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# Agronomic responses of soybean cultivars to narrow intra-row spacing in a cool region of northern Japan

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## ABSTRACT

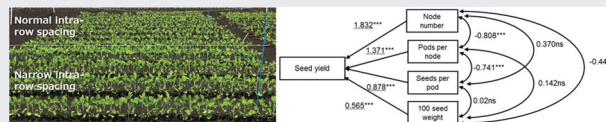
Combinations of cultivar selection and management practices are used to maximize soybean yields in Japan. High plant density could increase the amount of solar radiation intercepted by the canopy and thereby increase growth and yield, but both cultivar and environment affect the response to plant density. The mechanisms underlying responses of soybean to high plant density are not fully understood. This study tested the effects of high plant density by narrow intra-row spacing on agronomic traits in 2 years using three cultivars (Ryuhou, Okushirome, and Fukuibuki) in Morioka, Iwate, in the Tohoku region of Japan. Averaged across years and cultivars, narrow intra-row spacing increased seed yield. Interestingly, there was a significant year  $\times$  intra-row spacing  $\times$  cultivar interaction: In the first year, narrow intra-row spacing increased yield of Ryuhou and Fukuibuki but not of Okushirome; in the second year, however, it increased yield of all cultivars similarly. The lack of yield response of Okushirome to narrow intra-row spacing in the first year presumably resulted from an excessive leaf area index (LAI) and a higher lodging score. The results suggest that high plant density by narrow intra-row spacing is an effective option for increasing soybean yields in the Tohoku region, although agronomic traits such as LAI and susceptibility to lodging should be considered.

## ARTICLE HISTORY

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## 1. Introduction

In Japan, soybean (*Glycine max* (L.) Merr.) is an important crop used in traditional foods such as tofu, natto, and miso. However, the yield in Japan is much lower than those in other major producing countries such as the USA, Brazil, and China, and has stagnated over the past 30 years: the average (1982–2012) yield is only 1.65 t ha<sup>-1</sup> and has increased by less than 0.3 t ha<sup>-1</sup> since 1982 (MAFF, 2013). Generally, crop yield is determined by genetics, environmental conditions (soil, meteorology), management practices (sowing date, plant density, fertilization), and their interactions (Evans & Fischer, 1999; Rowntree et al., 2014; Van Roekel et al., 2015). At the local to regional scales in Japan, combinations of cultivar selection and management practices are used to maximize soybean yields (e.g., Kawasaki et al., 2018; Kumagai, 2018; Matsuo et al., 2018).

Among management practices, seeding rate (plant density) is one of the main factors controlled by growers

(Egli, 1988). In the USA, many studies of the effect of plant density on soybean yield have been conducted, but results have been inconsistent, with no response in some cases (Board, 2000; Cox et al., 2010), and positive responses to high plant density in other cases (De Bruin & Pedersen, 2008; Carciochi et al., 2019; Corassa et al., 2018; Egli, 1988; Gaspar & Conley, 2015; Gaspar et al., 2017; Holshouser & Whittaker, 2002; Thompson et al., 2015). The optimal density in these studies ranged from 7 to 60 plants m<sup>-2</sup>.

In the Tohoku region of northern Japan, which has a cool climate, paddy rice monoculture is the dominant cropping system, and the optimal transplanting time is mid to late May. The recommended sowing window for soybean is late May to early June, and the recommended density then is 8 to 15 plants m<sup>-2</sup> (Spaeth et al., 1987). The low plant densities are normal cultural practice in Japan because lodging and branch breakage, which reduce seed yield, are common at high planting density (Kamiya et al., 1980; Shimada & Hirokawa, 1984). In the

Tohoku region, studies of the response of soybean to high plant density (Kokubun, 1988; Mochida, 2016) showed that the optimal density was 9.2 to 38.1 plants  $m^{-2}$ , depending on maturity group (MG) (cultivars) and environments (years).

Several studies of US soybean cultivars evaluated genotypic differences in yield responses to plant density. Yield responses were explained by yield components, especially those of branches (Agudamu et al., 2016; Suhre et al., 2014), susceptibility to lodging (Cober et al., 2005), MG (Edwards & Purcell, 2005), and the interception and use of solar radiation by the canopy. Edwards et al. (2005) showed that cumulative intercepted solar radiation (CumIR) explained soybean yield responses to plant density among diverse MGs and environments. On the other hand, De Bruin and Pedersen (2008) showed that new soybean cultivars had greater seed yields than old cultivars across a wide range of densities from 4.9 to 44.5 plants  $m^{-2}$ , and a higher plant density at which yield plateaued (23.0 vs 19.9 plants  $m^{-2}$ ). Both new and old cultivars showed a similar increase of CumIR at high plant density, indicating that genetic gain from new cultivars is associated with higher radiation-use efficiency (RUE). A pioneer study by Kokubun (1988) in the Tohoku region showed that a high-yielding cultivar, Okushrome, had a higher optimal plant density than control cultivars of similar MG, due to high efficiency of dry matter production per leaf area. However, the importance of CumIR and RUE to cultivar differences in yield response to plant density in Japan is not fully understood.

The effect of plant density (seeding rate) on soybean yield depends not only on genetics but also on environment. Wells (1991) found that seed yield responded to plant density in less favorable environments but not in more favorable environments. Among the environmental factors in the currently recommended growing season in the Tohoku region, temperature, precipitation, and soil water content affect canopy development and thereby CumIR (Kumagai, 2018). Our studies with different sowing dates (from mid-May to mid-June) at normal plant density indicated that CumIR and dry matter production during the early reproductive stage determined seed yield, by determining pod and node numbers (Kumagai, 2018; Kumagai & Takahashi, 2020). Therefore, I hypothesized that a higher plant density than currently recommended would increase soybean yield by increasing CumIR in the Tohoku region.

