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Research Article
Effect of Different Growth Stages on Biomass Weight and Forage Quality of Different Growth Type Soybean (*Glycine max*)

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Abstract

Background and Objective: The dry matter yield, fiber content, crude protein content and crude protein yield should depend on the cutting stage of each different soybean cultivars. This study aimed to clarify the effect of different growth stages on biomass weight and forage quality of different growth type soybean (*Glycine max*) in temperate low warm region, Japan. **Materials and Methods:** Determinate growth (DET) soybeans (Fukuyutaka, Norin 2), Indeterminate growth (IND) soybeans (Moshidou Gong 503, Williams 82) and an unknown growing type soybean (Kohamadaizu) were sowed in the beginning of June 2019 and harvested at three stages (R_1, beginning of flowering stage, R_2, full flowering stage, R_5, beginning of seed development). The study was conducted as a randomized complete block design with three replications. **Results:** Fukuyutaka, Kohamadaizu and Norin 2 were reached flowering stage later than Moshidou Gong 503 and Williams 82. Longer time for growth stages of Fukuyutaka, Kohamadaizu and Norin 2 was resulting in high plant height, stem diameter, number of branches, number of leaves and dry matter weight. The amount of fiber content (ADF and NDF) was decreasing as stages developed for Fukuyutaka, Kohamadaizu and Norin 2. In another hand, fiber content of Moshidou Gong 503 and Williams 82 was increased as stages increasing. The CP weight was increased as advancing growth stages for all cultivars. **Conclusion:** Fukuyutaka and Kohamadaizu could be suitable in the utilization of soybean forage for feed livestock in the temperate low warm region in Japan.

Key words: Soybean, growth stages, growth type, biomass, nutrient quality

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

In Okinawa islands (subtropical region) and Kyushu (temperate low warm region), Japan, several tropical types of grass have been used as animal feed. For tropical forages such as Rhodes grass (*Chloris gayana* kunth.) and Guinea grass (*Panicum maximum*), in general, the yield of biomass is increasing as advancing maturity. In other hand, nutrient value (crude protein, non-fibrous carbohydrates) of grass decreased as advancing its maturity[1]. The forage production in the southwestern warm region in Japan often facing these issues and should be solved by developing of high-protein feed so that can reduce the cost of livestock production. Although the leguminous forage, namely alfalfa (*Medicago sativa* L.) has been attempted, it could not succeed and spread in Okinawa islands (subtropical region) and Kyushu (warm region). Because alfalfa prefers alkali soil and shows poor growth in acid soils[2,3].

In contrast with grass, protein value of soybean is increasing as advancing maturity stage because of the addition of seed component[4]. The vegetative stage and seed component of soybean (*Glycine max*) combined also make good forage. Thus, soybean can be a resource of high-protein forage for ruminant[5]. Studies with several soybean cultivars[6-8] for utilization as forage showed that harvested soybean after seed development had achieved high crude protein value.

As the previous reports[9,10], in northern cold region of Japan, cultivation of soybean that intercropped with Italian ryegrass sod as living mulch plant showed high whole-plant yield and crude protein content in northern part of Japan. In research case of southwestern part of Japan that developed soybean production technique by living mulch method with Rhodes grass suggest that the addition of soybean biomass could be useful for increasing the nutrition value of silage. However, it is necessary to choose an appropriate sowing date to increase dry matter yield of soybean biomass in Southwestern of Japan[11]. In addition, an appropriate date (growth stage) for harvest (cutting) has to be clarified. Soybean cultivars (strains) are classified into indeterminate growth (IND), semi-determinate growth and determinate growth form (DET). The stem length and leaf formation in IND continue to grow for a long period after flowering. In DET, stem growth is terminated when flowering begins or soon afterward[12]. Thus, dry matter yield, fiber content, crude protein content and crude protein yield should depend on the cutting stage of each different soybean cultivars. The objective of this study was to determine the duration of soybean cultivar, agronomic distribution and the yield and nutrient quality of each component (leaf and stem) soybean cultivars at different stages of maturity in the temperate low warm region in Japan.

