	28.88	28.91
10 - 15 cm	29.05	29.13	29.08	29.05	29.12
15 - 20 cm	29.67	29.66	29.59	29.57	29.56
20 - 25 cm	29.68	29.63	29.61	29.59	29.56

From Table 1, using regression function in the Microsoft Excel the moisture equation before irrigation obtained depend on the weather condition and soil characteristics and this equation can be used to predict the moisture before irrigation:

$$g(y) = 25.28203(y-1.5)^{0.057362}$$

With the correlation coefficient $R = 0.966073$, the coefficient of determination $R^2 = 0.933298$.

3.4. Defining the function of moisture increment $f(x,y)$ and the moisture function $F(x,y)$

When irrigated, the soil moisture will increase into the depth and over the width of soil layer. Based on this equation, crop density and soil characteristics the time of efficient irrigation with specific sprinklers discharge can be predicted:

The function of $f(x,y)$ has the following form

$$f(x,y) = k(x_0-x)^\alpha(y_0-y)^\beta \text{ when } 0 \leq x \leq x_0; 0 \leq y \leq y_0$$

$$f(x,y) = 0 \text{ when } x > x_0; y > y_0$$

To determine the coefficients k , α , β in the case experimental results were obtained immediately after irrigation, 1 day, 2 days, and 3 days after irrigation finished, we need to perform the following transformations:

From the function $f(x,y) = k(x_0-x)^\alpha(y_0-y)^\beta$ take logarithm on both sides we get:

$$\ln[f(x,y)] = \ln k + \alpha \ln(x_0-x) + \beta \ln(y_0-y)$$

Assuming that $z = \ln[f(x,y)]$; $u = \ln(x_0-x)$; $v = \ln(y_0-y)$; $\ln k = c$

$z = c + \alpha u + \beta v$ this is a linear function of z with two variables u and v

To find c , α , β we perform the following steps:

+ Determining the constants x_0 , y_0 : From the monitoring soil moisture experiment, we found $x_0 = y_0 = 25\text{cm}$

+ Finding out the moisture increment $f(x,y)$ at position $M(x,y)$

+ Finding the corresponding values $z = \ln[f(x,y)]$; $u = \ln(x_0-x)$; $v = \ln(y_0-y)$

+ Using Regression function to find c , α , β in the case of soil moisture monitoring

3.4.1. Soil moisture immediately after the drip irrigation

Table 2. The moisture after drip irrigation finished

Distance Depth	Irrigation point	x = 5 cm	x = 10 cm	x = 15 cm	x = 20 cm
0 - 5 cm	32.84	32.41	29.77	28.57	27.23
5 - 10 cm	32.31	32.26	29.64	29.49	29.08
10 - 15 cm	30.78	30.72	29.53	29.38	29.33
15 - 20 cm	30.23	30.15	29.65	29.59	29.48
20 - 25 cm	29.73	29.69	29.63	29.60	29.57

After irrigation finished, soil moisture in the surface layer was higher than in the deeper layers (Table 2). At the point of irrigation, soil moisture in the first layer and in the layer of 20-25 cm was 32.82% and 29.73%, respectively. Soil moisture was found to decrease when the measured position was farther from the irrigation point. The lowest moisture was 27.23%. Based on the variation of humidity, by performing transformations and using Regression Software, the moisture content function $F(x,y)$ at position $M(x,y)$ was identified with the coefficient of determination $R^2 = 0.946941$. The linear regression function for predicting the spread of soil moisture after irrigation is as follows:

$$F(x,y) = 8.9918.10^{-5}(25 - x)^{1.240306}(25 - y)^{2.311185} + 25.28203(y-1.5)^{0.057362}$$

3.4.2. Soil moisture at one day after drip irrigation

Table 3. The moisture at one day after the drip irrigation

Distance Depth	Irrigation point	x = 5 cm	x = 10 cm	x = 15 cm	x = 20 cm
0 - 5 cm	30.48	30.42	28.04	27.12	25.82
5 - 10 cm	32.14	32.01	29.12	29.09	29.00
10 - 15 cm	31.98	31.85	30.02	29.58	29.41
15 - 20 cm	30.85	30.26	30.21	30.05	29.66
20 - 25 cm	29.74	29.72	29.64	29.60	29.57

One day after the irrigation, the soil moisture tended to increase at the second layer (32.14%) and the third layer (31.98%), the last layer reached only 30.48% due to the impact of weather conditions (Table 3). Meanwhile, at the third layer and the fourth layer the soil moisture did not show any significant change as compared to itself before irrigation. After performing transformations and using the Regression software the moisture content $F(x,y)$ at position $M(x,y)$ was determined as follows:

$$F(x,y) = 0.0003325(25 - x)^{1.269}(25 - y)^{1.83194} + 25.28203(y-1.5)^{0.057362}$$

The coefficient of determination $R^2 = 0.9256$, the linear regression function is well accepted.

3.4.3. Soil moisture at two days after drip irrigation

Table 4. Soil moisture at two days after the drip irrigation

Depth \ Distance	Irrigation point	x = 5 cm	x = 10 cm	x = 15 cm	x = 20 cm
0 - 5 cm	27.11	27.09	26.73	26.33	25.12
5 - 10 cm	29.02	29.01	28.95	28.93	28.90
10 - 15 cm	29.18	29.14	29.10	29.07	29.04
15 - 20 cm	30.22	30.08	29.98	29.76	29.65
20 - 25 cm	29.77	29.73	29.67	29.61	29.58

Two days after irrigation, soil moisture at layers 1, 2 and even layer 3 are all affected by the evaporation process of the surface layer as well as by the subsoil layer. When performing transformations and using the Regression software, the coefficient of determination $R^2 = 0.559009$ can be determined according to the following equation:

$$F(x,y) = 0.00211(25 - x)^{1.315452} (25 - y)^{0.940766} + 25.28203(y-1.5)^{0.057362}$$

Therefore, using the assumptions to build the model is not satisfactory, and we need to further study to take into account climate factors into the equation.

3.4.4. The moment after the drip irrigation finished for three days

Table 5. Soil moisture at three days after the drip irrigation

Depth \ Distance	Irrigation point	x = 5 cm	x = 10 cm	x = 15 cm	x = 20 cm
0 - 5 cm	25.01	24.98	24.95	24.95	24.95
5 - 10 cm	29.02	29.01	28.95	28.93	28.90
10 - 15 cm	29.18	29.14	29.10	29.07	29.04
15 - 20 cm	30.22	30.08	29.98	29.76	29.65
20 - 25 cm	29.77	29.73	29.67	29.61	29.58

Three days after the irrigation, the soil moisture in the topsoil layer decreased very fast. At the irrigation point the soil moisture was only 25.01%, much lower compared to the moisture content after the irrigation finished (32.84% - Table 2). Performing transformations and using the software to determine the moisture content $F(x,y)$ at position $M(x,y)$ in this case with the coefficient of

determination $R^2 = 0.217065$, the linear regression function will not give good prediction. The function $F(x,y)$ is as follows:

$$F(x,y) = 0.019181(25 - x)^{0.755575} (25 - y)^{-0.13168} + 25.28203(y-1.5)^{0.057362}$$

From the monitoring data of the soil moisture at three days after irrigation shown in Table 5 we can see that the measured results were similar to the moisture before irrigation, meaning that after three days the soil moisture returns to its original state, and we need to make the next irrigation.

3.5. Validation

When using the data to setup the model in the section 3.4 we used only half of the experimental data measured on the right (north - south) of the experimental plot. The experimental data obtained from the left side was used to validate the objectivity of each model:

- The moisture before irrigation $g(y)$

Using the function $g(y) = 25.28203(y-1.5)^{0.057362}$ to evaluate the errors between the corresponding values of the function $g(y)$ and the value of the reference sample at the moisture measuring point (soil moisture values at the left plot), we found that none of the relative error (ratio f) of all the samples exceeded 5%. This shows that using the function $g(y) = 25.28203(y-1.5)^{0.057362}$ to determine the characteristics of soil moisture before irrigating is very good (Appendix table 1).

- The moisture determination functions immediately after irrigation and one day after irrigation $F(x,y)$

Validation of the level of errors between the corresponding values of moisture content determination function $F(x,y)$ at position $M(x,y)$ with the values of control samples is the assessment of the total error at this position of the moisture content increases $f(x,y)$ compared with measured values of moisture increase and errors between the experimental and the corresponding error of the moisture function before irrigation $g(y)$ compared with the value of humidity in $M(x,y)$ of the control sample. The results show that the errors between the modeled values and the values of the control data (ratio f) were mostly less than 10%. Only 2 samples had error exceeding 10% but not greater than 15%. Therefore, using the function $F(x,y)$ to determine the soil moisture immediately after the irrigation and one day after the irrigation is acceptable (Appendix table 2 and 3).

4. CONCLUSIONS

In the Red River alluvial soil condition, the maximum field capacity $\beta_{dr} = 32.41\%$; the average soil moisture content before irrigation was $\beta_0 = 28.42\%$ dry weight, then the standard irrigation was $M = 221.84 \text{ m}^3 \text{ ha}^{-1}$.

From the experiments on the Red River alluvial soil with the drip irrigation technique, a mathematical model on the spread of the soil moisture was preliminary constructed. A function for determining the soil moisture at the position $M(x,y)$ in the vertical and horizontal directions was established:

+ The function of moisture content before irrigation

$g(y) = 25.28203(y-1.5)^{0.057362}$; With the correlation coefficient $R = 0.966073$, the coefficient of regression determination $R^2 = 0.933298$

+ The function of moisture content immediately after the drip irrigation:

$F(x,y) = 8.9918 \cdot 10^{-5} (25 - x)^{1.240306} (25 - y)^{2.311185} + 25.28203(y-1.5)^{0.057362}$; the coefficient of determination $R^2 = 0.946491$.

+ The function of moisture content at one day after the drip irrigation:

$F(x,y) = 0.0003325(25 - x)^{1.269}(25 - y)^{1.83194} + 25.28203(y-1.5)^{0.057362}$ the coefficient of determination $R^2 = 0.9256$.

+ At two days and three days after irrigation the coefficients of determination were $R^2 = 0.559009$ and $R^2 = 0.217065$, respectively. The reason of having low R^2 values is, after the irrigation, soil moisture increased with depth leading to reverse permeation. Hence, using a linear regression function for this calculation is inappropriate. This result cannot allow prediction of the soil moisture evolutions.

The model validation by the functions resulted in acceptable errors, therefore the functions above can be used to simulate the soil moisture.

Further experiments should be continued in the field to construct a model that determines the moisture content more accurately based on physical characteristics of the soil and climate conditions.

ACKNOWLEDGEMENT

The authors are grateful to the members of Department of Water Resource, Faculty of Land Management, *Viet Nam National University of Agriculture, Viet Nam* for supplying the soil blocks for this study.

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APPENDIX

1. The moisture function before irrigation $g(y) = 25.28203(y-1.5)^{0.057362}$

Table 1. Evaluation of the error of the moisture function before irrigation $g(y)$

Zt	x	y	error 1	error	Ratio f
24.98	0	2.5	0.0120185	1.01209101	0.04051605
28.87	0	7.5	-0.0299301	0.97051338	0.03361667
29.05	0	12.5	-0.0013764	0.99862453	0.03437606
29.67	0	17.5	-0.0010012	0.9989993	0.03367035
29.68	0	22.5	0.01426048	1.01436264	0.03417664
24.98	5	2.5	0.0120185	1.01209101	0.04051605
28.89	5	7.5	-0.0306226	0.96984152	0.03357015
29.13	5	12.5	-0.0041265	0.995882	0.0341875
29.63	5	17.5	0.00034787	1.00034793	0.03376132
29.66	5	22.5	0.01493456	1.01504664	0.03422275
24.90	10	2.5	0.0152262	1.01534271	0.04077682
28.85	10	7.5	-0.0292371	0.97118618	0.0336633
29.08	10	12.5	-0.0024086	0.99759431	0.03430517
29.49	10	17.5	0.00508401	1.00509696	0.03408264
29.61	10	22.5	0.01662175	1.01676066	0.03433842
25.00	15	2.5	0.01121818	1.01128134	0.04045125
28.88	15	7.5	-0.0302764	0.97017733	0.0335934
29.05	15	12.5	-0.0013764	0.99862453	0.03437606
29.47	15	17.5	0.00576244	1.00577907	0.03412891
29.59	15	22.5	0.01729743	1.0174479	0.03438486
24.96	20	2.5	0.01281946	1.01290198	0.04058101
28.91	20	7.5	-0.0313146	0.96917058	0.03352371
29.12	20	12.5	-0.0037832	0.99622399	0.03421099
29.36	20	17.5	0.00950203	1.00954732	0.03438513
29.56	20	22.5	0.0183118	1.01848049	0.03445469

- Zt is the moisture before irrigation measured at the soil sample corresponds with the left plot
- Error 1 is the error between $\ln g(y)$ and $\ln(Zt)$.
- Error is the absolute error between $g(y)$ and ZT.
- The ratio f is the relative error between $g(y)$ and Zt.

2. The moisture function after the irrigation finished $F(x,y) = 8.9918 \cdot 10^{-5} (25 - x)^{1.240306} (25 - y)^{2.311185} + 25.28203 (y-1.5)^{0.057362}$

Table 2. Evaluation of the error of moisture function after the irrigation finished

Z0t	x	y	error 0	error 1	Total error	Ratio f
32.84	0	2.5	1.01209101	0.82703755	1.83912855	0.056002697
32.31	0	7.5	0.97051338	1.05714928	2.02766267	0.062756505
30.78	0	12.5	0.99862453	0.9658725	1.96449703	0.063823815
30.23	0	17.5	0.9989993	0.91631433	1.91531363	0.063358043
29.73	0	22.5	1.01436264	0.81011604	1.82447869	0.061368271
32.41	5	2.5	1.01209101	0.66336872	1.67545972	0.051695764
32.26	5	7.5	0.96984152	0.81820266	1.78804418	0.055426044
30.72	5	12.5	0.995882	0.79682841	1.79271041	0.058356459
30.15	5	17.5	1.00034793	0.7482129	1.74856083	0.057995384
29.69	5	22.5	1.01504664	1.02374551	2.03879215	0.068669321
29.77	10	2.5	1.01534271	0.70834522	1.72368792	0.057900165
29.64	10	7.5	0.97118618	1.49599867	2.46718485	0.083238355
29.53	10	12.5	0.99759431	1.9705141	2.96810841	0.100511629
29.65	10	17.5	1.00509696	1.70191818	2.70701514	0.091298993
29.63	10	22.5	1.01676066	1.07476471	2.09152537	0.070588099
28.57	15	2.5	1.01128134	0.58437004	1.59565138	0.055850591
29.49	15	7.5	0.97017733	1.91325701	2.88343435	0.097776682
29.38	15	12.5	0.99862453	1.62502614	2.62365067	0.089300568
29.59	15	17.5	1.00577907	1.37233344	2.37811251	0.08036879
29.60	15	22.5	1.0174479	1.29994693	2.31739483	0.078290366
27.23	20	2.5	1.01290198	0.38899378	1.40189576	0.051483502
29.08	20	7.5	0.96917058	2.9058058	3.87497637	0.133252282
29.33	20	12.5	0.99622399	1.0808548	2.07707879	0.070817552

- Z0t is the moisture after the irrigation finished measured at the left plot
- Error 0 is the error between the $\ln f(x, y)$ and $\ln(Z0t - Zt)$.
- Error 1 is the absolute error caused by $g(y)$.
- Error total is the total absolute error caused by $F(x, y)$.
- The ratio f is the relative error between the model and the control experimental value

3. The moisture function one day after the irrigation finished $F(x;y) = 0.00033248(25 - x)^{1.269}(25 - y)^{1.8319} + 25.28203(y-1.5)^{0.057362}$

Table 3. Evaluation of the error of the moisture function one day after the irrigation finished

Z1t	x	y	error 0	error 1	Total error	Ratio f
30.48	0	2.5	1.01209101	1.07764472	2.08973573	0.068560883
32.14	0	7.5	0.97051338	1.14380289	2.11431627	0.065784576
31.98	0	12.5	0.99862453	0.68919575	1.68782028	0.05277737
30.85	0	17.5	0.9989993	0.6713181	1.6703174	0.05414319
29.74	0	22.5	1.01436264	1.76453869	2.77890133	0.093439856
30.42	5	2.5	1.01209101	0.82083697	1.83292797	0.060254042
32.01	5	7.5	0.96984152	0.9031541	1.87299562	0.058512828
31.85	5	12.5	0.995882	0.55931803	1.55520003	0.048828886
30.26	5	17.5	1.00034793	0.94729954	1.94764747	0.064363763
29.72	5	22.5	1.01504664	1.32937864	2.34442527	0.078883758
28.04	10	2.5	1.01534271	0.98712955	2.00247226	0.071414845
29.82	10	7.5	0.97118618	2.01647441	2.98766059	0.100189825
30.02	10	12.5	0.99759431	1.12343477	2.12102909	0.070653867
30.21	10	17.5	1.00509696	0.66816571	1.67326266	0.055387708
29.64	10	22.5	1.01676066	1.8455537	2.86231437	0.096569311
27.12	15	2.5	1.01128134	0.87397989	1.88526123	0.069515532
29.29	15	7.5	0.97017733	2.85176735	3.82194469	0.130486333
29.58	15	12.5	0.99862453	1.1910592	2.18968373	0.074025819
29.20	15	17.5	1.00577907	0.5159031	1.52168217	0.052112403
29.41	15	22.5	1.0174479	0.47280646	1.49025436	0.050671688
29.66	20	2.5	1.01290198	0.89396465	1.90686663	0.064290851
29.57	20	7.5	0.96917058	1.67294417	2.64211475	0.089351192

- Z1t is the moisture after the irrigation finished ,measured at the left plot
- Error 0 is the error between the $\ln f(x, y)$ and $\ln(Z1t-Zt)$.
- Error 1 is the absolute error caused by $g(y)$.
- Error total is the total absolute error caused by $F(x, y)$
- The ratio f is the relative error between the model and the control experimental value

Growth and Sodium Absorption of Sweet Sorghum in Agricultural Land Damaged from Tsunami of the Great East Japan Earthquake

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ABSTRACT

The Great East Japan Earthquake on March 11, 2011, created a colossal tsunami that damaged agricultural land along the east coast of Japan, washing away topsoil, flooding areas with salt-water and causing poor drainage due to the destruction of aggregate structures. Cultivation experiments of sweet sorghum was carried out in an agricultural land at a distance of 2 km from the coastline in Miyagi, Japan in 2012 so as to obtain knowledge of the effect of sodium removal from soil by using sweet sorghum, which has a high dry matter production and salinity tolerance. Sweet sorghum was sown on May 26 and grew vigorously. The plant length was about 3.5 m at the end of September. After harvest, the amount of sodium absorbed by the plant and in soil was estimated using atomic absorption analysis. Lower internodes in the stems accumulated more sodium than higher ones. The addition of fertilizer on July 6 increased the internode length of the lower internodes, resulting in the increase of internode volume and dry matter and also the increase of amount of sodium in the stems. However, despite sweet sorghum's ability to remove sodium from the soil, it was not enough as most of the sodium carried inland by the tsunami still remained. This result suggested that plowing-in of organic matter produced by sweet sorghum would be useful for improving the aggregate structure of soil because outflow of sodium would be expected by large amounts of precipitation in Japan.