Many researchers in Japan have focused on high plant density by narrow inter-row spacing (<0.7 m) without inter-tillage and ridging to reduce labor (e.g., Matsuo et al., 2018). However, here I focused on high plant density by narrow intra-row spacing at conventional

inter-row spacing (0.7–0.75 m) with inter-tillage and ridging, which can prevent soybean from lodging (Shimada & Hirokawa, 1984). The objectives of this study were: (1) to test the hypothesis that high plant density by narrow intra-row spacing than normal would increase CumIR, dry matter production, and thereby increase seed yield in three soybean cultivars in the Tohoku region; and (2) to clarify what determines any responses of seed yield to narrow intra-row spacing. Despite positive yield responses to high plant density in previous studies, variables responsible for yield response, such as CumIR and RUE, are not fully understood. Field experiments were conducted in 2 years to quantify crop growth and yield, CumIR, and RUE of different cultivars sown at narrower intra-row spacing than normal (0.075 vs 0.15 m).

## 2. Materials and methods

### 2.1. Location, management, and cultivars

Experiments were conducted at the National Agriculture and Food Research Organization, Tohoku Agricultural Research Center, in Morioka, Iwate Prefecture, Japan (39°44'N, 141°7'E), in 2016 and 2017. Different upland fields were used between years. The soil in each field is an Andosol (Supplemental Table 1). Each field received 60 g  $m^{-2}$  fused phosphate fertilizer and 100 g  $m^{-2}$  magnesium lime a month before sowing each year, and 3 g  $m^{-2}$  N, 12.5 g  $m^{-2}$  P, and 5 g  $m^{-2}$  K as compound fertilizer 1 day before sowing each year. Fertilizers were applied and incorporated to a depth of ~15 cm by rotary tiller.

Three determinate soybean cultivars, Ryuhou, Okushrome, and Fukuibuki, were used for the experiment. Within the Tohoku region, Ryuhou is widely grown in Akita and Iwate Prefectures (Satou et al., 1998). Okushrome used to be widely grown in Aomori Prefecture (Tada et al., 1988). It had one of the highest yield records in the Tohoku region (649 g  $m^{-2}$ ; Spaeth et al., 1987) and responds well to high plant density (Kokubun, 1988). Fukuibuki was released in 2004 as a recommended new cultivar in Fukushima Prefecture. It also responds well to high plant density and was tolerant to lodging (Shimada et al., 2004).

Seeds were treated with a combined insecticide and fungicide (CruiserMaxx, Syngenta Co., Japan) at the rate recommended by the manufacturer. They were sown by hand on 30 May 2016 and 29 May 2017 at three seeds per hill. In 2016, the inter-row spacing (row width) was 0.70 m; at 0.15 m between hills at normal intra-row spacing plot, the target density was 9.5 plants  $m^{-2}$ , and at 0.075 m between hills at narrow intra-row spacing

plot, it was 19 plants  $\text{m}^{-2}$ . In 2017, the inter-row spacing (row width) was 0.75 m; at 0.15 m between hills at normal intra-row spacing plot, the target density was 8.8 plants  $\text{m}^{-2}$ , and at 0.075 m between hills at narrow intra-spacing plot, it was 17.8 plants  $\text{m}^{-2}$ . Just after sowing, a pre-emergence herbicide (Ecotop, Maruwa Biochemical Co., Japan; 1.0% dimethenamid-P + 1.4% linuron) was applied at the rate recommended by the manufacturer. After seedling establishment, plants were thinned to one per hill. Plants were not irrigated and relied entirely on rainfall. A month after sowing, the soil was tilled shallowly between the rows and ridged to control weeds and lodging. The pesticides were reapplied to maximize yield and seed quality. In each year, the experimental design used a split-plot arrangement; the main plot was intra-row spacing (normal vs. narrow) and the subplot was cultivar, with three replicates. A subplot consisted of five rows 4.2 m long in 2016 and six rows 4.2 m long in 2017. The total experimental area was 265  $\text{m}^2$  and 340  $\text{m}^2$  in 2015 and 2016, respectively. Plants were grown to maturity.

## 2.2. Measurements

In both normal and narrow intra-row spacing plots, I surveyed the phenology of 10 plants in the center row of each plot every 1 to 3 days and recorded the dates of each growth stage in the staging system of Fehr and Caviness (1977): VE, emergence; R2, full flowering; R5, beginning of seed filling; and R7, beginning of maturity. The dry weight of aboveground parts per  $\text{m}^2$  was determined by periodic sampling at V1 (first trifoliolate leaves fully developed), R2, and R5. On each date in 2016 (2017), the aboveground parts of 0.62 (0.90)  $\text{m}^2$  and of the center three (four) rows were harvested. At R2 and R5, leaves were separated from whole plants, and leaf area was measured with automatic area meter (AAM-8, Hayashi-Denko Co. Ltd., Japan). Leaf area index (LAI) was calculated as measured leaf area  $\div$  sampling area. Dry weights were measured after oven drying at 80°C for 3 days, and differences in the dry weights of aboveground parts (increase in aboveground biomass,  $\Delta\text{AGB}$ ) from V1 to R2 and from R2 and R5 were calculated. I estimated the solar radiation intercepted by using digital imaging techniques (GACS1; Kimura Ouyou-Kougei Co., Ltd., Japan) according to Kumagai and Takahashi (2020): The fractional canopy cover was determined from digital images taken at 1.5 m above the canopy at 1-week intervals from the early vegetative to late reproductive stages. Total daily solar radiation ( $\text{MJ m}^{-2} \text{d}^{-1}$ ) was measured with a pyranometer (CMP11, Campbell Scientific Inc., USA) and recorded on a data logger

(CR1000, Campbell Scientific) at a weather station near the field, and daily incident radiation above the canopy was computed as daily solar radiation  $\times$  fractional canopy cover. The CumIR from V1 to R2 and from R2 to R5 was computed as the sum of daily incident radiation above the canopy. RUE was determined as  $\Delta\text{AGB} \div \text{CumIR}$ . In addition to solar radiation, daily means of temperature and precipitation during the growing season were recorded at the weather station.

