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Evaluation of regrowth ability of soybeans for forage utilization under two-cutting systems

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Abstract

Soybean (*Glycine max*) has the potential as forage feed because of its high protein content and low fiber. Intercropping soybean with tropical forage grasses can improve forage quality and increase yield. However, their optimum cutting interval under mixed cropping system is still unknown. This study aimed to investigate regrowth ability and performance of soybean plant under two different cutting heights in Miyazaki, Southwestern of Japan, which has a humid subtropical climate with relatively high temperature and evenly distributed precipitation throughout the year. The experimental design was randomized block design arrange in 3 $\times$ 2 factorial scheme (soybean cultivars $\times$ cutting treatments). Three soybean accessions (*Glycine max* 'Fukuyutaka', *G. max* 'Kohamdaizu' and *G. gracilis* 'Moshidou Gong 503') with 5 replications were cultivated under single seeding condition. Soybeans were defoliated after 50 d of cultivation under low level cutting height (LC, 7.5 cm) and high level cutting height (HC, 12 cm). 'Fukuyutaka' achieved 100% regrowth rate in both LC and HC. Although the regrowth rate for 'Kohamadaizu' was 100% in the HC, it was only 20% in LC. 'Fukuyutaka' was more robust and high tolerant for defoliation stress than that of 'Kohamadaizu`. In other hand, no plants exhibited regrowth in both cutting heights for 'Moshidou Gong 503'. For 'Fukuyutaka' and 'Kohamadaizu', the number of branching nodes with re-emergent leaves was significantly higher in the HC. In both cutting height of 'Fukuyutaka', plant continued elongating and exhibited vigorous growth until 2nd cutting. The cutting height levels, height of cotyledonary node and growth stage of soybean plant when harvested at the 1st cutting significantly affected the regrowth ability of soybeans. In conclusion, 'Fukuyutaka' showed the ability for regrowth under LC and HC. HC provided significantly higher fresh matter and dry matter weight than that LC. Further study is needed to evaluate the performance of 'Fukuyutaka' under intercropping cultivation with tropical forage grasses. This is the first report to establish an approach for two-cutting soybean as forage.

Keywords: cutting height, two-cutting technique, soybean.
Abbreviations: LC_Low cutting, HC_High cutting.

Introduction

Tropical forage grasses are mainly cultivated in the southwestern region of Japan. Its cultivation will increase as global climate causes the summer depression of grassland zones to extend northwards (Sasaki et al., 2003). Even though tropical grasses exhibit high dry matter yield, the nutrient value such as crude protein, significantly decrease as the growth stage develop. This fall in CP is caused by two factors: first, as forage mature there is an increase in the proportion of leaf sheath and flowering stem, which have a lower CP than the leaf fraction, and second is the fall in CP of all plant fractions as forage grasses mature (Minson, 1990). Therefore, it is important to introduce new crops as alternative sources of high-protein forages to compensate for the shortcomings of tropical grasses.

Soybean (*Glycine max*) is a high-protein legume and can potentially be used as a forage in the southwestern region of Japan (Prasojo et al., 2021). Soybean plant has also ability to fix atmospheric nitrogen (Zapata et al., 1987) and reduce N_2O to N_2 emitted into the atmosphere via rhizobial symbiotic N fixation (Sanchez and Minamisawa, 2019). Therefore, soybean might play beneficial role for crops under intercropping cultivation system (Yang et al., 2018; Xu et al., 2020). Likewise, the addition of soybean plants improves the nutritional value of forage in the grass pasture (Acikgoz et al., 2013; Peiretti et al., 2017; Roger et al., 2017). Kaneko et al. (2011) and Uchino et al. (2016) reported that cultivation of soybean intercropped with Italian ryegrass sod as living mulch plant showed high whole-plant yield and crude protein content in northern part of Japan. In another report, Prasojo et al. (2019) reported the development of soybean production technique by living mulch method with Rhodes grass (*Chloris gayana* kunth.) in the southwestern region of Japan, and suggested that the addition of soybean biomass could be useful for increasing the nutrition value of silage.

