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Variation in Growth Attributes, Dry Matter Yield and Quality Among 6 Genotypes of Napier Grass used for Biomass in Year of Establishment in Southern Kyushu, Japan

[1,4]Lizah Khairani, [2]Yasuyuki Ishii, [2]Sachiko Idota, [3]Renny Fatmyah Utamy and [2]Aya Nishiwaki

[1]Graduate School of Agriculture, [2]Faculty of Agriculture, [3]Interdisciplinary Graduate School of Agriculture and Engineering, University of Miyazaki, Miyazaki 889-2192, Japan
[4]Faculty of Animal Husbandry, Pajajaran University, Indonesia

Corresponding Author: Yasuyuki Ishii, Faculty of Agriculture, University of Miyazaki, Miyazaki 889-2192, Japan

ABSTRACT

As for estimating the suitability of napier grass for biomass use in southern Kyushu, Japan, the objective of this study was to determine the most suitable genotype of napier grass for dry matter production and quality (fiber content) for biomass ethanol conversion in the year the plants were established. Six genotypes of napier grass, a dwarf early-heading type, a dwarf late-heading type, a hybrid of pearl millet with napier grass, a purple-foliage type and normal varieties of Merkeron and Wruk wona, were examined for growth attributes, dry matter yield, chemical composition and *in vitro* Dry Matter Digestibility (IVDMD) at the University of Miyazaki in southern Kyushu, Japan in 2009. Dry matter yield ranged from 13.4 to 33.5 Mg ha^{-1}, with the highest yielder being normal Wruk wona, followed by Merkeron. Hemicellulose and cellulose content of both Leaf Blade (LB) and stem inclusive of leaf sheath (ST) did not differ significantly among genotypes, except for cellulose content in ST which was significantly higher for the dwarf early-heading type than for the other genotypes. The dwarf late-heading type had significantly higher IVDMD in ST (65.8%) due to lower acid detergent lignin and cellulose content in ST than the other genotypes. Based on dry matter production, fiber (carbohydrate) content in the current study and theoretical conversion efficiency from cellulose and hemicelluloses of 0.29 and 0.23, respectively, Merkeron appears the most promising genotype for biomass production, with theoretical ethanol production potential of 5850 L ha^{-1} year^{-1} in the year of establishment.

Key words: Dry matter production, ethanol yield potential, napier grass, structural carbohydrate, variety

INTRODUCTION

By 2030, the Biomass Japan Comprehensive Strategy Promotion Council estimates that Japan will produce 6.0 million kL year^{-1} of bioethanol using domestic biomass resources, composed of 1.8-2.0 million kL year^{-1} from herbage as raw material (MAFF, 2007; Tanaka, 2007). It is very important to find the best candidate herbage and energy crop species in addition to crop by-products such as rice and wheat straw and sugarbeet and sugarcane tops which already are available and can also grow on currently unused agricultural lands (390 thousand ha in Japan) with no competition for cultivation of food crops (Maeda, 2007). However, for bioethanol purposes apart from Dry Matter (DM) production, plant materials should suit quality parameters to reach optimal energy efficiency which means high levels of fiber content and minimized environmental pollution which means low levels of water, N and ash content (Lemus *et al.*, 2002; McKendry, 2002).

The bioethanol industry will need a continuous and reliable supply of biomass that can be produced at a low cost and with minimal use of water, fertilizer and arable land. As many C_4 plants utilize light, water and nitrogen at high efficiency compared with C_3 species, some might be ideal for energy crops and one candidate that offers significant potential for biomass use and is already adapted to Japan is napier grass (*Pennisetum purpureum* Schumach). Normal varieties of napier grass have been grown in several areas (Ito and Inanaga, 1988; Ito *et al.*, 1988) for feedstock purposes under the cut-and-carry system (Ishii *et al.*, 1995) and a dwarf variety has recently been adapted to the cut-and-carry (Mukhtar *et al.*, 2003) and grazing system (Ishii *et al.*, 2005).

Napier grass is highly efficient at converting solar energy into chemical energy and is also efficient in fixing atmospheric CO_2, it is capable of accumulating 10-85 mg DM ha^{-1} $year^{-1}$ and its production potential is higher than other tropical grasses (Humphreys, 1994; Skerman and Riveros, 1990). However, yields of the grass vary depending on genotype (Schank *et al.*, 1993; Cuomo *et al.*, 1996), edaphic and climatic factors and management practice (Chaparro *et al.*, 1995, 1996; Woodard and Prine, 1993). Boonman (1993) reported that napier grass accumulated a large quantity of DM (29 Mg DM ha^{-1} $year^{-1}$) in a single cut per year when grown uninterruptedly for 3 years in Kenya while the yield varied from 10 to 40 Mg DM ha^{-1} $year^{-1}$ depending on soil fertility, climate and management (Schreuder *et al.*, 1993). Under four regimes of annual nitrogen (N) supply between 0 and 2000 kg ha^{-1}, annual DM yield in napier grass varied from 18.3 to 49.7 Mg ha^{-1} in Australia (Ferraris, 1980) and from 10.7 to 85.9 Mg ha^{-1} in Puerto Rico under seven levels of N supply (0-2223 kg N ha^{-1}) (Vicente-Chandler *et al.*, 1959; Skerman and Riveros, 1990). Napier grass must be grown uninterruptedly under a high level of fertilizer N supply in the year of establishment to optimize ethanol production from the plants.