Keywords: Growth, Sodium absorption, *Sorghum bicolor* Moench, sweet sorghum, the Great East Japan Earthquake, Tsunami.

1. INTRODUCTION

On March 11, 2011, the Great East Japan Earthquake created a colossal tsunami that damaged agricultural land along the east coast of Japan, e.g. washed away soil, caused flooding and poor drainage due to the destruction of aggregate structures. It was necessary to reduce the salt content of soil in agricultural lands as soon as possible. Repeated use of puddling and washing or underdrainage can be conducted using irrigation water to help remove the salt from paddy fields (Hoshi and Yusa, 2012). However, it is difficult to reduce the salt content in upland fields using the same methods. Lately, Sweet sorghum (*Sorghum bicolor* Moench) has garnered attention as a biomass energy crop since it accumulates a lot of sugar in the stem (Smith et al., 1987). Sweet sorghum also has a high dry matter production and is a medium salt tolerance plant (Almodares and Sharif, 2007). However, there is little information about the growth of sweet sorghum in the fields damaged by tsunami and the amount of sodium absorption by sweet sorghum. We evaluated the growth of sweet sorghum in damaged fields from seawater and the effect of sodium removal from the soil by growing sweet sorghum.

2. MATERIALS AND METHODS

2.1. Field condition

Field experiments were carried out in a field in Higashimatushima at a distance of 2 km from the coast in Miyagi Prefecture, Japan in 2012. On March 11 in 2011, after the tsunami, the field was

flooded with about 50 cm of seawater and about 3 cm of mud. In May of 2011, tillage was done without removing the mud and no crop was grown in the field that year. In 2012, there were cracks appeared in the soil's surface before tilling (Fig. 1a). After tillage for seeding, poor infiltration of rain water was observed (Fig. 1b). These occurrences are considered to have been caused by the destruction of aggregate structure by sodium in the seawater.



Fig. 1. Soil surface before tillage (a) and after tillage (b) for seeding in 2012

Note: Sodium in seawater destroyed aggregate structure soil, resulting in soil cracking and crust (a) and poor infiltration of rainwater (b).

2.2. Planting and measurement

Sweet sorghum (cv. KCS105, Super sugar sorgo) was sown on the 26th of May in 2012. The seedlings were thinned to one plant per hill with 0.75 and 0.15 m spacing between and within the rows. Fertilizer with 10g m^{-2} N, 9g m^{-2} K_2O , and 10g m^{-2} P_2O_5 were applied before seeding (control plot, Cont) and 5g m^{-2} N (ammonium sulfate) was applied at 41 days after seeding (DAS41, the 10th leaf stage) (additional fertilization plot, AF). Each plot has two replications. The plant height and the expanded leaf number were recorded about every 2 weeks from DAS22. At harvest (DAS116), length, diameter of internodes and head length, dry weight of head, leaf and stem were measured. Internode cross-section area was calculated by the major and minor of diameter in the middle of the internode as an ellipse (Nakamura et al. 1995). Internode volume was calculated by the product of the cross-section area and the length. The internode enclosed with the n th leaf sheath was defined as the n th internode (IN n) (Goto et al., 1994).

2.3. Na content in stem

Na concentration in plants, especially in the stems, were measured by atomic absorption analysis. The lower internode (at 10 cm above ground), the middle internode (at the middle of stem) and the upper internode (just under the head) of 3 main stems with an average length was analyzed for Na concentration (mg/g DW). The main stem was divided into 3 equal parts in length and dry weight of each part was calculated and then Na content in each part was obtained by Na concentration of 3 internode positions. Na content in a stem was estimated by the sum of 3 parts in stem.

2.4. Na content in soil

Na content in the soil at different depths were also measured by atomic absorption analysis. Soil samples at different depths (0-15 cm, 15-30 cm, 30-45 cm and 45-60 cm from the ground surface) in the field was obtained at DAS-30, DAS 16, DAS 40, DAS70 and DAS116 using a soil auger. Soil

samples from three different sections were mixed and air-dried in one plot so as to compare its Na content with soil samples obtained using the same method mentioned above from a Miyagi University experimental field not affected by the tsunami at DAS40 and DAS116. Soil samples (Fallow) were obtained from the sections of no cultivation in the field in Higashimatushima in order to elucidate the effect of sweet sorghum cultivation to the Na content in the soil.

3. RESULTS AND DISCUSSION

3.1. Plant growth

Sweet sorghum grew vigorously in the field in 2012 (Fig. 2). However, an abundance of plants with some prop roots elongating on the ground were observed (Fig. 3). This is due to the Na in the seawater having destroyed the aggregate structure of surface soil thus rendering it too hard for the prop roots to penetrate.



Fig. 2. Sweet sorghum plant growing vigorously in the field at DAS97 in 2012

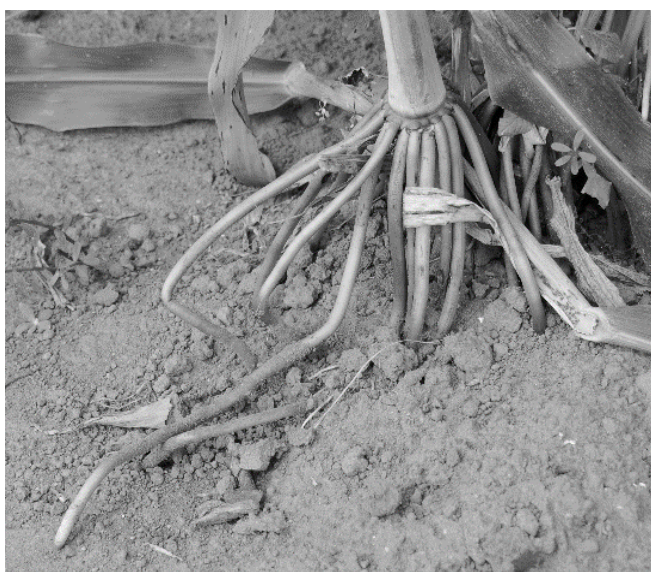


Fig. 3. Prop roots elongating on the ground surface because of hard surface soil (crust)

In both Cont and AF, the leaf number increased straightly from DAS27 to DAS84 (Fig. 4a). During this period, the leaf number per day was 0.25 and 0.26 in Cont and AF respectively. At harvest, there was no significant differences in the leaf number between 20.2 in Cont and 19.7 in AF (Table 1). The plant height increased 7.3 cm/day in Cont and 7.7 cm/day in AF from DAS41 through DAS 70 (Fig. 4b). Although the leaf number of AF was smaller than Cont, the plant length of AF was higher than Cont at harvest (DAS116). There were no significant differences in leaf number, plant length, stem length, head length and dry weight of main stem between Cont and AF (Table 1).

Table 1. Leaf number, plant length, stem length, head length and dry weight of head, leaf and stem of main stem at harvest

plot	leaf number	plant length (cm)	stem length (cm)	head length (cm)	Dry weight (g)			
					head	leaf	stem	total
Control	20.2 ± 0.3	365 ± 5	338 ± 5	29.4 ± 0.6	65.4 ± 6.1	39.1 ± 1.8	80.5 ± 6.0	185 ± 13
AF	19.7 ± 0.2	377 ± 4	349 ± 4	31.4 ± 0.4	56.3 ± 4.2	40.2 ± 1.4	79.8 ± 4.1	176 ± 9

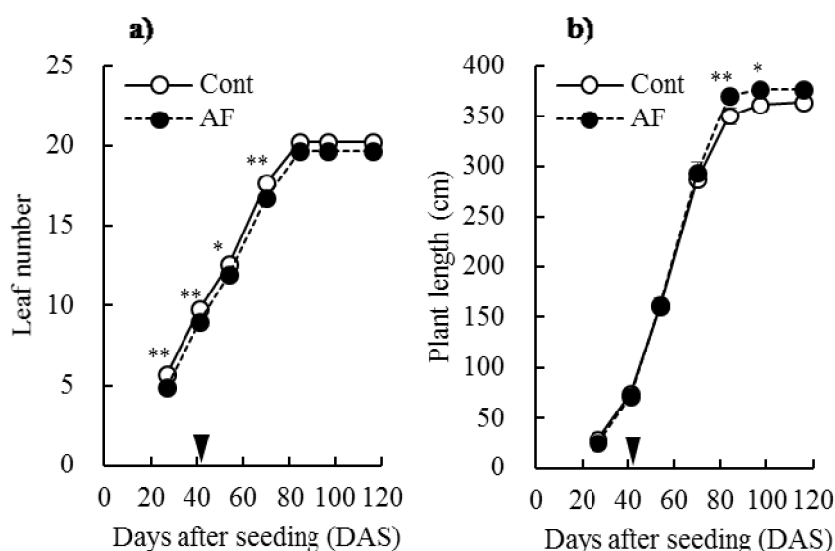


Fig. 4. Changes in leaf number (a) and plant length (b)

Note: An arrow indicates the time of additional fertilization (DAS41); * and ** represent significant differences at 5 %

3.2. Internodes characteristics

In both Cont and AF, the length of IN13 was the longest internode from IN 7 to IN18 (Fig. 5a). In AF, internode lengths were significantly longer than in Cont from the lower to the middle of the stem, but the internode diameter didn't increase (Fig. 5b). The volume of internodes in AF were larger than those in Cont in the lower to the middle of the stem (Fig. 5c) and the dry weight of internodes in AF were larger than those in Cont in the lower of the stem (Fig. 5d). Thus, the additional fertilizer significantly increased the internode length and the dry weight of the lower of the stem.

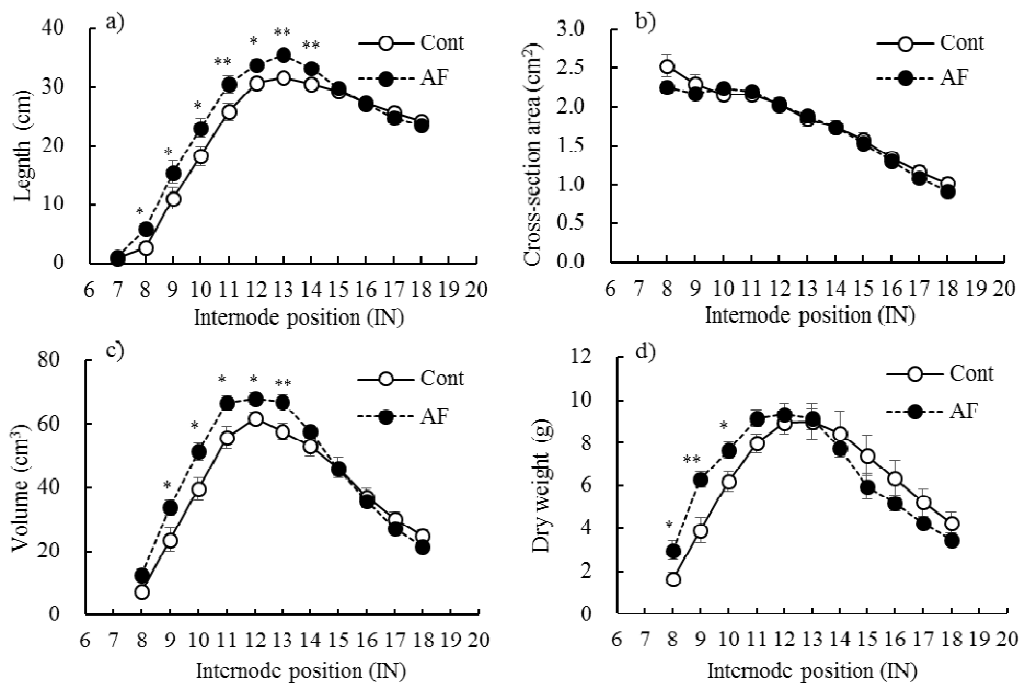


Fig. 5. Internode length (a), cross-section area (b), volume (c) and dry weight (d) based on the internode position (IN) from IN7 to IN18. The internode position including neck internode just below head was exclude for analysis of internode characteristics

Note: ** and * represent significant differences at 5 % and 1 % level respectively.

3.3. Na content in stem

Concentration of Na in the stem increased as plants grew (Fig. 6a, 6b). At harvest, concentration of Na increased with the lower internodes (Fig. 6b). In the middle and lower internode, Na concentration was significantly higher in AF than in Cont. Estimated content of Na in the stem was larger in AF than in Cont mainly due to larger dry weight (Fig. 5d) and higher concentration of Na in the lower internode in AF (Fig. 6c). The result suggested that additional fertilizer was effective in the increase of Na absorption.

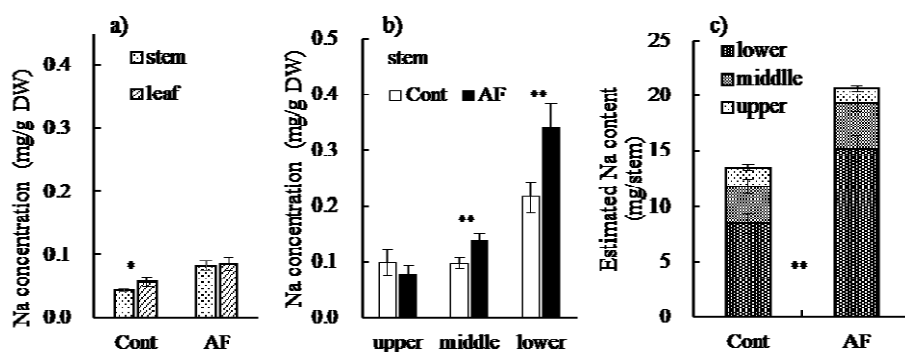


Fig. 6. Concentration of Na in stem and leaf at DAS70 (the 15 leaf stage) (a), in upper, middle and lower internode in stem at DAS116 (harvest) (b), and the estimated content of Na in upper, middle and lower part of stem at DAS116 (c)

Note: ** and * represent significant differences at 5 % and 1 % level respectively.

3.4. Na content in soil

Fig. 7 shows changes of Na content in soil at different depths. Soil infiltrated with seawater contained large quantities of Na compared with the field not affected by seawater (DAS40 and DAS116). Although variation of Na content was large during the growing season, Na content in the soil tended to decrease in all depths over harvest time. At DAS116, soil in AF contained lower Na. This result suggested that expansion of the roots by using additional fertilizer might absorbed more Na. However, Chiba et al. (2012) reported that the infiltration of rainwater and its discharge through underdrains eliminated considerable salt from the paddy fields. Our experimental field located in Higashimatsushima received 286 mm of precipitation from seeding to harvest in 2012. The decrease of Na content in all depths as shown in Fig. 7 might be attributed to the infiltration of rainwater though no drainage was installed in the field. In order to elucidate the effect of expansion roots through additional fertilizer on Na content in the soil, further experiments are needed.

