Dry Matter Digestibility of Warm-Season Forage Crops under Different Growing Conditions and a Rapid Enzymatic Assay of Fiber for Forage Breeding

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Introduction

Warm-season forage crops belonging to C_4 plants have the potential of producing a considerable amount of dry matter, and accordingly many studies have been made for increasing forage production by using these forage crops. However, many species of them have relatively lower feeding values compared to cool-season forage crops[2,13,17,18], and there are few studies on the causes of the lower feeding values, especially on the mechanism of formation of chemical components inducing lower feeding values. Therefore, it is necessary to study the production of warm-season forage crops with high quality for herbivorous animals. This paper gives a brief presentation of our studies, conducted, first, to investigate the possibility of producing warm-season forage crops with high quality by improving cultivation techniques, and secondly, to develop a rapid method for evaluation of digestibility as a technique useful in improving feeding value by plant breeding.

Factors reducing dry matter digestibility

(1) An excellent apparatus was developed for the analysis of carbohydrates, fibers and cell wall components of forage plants[6]. It has three functions; a boiling beaker, an incubating bottle and a filtrating crucible (Fig. 1). This apparatus is used for fiber analysis as follows[6]: A dried ground sample (0.5 g) is placed directly into this apparatus.

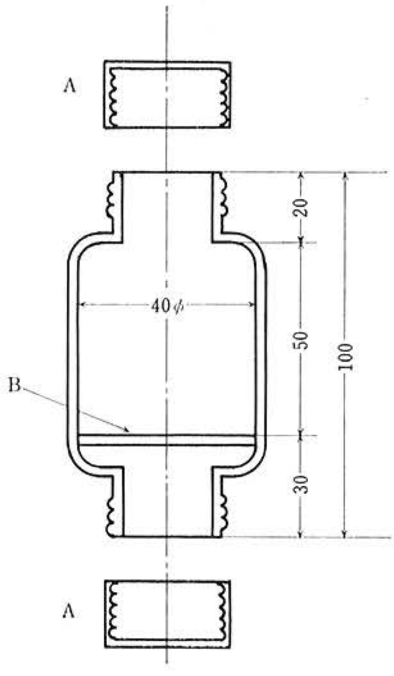

Fig. 1. Cross section of a sealed glass crucible for carbohydrates preparation
A: Sealing cap,
B: Glass filter, G3.

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Forty ml of neutral detergent solution is added and the mixture is boiled in a steam bath. After the heating the moisture is vacuumed to wash and remove dissolved substrates from the cell wall constituents (CWC). It is possible, without removing the specimen from this apparatus, to weigh CWC after drying and also to hydrolyze the specimen using callulase in the same apparatus.

Because of no necessity of transferring the sample from one apparatus to another, preparation for CWC determination and subsequent CWC hydrolysis is accomplished rapidly. Furthermore, the variation coefficients of this method in the determination of CWC and hydrolyzed CWC is lower and the reappearances of them are higher than those of the conventional method.

A part of the fiber analysis, including CWC, cellulose, hemicellulose and lignin were done by this apparatus in this study.

(2) *In vitro* dry matter digestibility (IVDMD) and chemical components affecting IVDMD in warm-season forage crops were measured to clarify the causes of changing feeding value[9]. It was made clear that high contents of CWC and low CWC digestibility (CWCD) decreased the feeding value of warm-season forage crops.

(3) Large increases in the proportion of lignin to CWC occurring with growth were found in sorghum (*Sorghum bicolor* M.), green panic (*Panicum maximum* Jacq. var. *trichoglume* Eyles) and fall panicum (*Panicum dichotomiflorum* Michx.), whereas little or no increase in the proportion was observed in bahiagrass (*Paspalum notatum* Flüegge), finger millet (*Eleusine coracana* Gaertn.) and maize (*Zea mays* L.)[9] (Fig. 2). Values of CWCD in various forage crop species showed low correlation with their lignin proportion to CWC at all the sampling dates except mature stage harvest (Table 1). This result suggests that lignin content in CWC is not a sole major factor of decreasing IVDMD of plants.

(4) The effect of maturation on digestibility of chemical components of the cell wall was examined by feeding a sorghum cultivar (Sudax) to sheep. Digestibility of lignin and/or hemicellulose was lower than that of cellulose. This result means that lignin and hemicellulose are the main factors of decreasing CWCD of sorghum.

