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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<sub>1</sub>, beginning of flowering stage, R<sub>2</sub>, full flowering stage, R<sub>3</sub>, 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<sup>1</sup>. 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<sup>2,3</sup>.

In contrast with grass, protein value of soybean is increasing as advancing maturity stage because of the addition of seed component<sup>4</sup>. 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<sup>5</sup>. Studies with several soybean cultivars<sup>6-8</sup> for utilization as forage showed that harvested soybean after seed development had achieved high crude protein value.

As the previous reports<sup>9,10</sup>, 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<sup>11</sup>. 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<sup>12</sup>. 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<sup>2</sup> single growing plot area (0.5'0.5 m). Manure (2.5% N, 4.0% P<sub>2</sub>O<sub>5</sub> and 2.1% K<sub>2</sub>O) was supplied at a rate of 1 t 10 a<sup>-1</sup> before the time sowing of all soybean cultivars. A basal fertilizer consisting of nitrogen (13% N), double superphosphate (13% P<sub>2</sub>O<sub>5</sub>) and potassium chloride (13% K<sub>2</sub>O) was applied to the plot area at a rate of 2.9 kg 10 a<sup>-1</sup>.

Stages of soybean development from sowing to R<sub>1</sub> (beginning flowering), R<sub>2</sub> (full flowering) and R<sub>5</sub> (beginning seed) growth stages were recorded according to Fehr and Caviness<sup>13</sup>. 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<sub>5</sub> 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 converting 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<sup>14</sup> 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/>)<sup>15</sup>.

## 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<sub>1</sub>) 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<sub>2</sub>) not more than 10 days after first flowering, whereas that of Fukuyutaka, Williams 82 and Kohamadaizu achieved at R<sub>2</sub> was later than 10 days. For entering the beginning of seed development (R<sub>5</sub>), 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<sub>2</sub> to R<sub>5</sub>. The result of Fukuyutaka in current study was the same with previous report by Uchino *et al.*<sup>10</sup> 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<sub>1</sub> 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<sup>13</sup>.

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

Cultivar	Type	Days			Total (Sowing-R <sub>5</sub> )
		Sowing-R <sub>1</sub>	R <sub>1</sub> -R <sub>2</sub>	R <sub>2</sub> -R <sub>5</sub>	
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<sub>1</sub>: Beginning of flowering, R<sub>2</sub>: Full flowering stage, R<sub>5</sub>: 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 <sub>1</sub>	77.2 <sup>ba</sup>	10.2 <sup>ba</sup>	8.7 <sup>baB</sup>	55.7 <sup>ca</sup>	
	R <sub>2</sub>	81.8 <sup>ba</sup>	12.8 <sup>baA</sup>	15.3 <sup>ba</sup>	93.3 <sup>ba</sup>	
	R <sub>5</sub>	95.7 <sup>aa</sup>	14.3 <sup>aa</sup>	15.7 <sup>aa</sup>	128.3 <sup>aa</sup>	176.7 <sup>A</sup>
Kohamadaizu	R <sub>1</sub>	59.5 <sup>bb</sup>	6.5 <sup>bb</sup>	9.3 <sup>aa</sup>	45.0 <sup>ba</sup>	
	R <sub>2</sub>	76.9 <sup>aa</sup>	7.2 <sup>bb</sup>	9.7 <sup>ab</sup>	63.0 <sup>bb</sup>	
	R <sub>5</sub>	79.3 <sup>aaB</sup>	10.7 <sup>aaB</sup>	11.0 <sup>abC</sup>	97.3 <sup>ab</sup>	194.0 <sup>A</sup>
Moshidou Gong 503	R <sub>1</sub>	18.8 <sup>bd</sup>	3.4 <sup>ac</sup>	4.3 <sup>bcB</sup>	12.7 <sup>cb</sup>	
	R <sub>2</sub>	25.6 <sup>bd</sup>	4.3 <sup>ac</sup>	7.0 <sup>abB</sup>	30.3 <sup>bc</sup>	
	R <sub>5</sub>	64.7 <sup>ab</sup>	4.4 <sup>ac</sup>	8.0 <sup>ac</sup>	52.3 <sup>ad</sup>	105 <sup>B</sup>
Norin 2	R <sub>1</sub>	38.8 <sup>bc</sup>	5.3 <sup>bbc</sup>	6.7 <sup>aaC</sup>	15.3 <sup>bb</sup>	
	R <sub>2</sub>	46.3 <sup>bb</sup>	5.5 <sup>bbc</sup>	7.7 <sup>ab</sup>	27.0 <sup>abC</sup>	
	R <sub>5</sub>	68.3 <sup>ab</sup>	7.6 <sup>abc</sup>	8.0 <sup>ac</sup>	30.0 <sup>ae</sup>	38.3 <sup>C</sup>
Williams 82	R <sub>1</sub>	21.7 <sup>bd</sup>	4.3 <sup>bbc</sup>	6.0 <sup>bbc</sup>	8.0 <sup>cb</sup>	
	R <sub>2</sub>	35.6 <sup>bc</sup>	5.0 <sup>bbc</sup>	7.3 <sup>bb</sup>	23.0 <sup>bc</sup>	
	R <sub>5</sub>	76.5 <sup>ab</sup>	8.5 <sup>ab</sup>	11.3 <sup>ab</sup>	77.0 <sup>ac</sup>	46.7 <sup>C</sup>