MATERIALS AND METHODS

Evaluation of agronomic traits: The field study was conducted in Sumiyoshi Livestock Science Station, Faculty of Agriculture, University of Miyazaki, Miyazaki prefecture which located to Southern part of Kyushu, Japan (39°59 N, 131°28 E, the elevation of 12 m above sea level). The soil type was characterized as sandy soil. The climate of Miyazaki prefecture according to the Koppen classification is Cfa that is humid subtropical climate, relatively high temperature and evenly distributed precipitation throughout the year. Precipitation and air temperature of the site during the experimental period were obtained from the database of the Geospatial Information Authority of Japan (URL: http://www.jma.go.jp/miyazaki/). This study was conducted as a randomized complete block design with 5 soybean cultivars (Fukuyutaka, Kohamadaizu, Moshidou Gong 503, Norin 2 and Williams 82) and three replications. These cultivars were provided from National Bio resource Project Soybean Lotus and Glycine Japan.

Fukuyutaka, Moshidou Gong 503, Norin 2, Williams 82 and Kohamadaizu were sowed on June 2019. All cultivars were sowed in a 0.25 m^2 single growing plot area (0.5'0.5 m). Manure (2.5% N, 4.0% P_2O_5 and 2.1% K_2O) was supplied at a rate of 1 t 10 a^{-1} before the time sowing of all soybean cultivars. A basal fertilizer consisting of nitrogen (13% N), double superphosphate (13% P_2O_5) and potassium chloride (13% K_2O) was applied to the plot area at a rate of 2.9 kg 10 a^{-1}.

Stages of soybean development from sowing to R_1 (beginning flowering), R_2 (full flowering) and R_5 (beginning seed) growth stages were recorded according to Fehr and Caviness[13]. Agronomic traits were investigated and soybean plants were cut 8 cm above ground level in each growth stages. Plant height was measured using a foldable ruler at the day of cutting for each harvest. Number of branches, leaves and stem diameter were also investigated for soybean plants on the same days of harvest in each specified growth stages. Number of pods was assessed when soybean plants reached R_5 stage.

Investigation of soybean plants nutritive value: Harvested soybean plants were fractionated into each plant fractions (stem and leaf) and dried at 60°C for 72 hrs prior to dry weight measurement. The dried samples of soybean were ground to pass through a 1 mm sieve. Total nitrogen content of each plant fractions was determined by NC-Analyzer (Model: Sumigraph NC-220F, Sumika Chemical Analysis Service, Ltd., Osaka, Japan) and the nutrient content was conversing to crude protein content by multiplying with conversion factor

6.25. The CP weight was calculated by multiplying dry matter weight with crude protein content. Acid Detergent Fiber (ADF) and Neutral Detergent Fiber (NDF) were determined by a detergent method[14] using ANKOM-200 fiber analyzer (Ankom Technology Corp., Macedon, NY). All percentages data were transformed to angular values.

Statistical analysis: Statistical analysis was conducted to compare the agronomical distribution and transformed data of nutritive value among soybean cultivars on each growth stages. Differences in means were evaluated by Tukey's test using R statistic program (URL: https://www.r-project.org/)[15].

RESULTS AND DISCUSSION

Growth stages and morphological traits: The duration of each growth stage varied in each soybean cultivars (Table 1). The duration from sowing to the beginning of flowering (R_1) in Fukuyutaka was longer than any other cultivar, followed by Kohamadaizu and Norin 2 (63, 55 and 40 days, respectively). Therefore, these cultivars had longer duration of the vegetative stage than IND cultivars (Williams 82 and Moshidou Gong 503). Moshidou Gong 503 and Norin 2 were achieved full flowering stage (R_2) not more than 10 days after first flowering, whereas that of Fukuyutaka, Williams 82 and Kohamadaizu achieved at R_2 was later than 10 days. For entering the beginning of seed development (R_5), Norin 2 (11 days) and Kohamadaizu (12 days) were shorter duration than those of another cultivar. In other hand, Fukuyutaka had longest duration (30 days) from R_2 to R_5. The result of Fukuyutaka in current study was the same with previous report by Uchino *et al.*[10] in the study about evaluation of eight soybean varieties for forage production in living mulch system, Fukuyutaka was extremely late-maturing variety which took 111 days after sowing to R_1 in no living mulch system. The growth stage duration data of Kohamadaizu, Moshidou Gong 503 and Norin 2 will contribute for the further studies related forage soybean production and breeding approach because that there is little published information. The factor that affecting differences on growth stage generally effected by variety, location and weather. Another study reported growth stage on indeterminate and determinate strains that were not affected by planting dates. Floral development is initiated by day and night length[13].