(5) The reduction of CWCD with maturation was investigated in sorghum[10]. Indigestible CWC increased until the dough

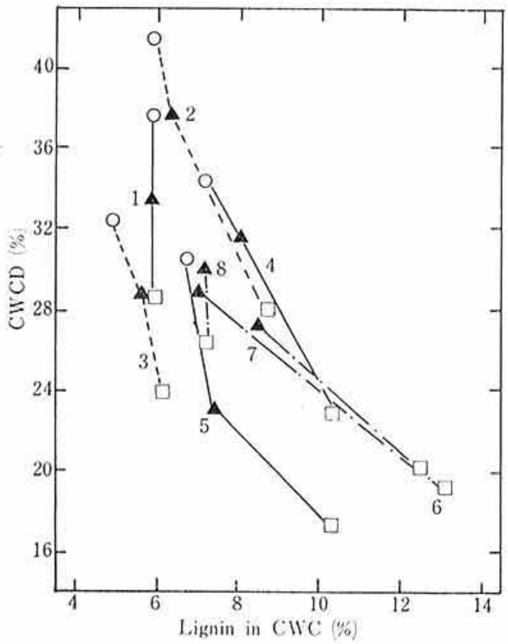


Fig. 2. Relationships between CWCD and proportion of lignin to CWC in warmseason forage crops
Species tested: 1. Bahia-grass, 2. Fall panicum, 3. Finger millet, 4. Green panic, 5. Rhodesgrass, 6. Sorghum (Sendachi), 7. Sorghum (Suzuho), 8. Maize.
Date of harvest: ○ ; July 24, ▲ ; July 31, □ ; Aug. 30.

Table 1. Correlations between CWCD and hemicellulose, cellulose, lignin or silica

Date of harvesting	Hemi-cellulose	Cellulose	Lignin	Silica
July 24	0.458	−0.574	−0.206	−0.288
July 31	0.321	−0.349	−0.476	−0.247
Aug. 30 (mature stage)	0.441	0.395	−0.772*	−0.027

* Significant at 0.05 level.

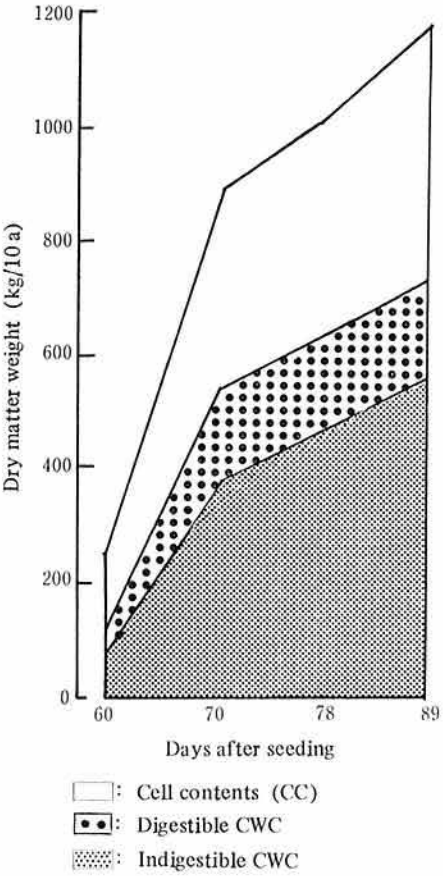


Fig. 3. Changes in the yields of CC and CWC with growth of sorghum

stage, while the proportion of digestible CWC decreased with maturation (Fig. 3). This phenomenon is the result of accelerated formation of lignin and hemicellulose as the indigestible constituents of sorghum plants.

(6) To investigate the effect of silica on IVDMD, sorghum, maize, green panic and fall panicum were grown in the media with or without silica addition by soilless culture[12]. Silica treatment decreased IVDMD and CWCD in the sorghum cultivars tested. CWCD of forage crops other than sorghum was scarcely affected by the treatment (Table 2). This result means that silica is one of the factors of reducing CWCD with sorghum, but not with the other forage crops.

These results confirmed that the low digestible dry matter and/or low digestible CWC increased due to the increase of lignin and hemicellulose.

Effects of cultivation techniques on digestible CWC production

(1) Investigation was undertaken to clarify the effect of planting density on the digestibility of sorghum cultivar (Sudax)[4]. With increasing planting density, dry matter production increased, but IVDMD and CWCD were decreased since the proportion of stem cortex, which was least digestible, increased.

