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The effects of the different sowing methods and planting density on the yield components of soybean (*Glycine max*) under intercropping condition with rhodes grass (*Chloris gayana* Kunth.)

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Abstract

In Japan, grass-legume intercropping systems are being developed to increase quality and yield of grain and forage. Hence, this study evaluated the effects of sowing methods and planting density on yield traits of forage soybean under intercropping condition with rhodes grass. The field experiment was laid on in a completely randomized block design with four treatments and four replications. Rhodes grass seeds were sown at 2.8 kg/10a in a field containing mono- and intercropping-plots. For soybeans, two levels of sowing densities (about 15 or 30 seeds/m^2: named "L" and "H") and two sowing methods (row or spray: named "R" and "S") were applied for a total of four different intercropping combinations. Rhodes grass DMY obtained the highest in low level of sowing density with rowing methods (LR) (338 kg/10a) while soybean DMY obtained the highest in high level of sowing density with rowing methods (HR) (251 kg/10a) and in high level of sowing density with spray methods (HS) (177 kg/10a). Intercropping at low density increased CP (12%) and CPY (40.4 kg DM/10a) in Rhodes grass, and CP (18.9%) in soybeans, whereas high sowing density increased soybean CPY in HR (41.5 kg DM/10a) and contributed to higher soybean CPY in DM percentage at 66% (HS) and 56% (HR). Overall, high density sowing increased the 1st total DMY while row sowing increased the 1st total CPY in both crops at the first cutting, while monocropping increased Rhodes grass yield and plant length at the second-cutting. Also, sowing soybean by a broadcast spreader could obtain sufficient crude protein yield as a forage soybean in southern Kyushu, Japan. Further studies are needed for soybean overseeding method and use of early-maturity soybean lines.

Keywords: Fukuyutaka; Intercropping; Rhodes grass; Sowing method.
Abbreviations: L_Low sowing density; H_High sowing density; R_Row sowing; S_Spray sowing; LR_Low density and row sowing; HR_High density and row sowing; LS_Low density and spray sowing; HS_High density and spray sowing; DMY_Dry matter yield; CP_Crude protein; CPY_Crude protein yield.

Introduction

Recently, feed prices have been steadily increasing globally. The market prices of imported raw materials are unstable, and furthermore, the mid- to long-term feed demand in many countries is expected to grow due to the expansion of the population. In view of this, there is an urgent need to increase domestic feed production in Japan and establish a system of feed self-sufficiency as much as possible for livestock. Cultivation of forage and crops will also be effective from the perspective of shifting rice paddies and preventing abandoned land to cultivation in Japan. Since the 1980s, various endeavors among researchers and stakeholders have tapped on the high productivity of tropical forage grasses as additional feed source especially in Southern Japan. Among these are oats, maize, Rhodes grass, Napier grass, Brachiaria grass, Bahiagrass, etc. Tropical grasses are known to exhibit high dry matter yield but their nutritional value such as crude protein, significantly decreases as the growth stage develops (Minson, 1990).

Soybean is a protein-rich legume that could fix atmospheric nitrogen into ammonia which contributes to the soybean plant's nourishment (Zapata et al., 1987; Sanchez and Minamisawa, 2019). Moreover, different maturity of soybean had different nutrient value (Prasojo et al., 2021). Reduction of nitrates by symbiotic *Rhizobia* in soybeans could improve soil fertility. As such, soybean could contribute in improving nitrogen efficiency (Yang et al., 2018; Xu et al., 2020) and increase the nutritional value of forage grasses in intercropping systems (Acikgoz et al., 2013; Peiretti et al., 2017; Roger et al., 2017). The cultivation of soybean with temperate grass (Italian ryegrass) sod as living mulch plant produced high whole-plant yield and crude protein content in northern part of Japan (Kaneko et al., 2011; Uchino et al., 2016) and some cultivar of soybean had advantages for regrowth ability with special cutting system (Prasojo et al., 2021b). Likewise, Prasojo et al. (2021a; 2022) first reported the development of soybean production technique using an intercropping system of soybeans with Rhodes grass (*Chloris gayana* Kunth.) and suggested that biomass inclusion could be useful for increasing the nutritional value of silage in the southwestern region of Japan. These previous researches, however, did not focus nor verify the seed density and seeding method in forage soybean cultivation under intercropped condition with tropical forage.

The aim of this study was to evaluate the effects of the different sowing densities and planting methods on the yield and regrowth traits of soybean cultivar 'Fukuyutaka' under intercropping condition with Rhodes grass.