In feasibility studies of biomass to ethanol production in Hawaii, napier grass was the highest yielding and lowest cost feedstock using simultaneous saccharification and fermentation conversion (Mielenz, 1997). In napier grass, 42 Mg DM ha^{-1} produced 12,099 L ha^{-1} compared with energy cane (*Saccharum* spp.) at 108.2 Mg DM ha^{-1}, yielding 44,300 L of ethanol ha^{-1}. In Florida, sugarcane and napier grass achieved the highest yielding and the lowest cost biomass, at 54.4 Mg ha^{-1} and \$21 Mg^{-1} and 44.5 Mg DM ha^{-1} and \$22 Mg^{-1}, respectively (Mielenz, 1997).

Dry matter yield and quality suitable for biomass use are expected to be variable, depending on the variation in growth attributes among genotypes of napier grass as affected by local climatic and edaphic factors at the examined site. High digestibility of feedstock facilitates enzymatic saccharification with cellulase (Kai *et al.*, 2010). Therefore, the objective of this study was to determine the most suitable genotype of napier grass for dry matter production and quality (fiber content) for biomass use in ethanol conversion and to find the growth attributes, including digestibility which were closely correlated with high yield and quality under a favorable fertilizer supply without interruption in the year of establishment in southern Kyushu, Japan.

MATERIALS AND METHODS

Experimental site and climatic conditions: The study was carried out in the growing and wintering seasons from 27th May 2009 to 27th May 2010 for a year in Kibana Field, University of Miyazaki, in southern Kyushu, Japan which was located on andosols at 31 m above sea level

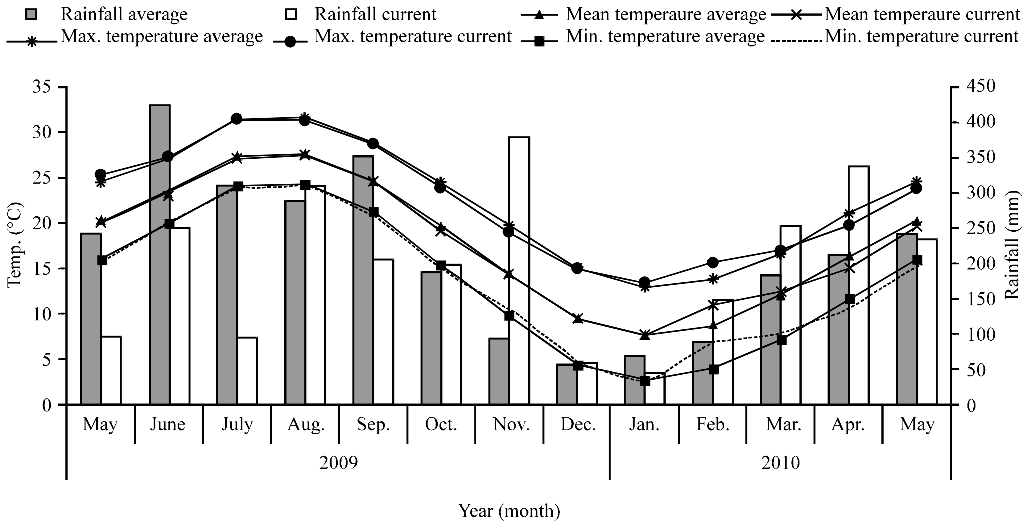

Fig. 1: Monthly rainfall (mm) and monthly mean of the daily mean, minimum and maximum temperatures (°C) in 2009-2010 (current) and in the 30-year average for 1980-2010 (average), Source: JMA, 2010

(31°50'N and 131°25'E). The temperature and rainfall data as shown in Fig. 1 were monitored from the Miyazaki Meteorological Observatory in addition to obtaining data on the 30 year average for 1980-2010 (JMA, 2010).

Treatments and design: Plots were arranged by a randomized complete design with 3 replications (main plots) and each subplot (genotype) having 4 rows 4 m long×5 m wide, with 1 m spacing between subplots and 2 m spacing between main plots. Genotypes of napier grass were selected having a wide range of phenotypic variation, such as a dwarf variety of an early-heading type (DE), a dwarf variety of a late-heading type (DL), a hybrid of pearl millet with napier grass (HY), a Purple-foliage type (PF) and the normal varieties of Merkeron (ME) and Wruk wona (WK). Plants were transplanted at a density of 2 plants m^{-2} on 27th May 2009 using nursery stock obtained from stem cuttings in the field-grown plants and had been grown in a glasshouse since November 2008. Plots were rain-fed and weeding was conducted by hand two times, on 16th June and 3rd July 2009. Basal fertilizer was supplied in the form of fermented cattle manure and dolomite at 400 and 20 g m^{-2}, respectively, on 20th May 2009 and additional fertilizer was supplied in the form of a chemical compound fertilizer at the rate of 55 g N, P_2O_5 and K_2O, each m^{-2} $year^{-1}$ by 7 split applications on 27th May, 3rd June, 24th June, 8th July, 22nd July, 21st August and 14th September 2009.