Forages (grasses) are harvested once or more during the growing season as whole plant while soybean plant can be harvested only once as seed. It will lose the chance to gain high-protein feed for grass after first defoliation. Most of the soybean varieties have standing and finite growth type, the subsequent regrowth is not performed after harvest at the optimum cutting time and cutting height. In legume, the nitrogen fixation in plant will reduced and ceases quickly immediately after defoliation (Sanderson et al., 1997). Therefore, it is necessary to select soybean varieties that are amenable for multiple cutting to incorporate into the production system and establishing multi-cutting system for tropical grass-soybean pasture. However, there is no information about dry matter yield in soybean regrowth after the initial cutting. In this study, we aimed to investigate the regrowth ability and dry matter yield of three soybean accessions with different growth types ['Fukuyutaka' (determinate), 'Kohamadaizu' (determinate) and 'Moshidou Gong 503' (indeterminate)] under two different cutting heights in the southwestern region of Japan.

Results

Agronomic traits and regrowth ability of soybean

The growth stages were different in each soybean accessions at the time of the first cutting (Table 1). 'Fukuyutaka' and 'Kohamadaizu' were at the vegetative stage, V6 (six number of nodes with full open leaf) and V4 (four number of nodes with full open leaf), respectively. Meanwhile, the growth stage of 'Moshidou Gong 503' was R5 (beginning of the seed development; mean number of flower: 44.5 and mean number of pods: 30.9) on the day of first cutting. The plant height of soybean was measured before cutting with two different measurements: LC and HC. For HC, the plant height of 'Fukuyutaka', 'Kohamadaizu', and 'Moshidou Gong 503' was 39.7 cm, 54.2 cm, and 37.8 cm, respectively. For LC, the plant height for 'Fukuyutaka', 'Kohamadaizu', and 'Moshidou Gong 503', was 43.2 cm, 50.6 cm, and 40.7 cm, respectively. There was no significant difference in the plant height between cutting treatments in all soybean accessions. The height of the cotyledonary node from ground level in LC and HC plots was 8.9 and 8.2 cm for 'Fukuyutaka', 5.6 and 5.4 cm for 'Kohamadaizu', respectively. For 'Moshidou Gong 503', no significant differences were also observed in the height of the cotyledonary nodes between LC (3.7 cm) and HC (4.1 cm) plots.

Table 2 summarizes the regrowth performance of soybean accessions at 7 days after the first cutting treatments. The average numbers of branching nodes with emergent leaves in the LC and HC plots were 1.8 and 3.6 for 'Fukuyutaka' and 2.0 and 3.4 for 'Kohamadaizu', respectively. For both 'Fukuyutaka' and 'Kohamadaizu', the number of branching nodes with emergent leaves was significantly higher in the HC plot ($p < 0.05$). In both LC and HC plots, 'Fukuyutaka' achieved a 100% regrowth rate. Although 100% regrowth rate was obtained for 'Kohamadaizu'in the HC plot, there was only 20% regrowth (1 plant per 5 replications) in the LC plot, and all regrown plants withered and died by 32 days after the 1st cutting treatment. No plants exhibited regrowth in both plots for 'Moshidou Gong 503', with all plants dying by 7 and 32 days after cutting in the LC and HC plots, respectively. Leaf emergence was observed in all plants in HC and LC plots for 'Fukuyutaka' (Figure 2a, 2c). For 'Kohamadaizu', leaf emergence was observed in all plants in the HC plot (Figure 2b, 2d) and only one plant in the LC plot. In contrast, no regrowth was recorded following the cutting plot for 'Moshidou Gong 503'. The branch elongation for 'Fukuyutaka' and 'Kohamadaizu' was observed at 14 days after first cutting (Figure 2c and 2d, respectively), while no elongation was observed in 'Moshidou Gong 503' (Figure 2e).

Fresh and dry matter weight of soybean plants

Fifty days after the 1st cutting, soybean plants were harvested for the 2nd cutting. In both plots for 'Fukuyutaka' and HC plot for 'Kohamadaizu', plant exhibited vigorous growth, with the growth stage reaching R5 (beginning of seed development) and R6 (Full seed development), for 'Fukuyutaka' and 'Kohamdaizu' by 50 days after the first cutting, respectively. Figure 3 shows changes in the plant height from the first cutting to day 50 (second cutting). In both cutting treatments for the 'Fukuyutaka', soybean plants continued elongating until the time of the second cutting. The plant height of 'Fukuyutaka' on day 50 was 40.7 and 48.7 cm in the LC and HC plots, respectively. Even though there was no significant difference, plant height of soybean under HC was higher than the LC plot. Meanwhile, plants scarcely elongated in the LC plot for the 'Kohamadaizu', with all plants dying by day 32. In contrast, the plant height reached 46.2 cm by day 32 in the HC plot and plateaued thereafter.