Results and Discussion

Germination

Germination rate of intercropped soybeans and their respective densities were summarized in Table 2. Although there were no significant differences in soybean germination rates among treatments, germination rates ranged from about 50% under the row sowing combinations (HR, high density-row sowing; LR, low density-row sowing) and about 70% germination rate under the spray sowing combinations (HS, high density-spray sowing; LS, low density-spray sowing). Soybean plant density was significantly higher in the high-density seeding combinations (HR and HS) than the low-density combinations (LR and LS). Overall, HS produced the highest soybean plant density.

Yield components in 1st cutting

Dry matter yield in crops depends on absorbed radiation and intercropping is more efficient in light interception which contributes to the production of higher crop biomass than in monocropping systems (Bedoussac et al., 2015). In this study, the biomass yield traits of Rhodes grass and soybean under different treatment combinations of sowing and planting density were evaluated (Table 3). No significant differences were observed in the plant length of Rhodes grass and soybean among different sowing combinations prior to the first cutting. The highest value for Rhodes grass FMY and DMY was obtained in LR at 1763 kg/10a and 338 kg/10a, respectively, while that for soybeans was obtained in HR at 1046 kg/10a and 251 kg/10a, respectively. Moreover, high density combinations (HR, HS) produced higher FMY in soybeans than in the low density combinations (LR, LS). Also, the intercropped soybeans planted in high density rows (HR) had better light distribution and produced a stable soybean population. On the other hand, it is inferred that soybean FMY was lowest in LR (329 kg/10a) because it was sown at a lower density and was unable to form a population sufficient to compete with Rhodes grass. No significant differences in DM were observed in monocropped Rhodes grass and all intercropping combinations. Moreover, the soybean mixing rate as a DM percentage against total dry matter yield was highest in HR (50%) and HS (39%). Interestingly, the percentage in LR (19%) was less than half of that in HR (50%), while that in LS (32%) was not significantly different from that in HS (39%). This may be due to a decrease in plant density of soybean in HS although it was not investigated in this study. Further research is needed to investigate change in plant density under different intercropping conditions.

The effects of the different seed density and sowing combinations on CP content and CP yield (kg DM/10a) of Rhodes grass and soybean under different cropping systems are summarized in Table 4. Based on all four intercropping combinations, CP content of Rhodes grass obtained the highest in LR. Among all sowing combinations, the highest CP content in Rhodes grass was obtained in LR at 12% while CP in soybeans obtained the highest in LR and LS with 18.9% and 18.6%. The highest CPY in Rhodes grass was obtained in LR at 40.4 kg DM/10a while soybean obtained the highest CPY in HR at 41.5 kg DM/10a. As such, intercropped sowing densities have effects on the CP content and CPY in both Rhodes grass and soybeans. In addition, there were no significant differences between the CPY of monocropped and intercropped spray seeding combinations in Rhodes grass. On the other hand, the total CPY of the combined Rhodes grass and soybean was highest in the HR and LR combinations by the strip seeding method, with relative ratio values of 300 and 261 compared to that of Rhodes grass monocropping which was 100. The contribution rate of soybean to CP yield for the 1st cutting was significantly higher in the high density seeding (HR: 66 and HS: 56) than that in the low density seeding (LR: 26 and LS: 47).

Planting densities and spatial structure (row or alternate strips) in intercrops increase competition for available nutrient but tends to dominate the dominant species or change light distribution (Bedoussac et al., 2015). In this study, it is possible that the high density in soybeans caused a competitive advantage over soil nitrogen absorption which was disadvantageous for Rhodes grass CPY. A similar trend in soybean intercropped with palisadegrass (*B. brizantha* cv Marandu) was reported to have reduced crude protein in soybean grain when palisadegrass was early planted or cultivated or the same time with soybean (Castagnara et al., 2014). Likewise, the availability of soil nitrogen also affects the protein concentration of intercropped plants, and yield advantages could be obtained when competition occurs partly among intercrops (Bedoussac and Justes, 2010).

Regrowth ability

One week after the first cutting, axillary bud development was observed on the main stem nodes (Table 5). In this study, no differences in regrowth ability (Ratio of plants with regenerated axillary buds, Number of nodes with axillary buds, Number of axillary buds per plant) were observed in the different intercropping combinations, and approximately 70–-80% individuals produced axillary buds (Table 5). New axillary buds regenerated at the stem node located at about 80 mm above the ground, and their stem diameter was about 5 mm. Thereafter, however, the experiment site had been under high drought stress with no rainfall for 7 days and was further damaged by insects (mainly grasshoppers), and the all the plants died without further growing.