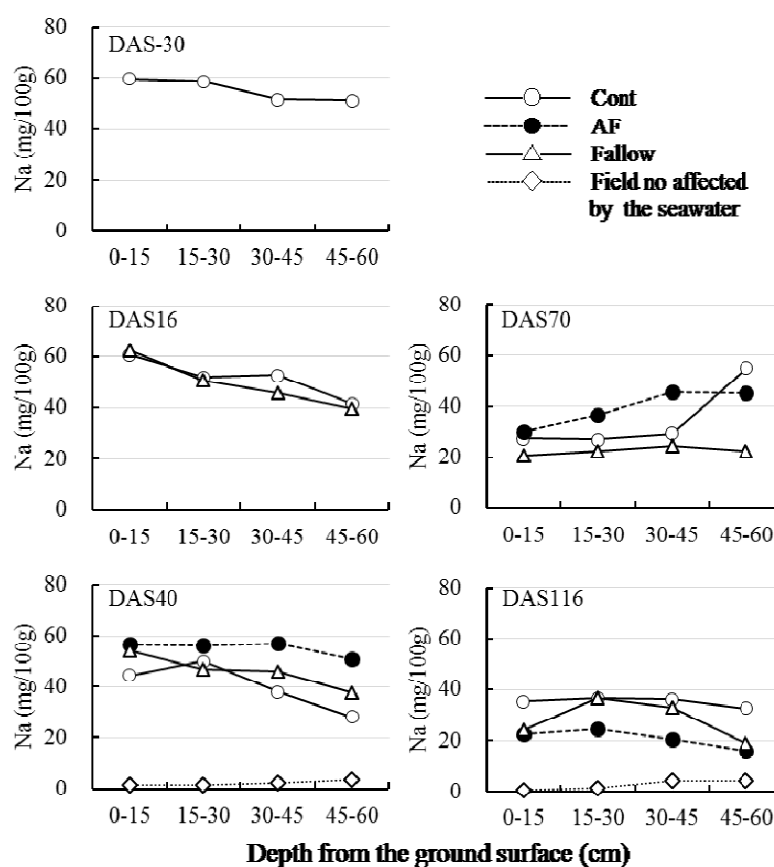


Fig. 7. Changes of Na content in soil (100g in air drying) at different depths from -30 days after seeding (DAS-30) to DAS116

4. CONCLUSIONS

In sweet sorghum, lower internodes in the stems accumulated more sodium than in the higher ones. The additional fertilizer increased the middle and lower internodes in length, resulting in the increase of internode volume and dry matter in lower internodes and also the increase of Na content in the stems in AF. However, the amount of sodium absorbed by only sweet sorghum was not enough to remove all the sodium caused by the tsunami from the soil in a short period of time. This result

suggested that, instead of salt removal, plowing-in of a large amount of organic matter produced by sweet sorghum would be useful for improving the aggregate structure of soil because outflow of sodium can be expected by the large amount of precipitation in Japan. When sweet sorghum is used as organic material for improving the aggregate structure in Japan, combining it with lime materials for removal of exchangeable sodium and winter crops with salinity tolerance such as barley would be more effective.

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Traits of Drought Resistance of NERICA with The Effects of Compost, Compared with Asian Rice and African Rice in The Field

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ABSTRACT

Recently NERICA (New Rice for Africa) was developed by a crossing of African rice (*Oryza glaberrima* Steud.) and Asian rice (*Oryza sativa* L.) in West Africa, and is considered to be drought resistant, but drought resistance of NERICA is not clarified enough. In this research, NERICA, Asian rice and African rice were cultivated in the field in Shizuoka University under drought to compare dry matter production, stomatal conductance, characteristics of absorbing water and leaf to clarify traits of drought resistance. Fertilization level was 4gN m⁻² and in compost plots compost levels were 2 and 4kg m⁻². Stomatal conductance, soil water content, SPAD value, leaf thickness and top dry weight at harvest were measured. Dry weight was higher in one of NERICA lines and NERICA 7. Ear weight was higher in Dular and one NERICA line. Effects of compost on ear weight were different between lines. In one NERICA line, compost increased ear weight but in another it decreased. In NERICA 7, Dular and *sativa* parent showed high stomatal conductance. Compost increased stomatal conductance. In soil water content in deep layer, Dular and IRAT 13 showed lower water content than NERICA. SPAD values of *sativa* parent and NERICA were higher than Dular and *glaberrima* parent. Leaf thickness of NERICA, *sativa* parent and Dular were higher than *glaberrima* parent. Cultivar differences in top dry weight were significantly correlated with those in stomatal conductance ($r=0.616^*$), cultivar differences in ear weight were significantly correlated with those in leaf thickness ($r=0.646^{**}$), SPAD value ($r=0.537^*$) and stomatal conductance ($r=0.534^*$).

Keywords: Compost, drought resistance, leaf thickness, NERICA, stomatal conductance, water use efficiency.

1. INTRODUCTION

Global water shortages are getting worse and drought is the major constraint to crop production and breeding for drought resistance in rice is important (Blum, 2009; Boyer, 2010; Kamoshita, 2011; Kumar et al. 2014; Serraj et al., 2011). Recently NERICA (New Rice for Africa) was developed by a crossing of African rice (*Oryza glaberrima* Steud.) and Asian rice (*Oryza sativa* L.) in West Africa (WARDA, 1999), and is considered to be drought resistant, but drought resistance of NERICA and the effects of compost under drought condition are not clarified enough.

Fujii et al. (2004, 2008 and 2013) have been investing the characteristics of NERICA under drought condition but key characteristics contribute to yield and the effects of compost under drought are still not clarified yet.

In this research, NERICA, Asian rice and African rice were cultivated in the field under drought condition to compare dry matter production, stomatal conductance, water absorbing characteristics and leaf characteristics to clarify traits of NERICA under drought condition. In two NERICA lines effects of compost under drought condition was tested.

2. MATERIALS AND METHODS

2.1. Materials

In this study four NERICA cultivars and three NERICA lines which showed superior drought resistant in our previous reports (Fujii et al., 2005, 2013) and their two parent cultivars as follows were tested. Three Asian rice (*O. sativa* L.) cultivars were also tested.

Four NERICA cultivars tested were NERICA 1, 2, 3 and 7. Three NERICA lines tested were WAB450-24-3-P3-1-HB, WAB450-I-B-P-82-2-1 and WAB450-I-B-P-157-2-1. Two parent cultivars of NERICA tested were *sativa* parent: WAB56-104 and *glaberrima* parent: CG14. Three Asian rice (*O. sativa* L.) cultivars, Koshihikari (japonica, lowland, Japan), Dular (indica, lowland-upland, India), IRAT13 (japonica, upland, Cote d'Ivoire), were also tested.

2.2. Methods

2.2.1. Cultivation

Rice cultivars and lines were seeded on June 13, 2011 in paper pots and planted at upland field in the vinyl house of Shizuoka University, Japan on July 4. Sides of vinyl house were kept open. Irrigation was applied on July 4, 5, 6, August 4, 5, 10 and 18, and after that no irrigation was applied.

Fertilization level was 4gN m⁻² by compound fertilizer (N:P₂O₅:K₂O = 8:8:8%) as basal treatment. In compost plots in WAB450-24-3-P3-1-HB and WAB450-I-B-P-82-2-1, compost made from rice straw was applied at the rate of 2 kg m⁻². In WAB450-I-B-P-82-2-1 compost application at the rate of 4 kg m⁻² was also created.

2.2.2. Measurements of dry weight

Plants were sampled at harvest on November 30. After dividing into leaf, dead leaf, stem and ear, dry weight was measured after desiccating in the drying oven at 80°C for 48 hours. Measurements were replicated 10 times in each plot.

2.2.3. Measurements of stomatal conductance

Stomatal conductance was measured at the center of abaxial side of topmost fully expanded leaves by dynamic diffusion porometer (AP4, Delta-T Devices Ltd., UK) on sunny days during the daytime. Measurements were made on September 13, 16 and October 9. Measurements were replicated six times in each plot.

2.2.4. Measurements of soil water content

Soil water content at individual depths were measured by TRIME-T3 tube access probe system (IMKO micromodultechnik, Germany) by TDR (Time domain reflectometry) method. Measurements were made at intervals of 10cm depth on September 14, October 5 and 25. Data presented are average of 20cm depth increments from surface to 60cm depth. Measurements were replicated four times in each plot.

2.2.5. Measurements of SPAD value

SPAD value was measured by SPAD meter (SPAD502, Konica Minolta, Tokyo, Japan) on September 15, 20 and October 6. Measurements were replicated 10 times in each plot.

2.2.6. Measurements of leaf thickness

Leaf thickness was measured by micrometer (No.193-111, Mitutoyo, Kawasaki, Japan) at the middle length of topmost fully expanded leaves avoiding midrib on September 15, 20 and October 9. Measurements were replicated 10 times in each plot.

2.3. Data analysis

Tukey's test was applied to determine any significant differences at the level of 5% between the means of the data. Significance of regression coefficient was analyzed at the level of 5% and 1%.

3. RESULTS

3.1. Top dry weight

Top dry weight at harvest on November 30 was maintained high in WAB450-I-B-P-82-2-1 (one of NERICA lines) and NERICA 7 (Fig. 1). Top dry weight in IRAT 13 and Dular (drought resistant cultivars in Asian rice) and CG14 (*glaberrima* parent of NERICA) also tended to be high.

Ear weight was highest in Dular (drought resistant cultivar in Asian rice) and followed by one of NERICA line (WAB450-I-B-P-82-2-1) which maintained highest top dry weight and tended to show higher ear weight than *sativa* parent of NERICA (WAB56-104).

Top dry weight of *glaberrima* parent of NERICA (CG 14) was almost as high as Dular but proportion of dead leaf was large and got no ear weight.

Effects of compost on ear weight were different between NERICA lines. In one NERICA line (WAB450-24-3-P3-1-HB), compost tended to increase ear weight but in another line (WAB450-I-B-P-82-2-1) it tended to decrease ear weight by the application rate of compost at the rate of 2kg m⁻² and at the rate of 4kg m⁻² in this line it tended to decrease ear weight more.

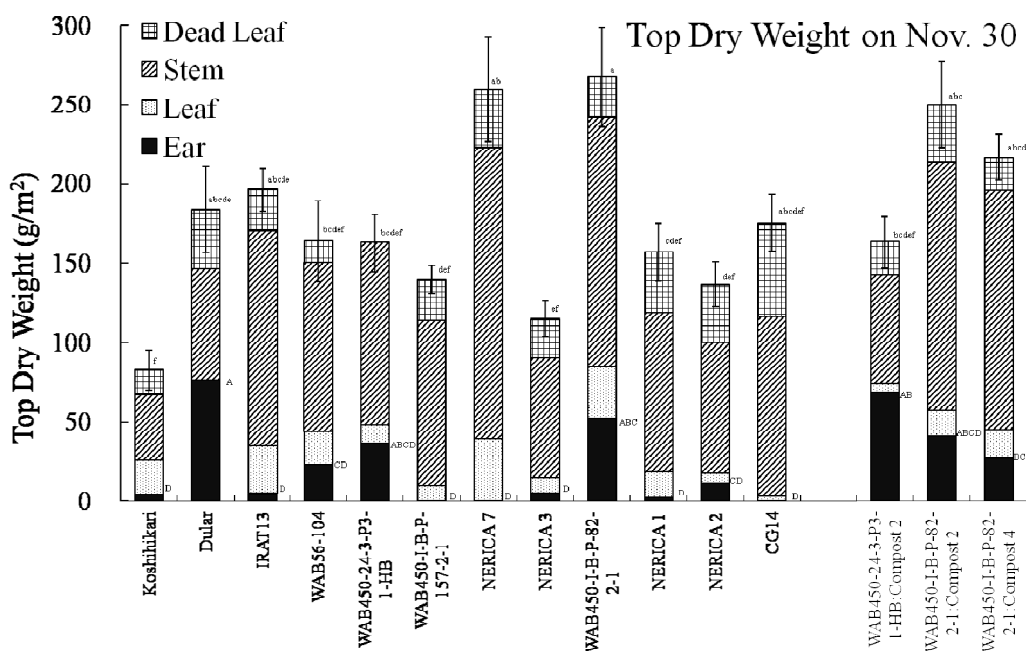


Fig. 1. Top dry weight of each organ at harvest on November 30

Note: Bars show standard error. Different letters indicate significant difference at 5% level (Tukey's test).

3.2. Stomatal conductance

NERICA 7, WAB450-I-B-P-82-2-1 (one of NERICA line that maintained highest top dry weight), *sativa* parent of NERICA (WAB56-104) and Dular tended to show high stomatal conductance under drought condition on September 16 (Fig. 2). Application of compost tended to increase stomatal conductance.

3.3. Soil water content

In soil water content at surface soil layer (0-20cm) on October 5, differences among cultivars and lines were small (Fig. 3).

At deep soil layer (40-60cm) on October 5, Dular, IRAT 13 and *sativa* parent of NERICA (WAB56-104) which are drought resistant Asian rice cultivars tended to show lower water content than NERICA cultivars and lines (Fig. 4).

Application of compost at the rate of 2 kg m⁻² tended to decrease soil water content at deep layer.

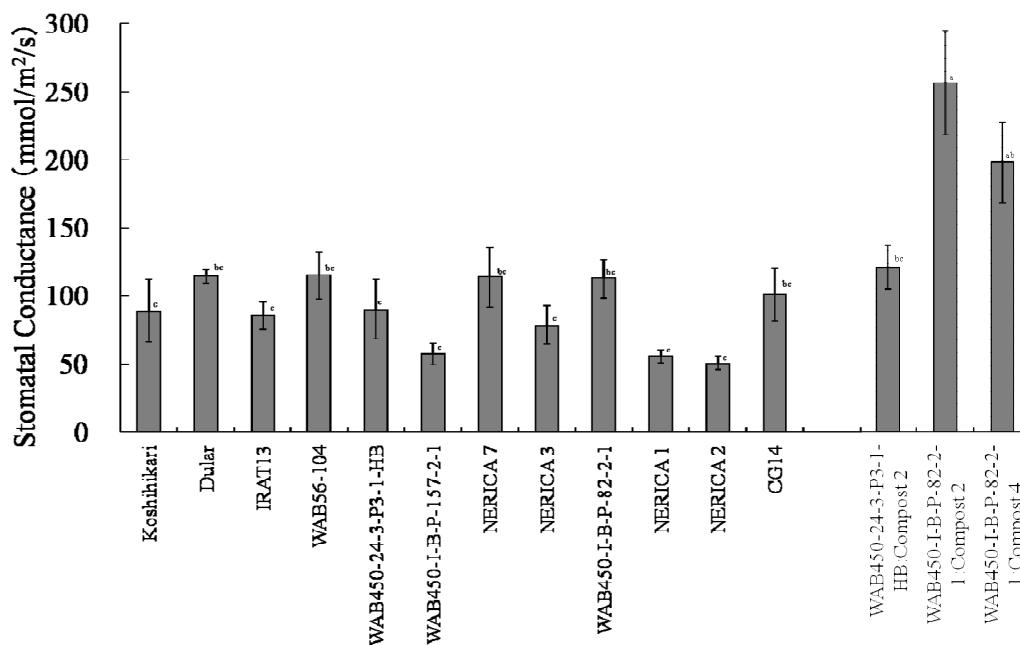


Fig. 2. Stomatal conductance measured on September 16

Note: Bars show standard error. Different letters indicate significant difference at 5% level (Tukey's test).

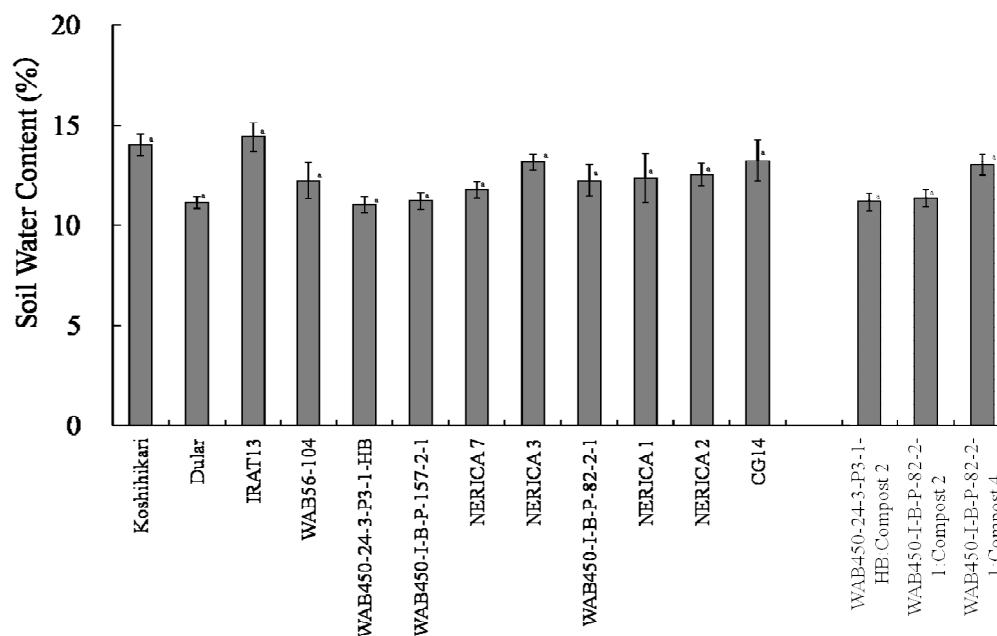


Fig. 3. Soil water content at surface layer (0-20cm) on October 5

Note: Bars show standard error.

