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# Effects of rice bean (*Vigna umbellata* (Thunb.) Ohwi & H. Ohashi) on growth of neighboring plants

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## Abstract

Rice bean (*Vigna umbellata* (Thunb.) Ohwi & H. Ohashi) is a legume crop cultivated in shifting cultivation systems in monsoon Asia, where soils are often deficient in available phosphorus (P). Due to its high capacity to utilize Al-bound P, rice bean has the potential to facilitate P uptake and promote the growth of neighboring crops. At the same time, it has been cultivated under conditions with intense weed pressure, suggesting a possible role of growth-inhibitory interactions such as allelopathy. This study aimed to evaluate the effects of rice bean on the growth of neighboring plants through three complementary experiments. In Experiment 1, maize was intercropped with rice bean in P-deficient soil collected from a shifting cultivation field. In Experiment 2, the allelopathic activity of rice bean was assessed using the sandwich method. In Experiment 3, weed suppression by rice bean was examined under field conditions. Intercropping with rice bean did not enhance P uptake or maize growth; instead, maize growth was reduced. The sandwich method revealed strong allelopathic activity of rice bean leaves and roots, comparable to or greater than that of other leguminous species. However, no significant weed suppression effect was observed under field conditions. These results suggest that rice bean has strong allelopathic potential, which may negatively affect neighboring crops under certain conditions, while its facilitative effect on P uptake may not be expressed under all environments. The discrepancy between laboratory and field results indicates that the ecological function of rice bean depends on environmental and management conditions.

**Keywords**: Allelopathy, intercropping, Phosphorus uptake, shifting cultivation

## Introduction

Plants influence one another in various ways through interactions with surrounding plants (Brooker *et al*. 2008). Incorporating crops with growth-promoting effects into intercropping systems or crop rotation systems can enhance the productivity of other crops. Numerous studies have been conducted on crops with growth-promoting effects and the mechanisms underlying them. Plants with high phosphorus (P) uptake capacity promote phosphorus uptake in other crops by converting unavailable P in the soil into an available form. Faba bean (*Vicia faba* L.) converted unavailable P bound to aluminum (Al) and iron (Fe) in the soil into an available form through organic acids secreted by its roots, thereby promoting P uptake and growth in intercropped maize (Li *et al*. 2007, Zhang *et al*. 2016a). Chickpeas (*Cicer arietinum* L.) also convert organic P into an available form by secreting acid phosphatase, thereby promoting P uptake in maize (*Zea mays* L.) when intercropped (Li *et al*. 2004). Therefore, incorporating crops with high nutrient use efficiency into cropping systems is expected to improve the nutrient efficiency of other crops and promote their growth in intercropping and rotation systems.

On the other hand, growth-inhibitory interactions between plants may reduce crop growth and yield. Allelopathy is known as a form of growth inhibition between plants (Fujii, 2000). Allelochemicals released by plants inhibit cell division (Cruz-Ortega *et al*. 1988), photosynthesis (Einhellig *et al*. 1993), and mineral absorption (Yu *et al*. 1997) in other plants, thereby suppressing their growth. Numerous reports have demonstrated reduced crop growth due to allelopathic activity from weeds and other crops. Phenolic compounds secreted by legume weeds (Zohaib *et al*. 2014) and secretions from spearmint (*Mentha spicata* L.) and peppermint (*Mentha* × *piperita* L.) remaining in the soil (Karkanis *et al*. 2019) inhibited the growth of rice and maize.

Rice bean (*Vigna umbellata* (Thunb.) Ohwi & H. Ohashi) was the most important legume in shifting cultivation, which was primarily based on millet cultivation in

monsoon Asia (Tomooka 2005). In Shiiba Village, Miyazaki Prefecture, Japan, it used to be cultivated with shifting cultivation. The soils of shifting cultivation in Shiiba Village are deficient in available P. Although the available P content in the soil increased slightly immediately after burning, it became extremely low 3-4 years after burning (Kondo and Saeki, 2017). Kondo and Koga (2021) reported that rice bean used to be cultivated 4-5 years after burning, when available P had almost disappeared, and it had a high capacity to absorb P from Al-bound P (Al-P). Therefore, similar to faba bean, which also excels at absorbing Al-P, it has the potential to improve the growth of other intercropped crops.