Growth characteristics: Ten plants were measured for growth attributes such as plant height, plant length and tiller number in each subplot with 3 replications. Two mature stalks were harvested manually from each subplot, using a sickle to cut them at a height of 10 cm above the ground level, on 10th November 2009 to obtain samples for measurement of Fresh Weight (FW). Leaf area was measured with an AAM-8 automatic area meter (Hayashi Denkoh Co. Ltd., Tokyo, Japan) to calculate the Leaf Area Index (LAI). Subsampled (ss) herbage at around 400 g of FW was hand-separated into Leaf Blades (LB), stems inclusive of Leaf Sheath (ST) and dead leaves (D) and dried in a forced-air oven at 70°C to measure Dry Weight (DW). The overwintering ability was determined by the Percentage of Overwintered Plants (POP) and Regrowing Tiller Number (RTN) for all plants excluding border plants on 27 May 2010. The POP was calculated as the number of plants that emerged one or more regrowing tillers divided by the total number of plants and RTN was calculated by multiplying plant tiller number by plant density.

DM yield: The DM yield was calculated according to Tarawali *et al.* (1995) as follows:

$$\text{DM yield (Mg ha}^{-1}\text{)} = \text{Tot FW} \times \frac{\text{DWss}}{\text{FWss}} \times 10^{-2}$$

Where:
Tot FW = Total fresh weight (g m^{-2})
DWss = Dry weight of the subsample (g)
FWss = Fresh weight of the subsample (g)

Chemical analyses of cell wall components and *in vitro* dry matter digestibility (IVDMD): Samples were ground by mill (Model D3V-10, Hsiangtai Machinery Industry Co. Ltd., Taipei, Taiwan) to pass through a 1 mm mesh and were analyzed in duplicate samples for IVDMD, Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF) and Acid Detergent Lignin (ADL) content using a detergent digestion protocol as described by Vogel *et al.* (1999). Samples were placed in preweighed bags and processed by a fiber extraction protocol (Vogel *et al.*, 1999) and the ANKOM procedure (ANKOM 220 Fiber Analyzer, Model 220v; ANKOM Technology Corp., Fairport, NY, USA). These procedures estimate NDF, ADF and ADL contents. The content of cell wall components, namely hemicelluloses and cellulose, was calculated using component concentrations expressed on a dry weight basis, as follows:

- On an oven-dry weight basis (g kg^{-1})
- Hemicelluloses = NDF-ADF
- Cellulose = ADF-ADL; (Jung and Vogel, 1992)
- IVDMD content was determined by the pepsin-cellulase digestion method (Goto and Minson, 1977)

Estimation of ethanol production: The conversion efficiencies from Badger (2002) were adopted to estimate ethanol production potential from 6 genotypes of napier grass as listed in Table 1. Then the ethanol yield summed up from both cellulose and hemicelluloses was converted from kg ha^{-1} $year^{-1}$ to L ha^{-1} $year^{-1}$ by multiplying by 1.1527.

Table 1: Ethanol conversion efficiencies, assumed after Badger (2002)

	Source of carbohydrate	
Step	Cellulose	Hemicelluloses
Dried napier grass	1 kg of DM	1 kg of DM
Mass fraction (a)	×% Cellulose	×% hemicelluloses
Enzymatic conversion efficiency (b)	×0.76	×0.90
Ethanol stoichiometric yield (c)	×0.51	×0.51
Fermentation efficiency (d)	×0.75	×0.50
Ethanol yield, kg = 1×(a×b×c×d)/100	= % Cellulose×0.29 kg, ethanol (P_1)	= % Hemicelluloses×0.23 kg, ethanol (P_2)
Total ethanol yield (kg)	= P_1+P_2	

Statistical analysis: Analysis of variance (ANOVA) was carried out using SPSS software (version 15.0) by one-way analysis procedures for growth attributes, DM yield, chemical components and IVDMD of napier grass in a randomized complete design. Mean separation was tested using the Least Significance Difference (LSD) method at the 5% level. Pearson correlation coefficients were calculated among growth attributes, DM yield, cell wall components, IVDMD and ethanol production potential using data from all genotypes (for each parameter, $n = 36$ except for LAI, $n = 18$). Correlation coefficients were considered high for $r = 0.75$, moderate for $r = 0.50$-0.75 and low for $r<0.50$. Multiple regression analyses by stepwise and forward selection and by stepwise and backward selection were performed for ethanol production potential ($n = 36$), where variables (predictors) were chemical composition and digestibility such as IVDMD, NDF, ADF and ADL content of LB and ST and the dependent variable was ethanol production potential; determination of variables was based on the significance of the standardized partial regression coefficient based on the t-value at the 5% level.