Same letters indicate differences were not significant at 5% level (Tukey's test).

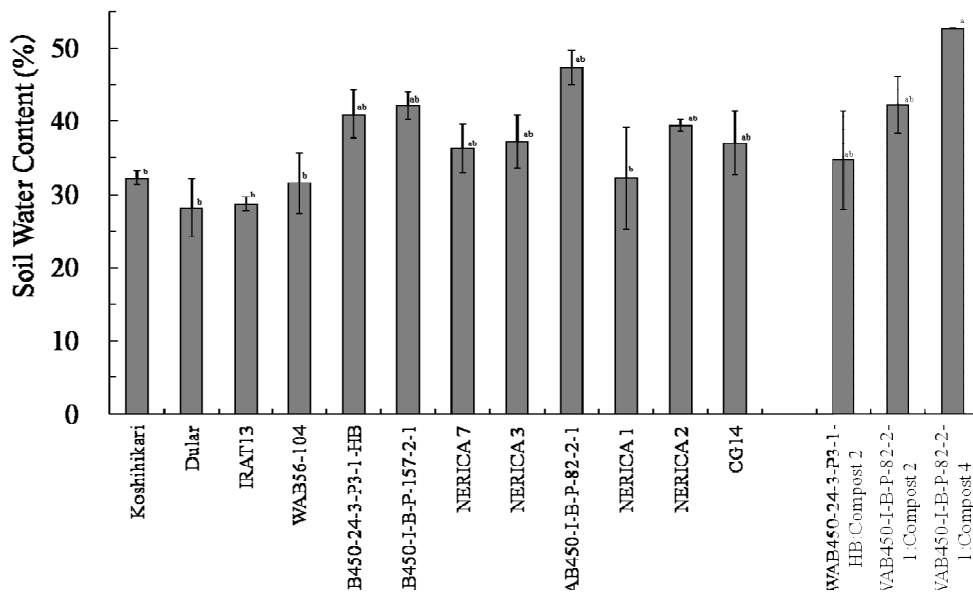


Fig. 4. Soil water content at deep layer (40-60cm) on October 5

3.4. SPAD value

Average SPAD values of *sativa* parent (WAB56-104), NERICA 3, IRAT 13, NERICA 1 and NERICA 2 tended to be higher than Koshihikari, *glaberrima* parent of NERICA (CG 14) and Dular (Fig. 5).

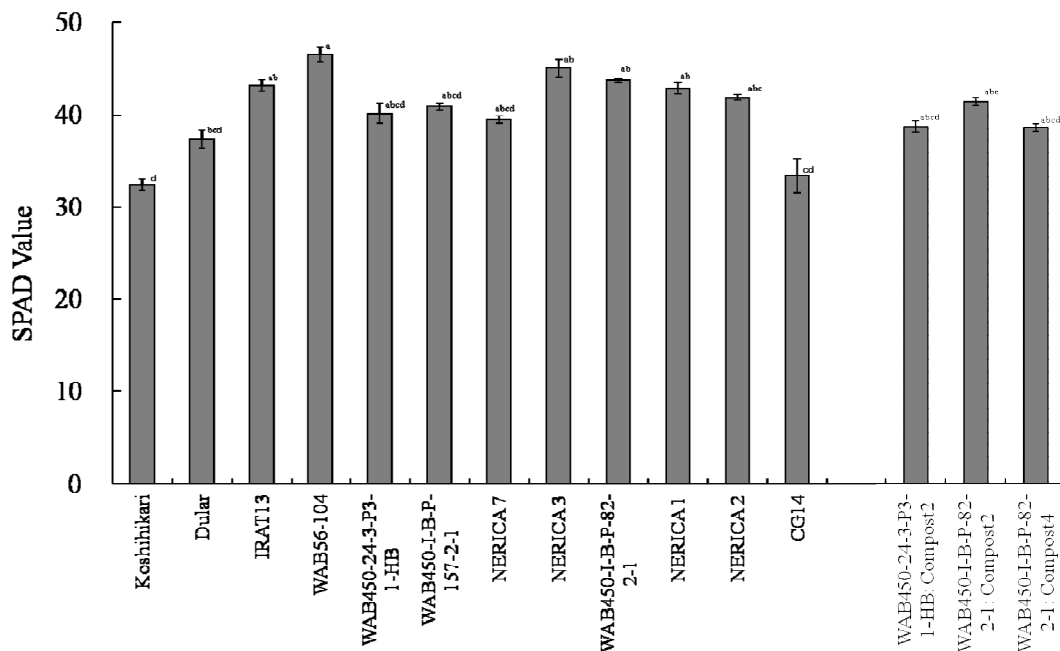


Fig. 5. Average SPAD value

Note: Bars show standard error.

Different letters indicate significant difference at 5% level (Tukey's test).

3.5. Leaf thickness

Leaf thickness of NERICA 3, *sativa* parent of NERICA (WAB56-104) and Dular were higher than *glaberrima* parent of NERICA (CG 14) and Koshihikari (Fig. 6). In one NERICA line (WAB450-24-3-P3-1-HB) compost tended to increase leaf thickness.

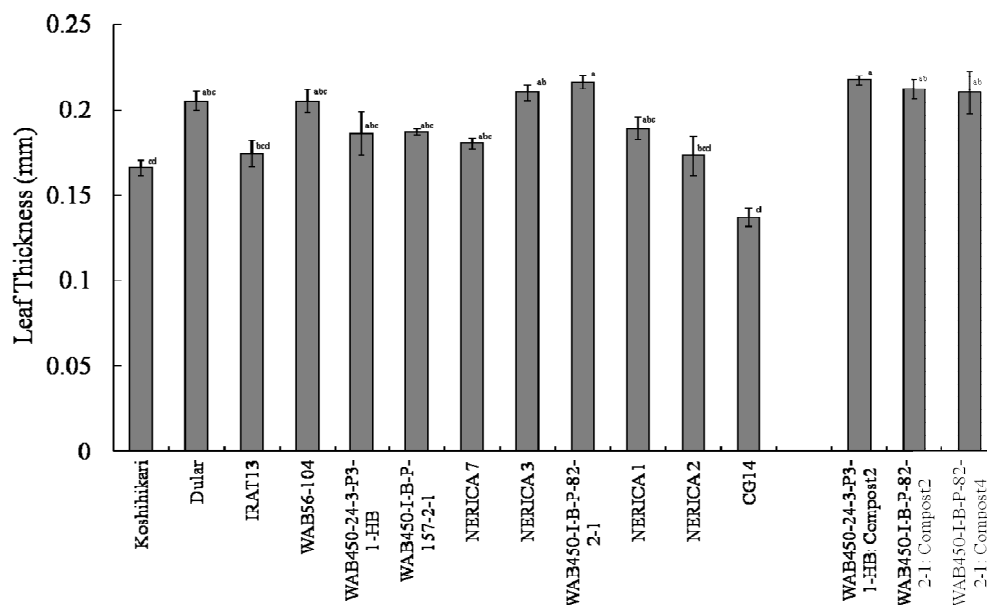


Fig. 6. Average leaf thickness

Note: Bars show standard error.

Different letters indicate significant difference at 5% level (Tukey's test).

3.6. Relationship between stomatal conductance and top dry weight

Cultivar and fertilization differences in top dry weight were significantly correlated with those in stomatal conductance at 5% level ($r=0.616^*$) (Fig. 7).

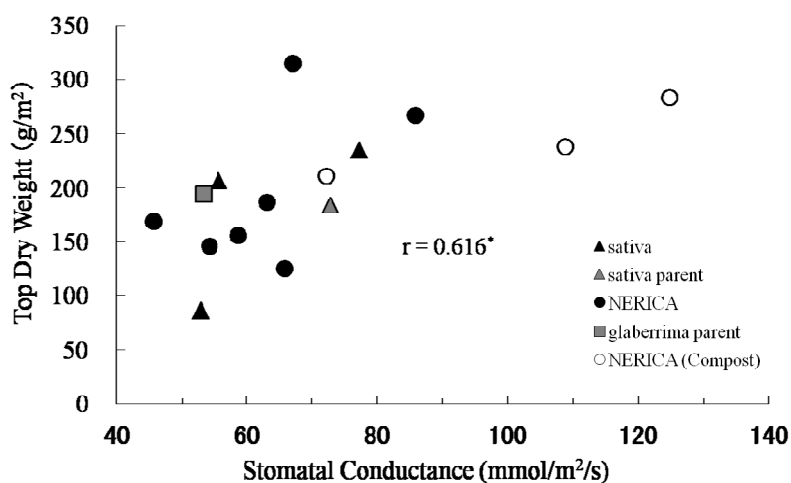


Fig. 7. Correlation between stomatal conductance and top dry weight

Note: * show significant at 5% level.

3.7. Relationship between leaf thickness and ear weight

Cultivar and fertilization differences in ear weight were significantly correlated with those in leaf thickness at 1% level ($r=0.646^{**}$) (Fig. 8).

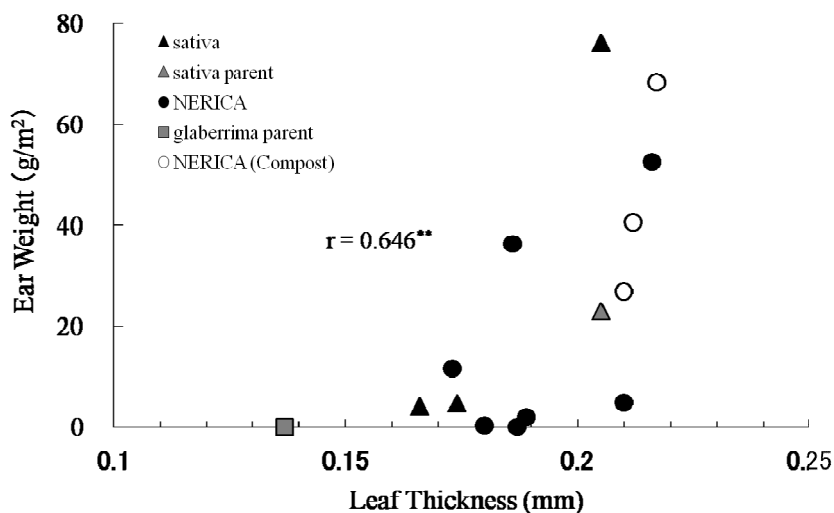


Fig. 8. Correlation between leaf thickness and ear dry weight

Note: ** show significant at 1% level.

3.8. Relationship between leaf thickness and SPAD value

Cultivar and fertilization differences in leaf thickness were significantly correlated with those in SPAD value at 5% level ($r=0.537^*$) (Fig. 9). In standard fertilizer plots correlation coefficient was 0.695^* .

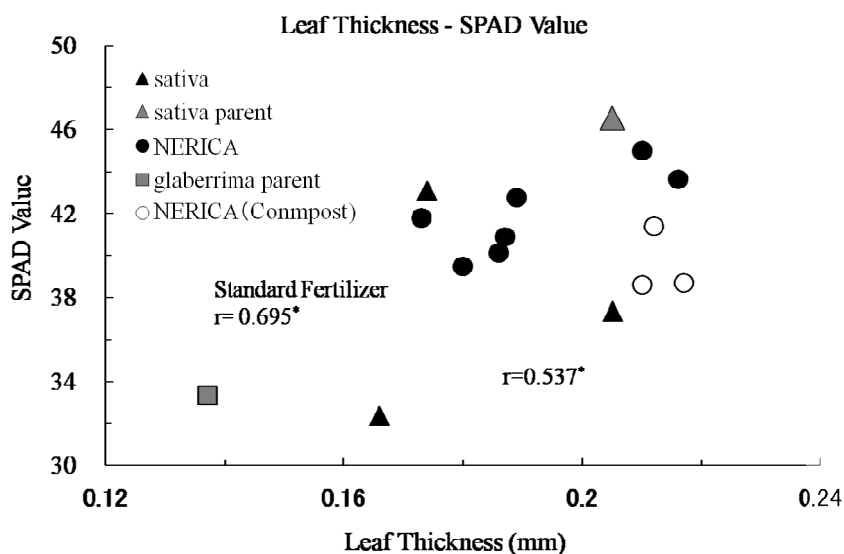


Fig. 9. Correlation between leaf thickness and SPAD value

Note: * show significant at 5% level.

3.9. Relationship between leaf thickness and stomatal conductance

Cultivar and fertilization differences in leaf thickness were significantly correlated with those in stomatal conductance at 5% level ($r=0.534^*$) (Fig. 10). In *sativa* cultivars correlation coefficient was high ($r=0.962^*$) and was significant at 5% level.

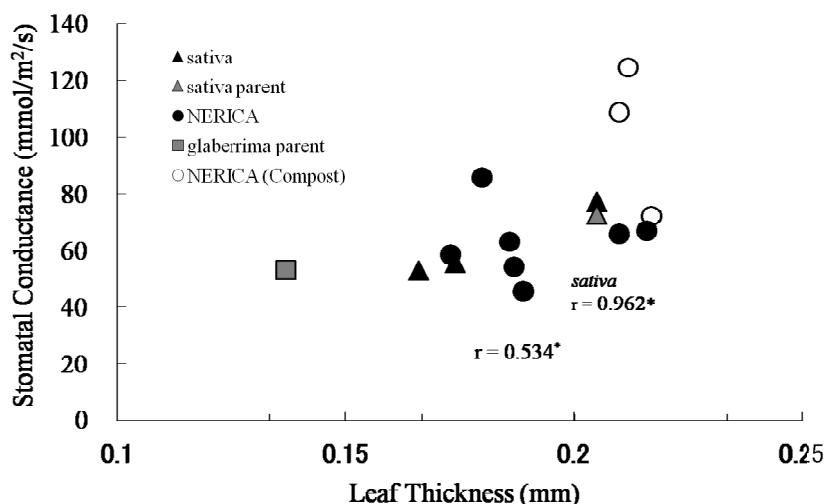


Fig. 10. Correlation between leaf thickness and stomatal conductance

Note: * show significant at 5% level.

4. DISCUSSION

Ear weight was highest in Dular, drought resistant cultivar in Asian rice and followed by one of NERICA line which showed higher top dry weight (Fig. 1). In drought resistant Asian rice cultivars (Dular, IRAT13, *sativa* parent of NERICA (WAB56-104)) soil water content at deep soil layer tended to be lower than other cultivars (Fig. 4). On the contrary in NERICA cultivars and lines soil water content tended to be maintained higher at deep soil layer. The tendency to maintain higher soil water content in NERICA compared with drought resistant Asian rice under drought condition are coincide with author's previous reports (Fujii et al. 2004, 2008, 2013).

Cultivars and lines that maintained high top dry weight showed high stomatal conductance (Fig. 7). Cultivar and line differences in top dry weight were significantly correlated with those in stomatal conductance ($r=0.616^*$) (Fig. 7). Cultivar and line differences in ear weight were significantly correlated with those in leaf thickness ($r=0.646^{**}$) (Fig. 8). Cultivar and line differences in leaf thickness were significantly correlated with those in SPAD value ($r=0.537^*$) (Fig. 9) and stomatal conductance ($r=0.534^*$) (Fig. 10).

In the author's previous reports (Fujii et al., 2004, 2008, 2013) correlation between stomatal conductance and top dry weight under drought condition and correlation between leaf thickness and dry weight at harvest were shown, but correlation between leaf thickness and yield (ear weight) have not shown.

Vadez et al. (2014) pointed out the importance of water availability at reproductive stage. It seems that in drought condition with little precipitation, consuming soil water conservative at vegetative stage and remaining much water at reproductive stage may be essential. Luo (2010) pointed out the importance of water-saving characteristics by high water use efficiency in rice.

High leaf thickness and SPAD values of NERICA seem to be effective to maintain dry matter production and yield under limited water condition with high water use efficiency by increasing the potential of photosynthesis. Condon et al. (2004) showed cultivar differences in water use efficiency in wheat. In rice NERICA seems to be appropriate for the cultivation under drought condition with the effective use of limited water for the sustainable crop production.

Effects of compost on ear weight were different between NERICA lines (Fig. 1). Compost increased stomatal conductance and as the results it seems to consume more water at vegetative stage and tended to decrease soil water content at deep layer at reproductive stage and in one NERICA line tended to decrease ear weight. Though there are possibilities to increase growth under drought condition, in this experiment the effects were different between NERICA lines and in some cases it tended to decrease ear weight. More research about appropriate cultivars to compost and adequate amount seem to be needed to clarify the effects of compost under drought.

5. CONCLUSIONS

Ear weight was highest in Dular, drought resistant cultivar in Asian rice and followed by one of NERICA line which showed higher top dry weight. In drought resistant Asian rice cultivars soil water content at deep soil layer tended to be lower than other cultivars. On the contrary in NERICA cultivars and lines soil water content tended to be maintained higher at deep soil layer.

Cultivars and lines that maintained high top dry weight showed high stomatal conductance. Cultivar and line differences in ear weight were significantly correlated with those in leaf thickness. Cultivar and line differences in leaf thickness were significantly correlated with those in SPAD value and stomatal conductance.

High leaf thickness and SPAD values of NERICA seem to be effective to maintain dry matter production under limited water condition with high water use efficiency. NERICA seems to be appropriate for the cultivation under drought condition with the effective use of limited water for the sustainable crop production.