In shifting cultivation at Shiiba Village, weed growth increased with each passing year following burning; the dry weight of weed per unit area in plots in the fifth year after burning was approximately 1.9 times that of plots in the first year after burning (Tanaka 2004). Furthermore, in shifting cultivation at northern Laos, where rice bean has been cultivated for many years, weed damage was a major limiting factor for crop production (Roder *et al*. 1995, Roder *et al*. 1997). Rice bean was cultivated in environments with vigorous weed growth. Therefore, it may possess a strong ability to promote its own growth while suppressing the growth of neighboring plants through potent allelopathic effects.

The objective of this study was to examine the effects of rice bean on the growth of neighboring plants and to evaluate its utility. In Experiment 1, soil collected from a shifting cultivation field in Shiiba Village, where available phosphorus is scarce, was used to intercrop rice bean and maize. In Experiment 2, the sandwich method, a technique for detecting allelopathic activity in plant residues, was used to determine the strength of the allelopathic activity of rice bean. In Experiment 3, rice bean was cultivated on a shifting cultivation field in Shiiba Village, where competition with weeds occurs, to determine the weed-suppressing effects of rice bean.

## Materials and methods

**Experiment 1:** Maize, rice bean, and adzuki bean (*Vigna angularis* (Willd.) Ohwi & H. Ohashi) were grown in a greenhouse at Kyoto University (35.0°N, 135.8°E) from July 3 to September 2, 2022. Adzuki bean was used in comparison with rice bean. The seed of rice bean collected in Gokase Town, Miyazaki Prefecture, in 2019 was used. Maize variety 'Gold Rush 88' and adzuki bean variety 'Tamba Dainagon' were used. Soil collected on April 26, 2022, from the top 10 cm of a shifting cultivation field that had been burned in August 2019 in Shiiba Village, Miyazaki Prefecture (32.5°N, 131.0°E) was used for cultivation. The physicochemical properties of the test soil are shown in Table 1. The methods used for physicochemical analysis are described later.

Six treatments were established: a maize monoculture, a rice bean monoculture, an adzuki monoculture, a maize–rice bean intercropping, a maize–adzuki bean intercropping, and an unplanted control. Six replicates were set up for each treatment. On July 1, seeds of each crop were sown and germinated in an incubator set to 30 °C. On July 3, the germinated seeds were transplanted into 1/5000-a Wagner pots filled with the soil described above. In the monoculture treatments, 3 seeds of each species were transplanted near the center of the pot. In the intercropping treatments, 3 seeds of each species were transplanted, for a total of 6 seeds. On July 11, in the monoculture treatments, only the single best-growing plant was retained. In the intercropping treatments, the single best-growing plant from each species was retained. The distance between the two retained plants was kept within 2 cm. The pots were spaced 5 cm apart and arranged in a 6-row by 6-column layout. For each treatment plot, a liquid fertilizer solution adjusted to 15 mM N ($NH_4NO_3$) and 8 mM K (KCl) was applied once a week at a rate of 200 mL per pot from July 13 to August 17. Since the objectives of this study were to determine the effect of intercropping on promoting P uptake in P-deficient soil, no P fertilizer was applied.

Plant height and leaf number of maize were measured on July 19, July 26, August 5, August 12, and August 21. For all plants, on August 5, August 12, and August 21, the length of the largest leaf among the third to fifth uppermost leaves was measured. In two bean plants, the length of a leaflet was measured as leaf length. On August 19 and August 31, the SPAD values, photosynthesis rate, and stomatal conductance were measured using a chlorophyll meter (SPAD-502Plus, Konica Minolta, Japan), a photosynthesis measurement device (MIC-100,

Masai International, Japan), and a leaf porometer (SC-1, METER Group, Inc., USA) in all plants. The measurement conditions of photosynthetic rate were as follows: PAR 1200 $\mu mol \cdot m^{-2} \cdot s^{-1}$, stabilization time 3 s, measurement start $CO_2$ concentration 400 ppm, and measurement $CO_2$ span 20 ppm. Measurements were conducted between 11:30 a.m. and 2:30 p.m.