RESULTS AND DISCUSSION

Climatic conditions: Changes in the monthly mean, minimum and maximum air temperatures and precipitation in the growing season showed that even though the current maximum and minimum temperatures were slightly higher than those in an average year, temperatures were not significantly different from those in an average year (Fig. 1). Precipitations in May-July 2009 were lower than the average rainfall, in contrast to a higher level than average in November 2009. Temperatures in the wintering season were slightly higher than average in January-March and rainfall was slightly higher than average in February-April 2010.

Growth attributes and DM yield: Values for the mean and standard deviation of growth attributes and yield at harvest in November 2009 (Table 2) showed that the HY and DE and the normal varieties of WK and ME had similar values for each attribute and yield, except for the highest plant height and DM yield and the lowest tiller number in WK among genotypes while DL and PF tended to have lower values than the other 4 genotypes, except for the highest tiller number and LAI in DL among the 6 genotypes. We estimated the correlation between lowest plant height in DL with highest tiller number and LAI from the largest amount of leafage in DL. Even though DE had lower plant height up to August than normal varieties (data not shown), the stem elongation triggered by floral induction in this genotype led to increasing plant height and yield components similar to other normal varieties such as WK and ME and HY at harvest in November. In contrast, PF had the lowest LAI, even falling in the middle rank of plant height which correlated with the lowest rank of yield components as well as DL. Therefore, fresh and DM yield among genotypes ranged from 90.1 to 156.7 and 13.4 to 33.5 Mg ha^{-1}, respectively, where WK was the highest yielder for both fresh and DM, followed by ME. The current study was in agreement with Utamy *et al.* (2009), who reported a similar DM yield for DL, the highest yielding variety at 13.6 Mg ha^{-1} among several sites in the year of establishment. Rajvanshi and Nimbkhar, 2008 also determined DM yield for HY as 24-40 Mg ha^{-1} in India. Anderson *et al.* (2008) reviewed the DM productivity of ME in the South of the United States at 27.76 Mg ha^{-1} in Georgia, 30-60 Mg ha^{-1} in southern and central Florida and 20-30 Mg ha^{-1} in the northern part of the region. However, HY has some variation in genotype which showed yields of 10-18 Mg DM ha^{-1} in a warm temperate climate being affected by planting density in Australia (Pearson and Anthony, 1977).

Table 2: Effects of genotypes on agronomic attributes and chemical compositions of napier grass genotypes in November of the year of establishment

	Agronomic attribute					
Genotype[†]	PH (cm)	TN (No m^{-2})	LAI	DMY (Mg ha^{-1})	PST (% DM)	Pdd (% DM)
DE	338.8±18.1^{b}	23.7±0.8^{b}	8.0±0.7^{a}	28.2±2.7^{b}	75.5±1.8^{ab}	9.4±1.8^{abc}
DL	160.2±11.5^{d}	74.7±6.2^{a}	8.5±0.6^{a}	13.4±1.9^{c}	50.1±5.9^{d}	11.5±8.1^{ab}
HY	305.0±63.4^{bc}	20.7±1.6^{bc}	7.2±1.9^{a}	26.9±4.0^{b}	75.0±2.3^{ab}	6.1±2.0^{c}
PF	247.3±103.5^{c}	20.7±3.3^{bc}	2.5±0.4^{b}	14.1±2.2^{c}	78.0±5.4^{a}	8.3±2.8^{bc}
ME	351.2±29.1^{b}	20.7±2.1^{bc}	8.1±2.2^{a}	30.9±7.1^{ab}	73.5±1.5^{b}	8.0±2.1^{bc}
WK	429.2±22.2^{a}	18.0±1.8^{c}	8.4±1.6^{a}	33.5±5.8^{a}	69.3±1.8^{c}	13.1±1.5^{a}
Significance‡	*	*	*	*	*	*

	Chemical composition							
	Hemicelluloses (% DM)		Cellulose (% DM)		ADL (% DM)		IVDMD (% DM)	
Genotype	LB	ST	LB	ST	LB	ST	LB	ST
DE	27.7±4.6	15.7±3.6	27.6±3.1	43.6±1.0^{a}	8.3±1.5^{bc}	9.7±0.8^{a}	70.4±7.9	39.8±3.1^{c}
DL	23.2±9.4	16.1±4.2	28.3±4.7	29.3±3.3^{c}	11.4±4.7^{ab}	6.5±1.1^{b}	66.8±7.6	65.8±8.0^{a}
HY	27.7±5.4	16.2±6.5	30.0±3.7	39.4±1.7^{b}	9.2±1.6^{abc}	9.8±0.9^{a}	70.6±4.3	49.0±8.0^{b}
ME	30.2±6.4	20.6±3.9	26.2±4.5	38.2±4.2^{b}	8.1±3.3^{bc}	9.5±1.3^{a}	65.6±6.1	46.7±6.6^{bc}
PF	24.5±6.1	17.0±2.5	26.2±2.9	40.1±2.9^{ab}	11.9±3.2^{a}	9.9±1.0^{a}	66.2±11.7	43.1±4.2^{bc}
WK	30.8±3.8	16.0±8.0	27.4±2.8	38.6±5.7^{b}	7.0±1.5^{c}	9.2±2.3^{a}	61.4±9.6	44.9±10.7^{bc}
Significance‡	ns	ns	ns	*	*	*	ns	*