At maturity, I measured the angle of the main stem of 10 plants per plot to score lodging. The angle was measured with a transparent acryl plate of 25 cm  $\times$  25 cm on which straight lines were drawn with a marker at 10°, 20°, 40°, and 60° to the upright. The lodging angle was scored 1 for 0°–10°, 2 for 10°–20°, 3 for 20°–40°, 4 for 40°–60°, and 5 for >60°.

Next, I manually harvested the aboveground parts from 3.2  $\text{m}^2$  of the center three rows of each plot in 2016 and from 3.4  $\text{m}^2$  in 2017. After the plants were completely air-dried, I removed the few remaining leaves and petioles and then weighed the remaining aboveground parts. AGB was the sum of the weights of the stems, pod shells, and seeds. The seed yield per  $\text{m}^2$  was adjusted to 15% moisture content, and the harvest index (HI) was determined as seed yield  $\div$  AGB. The yield components (numbers of nodes, pods, seeds per pod, pods per node, and 100-seed weight), main stem length, and lowest pod height were measured and averaged from eight representative plants in each plot. The 100-seed weight was also adjusted to 15% moisture.

For all variables except lodging score, I first conducted ANOVA with a split-split-plot design, with year as the main factor, intra-row spacing as the split factor, and cultivar as the split-split factor. Year, intra-row spacing, cultivar, and their interaction were each considered as fixed effects, and replicate (block) was considered as a random effect. Because lodging score is a categorical variable and the Shapiro–Wilk test showed that the data did not have a normal distribution, I used a Friedman nonparametric one-way ANOVA test to detect significant effects of year, intra-row spacing, and cultivar. Statistical significance was evaluated at  $\alpha < 0.1$ . I also examined the relationships among yield, yield components, and growth parameters ( $\Delta\text{AGB}$  and CumIR) by Pearson's simple correlation analysis. I examined the relative importance of yield components in the two-year data by multiple regression analysis by the response ratio of narrow to normal intra-row spacing. I used the natural logarithm transformation because the relationships between yield and its components were multiplicative. All procedures were performed in SPSS v. 24.0 software (IBM, Japan).

### 3. Results

#### 3.1. Weather conditions

In 2016, the 5-month mean temperature (from June to October) was 0.7°C higher than the 30-year mean (Table 1). The monthly mean temperatures were 1.2°C higher in August and 2.1°C higher in September. In 2017, the 5-month mean temperature was the same as the 30-year mean. However, the monthly mean temperatures were 2.3°C higher in July and 1.3°C lower in August. The five-month mean daily solar radiation in 2016 was 1.3 MJ m<sup>-2</sup> d<sup>-1</sup> higher than the 30-year mean in 2016 and 0.8 MJ m<sup>-2</sup> d<sup>-1</sup> higher in 2017. In 2016, solar radiation from July to October was higher than normal. In 2017, on the other hand, it was 2.9 MJ m<sup>-2</sup> d<sup>-1</sup> higher than the 30-year mean in July, 2.6 MJ m<sup>-2</sup> d<sup>-1</sup> higher in September, but 1.5 MJ m<sup>-2</sup> d<sup>-1</sup> lower in August. The 2016 and 2017 growing seasons had more precipitation than the 30-year mean (179.0 and 225.0 vs. 146.8 mm). In 2016, the monthly total precipitation was 52.0 mm higher than the 30-year mean in June, 77.5 mm higher in August, and 92.0 mm higher in October. In 2017, it was higher than the 30-year mean in all months, by as much as 190.5 mm in July. Overall, both years had normal temperature and abundant solar radiation and precipitation.

#### 3.2. Aboveground biomass, seed yield, harvest index, and yield components

The effects of year (Y), intra-row spacing (IS), and cultivar (CV) and their interactions on seed yield, AGB, HI, and yield components were analyzed by ANOVA (Table 2). Averaged over years and cultivars, narrow intra-row spacing increased seed yield by 9% and AGB by 15% ( $P < 0.01$ ). These enhancements were affected by cultivar ( $P = 0.077$  and  $P = 0.051$ , respectively, IS × CV) and by the year × cultivar interaction ( $P = 0.096$  and  $P < 0.05$ , respectively, Y × IS × CV). Seed yield did not differ between years, but AGB was significantly higher in 2016 ( $P = 0.051$ ) when averaged across cultivars and intra-row spacings. There was no significant year × intra-row spacing interaction in seed yield or AGB. Averaged