After the cultivation period ended on September 1, rhizosphere soil was collected. In the intercropping treatments, it was not possible to separate the roots of the two plants. Therefore, instead of collecting rhizosphere soil from each of the two plants individually, soil from both plants was collected together, making it difficult to distinguish the contribution of each species to the soil chemical properties. Using soils after cultivation, including soil from unplanted control, soil pH, EC, $NO_3$-N, and $NH_4$-N were measured in accordance with the standard soil environmental analysis method (Soil Analysis Method Editorial Committee 1997). Available P was also measured using Bray 2 method (Bray and Kurtz 1945). Soils before cultivation were also used for the same measurement, and additionally P absorption coefficient was measured.

After collecting the soil, the aboveground parts of the plants were harvested and dried in an oven at 70 °C for 3 days. After drying, the dry weight was measured. The aboveground parts were then ground using a mortar and pestle. Using the ground powder, N, P, K, Ca, and Mg content were measured with the same method as Kondo and Higuchi (2020).

**Experiment 2:** Rice bean seeds were the same as those used in Experiment 1. In addition, adzuki bean, faba bean, and velvet bean (*Mucuna pruriens* (L.) DC. var. *utilis*) were included for comparison. The adzuki bean variety was 'Tamba Dainagon', and the faba bean variety was 'Issun Nintoku'. The velvet bean variety is unknown. Seeds were sown on November 1, 2023, and the plants were cultivated in a plastic greenhouse at Kyoto University until December 3.

On December 3, five well-grown plants were selected from each species, and the leaves and roots were harvested separately. The leaves and roots were dried at 60 °C for 2 days. The leaves were cut into 5 mm squares. The roots were cut into 5 mm lengths. 10 mg of dried leaves or 10 or 50 mg of roots were placed in the wells of a 6-well plate, with a diameter of 3.6 cm and a height of 1.8 cm. Subsequently, 5 mL of 0.5 % (w/v) agar, which had been sterilized at 121 °C and then cooled to approximately 36 °C, was poured into the wells. The agar was allowed to solidify with the leaves or roots positioned on top of the agar. After solidification, an additional 5 mL of sterilized agar was added, and the agar was allowed to solidify again, sandwiching the dried leaves or roots between the two layers of agar. Agar without leaves nor roots was solidified as a control treatment.

Five seeds of the lettuce variety 'Legacy' were sown on agar plates. The plates were incubated in the dark at 20 °C for 72 hours. For each well, the hypocotyl and radicle lengths were measured for the three plants with the longest and shortest radicles excluded, and the average values were calculated. This average value was treated as one replicate, and data were collected for five replicates per treatment.

**Experiment 3**: In 2023, rice bean and adzuki bean were cultivated on a shifting cultivation field in Shiiba Village, Miyazaki Prefecture, that had been burned in August 2019. The beans were the same as those used in Experiment 2. On June 11, test plots were established with 11.5 m in length and 7 m in width (Figure 1). The average slope of the experimental plot was approximately 14.5 degrees. Weeding and tillage of the top 10 cm of soil were performed manually. Weeding was repeated on July 16. Within this plot, 18 square plots with 1 m by 1 m, arranged in 6 by 3 were settled. Three treatments, a rice bean cultivation plot, an adzuki bean cultivation plot, and an unplanted control plot, were established with six replicates each, and 42 seeds were sown in each plot on July 16. Additionally, to mitigate differences in light conditions due to plot location, an appropriate amount of adzuki beans were sown within a 1-meter range from the outer edge of the experimental plot inward.

On October 6, the fresh weights of the rice bean, adzuki bean, and weed aboveground parts were measured in each plot. The number of rice bean and adzuki bean plants and the total number of pods were counted. The number of seeds per pod was calculated, while some pods were immature, and the seed count could not be determined for those pods. Total seed numbers were estimated based on