Table 2 summarized the agronomic traits distribution of five soybean cultivars at the different growth stage. All

Table 1: Duration of each growth stage in five soybean cultivars

		Days			
Cultivar	Type	Sowing-R_1	R_1-R_2	R_2-R_5	Total (Sowing-R_5)
Fukuyutaka	DET	63	11	30	104
Kohamadaizu	Unknown	55	15	12	82
Moshidou Gong 503	IND	38	4	17	59
Norin 2	DET	40	8	11	59
Williams 82	IND	35	13	24	74

R_1: Beginning of flowering, R_2: Full flowering stage, R_5: Beginning of seed development, DET: Determinate and IND: Indeterminate

Table 2: Agronomic traits distribution of five soybean cultivars at different growth stage

Cultivar	Growth stage	Plant height (cm)	Stem diameter (mm)	Number of branches	Number of leaves	Number of pods
Fukuyutaka	R_1	77.2^{bA}	10.2^{bA}	8.7^{bAB}	55.7^{cA}	
	R_2	81.8^{bA}	12.8^{abA}	15.3^{bA}	93.3^{bA}	
	R_5	95.7^{aA}	14.3^{aA}	15.7^{aA}	128.3^{aA}	176.7^{A}
Kohamadaizu	R_1	59.5^{bB}	6.5^{bB}	9.3^{aA}	45.0^{bA}	
	R_2	76.9^{aA}	7.2^{bB}	9.7^{aB}	63.0^{bB}	
	R_5	79.3^{aAB}	10.7^{aAB}	11.0^{aBC}	97.3^{aB}	194.0^{A}
Moshidou Gong 503	R_1	18.8^{bD}	3.4^{aC}	4.3^{bCB}	12.7^{cB}	
	R_2	25.6^{bD}	4.3^{aC}	7.0^{abB}	30.3^{bC}	
	R_5	64.7^{aB}	4.4^{aC}	8.0^{aC}	52.3^{aD}	105^{B}
Norin 2	R_1	38.8^{bC}	5.3^{bBC}	6.7^{aAC}	15.3^{bB}	
	R_2	46.3^{bB}	5.5^{bBC}	7.7^{aB}	27.0^{abC}	
	R_5	68.3^{aB}	7.6^{aBC}	8.0^{aC}	30.0^{aE}	38.3^{C}
Williams 82	R_1	21.7^{bD}	4.3^{bBC}	6.0^{bBC}	8.0^{cB}	
	R_2	35.6^{bC}	5.0^{bBC}	7.3^{bB}	23.0^{bC}	
	R_5	76.5^{aB}	8.5^{aB}	11.3^{aB}	77.0^{aC}	46.7^{C}

^{abc}Means values by the different lowercase letter in the column are significantly different at $p<0.05$ between stages in the same cultivar and uppercase letter in the column are significantly different at $p<0.05$ between cultivars in the same stage, R_1: Beginning of flowering, R_2: Full flowering stage, R_5: Beginning of seed development

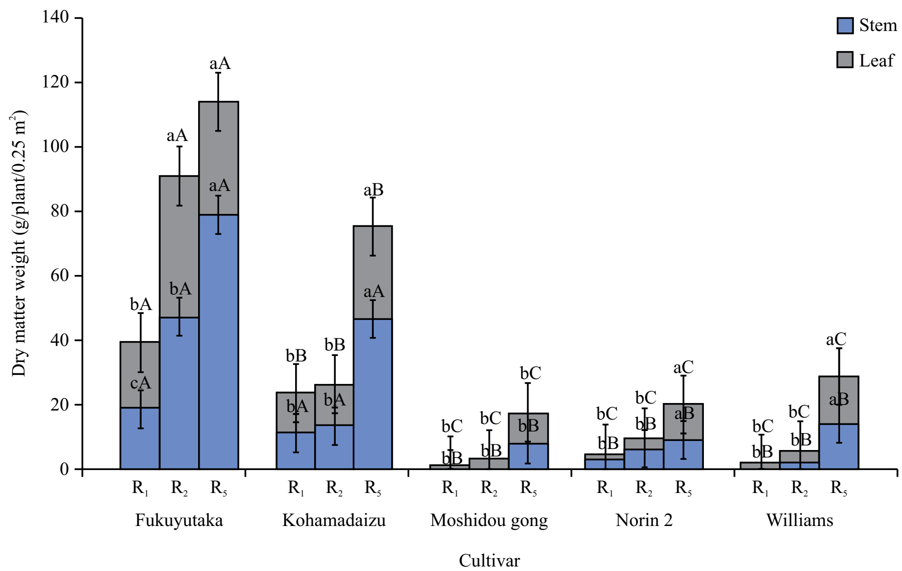

Fig. 1: Comparison of dry matter weight of five soybean cultivars, harvested at R_1: Beginning flowering, R_2: Full flowering and R_5: Beginning seed development