across years, yield and AGB increased more in Ryuhou and Fukuibuki (12%–21%) than in Okushirome (4%–7%). In 2016, narrow intra-row spacing increased seed yield and AGB in Ryuhou and Fukuibuki but not in Okushirome. In 2017, in contrast, it increased seed yield and AGB of all three cultivars similarly. Narrow intra-row spacing decreased HI by 5% on average over years and cultivars ( $P < 0.05$ ). Averaged across years, HI decreased more in Okushirome and Fukuibuki (5% and 7%) than in Ryuhou (2%), resulting in a significant intra-row spacing × cultivar interaction ( $P = 0.066$ , IS × CV). In contrast, HI was not affected by year or by year × intra-row spacing interaction. Averaged across years and cultivars, narrow intra-row spacing increased the total node number by 39% ( $P < 0.001$ ) but decreased pods per node by 17% ( $P < 0.001$ ) and seeds per pod by 2% ( $P = 0.093$ ). Intra-row spacing did not affect 100-seed weight. Moreover, there were no intra-row spacing × cultivar or year × intra-row spacing × cultivar interactions in total node number, seeds per pod, or 100-seed weight. Total node number and seeds per pod were higher but pods per node were lower in 2016 than in 2017 ( $P = 0.084$ ,  $P < 0.05$ , and  $P < 0.001$ , respectively). There was no year × intra-row spacing interaction in any yield components. However, there was a significant three-way interaction in pods per node ( $P = 0.065$ , Y × IS × CV). In 2016, narrow intra-row spacing decreased pods per node more in Ryuhou (by 20%) and Fukuibuki (by 25%) than in Okushirome (by 4%), whereas in 2017, it decreased pods per node in all three cultivars similarly (by 17%–21%).

Averaged across years and cultivars, main stem length and lowest pod height were higher in narrow intra-row spacing ( $P < 0.073$ ,  $P < 0.001$ , respectively, Table 3). Averaged between years, narrow intra-row spacing increased main stem length most in Fukuibuki, resulting in a significant intra-row spacing × cultivar interaction ( $P < 0.05$ , IS × CV). In contrast, there was no intra-row spacing × cultivar interaction in lowest pod height. Narrow intra-row spacing did not significantly affect lodging score, but year ( $P < 0.05$ ) and cultivar ( $P < 0.001$ ) did: averaged across cultivars and intra-row

**Table 1.** Monthly mean air temperature, solar radiation, and total precipitation during 2016 and 2017 growing seasons, and comparison with the 30-year mean values.

		Jun.	Jul.	Aug.	Sep.	Oct.	5-month mean	
Temperature (°C)	2016	18.1	21.6	24.1	20.3	11.2	19.0	
	2017	17.2	23.6	21.6	17.5	11.4	18.3	
	30-year mean	17.8	21.3	22.9	18.2	11.5	18.3	
		2016	16.4	16.9	18.2	12.5	11.6	15.1
Solar radiation (MJ m <sup>-2</sup> d <sup>-1</sup> )	2017	18.5	18.0	13.6	14.5	8.4	14.6	
	30-year mean	17.1	15.1	15.1	11.9	9.9	13.8	
	Precipitation (mm)	2016	160.0	131.0	258.5	160.5	185.0	179.0
		2017	123.0	386.5	216.5	193.5	205.5	225.0
30-year mean		108.0	196.0	181.0	156.0	93.0	146.8	

Table 2. Aboveground biomass, seed yield, harvest index, and yield components in the three soybean cultivars with normal and narrow intra-row spacing in 2016 and 2017.

Year	Cultivar	Seed yield (g m <sup>-2</sup> )		Aboveground biomass (g m <sup>-2</sup> )		Harvest index		Total node number (m <sup>-2</sup> )		Pods per node		Seeds per pod		100-seed weight (g)	
		Normal	Narrow	Normal	Narrow	Normal	Narrow	Normal	Narrow	Normal	Narrow	Normal	Narrow	Normal	Narrow
2016	Ryuhou	372	433	599	710	0.62	0.61	401	558	1.94	1.54	1.46	1.56	33.2	32.6
	Okushiro	363	348	625	643	0.58	0.54	478	560	2.02	1.94	1.45	1.28	26.2	25.1
	Fukuibuki	367	434	626	807	0.59	0.54	486	804	1.94	1.46	1.23	1.22	32.0	30.8
	Mean	368	405	617	720	0.60	0.56	455	641	1.97	1.65	1.38	1.35	30.5	29.5
2017	Ryuhou	350	391	595	683	0.59	0.57	388	538	1.55	1.26	1.66	1.63	35.3	35.4
	Okushiro	367	395	613	688	0.60	0.58	395	560	1.97	1.56	1.79	1.79	26.4	25.7
	Fukuibuki	368	391	596	668	0.62	0.59	441	578	1.61	1.34	1.71	1.68	30.3	30.2
	Mean	362	392	602	679	0.60	0.58	408	559	1.71	1.39	1.72	1.70	30.7	30.5
2-years-mean	Ryuhou	361	412	597	696	0.61	0.59	394	548	1.74	1.40	1.56	1.59	34.2	34.0
	Okushiro	365	372	619	665	0.59	0.56	437	560	2.00	1.75	1.62	1.54	26.3	25.4
	Fukuibuki	367	413	611	737	0.60	0.56	463	691	1.78	1.40	1.47	1.45	31.1	30.5
	Mean	365	399	609	700	0.60	0.57	431	600	1.84	1.52	1.55	1.53	30.6	30.0
ANOVA results															
Year (Y)		ns		0.051		ns		0.084		*		***		ns	
IS (Intra-row spacing)		**		**		*		***		***		***		ns	
CV (Cultivar)		0.083		ns		***		**		***		0.093		***	
Y × IS		ns		ns		ns		ns		ns		ns		ns	
Y × CV		*		*		***		ns		ns		***		**	
IS × CV		0.077		0.051		0.066		ns		ns		ns		ns	
Y × IS × CV		0.096		*		ns		ns		0.065		ns		ns	

\*\*\*P &lt; 0.001; \*\*P &lt; 0.01; \*P &lt; 0.05. Values indicate probabilities between 0.05 and 0.1. ns, not significant.

**Table 3.** Main stem length, lowest pod height, and lodging score in the three soybean cultivars with normal and narrow intra-row spacing in 2016 and 2017.