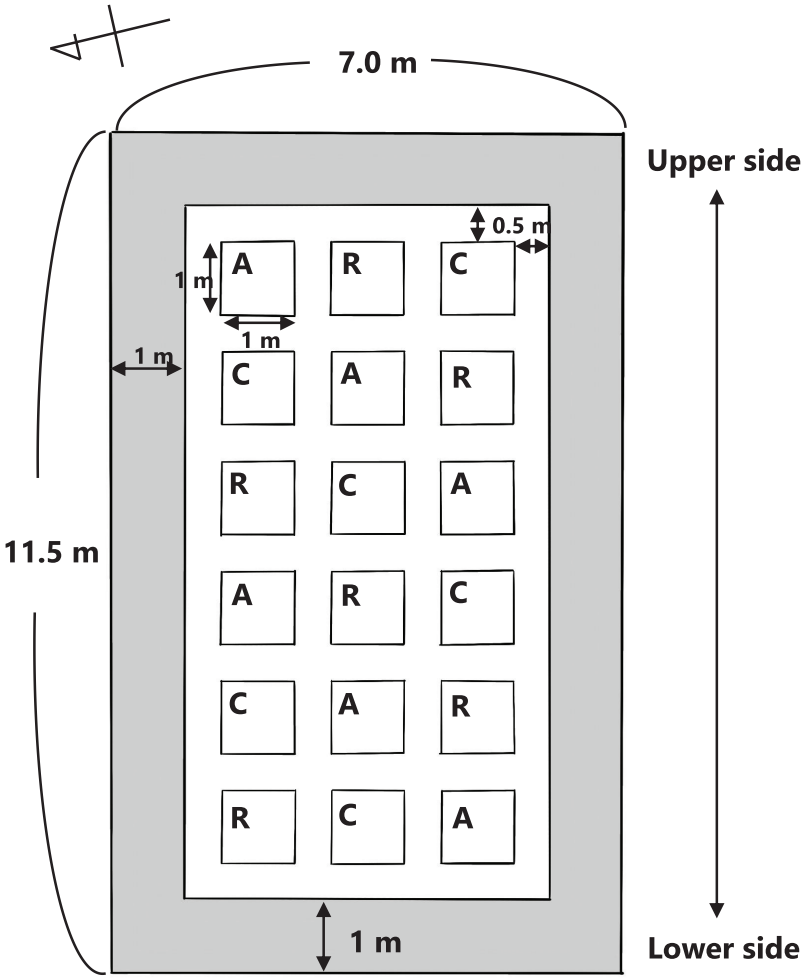



**Figure 1. Diagram of the field for the Experiment 3. The field was burnt in 2019. R: rice bean plot, A: adzuki bean plot, C: unplanted control plot. In the gray zone, adzuki beans were sown.**

the total number of pods, including mature and immature pods, and the number of seeds per pod. Furthermore, yield was estimated based on the total seed numbers and the seed weights of rice bean and adzuki bean, 75.7 mg and 205.1 mg, respectively, which had been measured prior to sowing.

**Statistical analysis:** Homogeneity of variances was assessed using Levene's test. When homogeneity of variances was assumed, one-way ANOVA was used to detect treatment effects among three or more groups; when significant differences were detected ($p < 0.05$), Tukey–Kramer's multiple comparison test was applied. For comparisons between two groups, a t test was used. When variances were unequal, Welch's one-way ANOVA was used, followed by the Games–Howell multiple comparison test when appropriate ($p < 0.05$).

## Results

The number of leaves and plant height of maize were not affected by intercropping until August 12, and decreased on August 21 by intercropping with rice bean (Figure 2, Tukey-Kramer test, $P < 0.05$). Leaf length was not affected by intercropping (Table 1). SPAD values on August 19 decreased by intercropping with rice bean, and SPAD values on August 31 were not affected by intercropping. On August 19, the photosynthetic rate was lower when intercropped with rice bean than when intercropped with adzuki bean (Tukey-Kramer test, $P < 0.05$). Stomatal