Effects of compost were different between NERICA lines and more research about appropriate cultivars and lines to compost and adequate amount seem to be needed to clarify the effects of compost under drought.

ACKNOWLEDGEMENT

I thank Emeritus Professor Horie and Professor Shiraiwa, Kyoto University, for kindly giving an opportunity to use seeds of NERICA lines in this study.

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Activity for Simulating Rice Productivity in Musi River Basin, Indonesia

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ABSTRACT

Rice production in Musi River Basin is conducted in 4 ecotypes: irrigated, rainfed, tidal swamp and freshwater swamp. The variation makes it difficult to estimate climate change impact on the rice production, because cultivation methods and water requirements were much different among ecotypes. This study firstly conducted investigation at 150 farmers' fields to characterize rice production in the ecotypes. Based on the research, parameters for rice growth model SIMRIW-rainfed were determined. To simulate weather and water availability, hydrological model WEB-DHM were combined with SIMRIW-rainfed. The simulating results were validated with the investigated data. The impact of climatic change on rice production will be estimated in future studies.

Keywords: Farmers' fields, fresh water swamp field, irrigated field, rainfed field, simulation model, Tidal swamp field.

1. INTRODUCTION

One of the impacts caused by climate change in Indonesia is considered to be a change of the water cycle. While rainfall tends to increase in Java, Bali, Nusa Tenggara and Papua in the rainy season especially, it tends to decrease in other regions. In the dry season, rainfall is projected to decrease in most of Java and South Sumatra. In addition, there are growing concerns about an increase of extreme events such as droughts and floods, owing to the increasing frequency of El Niño. To evaluate climate change on rice production in Indonesia, we focused on Musi River Basin in Sumatera Island, which occupies 60,000 km² as basin area in Sumatera Island. Here we reported data collection from farmer's fields, development of a simulation model and preliminary results of the simulation model.

2. MATERIALS AND METHODS

2.1. Study area and data collection from farmer's fields

Rice is planted in paddy fields which were agro-ecologically classified into 4 categories in Musi River Basin: tidal swamp, fresh water swamp, rainfed and irrigated. We selected 6 locations Telang, Upang (Tidal swamp), Rambutan (fresh water swamp), Leumping (rainfed), Belitang and Musi Rawas (irrigated) (Fig. 1). 25 farmer's fields were selected for each location, and total 150 fields were investigated. Because of the area and importance, we selected 2 tidal swamp and 2 irrigated locations. Biomass and nitrogen content in rice plant at 2 and 6 weeks after transplanting, heading and maturity were measured. Rice yield was measured at maturity. Soil chemical and physical properties were measured in laboratory. Cropping management was obtained by interviews to farmers.

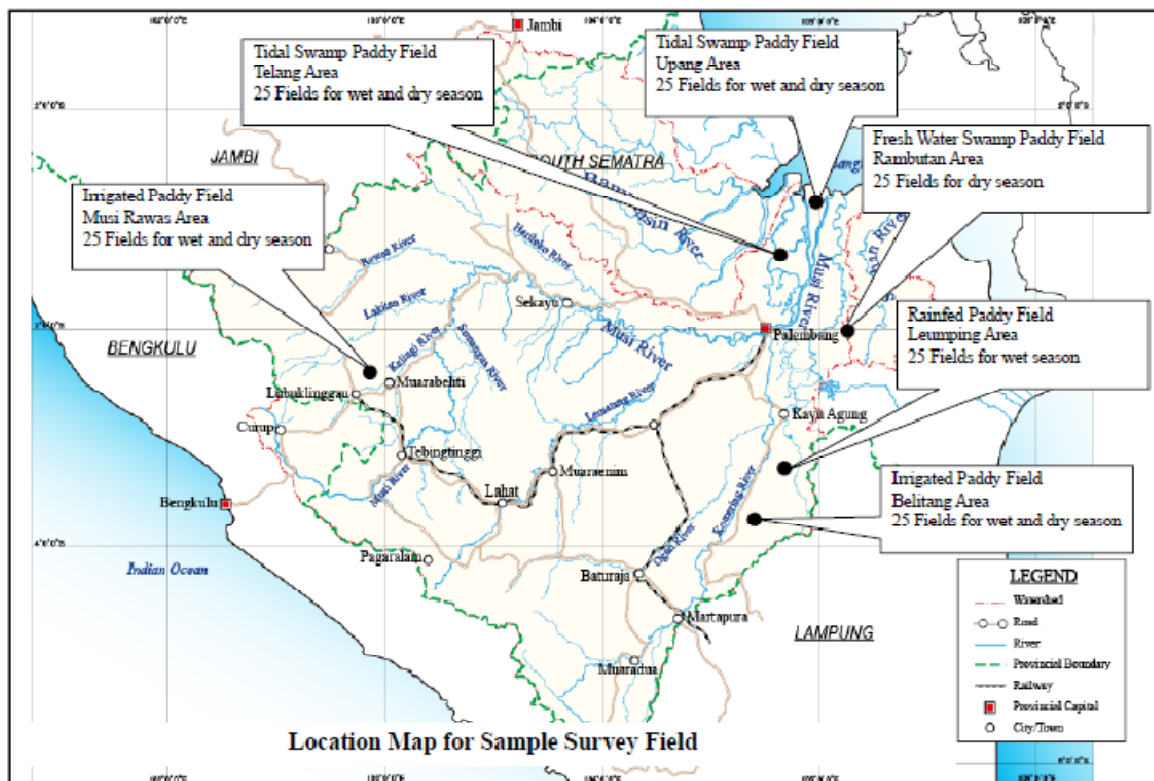


Fig. 1. Location map for investigated fields

2.2. Model development

We used a coupled model of hydrological model (water and energy budget-based distributed hydrological model; WEB-DHM; Wang et al., 2009), and crop model (simulation model for rice-weather relations for rainfed condition; SIMRIW-Rainfed; Homma and Horie, 2009) (Tsujiimoto et al. 2013). WEB-DHM can describe spatiotemporal distributions of soil moisture and river discharge with high accuracy and resolution at basin scale. The model of rice growth and production, SIMRIW-rainfed, can simulate the growth of rice as represented by LAI and rice production by considering yield decrease caused by stress from deficits of water and/or nitrogen. By dynamically coupling these two models, soil moisture distribution can be described by considering soil characteristics and topography of a river basin, and then the growth and production of rice responding to soil moisture can be calculated over the entire basin.

Parameters in the crop model are set using data from the target area obtained in 2.1. Based on the agricultural calendar and tables for characteristics of rice varieties, parameters such as for phenological development processes are established for each variety. Parameters such as for production properties are set for each variety based on yield data.

For coupling SIMRIW-rainfed and WEB-DHM, meteorological data such as air temperature, solar radiation and others, plus hydrologic data such as soil moisture and surface storage water depth output by WEB-DHM are input to SIMRIW-rainfed. Then the data of rice growth as represented by leaf area of rice is output from SIMRIW-rainfed to WEB-DHM. Interfaces between the two models are thereby developed.

The coupled model includes a module for irrigation management to investigate the effectiveness of water resource development. The irrigation-drainage system is regionally different and

complicated. Consequently, an irrigation system model suitable to the target river basin was developed by modifying the previously developed irrigation model4 based on the information obtained from field surveys of the target area, and then applied to the area.

3. RESULTS AND DISCUSSION

Crop management, e.x. water management and fertilizer application, varied among locations. Table 1 shows the amount of nitrogen fertilizer for example. Musi Rawas applied 120 N kg ha⁻¹ of nitrogen fertilizer, which was more than double of amount in Upang. Although the management was quite different, growth of biomass was similar among locations (Fig. 2).

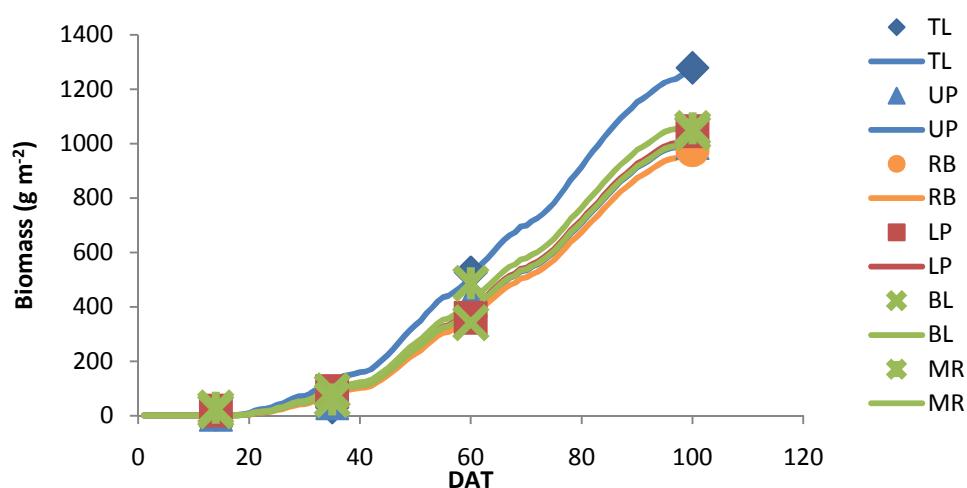


Fig. 2. Biomass change of rice grown in representative fields in the investigated locations (Symbols: measured; lines: simulated)

Table 1. Rice yield, harvest index (HI) and amount of nitrogen (N) fertilizer in each locations

Ecotypes	Location	Yield (t ha ⁻¹)	HI (t t ⁻¹)	Fertilizer (N kg ha ⁻¹)
Rainfed	Leumping	4.2	0.4	60.8
Irrigated	Belitang	4.2	0.4	62.8
	Musi Rawas	4.2	0.4	120.0
	Average	4.2	0.4	91.4
Tidal swamp	Telang	5.0	0.4	117.7
	Upang	2.1	0.2	50.6
	Average	3.6	0.3	84.2
Fresh water swamp	Rambutan	2.9	0.3	56.0

Telang recorded the highest yield on average among the research locations. Upang and Rambutan did not reach 3.0 t ha⁻¹ of yield on average. Lower yield in Upang was mainly caused by inadequate water supply, while that in Rambutan was by deep water. The drought and deep water decreased harvest index.

Using the investigated data of biomass and nitrogen uptake, the parameters in simulation model were optimized. One of the parameters corresponds to soil fertility (Homma and Horie, 2009), and roughly explained the variability of rice yield among farmers' fields (Fig. 2). The relation between parameter for soil fertility and soil properties will be analyzed in further study.

After developed the coupled model of WEB-DHM and SIMRIW-Rainfed, we have made some test runs for sub-basin "Padang". Since the input dataset and parameters for hydrology was not completed, the following results are merely examples and are not expected to be realistic. The land-use type in "Padang" is shown in Fig. 3. To examine the impact of irrigation during dry seasons, we have made runs by changing planting dates over Julian days 90-180 at 15-day intervals in 1986, for with and without irrigation. The results showed that if planting is after June, there is a difference of rice growth between rainfed and irrigated conditions (Fig. 4). However, if planting is before May, there is no difference because of adequate rainfall.

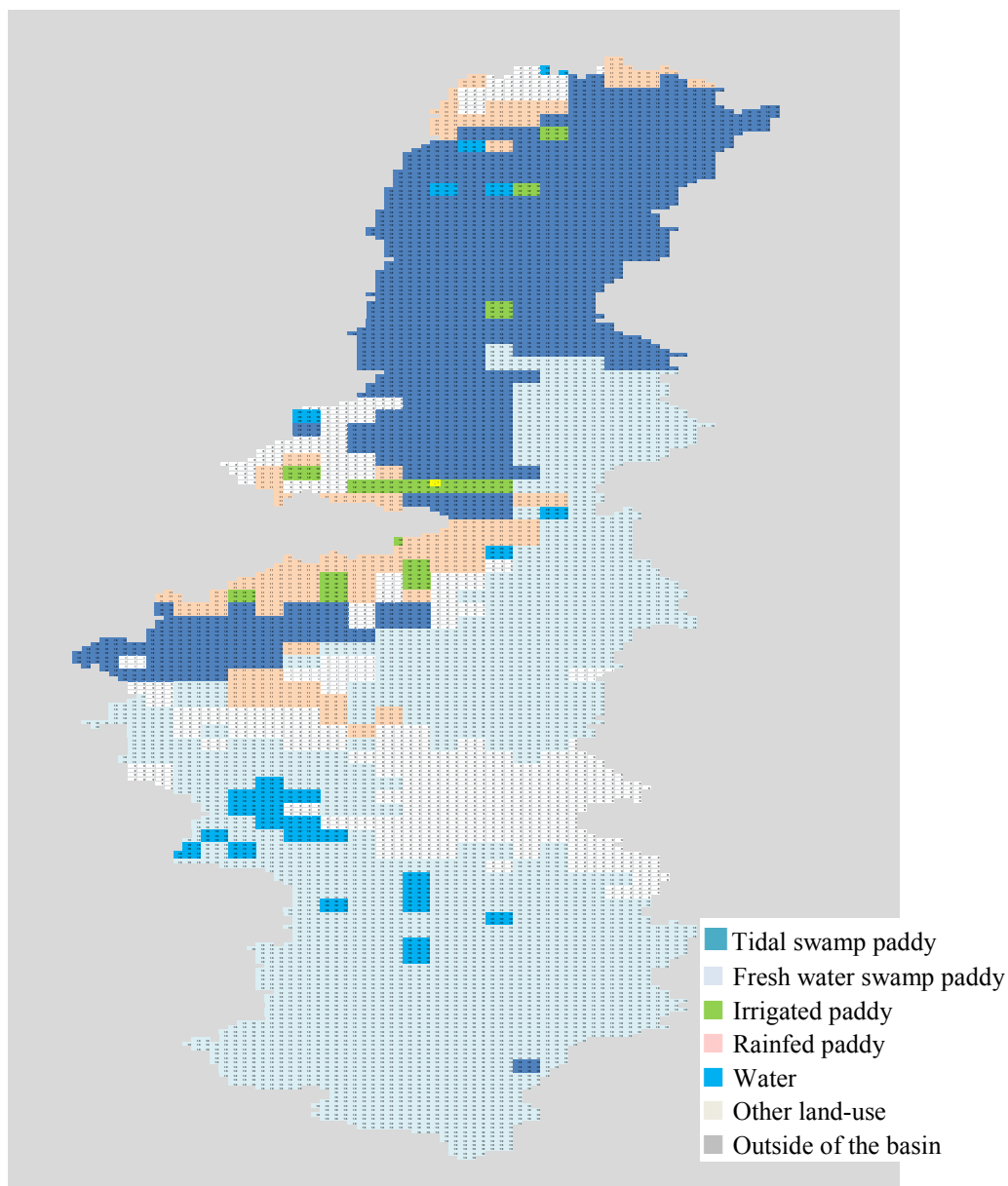


Fig. 3. Meshed map of Padang sub-basin in Musi River Basin

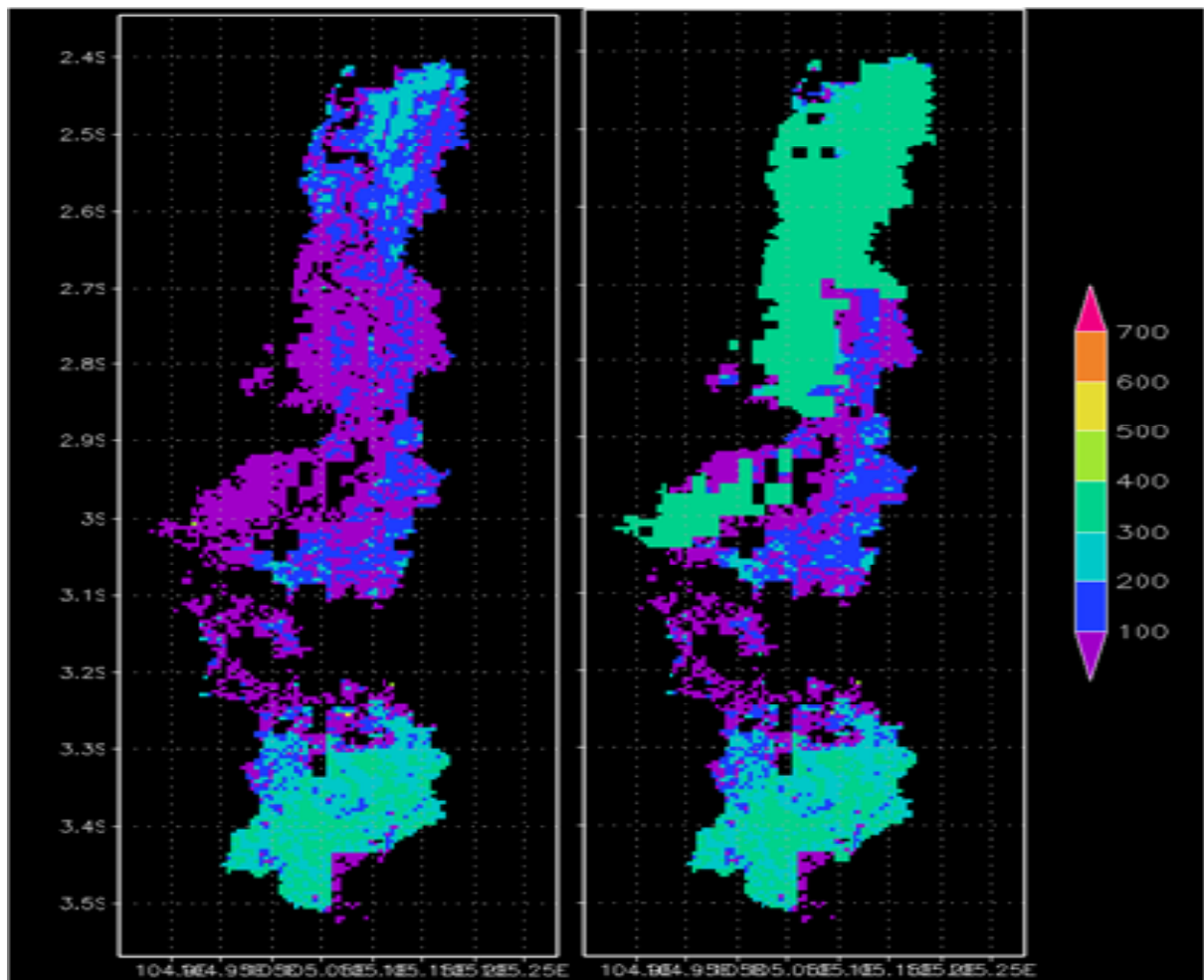


Fig. 4. One of the simulated result for Padang sub-basin. Simulation was conducted for planting date 29 June 1986; non-irrigated (left) and irrigated for irrigated paddy field and tidal swamp paddy field (right)

4. CONCLUSIONS

This study conducted research in Musi River Basin in Indonesia to simulate rice productivity under climatic change. The preliminary results by a test simulation suggested that the coupled model represented rice production in farmers' fields in each ecological categories. We are now revising climate data and calibrating parameters for hydrology. Climate change impact on rice production in Musi river basin will be evaluated in next study.