[abc]Means values by the different lowercase letter are significantly different at $p<0.05$ between stages in the same cultivar and uppercase letter are significantly different at $p<0.05$ between cultivars in the same stage

agronomic traits value was increased from R_1-R_5 for all cultivars, with Fukuyutaka being the tallest variety throughout the whole growth stages. Fukuyutaka had a significantly higher plant height (77.2 cm) than any other cultivar at R_1 stage. Moshidou Gong 503 and Williams 82 had short plant height at R_1 stage. Fukuyutaka had excess of stem diameter since at R_1 stage (10.2 mm). Fukuyutaka had highest number of leaves with high number of branches throughout the whole growth stage. The results related the duration of each growth stages and agronomic traits especially changes of plant height between R_2 and R_5 in Kohamadaizu, it seems to classified as DET and late-mature type similar to Fukuyutaka. In case of utilization soybeans as silage, the increase of plant height on soybean resulting in thicker plant stem and it may took longer curing time for making silage[16]. Higher percentage of stem was also had negative effect for ruminant, since ruminant tend to have less preference for stem[17]. When soybean plant used as silage, stems may be chopped to increase the palatability for livestock[16]. Blount *et al.*[16] also recommended that thinner stem may produce by cultivated soybeans in high population (1.5-2.0 bushel per acre of average size seed). Previous study related the drying characteristics of Rhodes grass have reported that the thinner and longer leaf part, in case of Rhodes grass, the quicker the drying rate[18]. Thus, the fundamental research on the evaluation of the drying characteristics in the temperate region of Japan (especially South part of Kyushu) has to be considered.

Dry matter weight and nutrient quality: Dry matter weight of all soybean cultivar was increased as growth stages developed (Fig. 1). Late-flowering soybean cultivars (Fukuyutaka and Kohamadaizu) had higher dry matter weight than that of early-flowering soybean cultivars (Moshidou Gong 503, Norin and Williams). Morse *et al.*[19] found that cutting soybeans at different growth stages has influenced to the yield, composition and the ease of curing the hay. They suggested that the crop is considered best fitted for hay production when the seeds are about half developed. Fehr and Caviness[13] have identified that yellowing in leaf and pod was sign for soybean plant mature. Harvesting soybean as forage should be completed no later than the R_7 stage of maturity due to the leaves agedness and decreasing the dry matter yields[20]. However, considering the harvest stage of intercrop-counterpart, Rhodes grass, which appropriate cutting time is around 60-70 days after sowing[21], the R_1 or R_2 stage of late-flowering soybean are suitable as cutting in subtropical (the Okinawa islands) and temperate areas (South part of Kyushu) in Japan. If the cutting stage of Fukuyutaka and Kohamadaizu is later R_5, it will take more than 100 days for growth of soybean, which excess the appropriate time for cutting of Rhodes grass. As Morse *et al.*[19] reported that if the cutting is delayed, the stems rapidly become hard and woody. Heavy loss in leaves will also occur, thus decreasing the palatability of hay.