Mean±Standard deviation, Agronomic attribute: PH: Plant height, TN: Tiller number, LAI: Leaf area index, DMY: Dry matter yield, PST: Percentage of stem inclusive of leaf sheath to total dry weight, Pdd: Percentage of dead leaves to total dry weight. Chemical composition: ADL: Acid detergent lignin, IVDMD: *In vitro* dry matter digestibility, LB: Leaf blade, ST: Stem inclusive of leaf sheath. [†]Genotype: DE: Dwarf variety of early-heading type, DL: Dwarf variety of late-heading type, HY: Hybrid of pearl millet with napier grass, PF: Purple-foliage type, ME: Normal varieties of Merkeron and WK: Wruk wona. *, $p<0.05$; ns, not significant ($p>0.05$), Values with different superscripts in the same column differ significantly by LSD method ($p<0.05$), ‡ Significance at 5% level by ANOVA

The LAI values ranging from 2.5 to 8.5 were in a good agreement with research carried out for DE, DL, ME and WK in Miyazaki (Mukhtar *et al*., 2003) and ME in Fukuoka (Kubota *et al*., 1994) in Kyushu, Japan.

Biomass quality: Values for mean and standard deviation of chemical composition of both LB and ST at harvest in November 2009 (Table 2) showed that the composition of several components of LB, such as hemicelluloses, cellulose content and IVDMD in LB, were not significantly different among the 6 genotypes. In contrast, in ST, cellulose, ADL content and IVDMD in ST were significantly different among the same genotypes, where DL had the lowest cellulose and ADL content and the highest IVDMD at 65.8% and DE had the highest cellulose content at 43.6% in ST.

Correlation analysis of growth attributes, yield and chemical compositions: Correlation coefficients of growth attributes, yield and chemical composition of both LB and ST among the 6 genotypes (Table 3) showed that plant height was positively correlated ($p<0.01$) with DM yield, hemicellulose content in LB and cellulose and ADL content in ST, influenced by the lower values of these attributes in DL and of DM yield in PF. Tiller number was negatively correlated ($p<0.01$) with percentage of stem to total dry weight (PST), influenced by the lowest value in DL. The DM yield was positively correlated with PST and negatively correlated with tiller number, ADL content

Table 3: Correlation coefficients of agronomic attributes and chemical compositions with ethanol production potential of six napier grass genotypes in the year of establishment

	n	Ethanol potential	PH	TN	LAI	PST	DMY	LB-Hemi	ST-Hemi	LB-Cellu	ST-Cellu	LB-IVDMD	ST-IVDMD	LB-ADL
PH	36	0.741**	-											
TN	36	-0.609**	-0.663**	-										
LAI	18	ns	ns	ns	-									
PST	36	0.408*	0.395*	-0.908**	-0.489*	-								
DMY	36	0.880**	0.770**	-0.554**	0.628**	0.361*	-							
LB-Hemi	36	0.454**	0.424**	ns	ns	ns	0.506**	-						
ST-Hemi	36	ns	ns	ns	ns	ns	Ns	ns	-					
LB-Cellu	36	ns	ns	ns	ns	ns	Ns	-0.673**	ns	-				
ST-Cellu	36	0.481**	0.507**	-0.707**	ns	0.703**	0.397*	ns	ns	ns	-			
LB-IVDMD	36	ns	ns	ns	ns	ns	Ns	ns	ns	ns	ns	-		
ST-IVDMD	36	-0.523**	-0.520**	0.726**	ns	-0.709**	-0.509**	ns	ns	ns	-0.760**	ns	-	
LB-ADL	36	-0.518**	-0.531**	ns	ns	ns	-0.543**	-0.789**	ns	0.334*	ns	ns	ns	-
ST-ADL	36	0.491**	0.467**	-0.681**	ns	0.650**	0.394*	ns	ns	ns	0.880**	ns	-0.724**	ns

†Agronomic attribute: PH: Plant height, TN: Tiller number, LAI: Leaf area index, PST: Percentage of stem to total dry weight, DMY: Dry matter yield. †Chemical composition: LB-Hemi: Hemicelluloses content in leaf blade, ST-Hemi: Hemicelluloses content in stem inclusive of leaf sheath, LB-Cellu: Cellulose content in leaf blade, ST-Cellu: Cellulose content in stem inclusive leaf sheath, IVDMD: *In vitro* dry matter digestibility, LB-IVDMD: IVDMD in leaf blade, ST-IVDMD: IVDMD in stem inclusive leaf sheath, ADL: Acid detergent lignin, LB-ADL: ADL content in leaf blade, ST-ADL: ADL content in stem inclusive leaf sheath, *Significant at $p<0.05$, **Significant at $p<0.01$ by Pearson correlation analysis ($n = 36$, except for LAI, $n = 18$), ns: Not significant at $p>0.05$

in LB and IVDMD in ST and was influenced by the lowest PST and the highest ST-IVDMD in DL. PST was a good indicator for chemical composition, since it was positively correlated ($p<0.01$) with cellulose and ADL content in ST and negatively correlated ($p<0.01$) with ST-IVDMD which was influenced by the lowest values of PST and cellulose and ADL content in ST and the highest values of ST-IVDMD in DL.