ACKNOWLEDGEMENT

The concept of this work was originally supported by the green network of excellence, Ministry of Education, Culture, Sport, Science and Technology, Japan. The study was substantially conducted for the project for assessing and integrating climate change impacts into the water resources management plans for Brantas and Musi river basins, funded by Japan International Cooperation Agency.

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The Number of Unemerged Leaves in Young Sucker of Sago Palm

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ABSTRACT

A branch of sago palm (*Metroxylon sagu* Rottb.) is called 'a sucker'. In sago palm cultivation, trunks accumulating a large amount of starch can be harvested persistently by having young suckers grow with mother palm. Young suckers are also used for propagation. They are cut off from the mother palm and nursed for about half year. After that, the suckers are transplanted to the field. Thus, suckers play an important role in sago palm cultivation, but there is little scientific knowledge about the growth and morphology of sucker. In this study, to obtain basic knowledge about the sucker, we investigated the number of unemerged leaf in suckers with proper size for transplantation. Suckers were collected in two sago garden in Mukah, Sarawak, Malaysia. The youngest leaf emerged from leaf sheath was defined as the spear leaf. The diameter at the base of the 5th leaf from the spear leaf was an average of 6 cm in the suckers used in this study. At least 6, up to 10 unemerged leaves were observed initiated inside the spear leaf. The number of unemerged leaf was significantly positively correlated with the diameter of the base of spear leaf at a 1 % standard.

Keywords: Sago palm, Sucker, unemerged leaf

1. INTRODUCTION

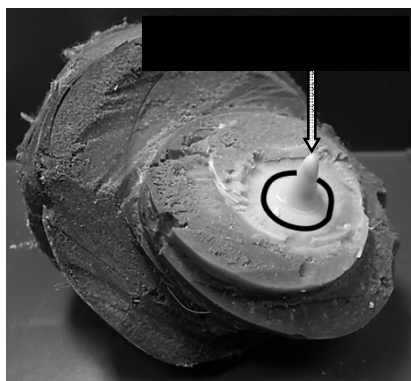
A branch of sago palm (*Metroxylon sagu* Rottb.) is called 'sucker'. In sago palm cultivation, trunks accumulating a large amount of starch can be harvested persistently by having young suckers grow with mother palm. Young suckers are also used for propagation (Sato et al., 1979). They are cut off from the mother palm and nursed for about half a year. After that, the suckers are transplanted in a field. Thus, suckers play an important role in sago palm cultivation, but there is little scientific knowledge about the growth and morphology of sucker. In this study, to obtain basic knowledge about the sucker leaf, we investigated the number of unemerged leaves in suckers in proper size for transplantation.

2. MATERIALS AND METHODS

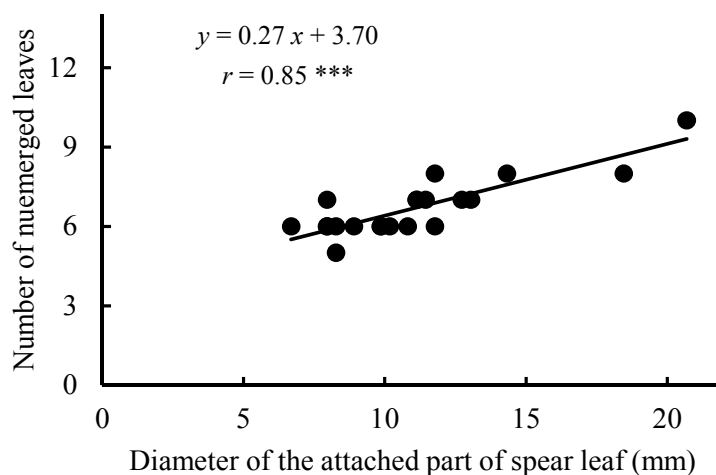
Suckers in proper size for transplantation were collected in two sago gardens in Mukah, Sarawak, Malaysia. Expanding leaves, a spear leaf (Jones, 1995) and unemerged leaves of the suckers were removed carefully up to the growth point. Regarding unemerged leaves, in the youngest hood-like leaf primordium covering the growth point, the leaf primordium which the tip of hood-like leaf was beyond the tip of growth point was assumed the first leaf primordium. The number of unemerged leaves inside a spear leaf was counted to the first leaf primordium using a stereomicroscope. In the sago palm, since a leaf sheath, which is a lower part of petiole, wraps around the stem, a part of leaf sheath attached appears to be a node like ring after removed of the leaf sheath (Fig. 1). Therefore, considering the part of leaf attached to be a circle, we measured the perimeter of the part of a spear leaf attached to the stem and calculated the diameter of the stem.

3. RESULTS AND DISCUSSION

On the suckers we investigated, unemerged leaves initiated inside of a spear leaf were at least 5, up to 10, an average of 6.7. The diameter of the part of a spear leaf attached ranged from 6.7 to 20.7 mm, an average of 11.1 mm. Fig. 2 shows relationship between the diameter of the part of a spear leaf attached and the number of unemerged leaves. The number of unemerged leaves was significantly positively correlated with the diameter of the part of a spear leaf attached at over 0.1 % level. This result suggests that the number of unemerged leaves inside of a spear leaf increases with increase of the diameter of the part of a spear leaf attached.



**Fig. 1. The part of a spear leaf attached to the stem and the unemerged leaf inside.
The solid line (-) shows the part of a spear leaf attached**



**Fig. 2. Relationship between the diameter of part of a spear leaf attached
and the number of unemerged leaves**

Note: *** represents significant difference at 0.1 % level (n = 21)

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Traits Related to Seed Yield in Early Varieties of Adzuki Bean in Hokkaido, Japan

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ABSTRACT

Seed yield-related traits of early varieties of adzuki bean [*Vigna angularis* (Willd.) Ohwi et Ohashi] were examined for introduction in Hokkaido regions with short frost-free seasons. An elite line with early maturity, Toiku No. 160; early varieties Sahoro-shozu and Kita-roman; and a medium variety, Erimo-shozu, were evaluated. Flowering and podding ceased earlier in Toiku No. 160 than in other varieties; the podding rate of Toiku No. 160 was approximately 10 % higher than that of the other varieties. Seed yield of Toiku No. 160 was similar to that of Erimo-shozu, a leading variety in Hokkaido. Sahoro-shozu showed lower biomass production and seed yield. The high seed yield of Toiku No. 160 may be useful for introducing early adzuki bean varieties in extremely cold regions of Hokkaido.

Keywords: Adzuki bean, early variety, flowering, podding, seed yield

1. INTRODUCTION

Adzuki bean [*Vigna angularis* (Willd.) Ohwi et Ohashi] is widely cultivated in East Asia and is the second most important leguminous crop in Japan after soybean [*Glycine max* (L.) Merr.] (Lumpkin and McClary, 1994). Adzuki bean is mainly used for producing sweet bean paste (Ann), which is a major ingredient in traditional Japanese sweets (Murata, 1999). Adzuki bean is mainly cultivated in Hokkaido, the northern region of Japan, and almost all adzuki bean varieties have been bred by Tokachi Agricultural Experiment Station in Hokkaido (Shimada, 2006). It is desirable to cultivate early adzuki bean varieties in extremely cold regions of Hokkaido owing to short frost-free seasons.

The previously released early Sahoro-shozu variety has a lower seed yield than the standard variety grown in Hokkaido (Aoyama, 2009). Therefore, it is important to identify the traits of early adzuki bean varieties with high yield potential. However, limited information is available regarding the varietal differences in traits associated with seed yield in adzuki beans grown in extremely cold regions. Knowledge of the flowering and podding habit is important in adzuki bean, as in many other leguminous crops, for determining a variety's earliness and seed yield (Kasajima et al., 2013). We investigated the growth and seed yield of early varieties, focusing on flowering and podding. This study describes the potential for cultivating early adzuki bean varieties as stable high-yielding varieties in the extremely cold regions of Hokkaido, Japan.

2. MATERIALS AND METHODS

2.1. Varieties

Four adzuki bean varieties (Toiku No. 160, Sahoro-shozu, Kita-roman, and Erimo-shozu) were used in this experiment. Toiku No. 160 is an elite line with early maturity, bred by Tokachi

Agricultural Experiment Station. Sahoro-shozu and Kita-roman are early varieties; the former are prone to soil-borne diseases, whereas the latter are tolerant against soil-borne diseases. Erimo-shozu, a medium variety, is a leading adzuki bean variety in Hokkaido.

2.2. Culture

A field experiment was conducted at the Tokyo University of Agriculture farm at Abashiri, Hokkaido, Japan (43°53'44"N, 144°21'45"E). The experimental site is in one of the coldest regions of Hokkaido. The soil was Andosol, and the preceding crop was sugar beet (*Beta vulgaris* L. var. *rapa* Dumort.). Four seeds per hill were seeded on June 1, 2011 with 16.6-cm hill spacing in approximately 3.5-m-long rows with 72-cm row spacing. Seedlings were thinned to 2 seedlings per hill 3 wks after seeding. Compound fertilizer (N:P₂O₅:K₂O = 5:25:14) was applied at 80 g m⁻² as the basal application. Eight experimental plots were prepared according to a randomized block design with 2 replications. Because variations in air temperature in 2011 were similar to the average over the preceding 5 yr, this experiment was performed under normal climatic conditions.

2.3. Investigation

The change in flower and pod numbers was investigated for 4 plant samples in each variety. The flowering date was recorded on a small label (9 × 22 mm) for each plant, which was wrapped around the flower pedicel. The labels were attached to all flowers that had formed pods by maturity. The podding rate was calculated from the flower and pod numbers during the entire flowering period based on the label data. Twenty standard plants of each variety, except for the labeled plants, were sampled at maturity from 10 hills. After air drying, the top dry weight, seed yield, and yield components, such as pod number, seed number per pod, and 100-seed weight were recorded.

3. RESULTS AND DISCUSSION

The change in flower and pod numbers per plant differed among varieties (Fig. 1). Flowering and podding of the early varieties (Toiku No. 160, Sahoro-shozu, and Kita-roman) began earlier than that of the medium variety Erimo-shozu. Flowering and podding ceased more quickly in Toiku No. 160 than in the other 3 varieties. Our results revealed that cumulative temperature had minimal effects on flowering and podding at the late flowering stage in Toiku No. 160. This trait of Toiku No. 160 is considered important for early maturity. In fact, the maturity date of Toiku No. 160 was September 18, whereas that of Sahoro-shozu, Kita-roman, and Erimo-shozu was September 16, 20, and 26, respectively. The highest flower and pod number per plant were observed in Sahoro-shozu, although the podding rate of Toiku No. 160 was approximately 10% higher than that of the other varieties, suggesting that Toiku No. 160 has highly efficient podding.

Table 1 shows the top dry weight, seed yield, and yield components for each variety. The top dry weight was highest for Toiku No. 160, followed by Kita-roman. The lowest seed yield was found in Sahoro-shozu, whereas few differences were observed between the other 3 varieties. The highest pod number per hill was observed in Sahoro-shozu, followed by Erimo-shozu and Toiku No. 160. The highest seed number per pod was observed in Toiku No. 160, followed by Kita-roman and Erimo-shozu. The 100-seed weights of Toiku No. 160 and Erimo-shozu were lower than those of Sahoro-shozu and Kita-roman.

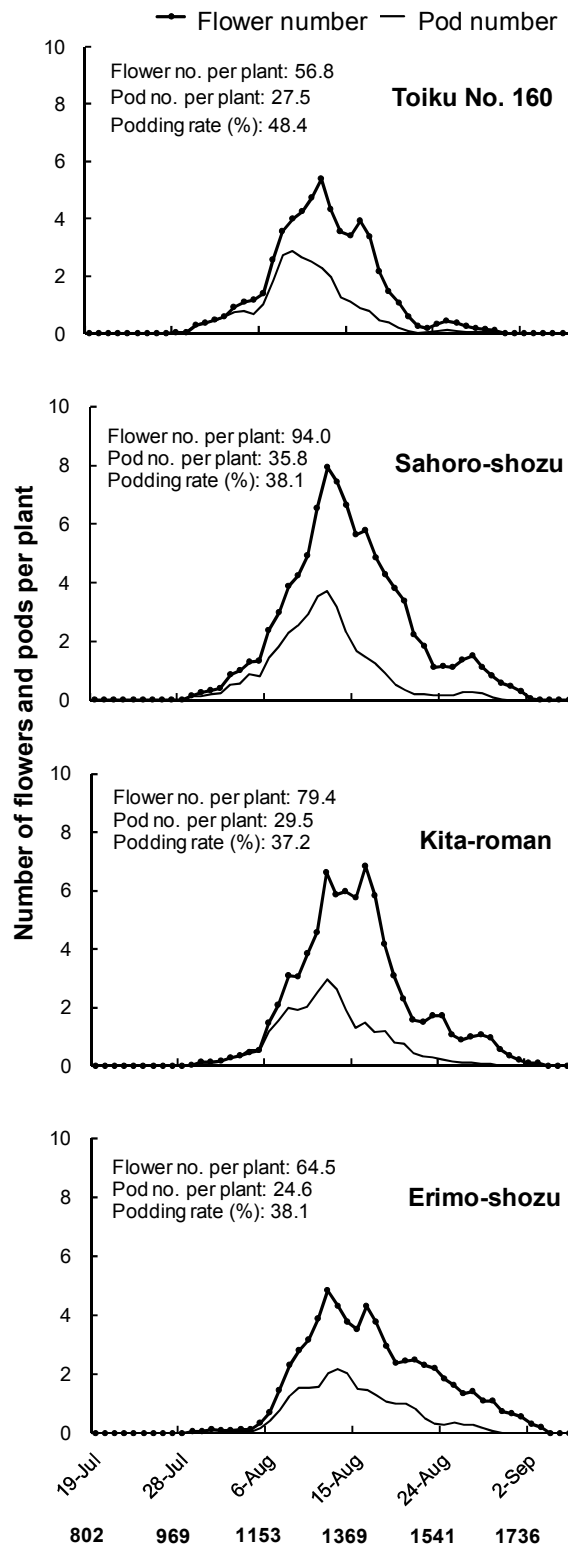


Fig. 1. Changes in flower and pod number per plant for each variety

Note: Values with the date on the abscissa indicate cumulative temperature (°C) from the seeding date. The plots show 5-d moving averages for 8 plants in 2 replicates. The flower number, pod number, and podding rate in each figure are the final values for the entire study period.

Table 1. Yield and yield components for each variety

Variety	Top dry weight (gm-2)	Pod no. per hill	Seed no. per pod	100-see weight (g)	Seed yield (g m ²)
Toiku No. 160	771.5 ± 26.8	62.8 ± 0.4	6.2 ± 0.1	13.5 ± 0.2	434.0 ± 12.5
Sahoro-shozu	656.5 ± 2.3	69.6 ± 1.6	5.2 ± 0.0	14.1 ± 0.4	418.8 ± 15.4
Kita-roman	733.3 ± 53.3	55.0 ± 1.6	6.0 ± 0.2	15.7 ± 0.6	437.5 ± 17.3
Erimo-shozu	719.6 ± 50.2	65.3 ± 2.3	5.7 ± 0.0	13.6 ± 0.4	437.8 ± 20.3
LSD ¹⁾	ns	9,27	0.72	ns	ns

Note: Data are mean ± standard error of 2 replicates.

¹⁾LSD shows significance level at $p < 0.01$; ns, not significant at $p = 0.05$.