Year (Y)	Cultivar (CV)	Main stem length (cm)		Lowest pod height (cm)		Lodging score (0–4)	
		Normal	Narrow	Normal	Narrow	Normal	Narrow
2016	Ryuhou	56.1	69.0	12.3	15.8	0.2	1.0
	Okushirome	73.3	78.5	11.9	14.3	1.7	1.4
	Fukuibuki	60.6	79.4	10.1	13.1	0.4	0.7
	Mean	63.3	75.7	11.4	14.4	0.8	1.0
2017	Ryuhou	57.5	66.4	14.5	20.3	0.0	0.2
	Okushirome	68.3	74.8	15.9	21.0	0.7	0.9
	Fukuibuki	50.3	64.7	12.9	18.2	0.0	0.2
	Mean	58.7	68.6	14.4	19.8	0.2	0.4
2-years-mean	Ryuhou	56.8	67.7	13.4	18.0	0.1	0.6
	Okushirome	70.8	76.6	13.9	17.6	1.2	1.2
	Fukuibuki	55.4	72.1	11.5	15.7	0.2	0.4
	Mean	61.0	72.1	12.9	17.1	0.5	0.7
ANOVA results							
Y		*		***		*	
IS		0.073		***		ns	
CV		***		*		***	
Y × IS		ns		*		nd	
Y × CV		**		ns		nd	
IS × CV		*		ns		nd	
Y × IS × CV		ns		ns		nd	

\*\*\* $P < 0.001$ ; \*\* $P < 0.01$ ; \* $P < 0.05$ . Values indicate probabilities between 0.05 and 0.1. ns, not significant. For lodging score, Friedman's test was used to detect significant effects of year, plant density and cultivar. nd, not detected.

spacings, lodging score was higher in 2016 than in 2017. Averaged across years and cultivars, Okushirome had the highest score, indicating its susceptibility to lodging.

### 3.3. Phenological development, LAI, canopy cover, dry matter production, and CumIR and RUE

Averaged across years and cultivars, narrow intra-row spacing did not affect phenology in any stage (Supplemental Table 2). In contrast, cultivar significantly affected all phases: averaged across years and intra-row spacing, Ryuhou had the shortest days in all stages. Averaged across years and cultivars, narrow intra-row spacing increased LAI at R2 by 47% ( $P < 0.05$ , Table 4). However, the effect differed across years and cultivars, with a significant year × intra-row spacing × cultivar interaction ( $P = 0.078$ , Y × IS × CV); narrow intra-row spacing increased LAI most in Ryuhou in 2016 (by 118%) and least in Ryuhou in 2017 (by 34%). At R5, averaged across years and cultivars, narrow intra-row spacing increased LAI by 18% ( $P < 0.05$ ). However, there were no significant intra-row spacing × cultivar or year × intra-row spacing × cultivar interactions at this stage.

Averaged across cultivars, canopy cover was greater in the narrow intra-row spacing plots than in the normal plots for most of the growth period [Day of year (DOY) 170 to 210] in both years (Supplemental Figure 1). Averaged between years, narrow intra-row spacing significantly increased  $\Delta$ AGB from early vegetative to R2 by 49% ( $P < 0.05$ ) and CumIR during the same phase by 37% ( $P < 0.01$ ), with a significant intra-row spacing × cultivar

**Table 4.** Leaf area index (LAI) at R2 and R5 in the three soybean cultivars in normal and narrow intra-row spacing in 2016 and 2017.

Year (Y)	Cultivar (CV)	LAI at R2 ( $m^2 m^{-2}$ )		LAI at R5 ( $m^2 m^{-2}$ )	
		Normal	Narrow	Normal	Narrow
2016	Ryuhou	2.2	4.8	5.3	7.3
	Okushirome	3.9	4.8	8.2	8.9
	Fukuibuki	3.3	4.5	6.0	7.4
	Mean	3.2	4.7	6.5	7.9
2017	Ryuhou	3.5	4.7	6.7	6.8
	Okushirome	4.1	6.1	7.2	8.5
	Fukuibuki	3.4	5.0	6.7	8.2
	Mean	3.7	5.3	6.9	7.8
2-years-mean	Ryuhou	2.9	4.8	6.0	7.0
	Okushirome	4.0	5.5	7.7	8.7
	Fukuibuki	3.4	4.7	6.4	7.8
	Mean	3.4	5.0	6.7	7.9
ANOVA results					
Y		0.064		ns	
IS		*		*	
CV		**		***	
Y × IS		ns		ns	
Y × CV		ns		ns	
IS × CV		ns		ns	
Y × IS × CV		0.078		ns	

\*\*\* $P < 0.001$ ; \*\* $P < 0.01$ ; \* $P < 0.05$ . Values indicate probabilities between 0.05 and 0.1. ns, not significant.

interaction in CumIR ( $P < 0.05$ , IS × CV; Table 5). The increase in CumIR was larger in Okushirome than in Ryuhou and Fukuibuki. Similarly, averaged across years and cultivars, narrow intra-row spacing significantly increased  $\Delta$ AGB from R2 to R5 by 15% ( $P < 0.05$ ) and CumIR during the same phase by 2% ( $P < 0.01$ ). There was no effect of intra-row spacing or year × intra-row spacing × cultivar interaction on RUE in the stages from early vegetative to R2 and from R2 to R5.

**Table 5.** Increases in aboveground biomass ( $\Delta$ AGB), cumulative intercepted irradiation (CumIR), and radiation-use efficiency for dry matter production (RUE) during specific stages (from vegetative to R2 and from R2 to R5) in three soybean cultivars in normal and narrow intra-row spacing in 2016 and 2017.