The fresh and dry matter weight (FMW and DMW) of the plants at the first and second cutting of plant fractions (leaf, stem and pod parts) are showed in Figure 4 and 5, respectively. The FMW and DMW at the first cutting were not significantly different among the accessions, nor were there any significant differences between HC and LC plots. Although among soybean accessions, the FMW and DMW were heaviest in both cutting treatments for 'Fukuyutaka', while in terms of cutting treatments, both 'Fukuyutaka' and 'Kohamadaizu' exhibited significantly higher values (FMW/DMW) in the HC plot. By plant part, the leaf part accounted for approximately 60% and stem and pod parts accounted for approximately 20% each in both cutting treatments for 'Fukuyutaka'. In the HC plot for 'Kohamadaizu', the leaf, stem and pod parts accounted for 55%, 10% and 25%, respectively. Harvesting soybean plants for forage at 12 cm resulted in high biomass and increased the possibility of soybean plants to regrowth after the 1st cutting.

Table 1. The agronomic traits of three soybean accessions before 1st cutting treatment.

Accessions name	Cutting treatment	Growth stage	Plant height (cm)	Number of flowers	Number of pods	Cotyledon node height (cm)
Fukuyutaka	High	V6	39.7	0.0	0.0	8.2
	Low	V6	43.2	0.0	0.0	8.9
Kohamadaizu	High	V4	54.2	0.0	0.0	5.4
	Low	V4	50.6	0.0	0.0	5.6
Moshidou Gong 503	High	R5	37.8	45.3	31.8	4.1
	Low	R5	40.7	44.2	30.0	3.7

†There is no significant differences between cutting treatment before 1st cutting (Two-way ANOVA test, $P > 0.05$).

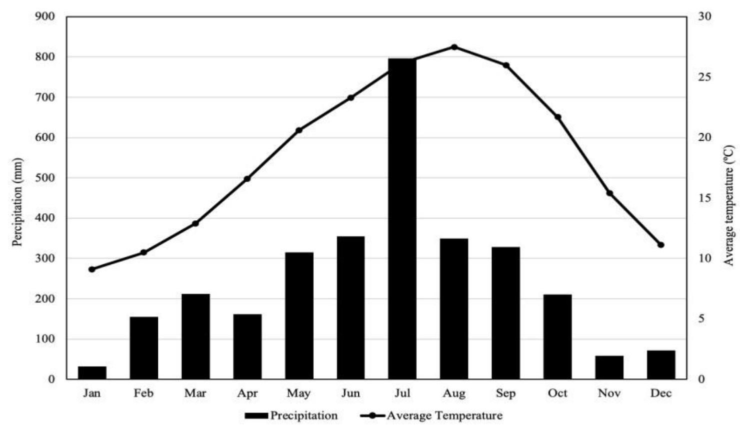

Fig 1. Average monthly temperature and precipitation during cultivation in 2019

Table 2. The agronomic traits of three soybean accessions after 1st cutting treatment.

Accessions name	Cutting treatment	Growth stage	Ratio of plants regenerated (%)	Average number of nodes with regenerated leaflet
Fukuyutaka	High	V6	100^{A}	3.6^{aA}
	Low	V6	100^{A}	1.8^{bA}
Kohamadaizu	High	V5	100^{aA}	3.4^{aA}
	Low	V5	20^{bB}	2.0^{bA}
Moshidou Gong 503	High	R1	0^{B}	0^{B}
	Low	R1	0^{C}	0^{B}

Means values by the different lowercase letters in the column are significantly different at $P<0.05$ between cutting treatment in the same cultivar and uppercase letter in the column are significantly different at $P<0.05$ among cultivars in the same cutting treatment by Tukey's HSD test.