Table 2. Effects of silica application on the dry matter weight, CWC composition, IVDMD and CWCD of warm-season forage crops

Attribute[b]	Sorghum (Sud)[a]		Sorghum (Suz)[a]		Corn		Green panic		Fall panicum	
	+Si	−Si	+Si	−Si	+Si	−Si	+Si	−Si	+Si	−Si
DM of plant top (g/pot)	23.2*	22.3*	24.1*	19.1*	15.0	15.8	34.8	38.5	21.0	19.2
CWC (%DM)	57.9*	54.8*	56.8*	53.7*	58.4	57.2	68.2	67.8	51.7	50.5
Hemicellulose (%DM)	24.4*	20.0*	24.2*	19.2*	23.7	21.5	27.7	27.0	21.0	20.3
Cellulose (%DM)	30.8*	33.3*	30.5*	33.0*	31.3	33.2	37.6	38.3	27.1	28.3
Lignin (%DM)	1.5	1.8	1.8	1.8	2.5	2.6	2.6	2.8	2.0*	3.4
Silicon (%DM)	2.79*	0.03*	2.64*	0.09*	2.55*	0.06*	1.45*	0.11*	0.78*	0.02*
IVDMD (%)	64.0*	70.8*	65.4*	72.3*	61.4	62.1	53.6	56.0	65.7	66.9
CWCD (%)	37.8*	46.7*	39.0*	48.5*	33.9	33.7	32.0	35.1	33.7	34.4

a): Sud; Sudax, Suz; Suzuho.
b): DM; Dry matter, CWC; Cell wall constituents, IVDMD; *In vitro* dry matter digestibility, CWCD; Cell wall constituents digestibility.
* Significant at 0.05 level.

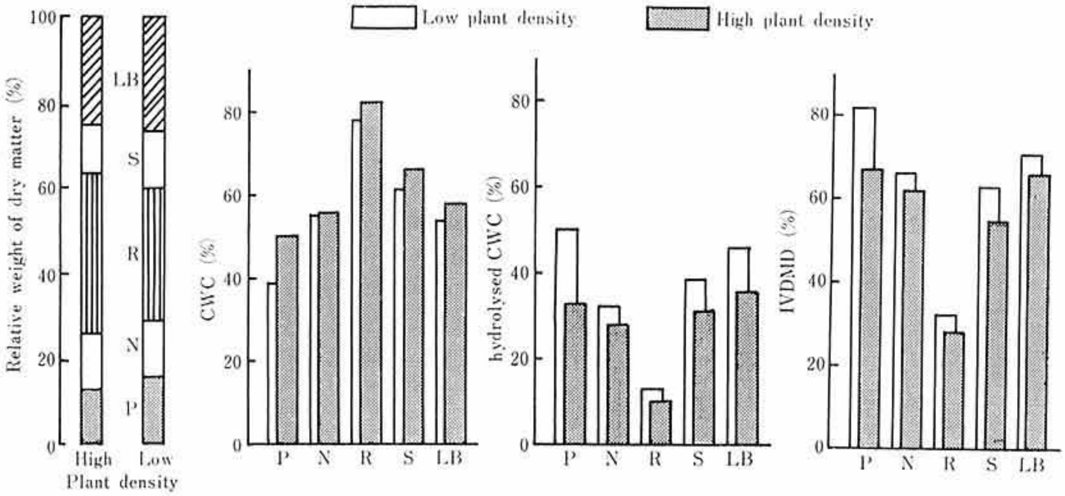


Fig. 4. Relative weight of dry matter, cell wall constituents (CWC), hydrolysed CWC by cellulase and *in vitro* dry matter digestibility (IVDMD) of individual plant composition at heading stage
P: Pith, N: Node, R: Rind, S: Sheath, LB: Leaf blade.