Year (Y)	Cultivar (CV)	From early vegetative to R2						From R2 to R5					
		$\Delta$ AGB ( $\text{g m}^{-2}$ )		CumIR ( $\text{MJ m}^{-2}$ )		RUE ( $\text{g MJ}^{-1}$ )		$\Delta$ AGB ( $\text{g m}^{-2}$ )		CumIR ( $\text{MJ m}^{-2}$ )		RUE ( $\text{g MJ}^{-1}$ )	
		Normal	Narrow	Normal	Narrow	Normal	Narrow	Normal	Narrow	Normal	Narrow	Normal	Narrow
2016	Ryuhou	136	291	211	316	0.63	0.91	270	311	302	318	0.90	0.98
	Okushirome	198	264	261	325	0.76	0.80	469	520	411	414	1.14	1.25
	Fukuibuki	185	261	219	325	0.84	0.82	380	465	445	451	0.85	1.03
	Mean	173	272	230	322	0.74	0.84	373	432	386	394	0.96	1.09
2017	Ryuhou	181	237	253	352	0.72	0.67	261	311	248	254	1.05	1.22
	Okushirome	207	322	316	406	0.66	0.79	290	327	272	274	1.06	1.19
	Fukuibuki	177	242	261	356	0.68	0.68	278	299	340	347	0.82	0.86
	Mean	189	267	277	372	0.69	0.72	276	312	287	292	0.98	1.09
2-years-mean	Ryuhou	159	264	232	334	0.68	0.79	265	311	275	286	0.97	1.10
	Okushirome	203	293	289	366	0.71	0.80	379	423	342	344	1.10	1.22
	Fukuibuki	181	252	240	340	0.76	0.75	329	382	392	399	0.84	0.95
	Mean	181	270	254	347	0.71	0.78	325	372	336	343	0.97	1.09
ANOVA results													
Y			ns	**		*		***		***			ns
IS		*		**		ns		*		**			ns
CV		0.089		***		ns		***		***			***
Y $\times$ IS		ns		ns		ns		ns		ns			ns
Y $\times$ CV		ns		**		ns		***		***			*
IS $\times$ CV		ns		*		ns		ns		*			ns
Y $\times$ IS $\times$ CV		ns		ns		ns		ns		ns			ns

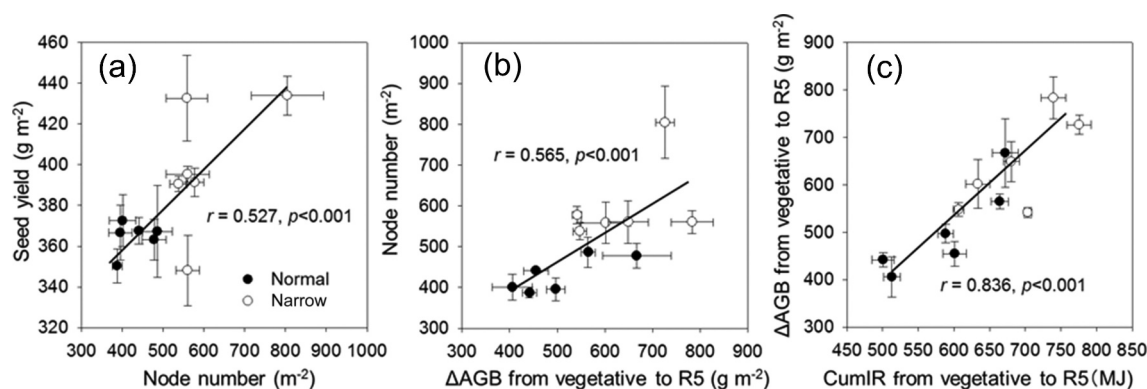
\*\*\* $P < 0.001$ ; \*\* $P < 0.01$ ; \* $P < 0.05$ . Values indicate probabilities between 0.05 and 0.1. ns, not significant.

### 3.4. Correlations between yield components and growth parameters

To explore the reason for yield variation across years, intra-row spacings, and cultivars, I examined the relationships among yield, yield components, and the growth parameters  $\Delta$ AGB and CumIR (Figure 1). When all data were pooled, seed yield was significantly positively correlated with total node number ( $P < 0.001$ , Figure 1(a)); total node number was significantly positively correlated with  $\Delta$ AGB from early vegetative to R5 ( $P < 0.001$ , Figure 1(b)); and  $\Delta$ AGB was closely related to CumIR from early vegetative to R5 ( $P < 0.001$ , Figure 1(c)).

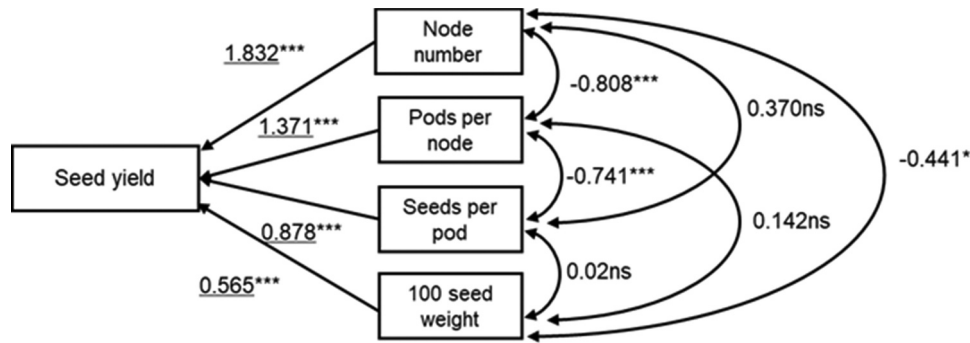
### 3.5. Multiple regression of response of seed yield to intra-row spacing against yield components