<sup>abc</sup>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<sub>1</sub>: Beginning of flowering, R<sub>2</sub>: Full flowering stage, R<sub>5</sub>: Beginning of seed development

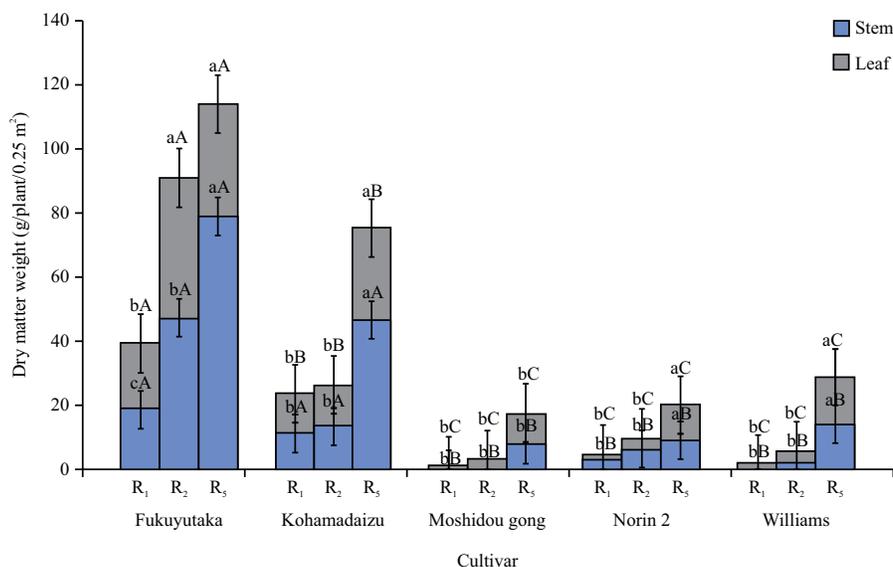


Fig. 1: Comparison of dry matter weight of five soybean cultivars, harvested at R<sub>1</sub>: Beginning flowering, R<sub>2</sub>: Full flowering and R<sub>5</sub>: Beginning seed development

<sup>abc</sup>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<sub>1</sub>-R<sub>5</sub> 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<sub>1</sub> stage. Moshidou Gong 503 and Williams 82 had short plant height at R<sub>1</sub> stage. Fukuyutaka had excess of stem diameter since at R<sub>1</sub> 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<sub>2</sub> and R<sub>5</sub> 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<sup>16</sup>. Higher percentage of stem was also had negative effect for ruminant, since ruminant tend to have less preference for stem<sup>17</sup>. When soybean plant used as silage, stems may be chopped to increase the palatability for livestock<sup>16</sup>. Blount *et al.*<sup>16</sup> 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<sup>18</sup>. 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.*<sup>19</sup> 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<sup>13</sup> 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<sub>7</sub> stage of maturity due to the leaves agedness and decreasing the dry matter yields<sup>20</sup>. However, considering the harvest stage of intercrop-counterpart, Rhodes grass, which appropriate cutting time is around 60-70 days after sowing<sup>21</sup>, the R<sub>1</sub> or R<sub>2</sub> 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<sub>5</sub>, 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.*<sup>19</sup> 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.