The relationship between the stem diameter of the soybean plants at harvest and their subsequent regeneration ability was shown in Table 6. It showed a significantly positive correlation (p<0.01) with both the number of nodes with axillary buds (correlation coefficient: 0.32) and the number of axillary buds (0.39). The thickened stem diameter of the individual soybean plants may have increased the regeneration rate due to the greater storage of assimilated products in the stem. Soybean plants with the thickest stem diameter tended to have shorter stems, and having a thicker stem may contribute to longer curing time for making soybean silage (Blout et al., 2009). On the other hand, a

Table 1. Monthly precipitation and average temperatures during experimental cultivation

Year	Precipitation (mm)					Temperature (°C)				
	June	July	August	September	October	June	July	August	September	October
2021	368	335	466	692	121	23.2	27.1	27.0	25.7	20.8
10 years average†	228	516	339	276	371	23.2	27.3	27.6	24.7	20.0

†2011–2020 average

Table 2. The germination rate and plant density of soybean.

Treatment	Germination rate	Plant density
	%	plants/m^2
Inter cropping		
HR	54±7	18.0^{b}±6.4
LR	53±12	8.8^{c}±4.1
HS	66±8	22.0^{a}±1.3
LS	69±7	11.4^{c}±6.8

a,b Values in the same column with different superscript letters differ significantly (*P* < 0.05) by Tukey-test. HR, high density and row seeding; HS, high density and spray seeding; LR. low density and row seeding; LS, low density and spray seeding.

Table 3. Yield traits of Rhodes grass and soybean under different sowing conditions.

	Plant length		FMY		DM		DMY			Soybean Mixing rate
Treatment	cm		kg/10a		%		kg/10a			DM%
	Rh	Soy	Rh	Soy	Rh	Soy	Rh	Soy	1st Total	
Mono- cropping	98±5.8	-	913^{b}±157	-		-	191^{b}±47	-	191^{b}±47	0
Inter-cropping										
HR	113±7.0	65±1.4	1167^{b}±251	1046^{a}±132	21.6	24.0	250^{ab}±54	251^{a}±32	503^{a}±56	50^{a}±7
LR	109±7.4	66±0.6	1763^{a}±364	329^{c}±149	19.2	23.0	338^{a}±34	76^{c}±34	413^{ab}±53	19^{b}±9
HS	115±3.7	67±1.1	1204^{b}±193	771^{b}±96	23.3	23.0	281^{ab}±45	177^{b}±22	458^{a}±37	39^{a}±6
LS	103±4.8	61±0.8	1121^{b}±198	483^{c}±116	19.1	21.1	214^{b}±38	102^{c}±25	316^{b}±23	32^{ab}±9

a,b Values in the same column with different superscript letters differ significantly (*P* < 0.05) by Tukey-test. HR, high density and row seeding; HS, high density and spray seeding; LR. low density and row seeding; LS, low density and spray seeding; Rh, Rhodes grass; Soy, soybean.

Table 4. Crude protein yield of Rhodes grass and soybean under different sowing conditions.

Treatment	CP		CPY			Relative ratio	Contribution rate of Soybean in CPY
	%		kg DM/10a				DM%
	Rh	Soy	Rh	Soy	1st CPY Total		
Mono-cropping	10.6^{ab}±2.2	-	21.0^{b}±10.0		21.0^{c}±10.0	100	-
Inter-cropping							
HR	8.6^{bc}±0.2	16.5^{b}±1.0	21.5^{bc}±4.8	41.5^{a}±6.4	63.0^{a}±5.1	300	66
LR	12.0^{a}±0.3	18.9^{a}±0.6	40.4^{a}±9.2	14.2^{c}±6.4	54.7^{a}±6.7	261	26
HS	8.3^{c}±0.1	16.5^{b}±0.2	23.1^{b}±3.5	29.2^{b}±3.9	52.4^{ab}±3.4	250	56
LS	10.2^{ab}±0.1	18.6^{a}±0.7	21.8^{b}±3.8	19.0^{bc}±5.1	40.8^{b}±3.4	195	47

^{a,b} Values in the same column with different superscript letters differ significantly (*P* < 0.05) by Tukey's test. CP, crude protein; CRY, crude protein yield; HR, high density and row seeding; HS, high density and spray seeding; LR. low density and row seeding; LS, low density and spray seeding; Rh, Rhodes grass; Soy, soybean.