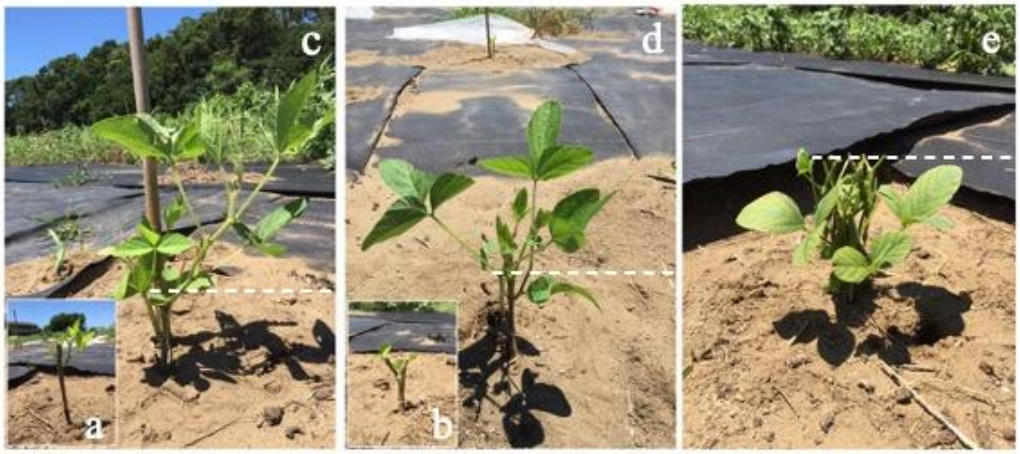

Fig 2. Regrowth performance at 7 days after 1st cutting (a. 'Fukuyutaka' and b. 'Kohamadaizu') and 14 days after first cutting (c. 'Fukuyutaka' and d. 'Kohamadaizu') of soybean plants under high level cutting. There was no elongation after 14 d from 1st cutting in 'Moshidou Gong 503' (e). Dotted white lines indicate the cutting place of soybean plant.

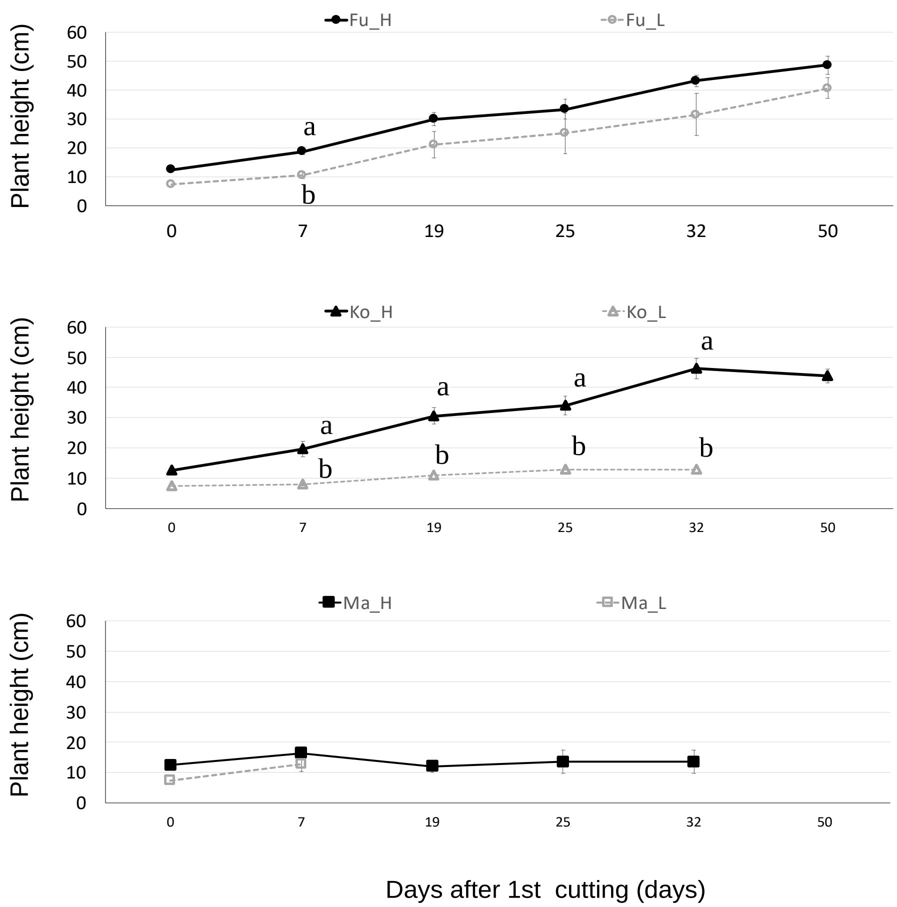

Fig 3. The transition of plant height after cutting treatment of three soybean accessions. Fu, 'Fukuyutaka': Ko, 'Kohamadaizu': Ma, 'Moshidou Gong 503': H, high level cutting height (12 cm above the ground): L, low level cutting height (7.5 cm above the ground).