Table 3. Effects of mineral supplements on the dry matter yield, cell wall constituents (CWC) and CWC digestibility (CWCD)

Mineral concentration in hydroponical solution	(mM)	Dry matter yield (g/pot)	CWC (%)	CWCD (%)	Difference between treatment & standard (%) Dry matter yield	CWC	CWCD
Standard		26.0	45.3	54.4	—	—	—
N	20.0	14.0	46.4 NS	49.4*	12.0	1.1	5.0
	0.4	14.0	55.3***	36.2***	12.0	10.0	18.2
P	10.0	22.6	47.3*	47.7**	3.4	2.0	6.7
	0.2	32.1	46.2 NS	45.5**	6.1	0.9	8.9
K	10.0	27.8	48.0**	50.7*	1.8	2.7	3.7
	0.2	18.0	47.9**	43.5***	8.0	2.6	10.9
Ca	10.0	23.3	51.5***	42.5***	2.7	6.2	11.9
	0.2	21.7	43.6**	50.8*	4.3	1.7	3.6
Mg	10.0	19.1	44.1 NS	50.9*	6.9	1.2	3.5
	0.2	28.5	45.9 NS	47.8*	2.5	0.6	6.6
Mean (%)		22.5	47.4	47.2	6.0	2.9	7.9
Standard deviation (%)		5.61	3.22	4.82	3.57	2.81	4.46
CV (%)		24.9	6.8	10.2	59.5	96.9	56.5

NS: Not significanct.
*, **, *** Siginificant at 0.05, 0.01, and 0.001 levels, respectively between standard and treatment.

Therefore, at flowering stage, there was almost no significant difference in digestible dry matter production between high and middle planting densities (Fig. 4). This suggests that the variation in plant production with planting density is one of the factors of decreasing CWCD.

(2) The effect of planting density on IVDMD was investigated by using two sorghum cultivars differing in tillering habit[9]. A major portion of dry matter was distributed into abundant tillers of the cultivar Sendachi, while it was found in main stems in the case of Hiromidori. However, variations in IVDMD and CWCD with increasing density were similar for the two cultivars, because high planting density reduced CWCD in the tillers as well as in the main stem. Then, it is considered that high planting density decreases IVDMD and CWCD in sorghum plants regardless of their tillering habits. The above two results suggest that suitable planting density would exist for digestible dry matter production in sorghum.

(3) The effect of mineral nutrients on IVDMD and chemical composition was investigated in a sorghum cultivar (Sendachi) by soilless culture. IVDMD was influenced by the amount of nitrogen (N). In either low or high N supply, dry matter yield decreased, while CWC, lignin and hemicellulose : cellulose ratio increased[11], and IVDMD and CWCD decreased in comparison with the standard N supply (Table 3). It seems that the formation of structural carbohydrates (cellulose and hemicellulose) and lignin was influenced by the levels of nitrogen nutrition.

(4) Eleven species and cultivars of warm-season forage crops were cultivated under two different soil moisture condition[11]. Dry matter yield was larger and IVDMD was lower under high moisture condition than those under low moisture condition in all forage crops. However, the decreasing rate of IVDMD was relatively smaller for the forage crops more adaptable to the high soil moisture. Selection of forage crops fitted to a given soil moisture condition is necessary

Table 4. IVDMD estimated from 2-step cellulase digestion method (dried and ground sample) and from whole leaves or 5 cm fresh leaf segments after 24 hr of digestion in cellulase and rumen fluid

	IVDMD (%)							
	4-leaf stage[a]		7-leaf stage			Flag leaf stage[b]		
	Cellulase	Rumen	Cellulase	Rumen	2-step	Cellulase	Rumen	2-step
First sample								
bl[c]	64.5	51.4	37.4	26.4	—	17.3	18.0	—
B	43.5	38.5	27.5	29.1	—	12.0	10.0	—
bmr-18	67.7	48.6	22.0	8.8	—	22.9	22.6	—
N-18	53.9	54.4	19.1	13.0	—	15.1	18.2	—
Average	57.4	48.2	26.5	19.3	—	16.8	17.2	—
Range	21.0	15.9	18.3	17.6	—	7.8	4.6	—
Second sample								
bl	64.6	47.6	19.3	22.3	76.5	24.8	30.2	73.0
B	47.3	41.2	8.7	7.3	69.5	17.3	27.0	69.0
bmr-18	52.1	69.6	(14.0)[d]	10.7	71.5	24.7	31.0	72.0
N-18	39.9	74.5	(11.0)	20.5	69.0	19.7	22.9	68.5
Average	51.0	58.2	13.3	15.2	71.6	21.6	27.8	70.6
Range	24.7	33.3	10.6	15.0	7.5	7.5	7.3	4.5