The seed yield response to narrow intra-row spacing was affected by year and cultivar and by a complex relationship among yield components. I used multiple regression to determine the relative contributions of node number, pods per node, seeds per pod, and 100-seed weight from the ratios of narrow to normal intra-row spacings. All four components significantly positively affected the yield response to narrow intra-row spacing (Figure 2). Node number contributed the most (standardized multiple regression coefficient  $\beta = 1.832$ ,  $P < 0.001$ ), followed by pods per node ( $\beta = 1.371$ ,  $P <$



**Figure 1.** Relationships between (a) node number and seed yield, (b)  $\Delta$ AGB from vegetative to R5 and node number, and (c) CumIR and  $\Delta$ AGB from vegetative to R5 of three cultivars at normal and narrow intra-row spacing in 2 years. Pearson's correlation coefficient ( $r$ ) was calculated with data combined across the two years ( $n = 36$ ).





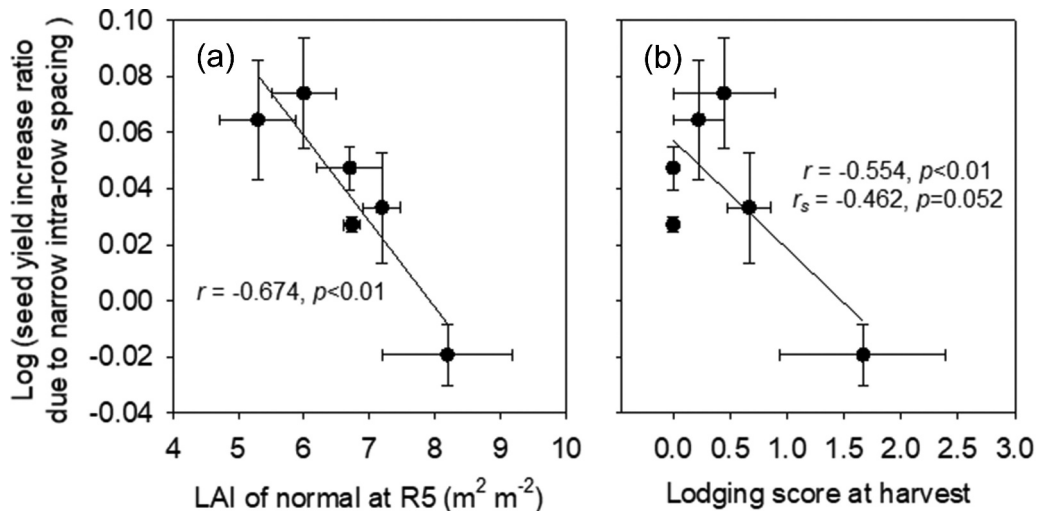
**Figure 2.** Results of multiple regression of the ratio of seed yield in narrow intra-row spacing to that in normal intra-row spacing against ratios of yield components, and correlations between variables, in two years using three cultivars. Because the relationship between yield and its components is multiplicative, I used ln-transformed data of all cultivars in each block ( $n = 18$ ). Values on single-headed arrows are standardized multiple regression coefficients, and those on double-headed arrows are Pearson's correlation coefficients. \*\*\* $P < 0.001$ ; \*\* $P < 0.01$ ; \* $P < 0.05$ ; ns, not significant.

0.001), seeds per pod and 100-seed weight ( $\beta < 1.0$  but  $P < 0.001$ ).

To identify the agronomic traits related to greater yield response to narrow intra-row spacing, I examined the correlation between the yield response ratio to narrow intra-row spacing and both LAI at R5 and lodging score at harvest at normal intra-row spacing (Figure 3). When all data were pooled, the increase in seed yield ratio was significantly negatively correlated with LAI at R5 ( $P < 0.05$ , Figure 3(a)), suggesting that cultivars with smaller LAI at normal intra-row spacing responded more to narrow spacing. Furthermore, when data were pooled, both Pearson's correlation coefficient ( $r$ ) and Spearman's rank correlation coefficient ( $r_s$ ) were significant for the relationship between the increased seed yield ratio by narrow intra-row spacing and lodging score ( $P < 0.01$ ,  $P = 0.052$ , respectively, Figure 3(b)).

#### 4. Discussion

In this study, because the inter-row spacing (row width) was differed between the 2 years (0.7 vs 0.75 m), the effect of year included the effects of inter-row spacing as well as weather and soil fertility. However, the difference of plant density due to inter-row spacing between years in the respective intra-row spacing plot is relatively minor (9.5 vs 8.8 plants  $m^{-2}$  in normal intra-row spacing and 19 vs 17.8 plants  $m^{-2}$  in narrow intra-row spacing) as compared to weather and soil conditions. Therefore, the difference of row width between years is seemingly negligible. The effect of high plant density by intra-row spacing on seed yield varied among combinations of year and cultivar, as evidenced by the significant year  $\times$  intra-row spacing  $\times$  cultivar interaction (Table 2): for all combinations of year and cultivar except



**Figure 3.** Relationships of the ratio of seed yield in narrow intra-row spacing to that in normal intra-row spacing with LAI at R5 (a) and lodging score (b) at harvest of normal intra-row spacing in two years. Pearson's correlation coefficient ( $r$ ) and Spearman's rank correlation coefficient ( $r_s$ ) were calculated with data combined across the two years ( $n = 18$ ).