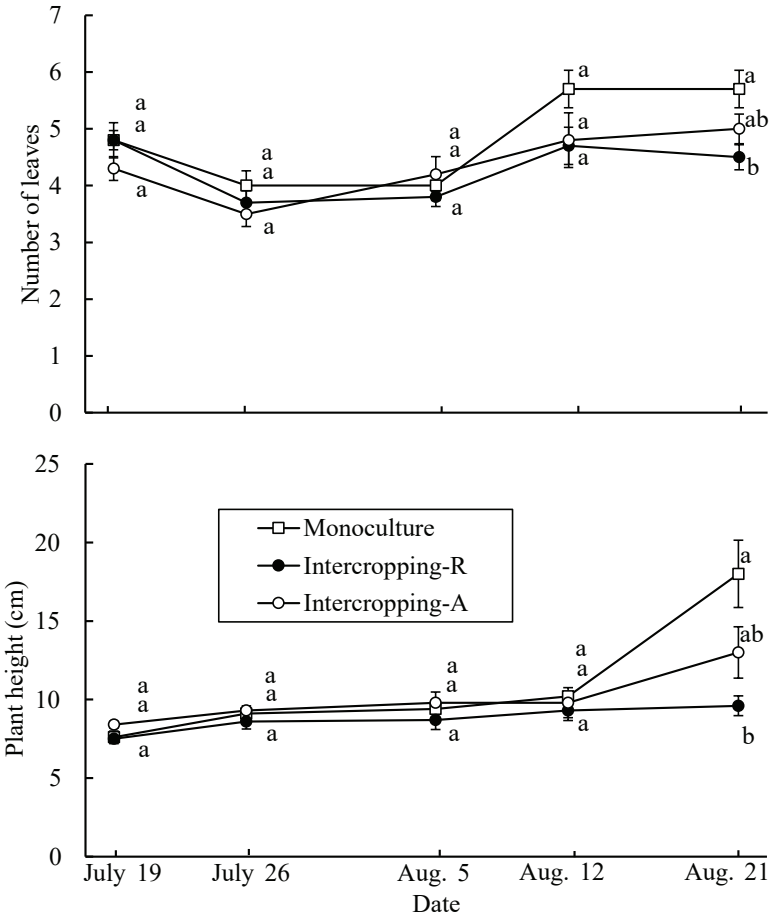



**Figure 2. Number of leaves and plant height in maize as affected by intercropping with rice bean (Intercropping-R) and adzuki bean (Intercropping-A). Different letters in the same date indicate statistical differences by Tukey-Kramer test at P<0.05. Data are means of 6 replicates.**

**Table 1. Leaf length, SPAD value, photosynthetic rate, stomatal conductance, and shoot dry weight in maize, rice bean, and adzuki bean as affected by intercropping of maize-rice bean(intercropping-R) or maize-adzuki bean (intercropping-A).**

| Species | Cropping method | Leaf length (cm) | | | SPAD value | | Photosynthetic rate (μmol·m$^{-2}$·s$^{-1}$) | | Stomatal conductance (mmol·m$^{-2}$·s$^{-1}$) | | Shoot dry weight (g) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Aug. 5 | Aug. 12 | Aug.21 | Aug. 19 | Aug.31 | Aug. 19 | Aug.31 | Aug. 19 | Aug.31 | |
| Maize | Monoculture | 38.2 a | 41.5 a | 42.3 a | 31.0 a | 32.1 a | 12.8 ab | 10.7 a | 307 a | 368 a | 4.4 a |
| | Intercropping-R | 36.8 a | 37.3 a | 36.3 a | 23.1 b | 25.6 a | 7.8 b | 6.3 a | 171 a | 336 a | 1.6 b |
| | Intercropping-A | 38.4 a | 44.3 a | 40.8 a | 29.2 a | 23.2 a | 14.2 a | 10.9 a | 268 a | 413 a | 3.1 ab |
| Rice bean | Monoculture | 8.6 a | 9.2 a | 10.1 a | 35.5 a | 31.5 a | 12.2 a | 6.7 a | 480 a | 319 a | 4.0 a |
| | Intercropping | 8.4 a | 8.8 a | 9.4 a | 38.6 a | 32.3 a | 11.6 a | 7.1 a | 546 a | 249 a | 4.1 a |
| Adzuki bean | Monoculture | 7.0 a | 7.8 a | 7.3 a | 38.4 a | 36.4 a | 5.9 a | 5.9 a | 349 a | 356 a | 2.6 a |
| | Intercropping | 6.9 a | 6.7 b | 7.3 a | 42.0 a | 33.3 a | 8.7 a | 4.8 a | 399 a | 334 a | 1.9 a |

Different letters in the same column for maize indicate statistical differences by Tukey-Kramer test at P < 0.05. Different letters in the same column for beans indicate statistical differences by t-test at P < 0.05. Data are means of 6 replicates.