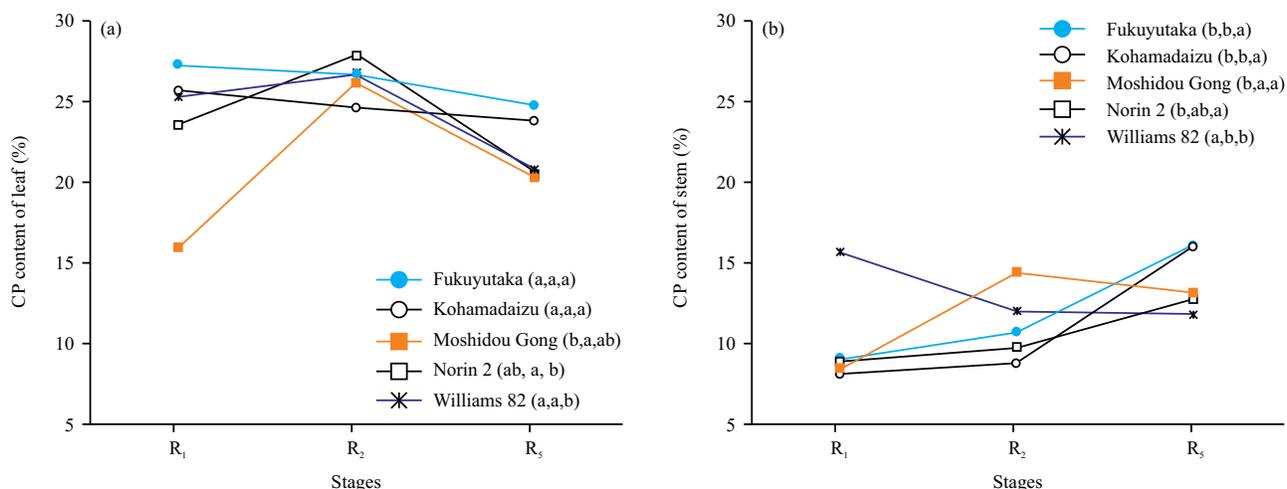


Fig. 2(a-b): Total crude protein of (a) Leaf (%) and (b) Stem (%) of five soybean cultivars, harvested at R<sub>1</sub>: Beginning flowering, R<sub>2</sub>: Full flowering and R<sub>5</sub>: Beginning seed development

<sup>abc</sup>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 <sub>1</sub>	29.5 <sup>aA</sup>	47.9 <sup>aA</sup>	35.2 <sup>aA</sup>	56.4 <sup>aA</sup>
	R <sub>2</sub>	26.1 <sup>bA</sup>	45.3 <sup>ab</sup>	38.9 <sup>aA</sup>	56.4 <sup>abAB</sup>
	R <sub>5</sub>	28.8 <sup>abA</sup>	40.2 <sup>bA</sup>	35.2 <sup>ab</sup>	48.2 <sup>bAB</sup>
Kohamadaizu	R <sub>1</sub>	28.2 <sup>aA</sup>	49.5 <sup>aA</sup>	34.0 <sup>bA</sup>	59.1 <sup>aA</sup>
	R <sub>2</sub>	26.6 <sup>aA</sup>	48.8 <sup>aA</sup>	35.7 <sup>aA</sup>	58.2 <sup>bAB</sup>
	R <sub>5</sub>	24.7 <sup>aAB</sup>	40.1 <sup>bA</sup>	45.1 <sup>aA</sup>	48.8 <sup>bAB</sup>
Moshidou Gong 503	R <sub>1</sub>	14.9 <sup>bC</sup>	-	19.3 <sup>bb</sup>	47.1 <sup>abC</sup>
	R <sub>2</sub>	-	-	25.3 <sup>abB</sup>	45.7 <sup>aC</sup>
	R <sub>5</sub>	21.3 <sup>ab</sup>	38.8 <sup>A</sup>	30.2 <sup>ab</sup>	44.1 <sup>ab</sup>
Norin 2	R <sub>1</sub>	22.4 <sup>abB</sup>	42.9 <sup>ab</sup>	23.6 <sup>bb</sup>	50.1 <sup>ab</sup>
	R <sub>2</sub>	25.4 <sup>aA</sup>	42.1 <sup>aC</sup>	25.2 <sup>abB</sup>	46.6 <sup>abC</sup>
	R <sub>5</sub>	19.7 <sup>bb</sup>	38.3 <sup>bA</sup>	30.3 <sup>ab</sup>	46.1 <sup>aAB</sup>
Williams 82	R <sub>1</sub>	22.1 <sup>ab</sup>	37.4 <sup>aC</sup>	22.7 <sup>ab</sup>	43.9 <sup>bc</sup>
	R <sub>2</sub>	23.3 <sup>aA</sup>	39.0 <sup>d</sup>	24.7 <sup>ab</sup>	45.8 <sup>abBC</sup>
	R <sub>5</sub>	20.3 <sup>ab</sup>	41.8 <sup>aA</sup>	29.4 <sup>ab</sup>	50.4 <sup>aa</sup>