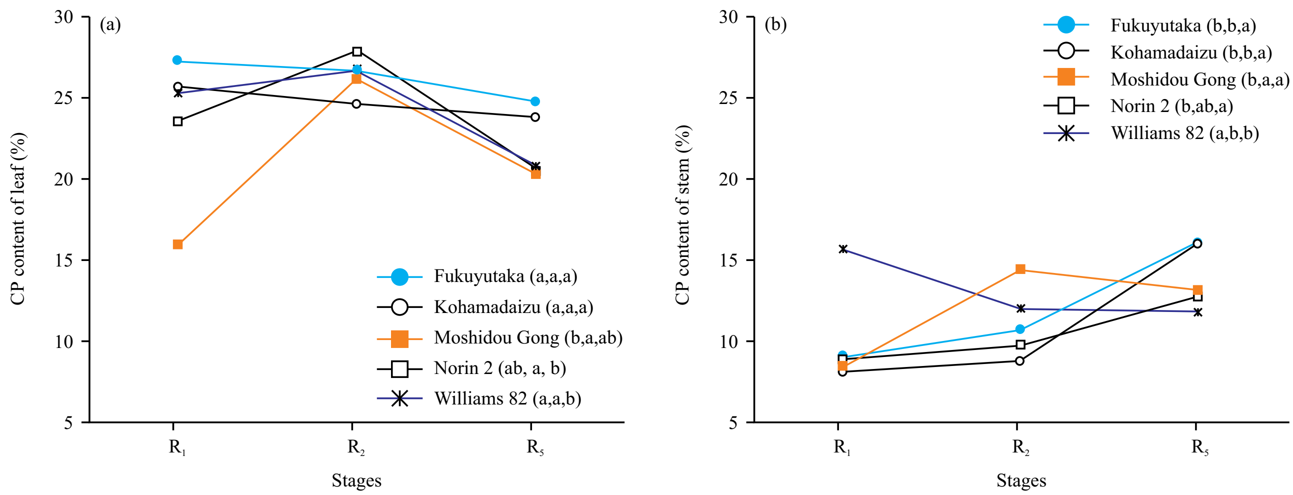

Fig. 2(a-b): Total crude protein of (a) Leaf (%) and (b) Stem (%) of five soybean cultivars, harvested at R_1: Beginning flowering, R_2: Full flowering and R_5: Beginning seed development
^{abc}Means values by the different letters inside legend are significantly different at p<0.05 between stages in the same cultivar

Table 3: Fiber quality (ADF and NDF) of five soybean cultivars

Cultivar	Growth stage	ADF (% DM)		NDF (% DM)	
		Leaf	Stem	Leaf	Stem
Fukuyutaka	R_1	29.5^{aA}	47.9^{aA}	35.2^{aA}	56.4^{aA}
	R_2	26.1^{bA}	45.3^{aB}	38.9^{aA}	56.4^{abAB}
	R_5	28.8^{abA}	40.2^{bA}	35.2^{aB}	48.2^{bAB}
Kohamadaizu	R_1	28.2^{aA}	49.5^{aA}	34.0^{bA}	59.1^{aA}
	R_2	26.6^{aA}	48.8^{aA}	35.7^{aA}	58.2^{bAB}
	R_5	24.7^{aAB}	40.1^{bA}	45.1^{aA}	48.8^{bAB}
Moshidou Gong 503	R_1	14.9^{bC}	-	19.3^{bB}	47.1^{aBC}
	R_2	-	-	25.3^{abB}	45.7^{aC}
	R_5	21.3^{aB}	38.8^{A}	30.2^{aB}	44.1^{aB}
Norin 2	R_1	22.4^{abB}	42.9^{aB}	23.6^{bB}	50.1^{aB}
	R_2	25.4^{aA}	42.1^{aC}	25.2^{abB}	46.6^{aBC}
	R_5	19.7^{bB}	38.3^{bA}	30.3^{aB}	46.1^{aAB}
Williams 82	R_1	22.1^{aB}	37.4^{aC}	22.7^{aB}	43.9^{bC}
	R_2	23.3^{aA}	39.0^{aD}	24.7^{aB}	45.8^{abBC}
	R_5	20.3^{aB}	41.8^{aA}	29.4^{aB}	50.4aa

R_1: Beginning of flowering, R_2: Full flowering stage, R_5: Beginning of seed development, ADF: Acid detergent fiber, NDF: Neutral detergent fiber, ^{abc}Means values by the different lowercase letter in the column are significantly different at p<0.05 between stages in the same cultivar and uppercase letter in the column are significantly different at p<0.05 between cultivars in the same stage

Table 3 summarized the fiber content (ADF and NDF) of five soybean cultivars at different growth stages. Interestingly, the amount of fiber (ADF and NDF) tended to be decreased with advancing stages for stem parts of DET soybean cultivars (Fukuyutaka, Norin 2) and Kohamadaizu. Hintz *et al.*[17] reported that the addition of pod fraction decreasing ADF and NDF concentration of the stem fraction. Thus R_7 has resulting in high nutritive value for soybean plant. On the other hand, the NDF fractions of leaf in IND soybean cultivars (Moshidou Gong 503 and Williams 82) increased from R_1-R_5.