**Table 2. Shoot mineral content in maize, rice bean, and adzuki bean as affected by intercropping of maize-rice bean(intercropping-R) or maize-adzuki bean (intercropping-A).**

| Species | Cropping method | N (%) | P (%) | K (%) | Ca (%) | Mg (%) |
|---|---|---|---|---|---|---|
| Maize | Monoculture | 2.4 a | 0.09 a | 2.8 a | 0.27 ab | 0.12 b |
| | Intercropping-R | 2.5 a | 0.08 a | 2.9 a | 0.29 a | 0.15 ab |
| | Intercropping-A | 2.2 a | 0.08 a | 2.9 a | 0.26 b | 0.15 a |
| Rice bean | Monoculture | 6.1 a | 0.10 a | 1.5 a | 1.01 a | 0.37 a |
| | Intercropping | 5.8 a | 0.11 a | 1.8 a | 0.94 a | 0.39 a |
| Adzuki bean | Monoculture | 5.4 a | 0.11 a | 2.6 a | 0.72 a | 0.30 a |
| | Intercropping | 5.2 a | 0.11 a | 2.2 b | 0.63 a | 0.24 b |

Different letters in the same column for maize indicate statistical differences by Tukey-Kramer test at $P< 0.05$. Different letters in the same column for beans indicate statistical differences by t-test at $P<0.05$. Data are means of 6 replicates.

conductance was not affected by intercropping. Above-ground dry weight decreased by intercropping with rice bean (Tukey-Kramer test, $P < 0.05$). Growth parameters of neither rice bean nor adzuki bean were affected by intercropping with maize. The N, P, and K contents in the maize aboveground part were not affected by intercropping (Table 2). The Ca content was higher when intercropped with rice bean than when intercropped with adzuki bean. The Mg content increased when intercropped with adzuki bean. The mineral content of the rice bean aboveground part was not affected by intercropping with maize. In adzuki bean, K and Mg content decreased by intercropping.

Soil pH was low in maize monoculture, adzuki bean monoculture, and unplanted control (Table 3). EC was high in maize monoculture, adzuki bean monoculture, and unplanted control, while it was low in maize-adzuki bean intercropping treatment. There were no significant differences in available P and $NH_4$-N among the treatments. $NO_3$-N was high in maize monoculture and unplanted control, while $NO_3$-N was low in maize-rice bean intercropping treatment.

Using 10 mg of dried leaves from rice bean and faba bean suppressed lettuce hypocotyl elongation compared to the control (Table 4, Tukey-Kramer test, $P < 0.05$). 10 mg of

**Table 3. Chemical properties of rhizosphere soil before and after cropping.**

| Cropping method | pH | EC ($mS \cdot cm^{-1}$) | Available P ($mg\ P \cdot kg^{-1}$) | $NH_4$-N ($mg\ N \cdot 100\ g^{-1}$) | $NO_3$-N ($mg\ N \cdot 100\ g^{-1}$) | P absorption coefficient ($mg\ P_2O_5 \cdot g^{-1}$) |
|---|---|---|---|---|---|---|
| Before cropping | 4.7 | 0.30 | 10.4 | 1.0 | 3.0 | 21.7 |
| Maize monoculture | 4.2 b | 1.19 a | 10.0 a | 12.1 a | 59.5 a | - |
| Maize - rice bean intercropping | 4.6 a | 0.52 b | 9.1 a | 6.1 a | 5.5 d | - |
| Maize - adzuki bean intercropping | 4.4 a | 0.64 ab | 11.7 a | 11.4 a | 18.9 bcd | - |
| Rice bean monoculture | 4.5 a | 0.59 ab | 10.4 a | 6.3 a | 13.6 cd | - |
| Adzuki bean monoculture | 4.3 b | 0.76 a | 10.9 a | 10.4 a | 26.4 abc | - |
| Unplanted control | 4.2 b | 0.70 a | 12.0 a | 9.1 a | 30.2 ab | - |

Different letters in the same column indicate statistical differences by Tukey-Kramer test (pH, available P) and by Games-Howell test (EC, $NH_4$-N, $NO_3$-N) at $P < 0.05$. Data are means of 6 replicates.