Ethanol production potential and future perspective: Significant differences in ethanol production potential of the 6 napier grass genotypes were detected from both LB and ST in the range 1826-5850 L ha^{-1} $year^{-1}$ (Fig. 2), where ME and WK were the highest producers of both tissues, followed by DE and HY and the lowest in PF and DL among genotypes.

If we could grow the highest potential genotype of ME in the whole area of abandoned cropland, a total of 386,000 ha in Japan (Matsumoto *et al.*, 2009), then ethanol production is expected to reach 2.258 millions kL $year^{-1}$ which equals 53.96 PJ of energy potential, corresponding to the target energy potential of domestic biofuel from herbage at 1.8-2.0 millions kL $year^{-1}$ in 2030 (MAFF, 2007; Tanaka, 2007). Our estimates of ethanol production could increase by achieving an optimized composition of structural carbohydrates (hemicelluloses and cellulose) by improved agronomic practices (Schmer *et al.*, 2008) and by optimizing the efficiency of hydrolysis and fermentation to more than 90% (Gunasekaran and Chandra Raj, 1999).

We examined these 6 genotypes in southern Kyushu using the Percentage of Overwintered Plants (POP) and Regrown Tiller Number (RTN) as indices of sustainability on 27 May 2010 (Table 4). Based on these results which are derived from overwintering ability only after the first year following establishment, it is difficult to accurately predict the sustainability of genotypes; however, POP was lower ($p<0.05$) in PF than the other 4 genotypes except for DE and RTN significantly differed ($p<0.05$) among genotypes, being the highest in DL, followed by DE, HY and ME and WK and PF. However, the overwintering ability was affected by the temperature

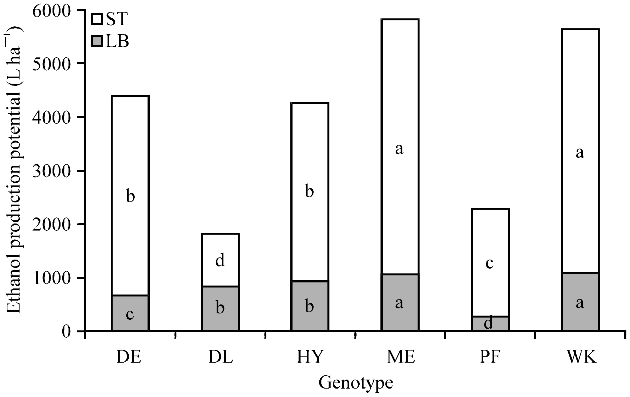

Fig. 2: Ethanol production potential in 6 genotypes of napier grass in the year of establishment Genotype: DE: Dwarf variety of early-heading type, DL: Dwarf variety of late-heading type, HY: Hybrid of pearl millet with napier grass, PF: Purple-foliage type and ME: Normal varieties of Merkeron and WK: Wruk wona; LB: Leaf blade; ST: Stem inclusive of leaf sheath. Values with different superscripts in each plant tissue differed significantly among genotypes by the LSD method (p<0.05)

Table 4: Percentage of overwintered plants and regrown tiller number of napier grass genotypes in May 2010

Genotype†	POP (%)	RTN (No m^{-2})
DE	90.0±30.51^{ab}	118.8±42.0^{b}
DL	100.0±0.00^{a}	157.1±50.7^{a}
HY	96.7±18.30^{a}	91.5±43.4^{c}
PF	83.3±37.90^{b}	29.5±13.9^{d}
ME	100.0±0.00^{a}	73.9^{c}±27.9
WK	100.0±0.00^{a}	42.3±15.1^{d}
Significance	*	*

†Genotype: DE: Dwarf variety of early-heading type, DL: Dwarf variety of late-heading type, HY: Hybrid of pearl millet with napier grass, PF: Purple-foliage type, ME: Normal varieties of Merkeron and WK: Wruk wona, POP: Percentage of overwintered plants, RTN: Regrown tiller number, Mean±Standard deviation; Values with different superscripts in the same column differ significantly (p<0.05) by LSD method, *Significance at 5% level by ANOVA

conditions in the wintering season (Ishii *et al.*, 2000) and the current study period of January-March 2010 had higher temperatures than the average over the past 30 years. Therefore, we need to monitor the sustainability of 6 genotypes in subsequent years.