In previous studies (Aoyama et al., 2009; Yamazaki et al., 2010), seed yield of early adzuki bean varieties was lower than that of medium varieties. From the results of our experiment, the top dry weight and seed yield of Sahoro-shozu was lowest among the varieties. Furthermore, the lowest pod number per hill was observed in Kita-roman. However, the seed yield of Toiku No. 160 was almost similar to that of the standard variety Erimo-shozu, although flowering and podding in the former ceased earlier than those in the other varieties. This result may be attributed to the higher podding rate of Toiku No. 160. These traits observed in Toiku No. 160 may be useful for introducing early adzuki bean varieties in the extremely cold regions of Hokkaido. By allowing expansion of the cultivation area, the development of early varieties such as Toiku No. 160 may promote stable adzuki bean production. In conclusion, we suggest extensive adzuki bean cultivation in the extremely cold regions of Hokkaido using early varieties.

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Dihaploid Rice Line Production through Anther Culture to Accelerate the Development of New Rice Varieties

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ABSTRACT

Crop improvement to environmental stresses is the key to agriculture adaptation due to the changing climates and less favourable soil conditions. Anther culture is one of rice breeding methods which can be used to achieve homozygous pure lines in 2-3 generations, while conventional rice breeding usually needs more than 5 generations. Thus anther culture can reduce the time, costs, and labours in developing new rice varieties. The objective of this research was to obtain spontaneous doubled-haploid or dihaploid plants (DH) produced from *indica* rice anther culture. The explants used for the experiment consisted of several kinds of F1s from crosses between lowland rice (high yielding, resistant to bacterial leaf blight) and upland rice (high yielding, tolerant to shade and drought, moderately tolerant to Al toxicity, resistant to blast), i.e. IR83821-99-2-2-2/I5-10-1-1, IR85640-114-2-1-3/I5-10-1-1, IR83821-99-2-2-2/O18b-1, IR85640-114-2-1-3/O18b-1, Bio-R81/I5-10-1-1, Bio-R82-2/I5-10-1-1, Bio-R81/O18b-1, and Bio-R82-2/O18b-1. Callus induction and regeneration media were N6 and MS medium. Observations were conducted on number of anther inoculated, calli produced and producing plantlets, green plantlets, and albino plantlets. The result from this research were 608 green plantlets (15.9% from the total plantlets) consisted of 66, 60, 26, 12, 91, 15, 140, and 198 green plantlets from IR83821-99-2-2-2/I5-10-1-1, IR85640-114-2-1-3/I5-10-1-1, IR83821-99-2-2-2/O18b-1, IR85640-114-2-1-3/O18b-1, Bio-R81/I5-10-1-1, Bio-R82-2/I5-10-1-1, Bio-R81/O18b-1, and Bio-R82-2/O18b-1, respectively. The first generation of spontaneous doubled-haploid (185 DH) plants were grown to be further selected to speed up the rice breeding program.

Keywords: Doubled-haploid, *indica*, lowland rice, upland rice.

1. INTRODUCTION

Rice yield has increased dramatically with the development of new high yielding varieties resistant to lodging, pests and diseases. Although rice farmers commonly attain high yield, yield fluctuations sometimes occur because of unfavorable weather conditions during the rice cropping season as well as less favourable soil conditions in particular areas. Therefore, crop improvement to environmental stresses is the key to agriculture adaptation (Kochian et al., 2004; Vinocur and Altman, 2005; Naylor et al., 2007).

Anther culture is one of the *in-vitro* techniques that can be used to accelerate the obtainment of pure lines in rice breeding programs through production of doubled-haploid (DH) plants. Anther culture will shorten the breeding cycle significantly by rapid development of completely homozygous lines (in 2-3 generations), instead of the conventional inbred line development process which takes at least 6-8 generations to derive lines with ~99% homozygosity (Forster and Thomas, 2005). Thus anther culture can reduce the time, costs, and labours in developing new rice varieties. However, the technique should allow production of large number of genetically stable spontaneous DH plants from a wide range of genotypes for effective utilization in rice breeding programs (Dewi and Purwoko, 2008).

Rice subspecies *indica* is generally used for rice variety development in Indonesia. Early anther necrosis, poor callus proliferation and high albino-plant regeneration are recognized as the major problems in *indica* rice varieties (Dewi and Purwoko, 2008). The objective of this research was to obtain spontaneous DH or dihaploid plants produced from rice anther culture of several F1s from *indica* x *indica* crosses.

2. MATERIALS AND METHODS

2.1. Materials

F1 plants from crosses between lowland rice which is high yielding, resistant to bacterial leaf blight (Purwoko et al., 2010) and upland rice which is high yielding, tolerant to shade and drought, moderately tolerant to Al toxicity, resistant to blast (Herawati et al., 2010; Safitri et al., 2011) i.e. IR83821-99-2-2-2/I5-10-1-1, IR85640-114-2-1-3/I5-10-1-1, IR83821-99-2-2-2/O18-b-1, IR85640-114-2-1-3/O18b-1, Bio-R81/I5-10-1-1, Bio-R82-2/I5-10-1-1, Bio-R81/O18b-1, and Bio-R82-2/O18b-1 were used in this study. These anther donor rice plants were grown in green house.

2.2. Methods

2.2.1. Preparation of Planting Materials

Panicles at booting stage were collected when the anthers occupy 1/3 to 1/2 of the spikelet length or when the auricle distance between the flag leaf and subtending leaf of the primary and secondary tillers reach 5-10 cm depending on genotypes. The panicles were placed inside the refrigerator with an average temperature of 7-10°C for 8 days. After cold treatment, panicles were surface sterilized in 20% commercial bleach containing sodium hypochlorite for 20 minutes under aseptic condition. Individual spikelets were cut to expose the anthers.

2.2.2. Callus Induction and Plant Regeneration

The media used for callus induction and plant regeneration in anther culture was according to Dewi and Purwoko (2008). Anthers containing pollen at the mid-uninucleate to early binucleate stage of development were plated onto the N6 medium supplemented with 2.0 mg/L naphthalene acetic acid (NAA) + 0.5 mg/L Kinetin + 10^{-3} M putrescine and 6% sucrose. Induced calli were subculture onto the plant regeneration medium consisting of MS medium supplemented with 0.5 mg/L NAA + 2.0 mg/L Kinetin + 10^{-3} M putrescine and 3% sucrose. Cultures were kept under dark conditions for callus induction and under 16 hrs daily illumination with 80-watt fluorescent bulb for plant regeneration.

Observation was done on number of anther plated, number of calli produced, number of calli producing plantlet, number of green and albino plantlets.

The rate of callus induction and regeneration was calculated as follows:

- % formed calli (CF) = (No. calli/No. plated anthers) x 100
- % calli forming plantlets (CFP) = (No. calli forming plantlet/No. formed calli) x 100
- % calli forming green plantlets (CGP) = (No. calli forming green plantlets/No. formed calli) x 100
- % calli forming albinoplantlets (CAP) = (No. calli forming albino plantlets/No. formed calli) x 100
- % of green plantlets (GP) = (No. of green plantlets/No. of plantlets) x 100
- % of albino plantlets (AP) = (No. of albino plantlets/No. of plantlets) x 100
- % of anther culture ability (ACA) = (No. of green plantlets/No. of plated anthers) x 100
- % Doubled haploid (DH) plants = (No. of DH plants/No. Grow out plants) x 100

3. RESULTS AND DISCUSSION

3.1. Callus Induction and Regeneration

There were strong genotypic effects on callus induction frequency among 8 crosses. Callus induction started when anther colour changed from yellow to brown three to four weeks after culture. Embryogenic calli were compact, globular in shape, rapidly growing and with clearly formed somatic embryos. Callus formation varied between 11.8 to 26.2% depending upon the genotype (Table 1). The data indicated that anthers from Bio-R81/I5-10-1-1 produced the highest percentage of calli, while the lowest induced from anthers of IR83821-99-2-2-2/I5-10-1-1.

The ability of calli to regenerate plantlet was varied between 1.0-12.7 % (Table 1). Percentage of calli producing green plantlet ranged between 0.2-2.7%, while for calli producing albino plantlet the range were between 3.5-14.6%. Therefore, high callus formation did not result in high levels of green plant regeneration. The result was similar to other *indica* rice anther culture (Niroula and Bimb, 2009).

Table 1. Callus formation and ability to regenerate plantlets in rice anther culture of F1s

Genotypes (F1s)	CF (%)	CP (%)	CGP (%)	CAP (%)
1. IR83821-99-2-2-2/I5-10-1-1	11.8	5.2	1.7	3.5
2. IR85640-114-2-1-3/I5-10-1-1	20.8	12.7	1.5	11.2
3. IR83821-99-2-2-2/O18b-1	13.8	1.1	2.2	5.4
4. IR85640-114-2-1-3/O18b-1	19.7	1.0	0.2	4.9
5. Bio-R81/I5-10-1-1	26.2	3.1	2.7	9.0
6. Bio-R82-2/I5-10-1-1	12.8	2.1	1.5	14.6
7. Bio-R81/O18b-1	13.1	2.0	4.1	11.2
8. Bio-R82-2/O18b-1	25.5	2.7	3.2	7.4

Note: AP= Anther plated; CI= calli formed; CP= calli producing plantlets; CGP= calli producing green plantlets; CAP= calli producing albino plantlets.

The plant regeneration started after 5 days to 2 weeks after transferring calli onto regeneration medium. Some calli differentiated only into green plantlets or albino plantlets. Some calli differentiated into both green and albino plantlets. The result showed that 2-4 mm callus with soft shape, light yellow colour was given high green plant regeneration, while more than 4 mm callus with hard shape, yellow colour was gradually browning and died after transfer to plant regeneration medium.

Table 2. Plant regeneration in rice anther culture of F1s

Genotypes (F1s)	PF	GP (%)	AP (%)	ACA (%)
1. IR83821-99-2-2-2/I5-10-1-1	188	35.1	64.9	1.5
2. IR85640-114-2-1-3/I5-10-1-1	948	6.3	93.7	1.0
3. IR83821-99-2-2-2/O18b-1	213	12.2	87.8	1.1
4. IR85640-114-2-1-3/O18b-1	423	2.8	97.2	0.2
5. Bio-R81/I5-10-1-1	444	20.5	79.5	2.0
6. Bio-R82-2/I5-10-1-1	236	6.4	93.6	1.0
7. Bio-R81/O18b-1	673	20.8	79.2	2.0
8. Bio-R82-2/O18b-1	702	28.2	71.8	3.5

Note: PF= Plantlet formed; GP= Green plantlet; AP= Albino plantlet; ACA= Anther culture ability

Number of plantlet regenerate from cultured callus derived rice anther culture was varied depending upon the genotypes. Green plant regeneration showed differences between the genotypes. Green plant regeneration rates varied from 2.8 to 35.1% (Table 2). Experiment by Gioi (2004) showed the results of anther culture of F1 plants from crosses between IR64 and new plant types cultivars reached the highest green plant regeneration of 5.72%. In this experiment, the genotypes that give more than 20% rates of green plant regeneration were IR83821-99-2-2-2/I5-10-1-1 (35.1%), Bio-R82-2/O18b-1 (28.2%), Bio-R81/O18b-1 (20.8%), and Bio-R81/I5-10-1-1 (20.5%). Anther culture ability (ACA) for these genotypes relatively high ranged between 1.5-3.5%. Thus, the results of this experiment indicated the ability to form green plant regeneration in all genotypes, except for IR85640-114-2-1-3/O18b-1, was superior.

However, high rate of albino plant regeneration was also occurred in this experiment (Table 2). Percentage of albino plant ranged between 64.9-97.2%. According to Dewi and Purwoko (2008) compared to *japonica*, the low androgenic response of rice subspecies *indica* in anther culture due to early necrosis or senescence of anthers may results from a higher rate of ethylene productions by anthers of *indica*. In this experiment a senescence inhibitor, putrescine, was used in both callus induction and regeneration media but also could not nullified albino plant regeneration.

3.2. Dihaploid Plants Production

All green plantlets (608 plantlets) were acclimatized and survival plants (589 plants) were planted in the green house. Spontaneous doubled-haploid or dihaploid (DH) plants obtained from the experiment were 185 plants or approximately 31.4%. Bio-R82-2/O18b-1 had the highest number of DH plants, followed by Bio-R81/I5-10-1-1, IR85640-114-2-1-3/I5-10-1-1, and Bio-R81/O18b-1 (Table 3). Those DH plants can be used further to ease selection of phenotypes for quantitative characters (Datta 2005; Herawati et al., 2010).

Table 3. Dihaploid plants obtained from rice anther culture of F1s

Genotypes	No. of Plants			DH (%)
	GP	GOP	DH	
1. IR83821-99-2-2-2/I5-10-1-1	66	55	14	25.5
2. IR85640-114-2-1-3/I5-10-1-1	60	60	33	55.0
3. IR83821-99-2-2-2/O18-b-1	26	18	3	16.7
4. IR85640-114-2-1-3/O18b-1	12	12	4	33.3
5. Bio-R81/I5-10-1-1	91	91	34	37.4
6. Bio-R82-2/I5-10-1-1	15	15	5	33.3
7. Bio-R81/O18b-1	140	140	31	22.1
8. Bio-R82-2/O18b-1	198	198	61	30.8
Total	608	589	185	31.4

Note: GP= Green plant, GOP= Grow out plant; DH= Doubled-haploid or dihaploid

4. CONCLUSIONS

There were strong genotypic differences for anther culture ability both for callus induction and plant regeneration. Callus induction and plant regeneration from anther culture were found to be independent of each other. 185 DH plants were obtained from IR83821-99-2-2-2/I5-10-1-1, IR85640-114-2-1-3/I5-10-1-1, IR83821-99-2-2-2/O18-b-1, IR85640-114-2-1-3/O18b-1, Bio-R81/I5-10-1-1, Bio-R82-2/I5-10-1-1, Bio-R81/O18b-1, and Bio-R82-2/O18b-1.

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The Effects of Phosphorus Application on Salinity Tolerance under Different Fertility Upland Conditions in The Interspecific Rice Cultivar NERICA1

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ABSTRACT

NERICA is interspecific hybrid rice developed to increase rice production in Africa. Cropping area has increased, but grain yield has remained low due to infertile soil and progressive salinization. Phosphorus (P) application improves rice growth. However, the effects of P application on rice have not been explored in saline soils. This study examined whether P application improves rice growth in saline soils with different fertilities under upland conditions. NERICA1 seeds were sown in 14-L pots filled with fertile paddy soil or infertile decomposed granite soil (masa soil). P was applied to half the pots containing each soil. NaCl was applied at 0, 13, 26, or 39 g pot⁻¹, fourteen days after sowing. Electrical conductivity of soil solution (ECs) was recorded. Plants were harvested 114 days after sowing. Shoot dry weight was determined, and Na and P content in the shoots was analyzed. Plant dry weight was larger in the paddy soil than in the masa soil, and decreased as the NaCl levels rose, and ECs, increased. The decrease was larger in masa soil. Shoot P concentration increased with P application, while shoot dry weight changed with salinity levels. P application increased shoot dry weight in the two soils at low salinities, but decreased it at high salinities. Therefore, P fertilization efficiency was negative at high salinities. Therefore, P application may improve rice growth in low saline soils, but not in high saline soils. Rice salinity tolerance may be reduced by P application, whereas increased fertility may enhance tolerance.

Keywords: Electrical conductivity, Fertilization, Mineral soil, Sodium accumulation.

1. INTRODUCTION

In the past, rice was only grown over a limited area in Africa, but it is now becoming an important food crop and is grown over a much wider area (Balasubramanian et al., 2007). Demand for rice has risen due to population growth and a shift in consumer preference for rice, especially in urban areas, but domestic production is far below demand. NERICA was developed by the Africa Rice Center (formerly the West Africa Rice Development Association) to improve rice production (Jones et al., 1997). It was derived from a cross between two rice species: *Oryza sativa* L. and *O. glaberrima* Steud., and the end cultivar line combined the useful traits from both species. NERICA has a high-yield potential; therefore, it has been disseminated extensively. However, its actual yield remains low.

Soil infertility and salinity are the major soil constraints in Africa (Korb et al., 2000; Ash and Woperies, 2001; Balasubramanian et al., 2007). NERICA1, one of the NERICA cultivars, was identified as not being salt tolerant (Sone et al., 2010), so NERICA1 yield should increase with improved cultivation management of infertile saline soils. Phosphorus (P) deficiency is one of the major limiting factors for crop production in the acid soils throughout Africa because available P is reduced by the reaction of soluble P with iron. A previous study showed that the growth and yield of upland rice was improved by P fertilizer in these unfavorable soils (Sahrawat et al., 1995). However, the responses of NERICA cultivars varied with soil fertility (Sone et al., 2011). This study examined

the responses of NERICA1 to P application under various soil salinity stress conditions in soils with different levels of fertility.

2. MATERIALS AND METHODS

2.1. Materials

The upland cultivar: NERICA1 was used. Its salinity tolerance is reported to be comparable to japonica upland rice cultivars (Sone et al., 2010).