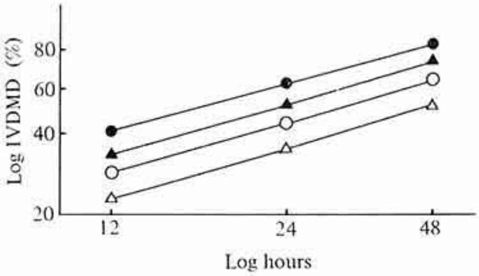
a): Data of 4-leaf stage of first samples were obtained from 12 hr digestion.
b): Sampling time at flag leaf stage was slightly different between first and second samples.
c): bl: bloomless Combine Kafir 60, B: bloom Combine Kafir 60, bmr-18: brown midrib-18, N-18: normal-18.
d): The data in parentheses were calculated by the method of Snedecor and Cochran[14].

for producing high quality forage.

(5) From the above results, high planting density, low or high N supply and high soil moisture are considered to accelerate indigestible CWC synthesis and decrease CWCD. IVDMD of warm-season forage crops would be improved if the cultivation techniques shown above as effective in increasing IVDMD are practiced properly.

Development of a simple digestion method for evaluation of IVDMD

(1) Fresh leaf samples kept in their original form were directly digested by the cellulase solution or by the rumen fluid[3,15]. Although IVDMD values of fresh leaves determined by using such a simple digestion method were lower than those of dried and ground samples determined by the 2-step cellulase digestion method[1], as shown in Table 4, the simple digestion method was usable to compare the variations of IVDMD values among sorghum lines. Especially, IVDMD values determined by the simple digestion method using cellulase were correlated well with those by the 2-step cellulase digestion method (Fig. 5).



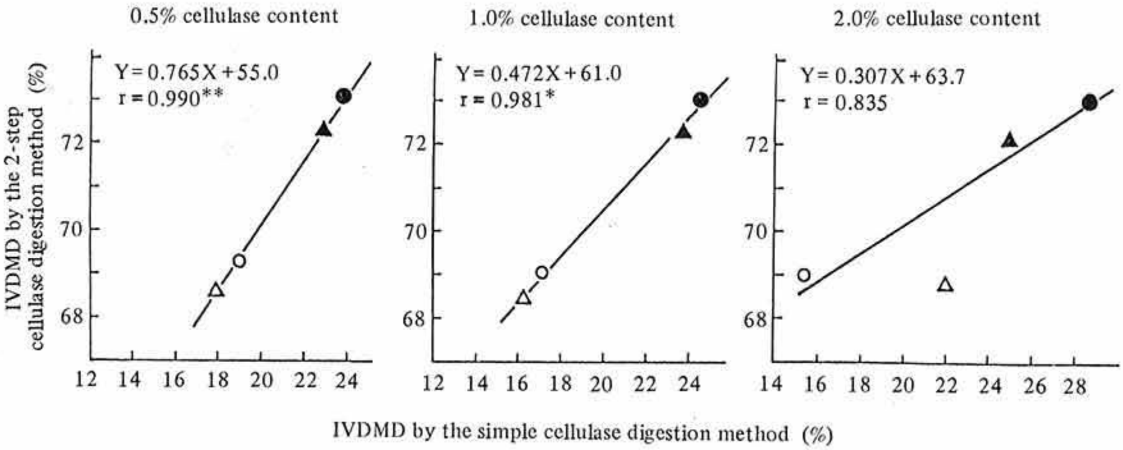
●: bl, ○: B, ▲: bmr-18, △: N-18.

Fig. 5. Relationships between digestion periods and IVDMD of fresh leaf, sampled at fourth leaf stage and digested in 1% cellulase solution, plotted on logarithmic graph paper

Regression and correlation coefficeint for each sorghum line were as follows:

bl:	$Y=0.884X+34.75$	$r=0.905$
B:	$Y=0.848X+24.65$	$r=0.992$
bmr-18:	$Y=1.091X+24.05$	$r=0.997^*$
N-18:	$Y=0.712X+17.50$	$r=0.941$

(2) The simple digestion method using fresh leaves was investigated to know the suitable cellulase concentration and digestion period[5]. With increasing cellulase concentration, differences in IVDMD values among sorghum lines increased, but correlation coefficient between IVDMD by the simple diges-



●: bl, ○: B, ▲: bmr-18, △: N-18.