Multiple regression analysis: Multiple regression analyses for ethanol production potential showed that in stepwise and forward selection, NDF content in ST (X_1) and ADL content in LB (X_2) could be used as variables to estimate ethanol production potential (Y) as follows:

$$Y = 1012.6+129.4\,X_1-406.6\,X_2,\ \text{adjusted } R^2 = 0.489\ (p<0.01),\ SSE = 1181.91$$

Anderson *et al.* (2010) demonstrated that NDF content is also the best predictor among cell wall components to estimate ethanol production in bermuda grass (*Cynodon dactylon*). However, stepwise and backward selection showed that predictors could be ADL content in LB (X_1) and in ST (X_2), estimating ethanol production potential (Y) as follows:

$$Y = 2306.9-450.9\ X_1+795.5\ X_2,\ \text{adjusted}\ R^2 = 0.467\ (p<0.01),\ SSE = 1207.3$$

Isci *et al.* (2008) also pointed out that ADL content could be the sole predictor to estimate ethanol yield from corn stover samples which is in a good agreement with our analysis.

CONCLUSION

We found that napier grass has a wide range of genotypic variation in DM yield, structural carbohydrate content and digestibility which results in variation of ethanol production potential among genotypes. The normal variety of ME was identified as the most promising genotype to produce bioethanol from napier grass, with a yield of 5850 L ha^{-1} $year^{-1}$ in the year of establishment in southern Kyushu under the assumption of theoretical conversion efficiency of 0.29 and 0.23 from cellulose and hemicelluloses, respectively.

REFERENCES

Anderson, W.F., B.S. Dien, H.J.G. Jung, K.P. Vogel and P.J. Weimer, 2010. Effects of forage quality and cell wall constituents of Bermuda grass on biochemical conversion to ethanol. Bioenerg. Res., 3: 225-237.

Anderson, W.F., B.S. Dien, S.K. Brandon and J.D. Peterson, 2008. Assessment of bermuda grass and bunch grasses as feedstock for conversion to ethanol. Applied Biochem. Biotechnol., 145: 13-21.

Badger, P.C., 2002. Ethanol from Cellulose: A General Review. In: Trends in New Crops and New Uses, Janick, J. and A. Whipkey, (Eds.). ASHS Press, Virginia, USA., pp: 7-21.

Boonman, J.G., 1993. Elephant grass Husbandry. In: East Africa's Grasses and Fodders: Their Ecology and Husbandry, Boonman, J.G. (Ed.). Kluwer Academic Publisher, Netherlands, pp: 235-257.

Chaparro, C.J., L.E. Sollenberger and C.S. Jr. Jones, 1995. Defoliation Effects on `Mott` Elephant grass Productivity and Leaf Percentage. Agron. J., 1995: 981-985.

Chaparro, C.J., L.E. Sollenberger and K.H. Quesenberry, 1996. Light interception, reserve status and persistence of clipped Mott elephant grass swards. Crop Sci., 36: 649-655.

Cuomo, G.J., D.C. Blouin and J.F. Beatty, 1996. Forage potential of dwarf napier grass and a pearl millet x napier grass hybrid. Agron. J., 88: 434-438.

Ferraris, R., 1980. Effect of harvest interval, nitrogen rates and application times on *Pennisetum purpureum* grown as an agroindustrial crop. Field Crops Res., 3: 109-120.

Goto, I. and D.J. Minson, 1977. Prediction of the day matter digestibility of tropical grasses using a pepsin-cellulase assay. Anim. Feed Sci. Technol., 2: 247-253.

Gunasekaran, P. and K. Chandra Raj, 1999. Ethanol fermentation technology-*Zymomonas mobilis* (Review). Curr. Sci., 77: 56-68.

Humphreys, L.R., 1994. Tropical Forage: Their Role in Sustainable Agriculture. Harlow, Longman, UK.

Isci, A., P.T. Murphy, R.P. Anex and K.J. Moore, 2008. A rapid Simultaneous Saccharification and Fermentation (SSF) technique to determine ethanol yields. Bioenerg. Res., 1: 163-169.

Ishii, Y., K. Ito and H. Numaguchi, 1995. Effects of cutting date and cutting height before overwintering on the spring regrowth of summer-planted napier grass (*Pennisetum purpureum* Schum). J. Jpn. Grassland Sci., 40: 396-409.

Ishii, Y., K. Ito and K. Fukuyama, 2000. Effect of several cultivation factors on the overwintering ability of napier grass in southern Kyushu. Jpn. J. Crop Sci., 69: 209-216.

Ishii, Y., M. Mukhtar, S. Idota and K. Fukuyama, 2005. Rotational grazing system for beef cows on dwarf napier grass pasture oversown with Italian ryegrass for 2 years after establishment. Grassland Sci., 51: 223-234.

Ito, K. and S. Inanaga, 1988. Studies on dry matter production of napier grass, 1: Comparison of dry matter productivities and growth parameters between Tokyo and Miyazaki. Jpn. J. Crop Sci., 57: 90-96.

Ito, K., Y. Murata, S. Inanaga, T. Ohkubo and T. Takeda *et al.*, 1988. Studies on dry matter production of napier grass. II. Dry matter productivities at six sites in southern area of Japan. Jpn. J. Crop Sci., 57: 424-430.

JMA, 2010. Previous climatic data. Japan Meteorological Agency.