2.2. Methods

Fertile organic soil from the experimental field at Okayama University and infertile decomposed granite soil were used as rooting media, and are referred to as paddy and masa soils, respectively. The soils were packed in 14-L pots with pumice stone at the bottom. Nitrogen and potassium were mixed with the soils as a basal fertilizer at a rate of 1.6 g pot⁻¹. Phosphate was applied to a half the pots containing each soil to create pot treatment (P) and not applied to the other half to create pot treatment (N). The amount of phosphate was 1.6 g pot⁻¹ resulting in 0.44 g pot⁻¹ for P. NERICA1 seeds were sown on May 31, 2013. A drain hole at the bottom of the side wall was connected to a storage tank via tubing. Surface irrigation was conducted and water draining into the tank was used for irrigation. The plants were grown under upland conditions. Leaf age, number of stems, and plant length were assessed every week.

Salinization treatment began 21 days after sowing. Sodium chloride (NaCl) was dissolved in the irrigation water and applied to pots at a rate of 13 g pot⁻¹ for the saline pots. The same amount of NaCl was added every two days. The different salinization treatments were 13, 26, and 39 g NaCl pot⁻¹. NaCl was not applied to the control pots. The electrical conductivity of soil solution (ECs) was recorded. Plant shoots were harvested 114 days after sowing and dried at 80°C for three days. Then, their dry weights (DW) were determined.

Dry material (0.2 g) was extracted with 50 mL distilled water and 1 mL nitric acid at 80°C over 24 h. The extract solution was filtered and then analyzed with a flame spectrophotometer (Flame Photometer, BWB Technologies Ltd. UK). The same amount of dry material was subjected to dry ashing at 400°C for 24 h and then dissolved in nitrate. The solution was analyzed for P by vanado-molybdate absorption spectrometry using a spectroscopy photometer (DU530, Beckman Coulter Inc. US) at $\lambda = 420$ nm.

Phosphorus fertilization efficiency was calculated as follows: DW/amount of P applied, where DW is the increase in shoot dry weight after a given level of P application at the same salinity.

2.3. Data analysis

The experiment included three factors, i.e., soil type, P application, and salinity, with five replications, where one pot was one considered to be a replicate. The pots were arranged as a randomized complete block design. The data were subjected to analysis of variance and differences of means were examined by Tukey's test, with a significance level at 5%, using the Ekuseru-Toukei 2012 software (Social Survey Research Information Co., Ltd. Japan).

3. RESULTS

Electrical conductivity of the soil solution (ECs) increased as the amount of NaCl applied increased (Fig. 1). At the same NaCl application level, ECs was higher in masa soil than in paddy soil. P application had no significant effect on ECs.

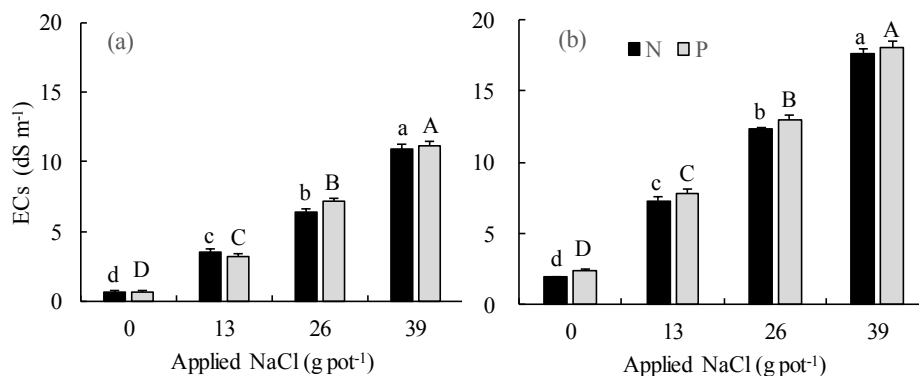


Fig. 1. Effects of NaCl and P application on the electrical conductivity of the soil solution (ECs) in paddy (a) and masa (b) soils

Note: Values are the mean and standard error of five replications. The same lowercase and uppercase letters indicate no significant differences for N (no P application) and P (P application), respectively (Tukey's test, $P < 0.05$).

Plant growth i.e. leaf age, plant length and number of stems per plant, was suppressed by high salinity, but the effects of P application were complex. Figure 2 shows the changes in the number of stems as an example. P application increased stem numbers at low salinity levels, whereas it decreased stem numbers at the high salinity levels. The number of stems was about three-fold greater in the paddy soil than in the masa soil. Plants died about 90 days after sowing in NaCl-39 g treated masa soil.

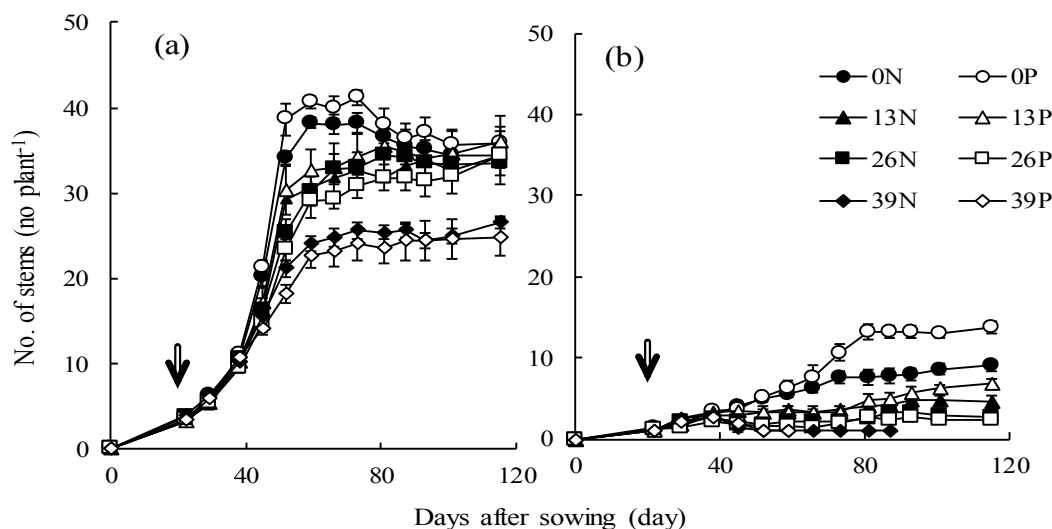


Fig. 2. Effect of NaCl and P application on number of stems in paddy (a) and masa (b) soils

Note: Values are the mean and standard error of five replications. Arrows indicate application of NaCl. Numbers attached to the symbols indicate the amount of NaCl applied to a pot ($g\ pot^{-1}$). N is no P application and P is P application.

Na concentration in the shoots was hardly affected by NaCl and phosphate application, except for the NaCl-39 g treated paddy soil and the NaCl-26 g treated masa soil where it was about 10 mg

g^{-1} (Fig. 3). P concentration in the shoots was averaged 2.5 mg g^{-1} without significant effects of NaCl or P application in paddy soil (Fig. 4). In the masa soil, P concentration in the shoots was not affected by salinity, but there was a P application effect. Without P application, it was as low as 1.2 mg g^{-1} , but increased by 3.7 mg g^{-1} when P was applied.

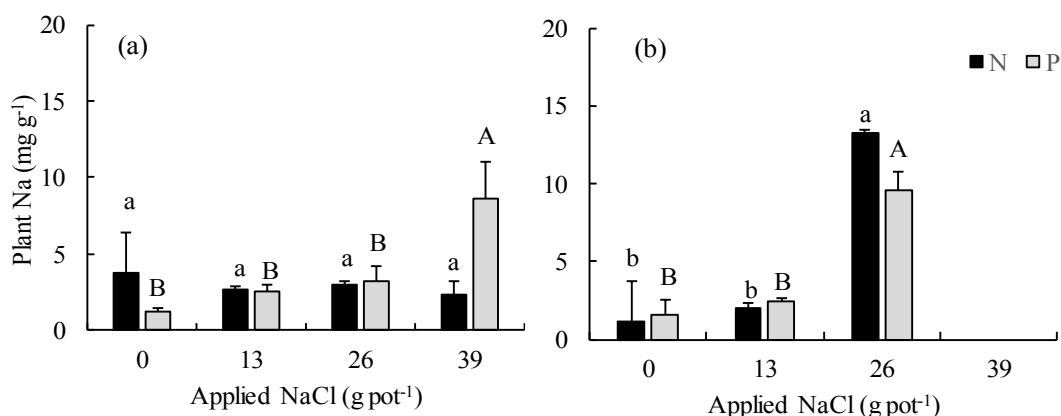


Fig. 3. Effect of NaCl and P application on Na concentration in the shoot (Plant Na) in paddy (a) and masa (b) soils

Note: Values are the mean and standard error of five replications. The same lowercase and uppercase letters indicate no significant differences between applied NaCl for N (No P application) and P (P application), respectively (Tukey's test, $P < 0.05$)

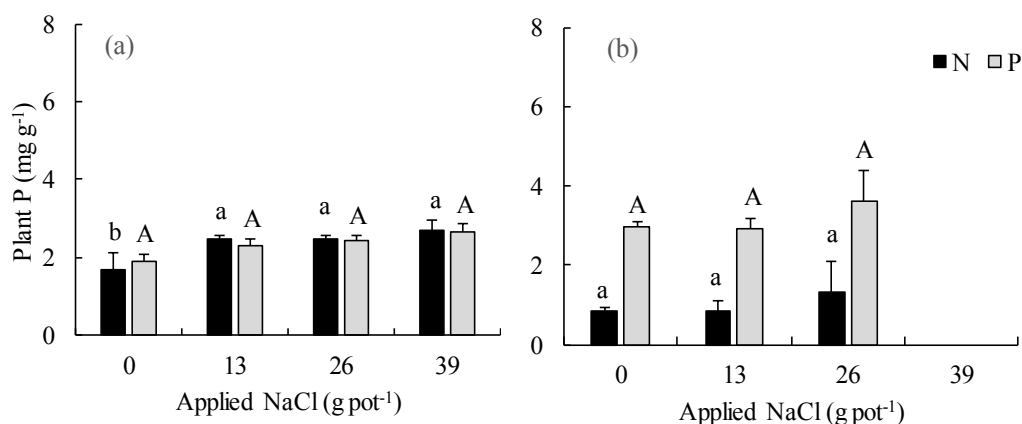


Fig. 4. Effect of NaCl and P application on P concentration in the shoot (Plant P) in paddy (a) and masa (b) soils

Note: Values are the mean and standard error of five replications. The same lowercase and uppercase letters indicate no significant differences between applied NaCl for N (No P application) and P (P application), respectively (Tukey's test, $P < 0.05$)

Generally, shoot dry weight declined as the amount of NaCl applied increased, but the effect of P application on dry weight was complex. There were interactions between soil type, salinity, and P application (Fig. 5). Dry weight was greater for plants grown in the paddy soil compared to those grown in the masa soils. It was increased by P application at low salinities but P application decreased dry weight at high salinities. As a result, the P fertilization efficiency, i.e. the increase in dry weight per 1 g P application, was large in the low salinity treatments, but negative in the high salinity treatments (Table 1).

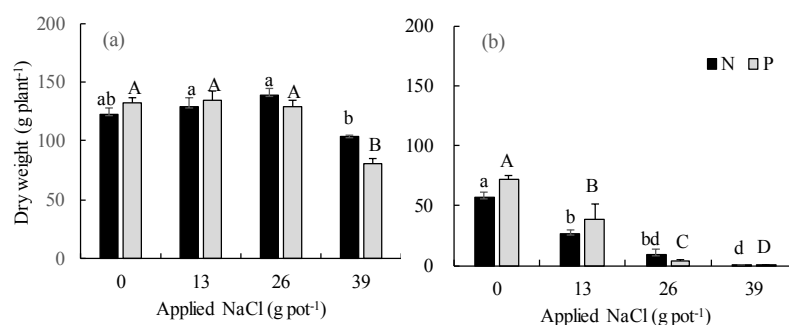


Fig. 5. Effect of NaCl and P application on shoot dry weight in paddy (a) and masa (b) soils

Note: Values are the mean and standard error of five replications. The same lowercase and uppercase letters indicate no significant differences between applied NaCl for N (No P application) and P (P application), respectively (Tukey's test, P , 0.05)

Table 1. Effect of NaCl application on phosphorus fertilization efficiency (PFE) in paddy and masa soils

Applied NaCl (g pot ⁻¹)	P FE*	
	Paddy	Masa
0	6.6	9.1
13	3.7	7.5
26	-6.1	-3.2
39	-14.5	-0.2

Note: *Fertilization efficiency of phosphorus. Increase in shoot dry weight due to fertilization is divided by the amount of phosphorus applied.

To examine the factors responsible for the decrease in shoot dry weight at higher salinities, the P relative value against NaCl-0 g (no application of NaCl) was calculated. Relative dry weight decreased as salinity increased i.e. amount of NaCl applied, ECs and sodium concentration in the shoot (Fig. 6). The size of the reduction was always larger in masa soil than in paddy soil, which indicated that the plants seemed more salt tolerant in paddy soil.

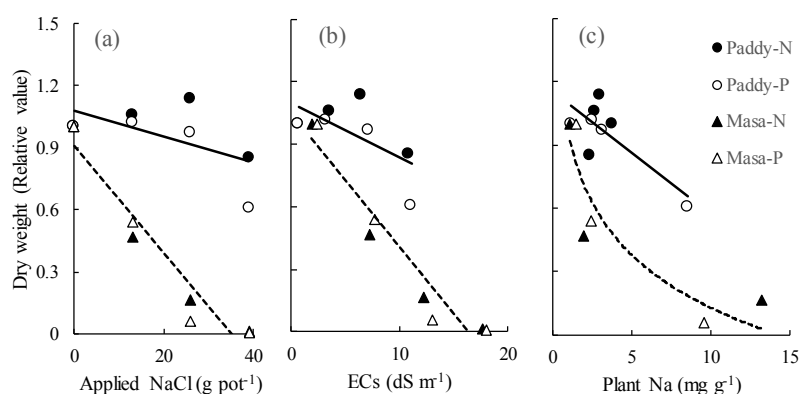


Fig. 6. Relationships between applied NaCl, electrical conductivity of the soil solution (ECs), Na concentration in the shoots (Plant Na) and shoot dry weights in salinized paddy (a) and masa (b) soils with and without phosphorus fertilization

Note: Values are the mean of five replications.

4. DISCUSSION

Soil salinity has hazardous effects on crop production (Ghassemi et al., 1995). In a similar manner to previous studies (Makihara et al., 1999; Wang et al., 2007; Sone et al., 2010), shoot dry weight decreased as soil salinity increased (Fig. 5). However, the degree of decrease with respect to the amount of NaCl applied was different for the two soils, in that the decrease was smaller in the paddy soil. Soil salinity should be expressed using soil electrical conductivity (ECs) instead of the amount of NaCl applied. The ECs for the two soils were not the same when a comparable amount of NaCl was applied (Fig. 1). This suggests that relative dry weight was related to ECs (Fig. 6). Although relative dry weight decreased as ECs increased, the degree of decrease was still smaller in the paddy soil. Furthermore, relative dry weight was related to Na concentration in the shoot (Fig. 6) because Na accumulation in plants is often responsible for reductions in dry matter production. In this study, relative weight decreased as sodium concentration increased, and the degree of decrease was smaller in the paddy soil. Shoot dry weight was larger in the paddy soil (Fig. 5), which indicates that the productivity of paddy soil was higher than that of masa soil. These results strongly suggest that plants grown in fertile soil may be salt tolerant in terms of soil salinity and accumulation of Na in plants. This confirmed the results reported by Sone et al. (2011).

Sone et al. (2011) indicated that there was a large variation in response to P application at a given salinity between NERICA strains. Additionally, this study found that the effect of P application on dry matter production changed, depending on the salinity level: it raised dry matter in the low soil salinity treatments, whereas it reduced dry matter production at high salinity levels in the two soils (Fig. 5). Stem numbers also followed this trend (Fig. 2). As P concentration in the shoot increased by generally the same amount under both low and high soil salinities, it could not be responsible for the complex changes in dry weight. P concentration did not seem to affect salt tolerance (Fig. 6). Therefore, the role of phosphorous in dry matter production under saline conditions should be explored in the future.

Shoot dry weight increased with P application (Fig. 5) in non-saline soils, which confirms the results reported by Sahrawat et al. (1995). However, fertilization efficiency may decline as salinity levels rise (Table 1). Recently, fertilizers are being increasingly used by small farmers; therefore, farmers are being urged to use fertilizers more appropriately (Kamara et al., 2010; Saito et al., 2010; Vanlauwe et al., 2014). However, the price of fertilizer is still not within the means of many small farmers. Therefore, fields that could benefit from P fertilization should be evaluated carefully, especially for saline soils. This would reduce the cost and inappropriate use of P fertilizers.

5. CONCLUSIONS

Salinity tolerance in rice may be greater in fertile soil than in infertile soil. P application may increase plant P concentration, but changes in dry matter production vary, depending on the salinity of the soils. This suggests that P application should be carefully evaluated before it is added to the soil.

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Publication Responsible
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VU DINH HOA

Cover designer
DO LE ANH

ISBN: 978-604-924-199-4

Printed 100 copies in Anh Duong Printing Co. ltd.

Publishing licence No 3333-2015/CXBIPH/01-06/ĐHNN.

Publishing decision No 28/QĐ-NXB-HVN issued on 30 December 2015.

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