Table 5. Regrowth ability of soybean after 1st cutting.

Treatment	Ratio of plants with axillary bud	Number of nodes with axillary bud	Number of axillary buds per plant	Height of stem node with axillary bud*	Stem diameter
	%	no/plant	no/plant	mm	mm
Inter cropping					
HR	82.5±17.1	1.7±0.8	2.4±1.4	76.9±15.4	5.5±0.5
LR	75.0±12.9	1.6±0.3	1.7±0.7	78.3±5.8	5.1±0.3
HS	82.5±15.0	1.4±0.5	1.6±0.6	79.3±4.5	4.9±0.5

There is no significant differences among cultivation treatments by Tukey's test. HR, high density and row seeding; HS, high density and spray seeding; LR. low density and row seeding; LS, low density and spray seeding. *Height of node with axillary bud as the lowest located.

Table 6. Correlations between two regrowth ability traits and stem diameter of soybean plant.

	Number of nodes with axillary bud		Number of axillary buds per plant	
	Co	p-value	Co	p-value
Stem diameter	0.32	<.01	0.39	<.01

Table 7. Yield traits of 2nd cutting Rhodes grass under different cultivation system.

Treatment	Plant length	FMY	DM	DMY	DMY Year Total 1st+2nd	Relative ratio
	cm	kg/10a	%	kg/10a	kg/10a	
Mono cropping	167^{a}±8	2969^{a}±280	28.0	841^{a}±71	1032^{b}±134	100
Inter cropping						
HR	148^{b}±10	1881^{c}±109	31.0	585^{c}±93	1088^{ab}±134	105
LR	146^{b}±13	2169^{c}±75	30.1	653^{bc}±33	1066^{ab}±74	103
HS	158^{b}±15	2688^{ab}±97	29.7	797^{ab}±56	1254^{a}±77	122
LS	150^{b}±12	2538^{b}±148	30.0	765^{ab}±101	1081^{b}±83	105

^{a,b} Values in the same column with different superscript letters differ significantly (*P* < 0.05) by Tukey-test. HR, high density and row seeding; HS, high density and spray seeding; LR. low density and row seeding; LS, low density and spray seeding.

higher stem to leaf ratio in soybeans could negatively affect the forage intake of ruminants because they tend to have less preference for stems (Hints et al., 1994).

Yield components in 2nd cutting

The yield traits of Rhodes grass under different intercropping combinations after the 2nd cutting are summarized in Table 7. Here, the plant length of Rhodes grass before 2nd cutting was highest under monocropping (167 cm) and no significant differences were observed in each of the other intercropping combinations. In Rhodes grass, monocropping obtained the highest FMY (2969 kg/10a), followed by the spray sowing conditions (HS: 2688 kg/10a and LS: 2538 kg/10a). Row sowing conditions (HR: 1881 kg/10a; LR: 2169 kg/10a) had significantly lower values than the spray sowing conditions (HS and LS). The same trend was observed for Rhodes grass DMY. Based on the results of plant length and yield traits in the 2nd cutting, the growth conditions of Rhodes grass after the first cutting were considered to be favorable in the order of Rhodes grass monocropping > Rhodes grass-soybean intercropping (spray sowing) > Rhodes grass-soybean (row seeding). This might be mainly due to the effect of plant density, light competition condition among plants, and soil nitrogen status. These factors might have been influenced by the degree of soybean occupation until the 1st cutting (Table 3). The annual dry matter yield, which is the sum of the dry matter yield of the 1st cutting (Rhodes grass, soybean) and 2nd cutting (Rhodes grass), was highest in HS (1254 kg/10a), with a relative value of 122 compared to the Rhodes grass monocropping (1032 kg/10a) with a relative value of 100 (Table 7).

Harvesting soybean forage at an advanced maturity stage (from R4 to R6) is most beneficial for making soybean silage because it significantly increases protein, fat, and degradable NDF contents (Spanghero et al., 2015). Moreover, the wilting soybean forage has to be targeted to achieve a DM content of ensiled forage of about 440 g/kg (between about 410 and 480 g/kg, according to the maturity stage). Further wilting would indicate no fermentative improvements at ensiling or an absence of relevant modification in nutritional contents of the silage. In order to significantly increase the crude protein content of soybean plants during the growing period of Rhodes grass (60–70 days) before the first cutting, several soybean lines with earlier maturity will be needed.

Based on the above findings, focus is needed on not only soybean regeneration method but also on the soybean overseeding method. Moreover, the introduction of early maturity soybean lines would help establish a multi cutting management that can adapt flexibly even under unstable weather conditions caused by the global warming.