Jung, H.J., K.P. Vogel, 1992. Lignification of switchgrass (*Panicum virgatum*) and big bluestem (*Andropogon gerardi*) plant parts during maturation and its effect on fiber degradability. J. Food Sci. Agric., 59: 169-176.

Kai, T., T. Tanimura, N. Nozaki, M. Suiko and K. Ogawa, 2010. Bioconversion of soft cellulosic resources into sugar and ethanol. Seibutsu-kogaku Kaishi, 88: 66-72.

Kubota, F., Y. Matsuda, W. Agata and K. Nada, 1994. The relationship between canopy structure and high productivity in napier grass, *Pennisetum purpureum* Schumach. Field Crops Res., 38: 105-110.

Lemus, R., E.C. Brummer, K.J. Moore, N.E. Molstad, C.L. Burras and M.F. Barker, 2002. Biomass yield and quality of 20 switchgrass populations in southern Iowa, USA. Biomass Bioenergy, 23: 433-442.

MAFF, 2007. The biomass Japan comprehensive strategy promotion council. Prime Minister's Report, The Ministry of Agriculture, Forestry and Fisheries (MAFF), Tokyo.

Maeda, S., 2007. Research and development trends in energy crops and biofuel conversion technologies. Science and Technology Trends, Japan. http://www.nistep.go.jp/achiev/ftx/eng/stfc/stt025e/qr25pdf/STTqr2504.pdf

Matsumoto, N., D. Sano and M. Elder, 2009. Biofuel initiatives in Japan: Strategies, policies and future potential. Applied Energy, 86: S69-S76.

McKendry, P., 2002. Energy production from biomass (part 1): Overview of biomass. Bioresour. Technol., 83: 37-46.

Mielenz, J.R., 1997. Feasibility studies for biomass-to-ethanol production facilities in Florida and Hawaii. Renewable Energy, 10: 279-284.

Mukhtar, M., Y. Ishii, S. Tudsri, S. Idota and T. Sonoda, 2003. Dry matter productivity and overwintering ability of the dwarf and normal napier grass as affected by the planting density and cutting frequency. Plant Prod. Sci., 6: 65-73.

Pearson, C.J. and D.T.W. Anthony, 1977. Hybrid pennisetum in a warm temperate climate: Effects of plant density on summer production. Austr. J. Exp. Agric. Anim. Husbandry, 17: 284-289.

Rajvanshi, A.K. and N. Nimbkhar, 2008. Sweet sorghum R and D at the Nimbkar Agricultural Research Institute (NARI). Nimbkar Agricultural Research Institute (NARI), Maharashtra, India. http://www.nariphaltan.org/sorghum.pdf

Schank, S.C., D.P. Chynoweth, C.E. Turick and P.E. Mendoza, 1993. Napier grass genotype and plant parts for biomass energy. Biomass Bioenergy, 4: 1-7.

Schmer, M.R., K.P. Vogel, R.B. Mitchell and R.K. Perrin, 2008. Net energy of cellulosic ethanol from switchgrass. Proc. Natl. Acad. Sci. USA, 105: 464-469.

Schreuder, R., P.J.M. Snijders, A.P. Wouters, A. Steg and J.N. Kariuki, 1993. Variation in OM digestibility, CP, yield and ash content of Napier grass (*Pennisetum purpureum*) and their prediction from chemical and environmental factors. Research Report, National Animal Husbandry Research Station, Naivasha, Kenya, pp: 1-62.

Skerman, P.J. and F. Riveros, 1990. Tropical Grasses. Food and Agriculture Organization, Rome, Italy, ISBN: 9789251011287, Pages: 832.

Tanaka, R., 2007. Biofuels in Japan. Science and Innovation Section, British Embassy Tokyo.

Tarawali, S.A., G. Tarawali, A. Larbi and J. Hanson, 1995. Methods of Evaluation of Forage Legumes, Grasses and Fodder Trees for use as Livestock Feed. ILRI, Nairobi, pp: 51.

Utamy, R.F., Y. Ishii, A. Wadi, S. Idota, N. Harada and K. Fukuyama, 2009. Adaptibility and sustainability of DL napier grass under cut-and-carry and grazing systems for small holder farmers in southern Kyushu Japan. Proceedings of the 3rd Korea-China-Japan Joint Symposium on Grassland Agriculture and Livestock Production, August 10-14, 2009, Konkuk University, Seoul, Korea, pp: 62-63.

Vicente-Chandler, J., S. Silva and J. Figarella, 1959. The effect of nitrogen fertilization and frequency of cutting on the yield and composition of three tropical grasses. Agron. J., 51: 202-206.

Vogel, K.P., J.F. Pedersen, S.D. Masterson and J.J. Toy, 1999. Evaluation of a filter bag system for NDF, ADF and IVDMD forage analysis. Crop Sci., 39: 276-279.

Woodard, K.R. and G.M. Prine, 1993. Dry matter accumulation of elephant grass, energycane and elephantmillet in the subtropical climate. Crop Sci., 33: 818-824.