R<sub>1</sub>: Beginning of flowering, R<sub>2</sub>: Full flowering stage, R<sub>5</sub>: Beginning of seed development, ADF: Acid detergent fiber, NDF: Neutral detergent fiber, <sup>abc</sup>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.*<sup>17</sup> reported that the addition of pod fraction decreasing ADF and NDF concentration of the stem fraction. Thus R<sub>7</sub> 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<sub>1</sub>-R<sub>5</sub>.

Figure 2a showed that CP content in leaf of IND soybeans (Moshidou Gong 503 and Williams) was increased between R<sub>1</sub> and R<sub>2</sub>, however, they were decreased between R<sub>2</sub> and R<sub>5</sub>. Decreasing trend of CP in leaf between R<sub>2</sub> and R<sub>5</sub> was showed in all cultivars. In another hand, CP content in stem was increased between R<sub>2</sub> and R<sub>5</sub> for DET soybean (Fukuyutaka and Norin 2) and Kohamadaizu (Fig. 2b). The CP content for R<sub>5</sub> was in good agreement with previous report that found the increasing nitrogen (N) in R<sub>5</sub>. The increasing trend in stage R<sub>5</sub> because coincides with a time when N is being redistributed to the seed and would thus be in transit in the vascular tissue

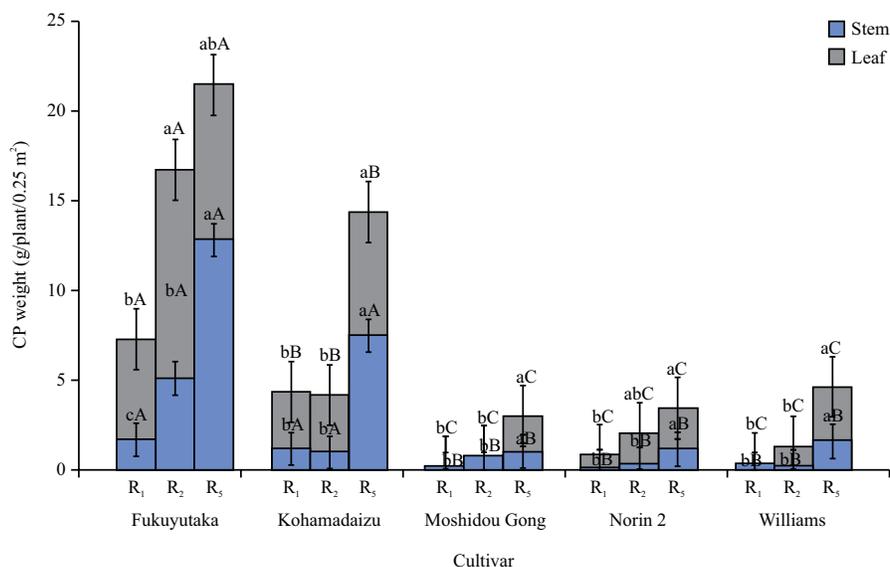


Fig. 3: Comparison of crude protein weight of five soybean cultivars, harvested at R<sub>1</sub>: Beginning flowering, R<sub>2</sub>: Full flowering and R<sub>5</sub>: Beginning seed development

<sup>abc</sup>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<sup>22-28</sup>. There were also positively correlated between cultivar differences with the amount of N in the plant at R<sub>5</sub><sup>24,29,30</sup>. Study about N remobilization in different beans reported that N remobilization may be an important drought adaptation for legume plant under water deficits<sup>31</sup>. 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<sub>1</sub> and R<sub>2</sub>, however, it was increased between R<sub>2</sub> and R<sub>5</sub>.

## 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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