**Table 4. Hypocotyl and radicle length of lettuce seedlings grown on agar gel containing dried leaves or roots of rice bean, adzuki bean, faba bean, or velvet bean.**

| Species | 10 mg of dried leaves | | 10 mg of dried roots | | 50 mg of dried roots | |
|---|---|---|---|---|---|---|
| | Hypocotyl length (cm) | Radicle length (cm) | Hypocotyl length(cm) | Radicle length (cm) | Hypocotyl length (cm) | Radicle length (cm) |
| Rice bean | 1.7 c | 3.6 c | 5.6 a | 7.6 bc | 3.9 b | 3.9 b |
| Adzuki bean | 4.3 abc | 5.5 bc | 8.4 a | 10.9 ab | 4.9 b | 4.5 b |
| Faba bean | 3.5 bc | 5.6 bc | 5.5 a | 4.9 c | 2.9 b | 2.7 b |
| Velvet bean | 6.6 ab | 8.9 ab | 7.9 a | 11.7 ab | 5.9 ab | 5.1 b |
| Control | 5.4 a | 11.9 a | 7.4 a | 14.3 a | 9.3 a | 15.2 a |

Different letters in the same column indicate statistical differences by Games-Howell test at $P< 0.05$ for leaves or Tukey-Kramer test at $P < 0.05$ for roots. Data are means of 5 replicates.

**Table 5. Effect of rice bean or adzuki bean cultivation on weed fresh weight and growth parameters of beans.**

| Cultivated plant | Fresh weight of weed ($g \cdot m^{-2}$) | Growth parameters of rice bean and adzuki bean | | | | | |
|---|---|---|---|---|---|---|---|
| | | Population ($g \cdot m^{-2}$) | Shoot fresh weight ($g \cdot m^{-2}$) | Number of pods/$m^2$ | Number of seeds/pod | Number of seeds/$m^2$ | Yield ($g \cdot m^{-2}$) |
| Rice bean | 852 a | 32.0 a | 201 b | 108 a | 6.1 a | 662 a | 50.1 a |
| Adzuki bean | 919 a | 32.0 a | 325 a | 51 b | 5.6 b | 283 b | 57.9 a |
| Unplanted control | 914 a | – | – | – | – | – | – |

Different letters in the same column indicate statistical differences by Tukey-Kramer test at $P < 0.05$ for fresh weight of weed and t-test at $P<0.05$ for remains perametters. Data are means of 6 replicates.

dried leaves from the 3 species other than velvet bean also suppressed lettuce radicle elongation (Tukey-Kramer test, $P < 0.05$). On the other hand, 10 mg of dried roots from any of these species did not suppress lettuce hypocotyl elongation. Using 10 mg of dried roots from rice bean and faba bean suppressed lettuce radicle elongation. With 50 mg of dried roots, hypocotyl length growth was suppressed in the three species other than velvet bean, and radicle growth was suppressed in all species (Tukey-Kramer test, $P < 0.05$). There were no differences among species.

There were no significant differences in fresh weed weight among the three plots (Table 5). There were no differences in plant population and yield between rice bean and adzuki bean. The fresh weight of the aboveground parts of adzuki bean was greater than that of rice bean. The total number of pods, seeds per pod, and total number of seeds were higher for rice bean than for adzuki bean.

## Discussion

Maize growth was reduced by intercropping with rice bean in Experiment 1. Furthermore, neither the P content in the maize aboveground part nor the results for available P in the soil after cultivation showed any evidence of enhanced P uptake by maize. Therefore, under the conditions of this experiment, it can be concluded that there was no increase in P uptake or growth-promoting effect from intercropping with rice bean, as has been reported for other leguminous crops (Li *et al*. 2004 2007, Zhang *et al*. 2016a). Furthermore, unlike the report by Kondo and Koga (2021), no decrease in soil available P was observed with rice bean cropping, both monoculture and intercropping. The P content of rice bean in the aboveground parts was not higher than that of adzuki bean. Therefore, it is considered that the superior P uptake capacity of rice bean was not demonstrated under the conditions of this experiment. This is thought to be one of the reasons why maize growth was not promoted. The reason why P could

not be absorbed from Al-P under the conditions of this experiment remained unclear. The secretion of organic acids by roots, which play a key role in P uptake from Al-P, fluctuated under the influence of temperature and soil water stress (Pramanik *et al*. 2000, Henry *et al*. 2007). It is necessary to determine the effect of environmental conditions, such as temperature and water stress, on the amount of organic acid or other organic matter secreted by rice bean and P uptake capacity.