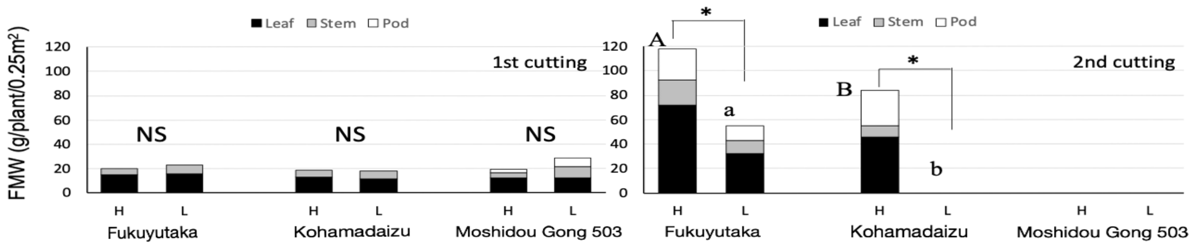

Fig 4. The fresh matter weight (FMW) of three soybean accessions at 1st and 2nd cutting. Asterisk indicates the significant difference between high level cutting height (H) and low level cutting height (L) of each accessions by t-test (P<0.01). Different letters indicate the significant differences between accessions with the same cutting treatment by t-test (P<0.01).

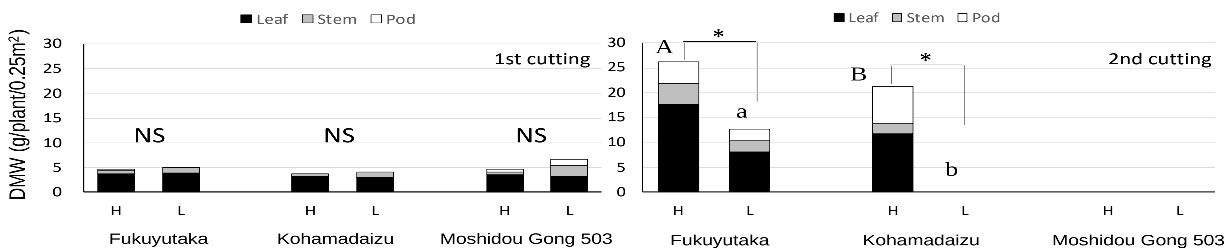

Fig 5. The dry matter weight (DMW) of three soybean accessions at 1st and 2nd cutting. Asterisk indicated the significant different between high level cutting height (H) and low level cutting height (L) at same accessions by t-test (P<0.01). Different letters indicate the significant differences between accessions with the same cutting treatment by t-test (P<0.01).

Discussion

Regrowth ability of soybean plant

The cutting height levels, height of cotyledonary node and growth stage of soybean plant when harvested at the 1st cutting significantly affected the regrowth ability of soybeans until the 2nd cutting. Soybean plants that were harvested by cutting 12 cm above the ground and at the vegetative stage tended to have higher number of nodes with regenerated leaflets on the main stem. Functional green leaf tissue and cotyledons are important in generating energy to drive regrowth (Hardman and Gunsolus, 1991). Leyser and Day (2003) noted that branches can develop from axillary buds, located at the upper-angle junction between the main stem and leaf petiole. In the study about regrowth in alfalfa by Dhont et al. (2006) confirm that the root nitrogen (N) reserves, in the form of free amino acids and soluble proteins, play important role for the spring regrowth of field-grown alfalfa. When a plant is not permitted to regrow leaves and roots before being cut again, it will eventually die (Stichler, 2002). In this study, 'Fukuyutaka' was more robust and high tolerance for defoliation stress that that of 'Kohamadaizu' and 'Moshidou Gong 503'. The cutting height level in this study was decided regarding the usual cutting height done by farmer in southwestern region of Japan to cut the forage grass by using cutting machine.

Fresh and dry matter weight of soybean plants

Harvesting soybean plants for forage at 12 cm from the ground resulted in high biomass and increased the possibility of soybean plants to regrowth after the 1st cutting. Even though there was no regrowth for 'Moshidou Gong 503' after the 1st cutting, harvesting such accession with early-flowering and early-maturing type of soybean might gain a higher protein due to the addition of flower and pods. On the other hand, 'Fukuyutaka' and 'Kohamadaizu' may possibly produce high-protein forage in the 2nd cutting. The result in this study showed that biomass yield was even higher in 2nd cutting than that in 1st cutting with the possibility to provide higher-protein content in forages. The increasing crude protein in stage R5 or R6 coincides with a time when N is being redistributed to the seed and would thus be in transit at the vascular tissue of the stem (Egli et al., 1978; Streeter, 1978).