In conclusion, intercropping have a greater effect on the yield and agronomic traits of soybean and Rhodes grass under the climatic condition of southern Kyushu. Increased cropping density enhanced forage yield parameters (FMY and DMY) in both soybeans and Rhodes grass at the first cutting, except for plant length and DM. Conversely, monocropping increased the yield parameters (FMY and DMY) of Rhodes at the second cutting, except for DM. Intercropping at low density increased CP and CPY in Rhodes grass, and CP in soybeans. High density increased CPY in soybeans and contributed to higher soybean CPY in DM percentage. Although axillary bud development was observed on the main stem node, no individual soybean plants grew due to drought stress and insect damage, and only Rhodes grass was harvested in the second cutting. Regarding seeding methods, the results in this study suggested that it is possible to obtain sufficient crude protein yield in a forage soybean production system in the southern Kyushu by spreading with broadcaster, which is generally required for forage production.

Material and Methods

Plant material

Rhodes grass (*Chloris gayana* Kunth. cv. Callide) and soybean (*Glycine max* (L.) Merr. cv. Fukuyutaka, late-maturing and erect type) were used for this study.

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 regosol 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/).

Climatic conditions

Monthly precipitation and average temperature during the experiment in 2021 and the average precipitation and rainfall for a 10-year period (2011–2020) are summarized in Table 1. The precipitation in 2021 was lower than the 10-year average for July and October. On the other hand, June, August, and September in 2021 had higher precipitation than the 10-year average. Monthly temperatures exhibited similar trend with the 10-year average.

Experimental design and cultivation

The field experiment was laid on in a completely randomized block design with four treatments and four replications. Each replicate had a plot area of 12 m^2 (3m × 4m) and a combined total of 20 plots were made from Mono- (as control) and intercropping plots (treatments). Rhodes grass used in monocropping plots as control was sown at a rate of 2.8 kg/10a in 7 June 2019. Under monocropping condition, Rhodes grass was sown at the same rate as that in monocropping while soybean seeds were subjected to a combination of each of the two factors: sowing densities (about 15 or 30 seeds/m^2: named “L” and “H”) and two sowing methods (row or spray: named “R” and “S”); thus, making a total of four different sowing treatments (LR, LS, HR and HS). For row sowing, soybean seeds were manually sown with the following conditions: 3 m row length, 33 cm row space, 5 rows (for 15 seeds/m^2) or 10 rows (for 30 seeds/m^2), 8 cm between individuals, and 3–4 cm depth for two sowing densities, respectively. For the broadcast sowing, soybean seeds were manually sprayed, and covered with soil to a depth of 2–3 cm for two sowing densities (about 15 or 30 seeds/m^2), respectively. Rhodes grass and Soybean seeds were planted in 7 June 2019 and 8 June 2019, respectively.

Manure (2.5% N, 4.0% P_2O_5, and 2.1% K_2O) was supplied at a rate of 2.7 t/10a before the time of sowing of all soybean accessions. A basal fertilizer consisting of nitrogen (14% N), double superphosphate (12% P_2O_5) and potassium chloride (10% K_2O) was applied to the plot area at a rate of 43 kg/10a each.

Measurements of agronomic and nutritive value

The number of germinated seeds was counted at 7 days after sowing. Plant height of five randomly selected plants in all plots was measured before each cutting (1st and 2nd). Plant sampling (Rhodes grass and soybean) at 12.5 cm above ground level were taken from 6 m^2 (2 m×3 m) in all plot on 27th July (1st) and 5th October (2nd). The cut plants were measured for fresh matter and were dried at 60 ºC for 48 hr to determine dry matter ratio and dry matter yield. The dried sample of Rhodes grass and soybean plants 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.
Regrowth ability of soybean was investigated at 7 days after the 1st cutting. A 1 m^2 sampling area was randomly set in each plot, and 10 individual plants were randomly selected from it for the regrowth ability investigation. The number of individuals with confirmed axillary buds that regenerated from the main stem were counted. Likewise, the number of nodes with regenerated axillary buds and the height of the lowest axillary bud development node located and the stem diameter were measured.

Statistical analysis

Statistical analysis was conducted to compare the yield and regrowth ability traits among different sowing conditions. The relationships between two regrowth ability traits and the stem diameter of soybean plant were assessed by calculating the correlation coefficient. Differences in means were analyzed by Tukey's test using R static program (https://www.r-project.org) (Version 3.1.1, R Core Team 2014).

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