Figure 2a showed that CP content in leaf of IND soybeans (Moshidou Gong 503 and Williams) was increased between R_1 and R_2, however, they were decreased between R_2 and R_5. Decreasing trend of CP in leaf between R_2 and R_5 was showed in all cultivars. In another hand, CP content in stem was increased between R_2 and R_5 for DET soybean (Fukuyutaka and Norin 2) and Kohamadaizu (Fig. 2b). The CP content for R_5 was in good agreement with previous report that found the increasing nitrogen (N) in R_5. The increasing trend in stage R_5 because coincides with a time when N is being redistributed to the seed and would thus be in transit in the vascular tissue

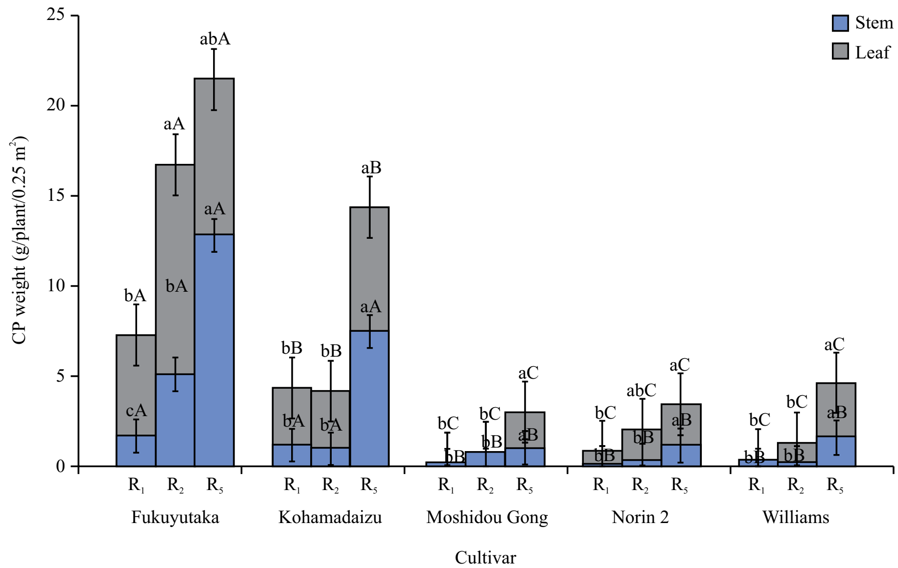

Fig. 3: Comparison of crude protein weight of five soybean cultivars, harvested at R_1: Beginning flowering, R_2: Full flowering and R_5: Beginning seed development

[abc]Means values by the different lowercase letter are significantly different at p<0.05 between stages in the same cultivar and uppercase letter are significantly different at p<0.05 between cultivars in the same stage

of the stem[22-28]. There were also positively correlated between cultivar differences with the amount of N in the plant at $R_{524,29,30}$. Study about N remobilization in different beans reported that N remobilization may be an important drought adaptation for legume plant under water deficits[31]. For aspect of CP weight, the CP weight of all soybean cultivars was increased as growth stage increased (Fig. 3). The CP weight of Kohamadaizu was decreased between R_1 and R_2, however, it was increased between R_2 and R_5.

CONCLUSION

In conclusion, late-maturing soybean cultivars (Fukuyutaka and Kohamadaizu) produced higher amount of dry matter than those of early-maturing soybean cultivars (Moshidou Gong 503 and Williams 82) with indeterminate (IND) growth type. Fiber content (ADF and NDF) was decreased as advancing growth stages for late-maturing cultivar with DET growth type. On the other hand, CP content increased as advancing growth stages for late-maturing cultivar with DET growth type resulting higher CP weight. Late-maturing soybean cultivar could be harvested since beginning flowering time and best for cutting when reached beginning seed development. In addition, considering best cutting height for Rhodesgrass was 70 days after sowing, late-maturing cultivar could be intercropped with Rhodesgrass for hay or silage making.

SIGNIFICANCE STATEMENT

This study discovered the importance of growth stage and growth type in different soybean cultivars and its effect to biomass and quality that can be beneficial for researchers and farmers to choose appropriate harvesting time for soybean as forage. This study will help the researcher to uncover the critical areas of soybean as forage in Japan that many researchers were not able to explore. Thus a new theory on appropriate cutting time of soybean as forage in Japan may be arrived at.

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