Two possible causes for the reduced growth of maize when intercropped with rice bean are competition for light, nutrients, and water, and the effects of allelopathy. The N, P, K, Ca, and Mg contents of both maize and rice bean did not decrease in the intercropping treatment. Therefore, the likelihood of nutrient competition is low. Although the $NO_3$-N content in the rhizosphere soil after the experiment was lower in the maize-rice bean intercropping treatment than in the maize monoculture treatment, the N content of the maize did not decrease, suggesting that the likelihood of N deficiency is low. In this experiment, the distance between pots was approximately 5 cm, and the plants in the monoculture treatments were also close to one another; thus, it is believed that there was no significant difference in the degree of light competition between the intercropping and monoculture treatments. Since the plants were irrigated daily, the likelihood of water competition was also low. Therefore, it was likely associated with allelopathic effects. Although six replicates are commonly used in greenhouse pot experiments, the present experiment should be regarded as exploratory with respect to detecting subtle interaction effects among multiple traits.

Therefore, we investigated the allelopathic activity of the leaves and roots of rice bean using the sandwich method, developed by Fujii (1994). The results showed that they exhibited allelopathic activity comparable to or stronger than that of adzuki bean, faba bean, and velvet bean. Velvet bean has been reported to have one of the strongest allelopathic effects among legume crops (Fujii *et al*. 1991). In a study involving 62 plant species, only one plant was found to inhibit the growth of lettuce hypocotyls and radicle to the same extent as rice bean in our study (Itani *et al.* 2020). Therefore, it can be concluded that rice bean has extremely strong allelopathic activity. Various substances have been identified as allelochemicals in velvet bean and adzuki bean (Nakahisa *et al*. 1994, Fujii 2003, Song *et al*. 2016). Future studies should focus on identifying the allelochemicals in rice bean and measuring their secretion levels. Furthermore, while this study used dried leaves and roots, it is also necessary to examine the allelopathic effects in living plants.

On the other hand, in a shifting cultivation field, rice bean did not suppress weed growth. In Experiment 3, the number of pods per plant was 3.3 for rice bean and 2.6 for adzuki bean, and the fresh weight of the aboveground biomass was 201 and 325 $g \cdot m^{-2}$, respectively. In contrast, Kondo and Koga (2021) reported pod numbers per plant of 17.7 and 8.3, respectively, with soil collected from a shifting cultivation field in Shiiba Village. The fresh weight of aboveground biomass for adzuki 'Tamba Dainagon' cultivated by Koroda *et al*. (2013) was 797 $g \cdot m^{-2}$. Therefore, it can be said that the growth of rice bean and adzuki bean in Experiment 3 was extremely poor. As of October 6, many rice bean and adzuki bean plants were grown under weeds, and it is highly likely that growth was strongly suppressed due to light competition. According to village lore and previous interviews, it was said that weeding was not necessary for rice bean cultivation. Since these testimonies may be consistent with the results of Experiments 1 and 2, it is highly likely that rice bean was able to grow without weeding. Although the reason for the lack of the allelopathic effect in Experiment 3 remains unclear, it was possible that rice bean could not exhibit the allelopathic effects observed in Experiments 1 and 2 because the rice bean plants remained extremely small due to competition with weeds during the early growth stage. Moreover, the growth-inhibiting effect varies significantly depending on seeding density (Boyd *et al*. 2009, Kristensen *et al*. 2008), weed species (Fujii 2000, Seal and Pratlay 2010, Zhang *et al*. 2016b), and the growth stage of the plants (Oveisi *et al*. 2008). Furthermore, since this is a one-year trial conducted at a single location, it is difficult to conclude that there is no allelopathic effect at the field level. It will be necessary to elucidate these factors in detail in future studies.

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