Moreover, the fact that the cotyledonary node height was higher in both cutting levels and resulted in higher biomass at the 2nd cutting suggests that the plants nutritional condition was exceptionally good in this study, probably because the study was conducted in a single seeding plot. Competition for soil nutrients is unavoidable when soybeans is subjected to mixed seeding with warm-season grasses. Soybean intercropped with *Brachiaria* grass was reported to have reduced crude protein in soybean grain when *Brachiaria* was early planted or cultivated at the same time with soybean (Castagnara et al., 2014). Light penetration into canopy is one of the environmental stress that could affect the agronomic quality of soybean. In the experiment about soybean and living mulch, competition for nutrients reduced the yield of whole soybean plant at the R1 stage (Uchino et al., 2016). Therefore, future studies need to clarify the regrowth traits of soybeans under such mixed seeding conditions.

Materials and Methods

Plant material

Three soybean accessions (*Glycine max* 'Fukuyutaka', *G. max* 'Kohamdaizu' and *G. gracilis* 'Moshidou Gong 503') were used in this study. This study was conducted as a randomized complete block design with five replications These accessions were obtained from the National BioResource Project (NBRP) for *Lotus/Glycine* in Japan (https://www.legumebase.brc.miyazaki-u.ac.jp/). 'Moshidou Gong 503' is an early-flowering with indeterminate growth type, while 'Fukuyutaka' and 'Kohamadaizu' are late-flowering with determinate growth type soybeans.

Experimental site

The field trials were carried out at the Sumiyoshi Livestock Science Station, University of Miyazaki, Southern Kyushu, Japan (39°59'N, 131° 28'E, 12 m asl). The soil type was characterized as sandy regosols based on the soil classification system in Japan (Obara et al., 2015). The climate of Miyazaki according to the Köppen classification is Cfa, which has a humid subtropical climate with relatively high temperature and evenly distributed precipitation throughout the year. Precipitation and air temperature at the site in 2019 (Figure 1) were obtained from the data base of the Geospatial Information Authority of Japan (URL: http://www.jma.go.jp/miyazaki/). 'Fukuyutaka', 'Kohamadaizu' and 'Moshidou Gong 503' were sowed in 22 May 2019. All accessions were sowed in a 0.25 m^2 single growing plot area (0.5 m × 0.5 m). Manure (2.5% N, 4.0% P_2O_5 and 2.1% K_2O) was supplied at a rate of 1t/10a before the time of sowing of all soybean accessions. 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/10a each, with an additional annual application at the same rate after cutting. The soybean plants were harvested on 25 July and 13 September in 2019 for the 1st and 2nd cutting, respectively. The soybean plants were cut manually by hand at 7.5 cm (low level cutting height, LC) and 12 cm (high level cutting height, HC) from the ground level and biomass was weighed for both the 1st and 2nd cutting.

Measurements

Soybean development from sowing to V4, V6 (Vegetative stages; with 4 and 6 number of nodes on the main stem) and R5 (Reproductive stages; beginning of the seed development) were recorded according to Fehr and Caviness (1977). Plant height, number of branched nodes, number of flower buds, number of pods, and the height of cotyledonary node from ground level were measured before harvest for the 1st cutting. In addition, the plant height, average number of nodes with regenerated leaflet, and the ratio of plant regrowth were measured before harvest for the 2nd cutting. After each cutting, the leaves, stems, and pods were sorted, and the fresh weight and dry matter weight (after air drying at 60°C for 72h) of each plant fraction were measured.

Experimental design

The experimental design was a randomized block design arraged in a 3 × 2 factorial scheme (soybean cultivars × cutting treatments). The cutting treatments consisted of two cutting heights (low cutting height and high cutting height). The weed and pest insect control were carried out in accordance with the technical recommendations for the soybean in Japan.

Statistical analysis

Statistical analysis was conducted to compare the agronomical distribution and dry matter weight among soybean accessions in the 1st and 2nd cutting. Differences in means were evaluated by Tukey's test using R statistic program (URL.https://www.r-project.org/).

Conclusion

In conclusion, 'Fukuyutaka' performed best among soybean accessions in both cutting levels with higher dry matter weight after the 1st cutting. This is the first study of soybean research to discover that soybean plant can achieve regrowth ability under different cutting treatments to establish sustainable two-cutting techniques of forage soybeans in southwestern region of Japan. Further studies need to clarify the regrowth ability of soybean plant, particularly 'Fukuyutaka' and 'Kohamadaizu', under mixed seeding condition with tropical grasses.

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