Fig. 6. Relationships between IVDMD by the 2-step cellulase digestion (dried and ground leaf) and by digesting fresh leaf in each of 0.5%, 1.0% and 2.0% cellulose solution for 24 hr, in which leaves were sampled at flag leaf stage

Table 5. A proposal of the simple cellulase digestion method for evaluation of IVDMD in forage crops

1.	Material	Fully expanded top leaves from the seventh to heading leaf stages.
2.	Leaves as a sample	Several pieces of leaf blade either whole or 7 cm long segments cut from the center portion (0.5 to 1.0 g of dry weight).
3.	Digestion	Dried leaves are digested in 1% cellulase solution (55 ml) at 40°C for 48 hr.
4.	IVDMD calculation	The digested leaves are washed with distilled water, dried and weighed. IVDMD is then calculated as follows: $\text{IVDMD}(\%)=\frac{\text{Pre-dig. dry wt.}-\text{Post-dig. dry wt.}}{\text{pre-dig. dry wt.}}\times 100$

tion method and that by the 2-step cellulase digestion method became smaller and it was not significant when 2% cellulase was used (Fig. 6).

Thus, 1% cellulase solution was recognized as the optimum enzyme concentration. IVDMD of fresh leaves was increased by longer digestion periods, at least until 48 hr. With the digestion period from 12 to 48 hr, IVDMD values of the lines showed the consistent mutual relation. The digestion for 24–48 hr in 1% cellulase solution is regarded as suitable for the simple digestion method.

(3) It was found that IVDMD values of the fresh leaves were occasionally distorted presumably due to varied freshness of the leaves. Therefore the digestion of leaf sections after drying was studied[7]. The result indicated that dried leaves can be used instead of fresh leaves to determine IVDMD with the simple digestion method and that the digestion procedure does not require shaking of the cellulase solution.

(4) A further study to know the most suitable condition for determining IVDMD of dried leaves in the simple digestion method was carried out[16]. The use of 0.5–1.0 g (dry weight) of 7 cm long leaf segments, 1% cellulase (about 55 ml) solution, and 24–48 hr of the digestion period at 40°C of digestion temperature was found to be optimal. This method was named "the simple cellulase digestion method" (Table 5). In the studies using fully expanded top leaves, high correlation coefficients were found between IVDMD values determined by the simple cellulase digestion method and those determined by the 2-step cellulase digestion method with 22 sorghum

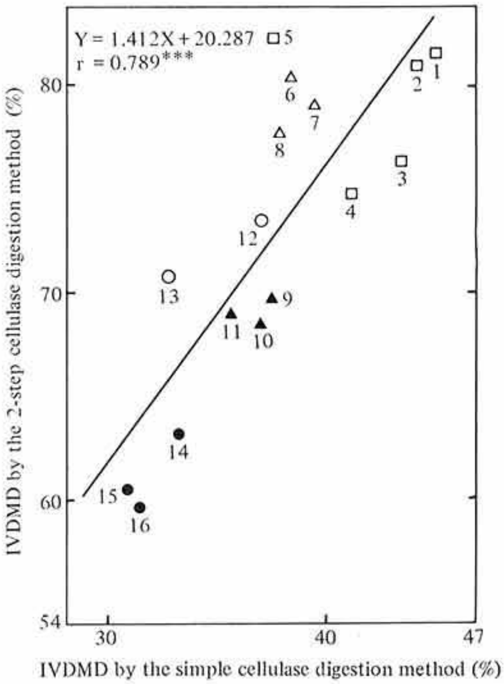


Fig. 7. Relationships between IVDMD of fully expanded top leaves by the simple cellulase digestion method and that by the 2-step cellulase digestion method
The crops were harvested on July 17.
*** Significant at 0.001 level.
□ : Finger millet (1. Iya, 2. Kyo-127, 3. IE-849, 4. Yukijirushi, 5. 73-349).
△ : Guineagrass; *Panicium maximum* Jacq. (6. Gatton, 7. Dar es Salaam, 8. Makueni).
▲ : Rhodesgrass (9. Tochiraku, 10. M. Barara, 11. FK-819).
○ : Japanese millet; *Echinochloa frumentacea* Link (12. Miyazaki, 13. Hida).
● : Maize (14. Cho ko-9, 15. Cho ko-590, 16. Cho ko-3).

cultivars ($r = 0.726^{***}$) and 16 warm-season forage crops ($r = 0.789^{***}$) (Fig. 7)[8]. It was concluded that the simple cellulase digestion method was useful because of its simplicity and rapidity for the comparison of IVDMD of crop species, cultivars, and other plant breeding materials.

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