for Okushirome in 2016, narrow intra-row spacing increased seed yield. Previous studies have reported genotypic differences in yield response to higher plant density and its determinants (e.g., Cober et al., 2005). Here, the year  $\times$  intra-row spacing  $\times$  cultivar interaction could not be explained fully by the response of any yield component except pods per node (Table 2). Multiple regression analysis conducted to determine the relative contributions of each yield component response to intra-row spacing to seed yield response showed that the variation in seed yield enhancement ratio due to narrow intra-row spacing among years and cultivars was determined in a complex manner, most strongly by node number, followed by pods per node (Figure 2). When all data were pooled, the results confirmed the good associations between seed yield and node number, between node number and  $\Delta$ AGB until R5, and between  $\Delta$ AGB and CumIR until R5 (Figure 1). Node number per m<sup>2</sup> was most closely associated with higher yield, as reported in a previous study of the relationship between yield and its components under a wide range of plant densities (Ball et al., 2001). Previous studies which evaluated the soybean yield response to plant density among diverse environments reported the contribution of CumIR until the mid-reproductive stage to yield determination (Edwards et al., 2005; Gaspar & Conley, 2015). The results here reveal that the yield increase at high density by narrow intra-row spacing was due mainly to the increase in node number, which in turn resulted from the increase in the  $\Delta$ AGB associated with the increase in CumIR during the vegetative to mid-reproductive stage (R5). This confirms the hypothesis that high plant density by narrow intra-row spacing increases seed yield by increasing  $\Delta$ AGB and CumIR. Although the appearance of main stem nodes stops soon after R2, the number of branch nodes continue to increase from R2 to R5 in determinate soybean cultivars (Board & Harville, 1993). Therefore, the node number is determined by stage R5 in determinate soybean. The increases of CumIR and  $\Delta$ AGB and the associated increased total number of nodes were important in enhancing soybean yield with narrow intra-row spacing in the Tohoku region.

Previous reports emphasized the importance of RUE in the determination of seed yield response to plant density (De Bruin & Pedersen, 2008; Kokubun, 1988). In contrast, the effects of intra-row spacing and of the intra-row spacing  $\times$  cultivar interaction on RUE were not detected here (Table 5).

One of the interesting findings here is related to the agronomic traits which determine the yield response to narrow intra-row spacing. The present study confirmed the negative associations of seed yield increase due to

narrow intra-row spacing with LAI at R5 at normal intra-row spacing and lodging score at normal intra-row spacing (Figure 3). This indicates that soybean cultivars such as Okushirome, with greater LAI and greater susceptibility to lodging, respond less to narrow intra-row spacing. The optimum LAI required to maximize seed yield in soybean depends strongly on cultivar and environment. The value for Okushirome during late flowering to pod filling ranged from 4.9 to 5.7 mm<sup>-2</sup> (Kokubun, 1988). A larger LAI than this appears to be not beneficial for CumIR and RUE of Okushirome in this study. Although there was no interaction between year and cultivar, Okushirome in 2016 showed a slightly increased lodging score of 1.4–1.7 at harvest (Table 3). It lodged severely after heavy rainfall (63.5 mm) on 17 August 2016 (Supplemental Figure 2), during the mid-reproductive stage. LAI is a function of the total water storage capacity of a canopy (Drewry et al., 2010). Therefore, a heavier canopy may partly explain the high lodging score, along with other complex factors. Severe lodging before maturity could disturb the canopy structure, decreasing the radiation interception and use efficiencies for yield formation (Saitou et al., 2012). Excessive LAI (>8.2) at R5 of Okushirome in 2016 might have resulted in a higher lodging score and thus less responsiveness to narrow intra-row spacing. These results suggest the importance of tolerance to lodging for greater yield enhancement at narrow intra-row spacing. Ample evidence from soybean breeders suggests that yield increases associated with year of release of new soybean cultivars are correlated with slight decreases in LAI and lodging score in Canada (Morrison et al., 2000) and China (Jin et al., 2010). Cober et al. (2005) showed that new soybean cultivars had better tolerance to plant population stress than older cultivars. Similar approaches with selection of cultivar with smaller LAI and higher tolerance to lodging might be needed if high planting density became popular in Japan. Since only three cultivars were tested here, further investigations of differences in yield response to high plant density among many accessions will be needed to guide breeding for high-yielding cultivars under high plant density.

Another interesting finding was an increase in the lowest pod height without an increase in lodging score in narrow intra-row spacing (Table 3). This increase will be beneficial by reducing mechanical harvest losses.

Yield responses to plant density vary among environments (e.g., Wells, 1991). However, the results here did not detect an effect of year (weather and soil fertility) or an interaction between year and intra-row spacing on seed yield (Table 2). The average seed yield (382 g m<sup>-2</sup>) here was similar to or greater than my group previously found in studies with

sowing at the normal date (384 g m<sup>-2</sup>, Kumagai, 2018; 300 g m<sup>-2</sup>; Kumagai & Takahashi, 2020). The 2016 and 2017 growing seasons had normal temperature and abundant solar radiation and precipitation. Therefore, the lack of a year × intra-row spacing interaction on seed yield was presumably due to the favorable conditions. Carciochi et al. (2019) evaluated 78 responses of yield to plant density in different regions of the USA and Canada and found that yield response depends on yield environment: low yield environments (<400 g m<sup>-2</sup>) required higher plant densities than high yield environments (>430 g m<sup>-2</sup>). Corassa et al. (2018) found similar results in 109 field trials in southern Brazil. Since my dataset is limited by the combination of 2 years and two plant density at one site, more comprehensive and sophisticated investigations of the effect of plant density on soybean under different management practices and environmental conditions are required in future research to develop a prescription for the optimal plant density (seeding rate) at both local and regional levels in Japan.

## 5. Conclusions

The two-year field experiment revealed a positive effect of narrow intra-row spacing on seed yield in all combinations between years (2016 and 2017) and cultivars (Ryuhou, Okushirome, and Fukuibuki) except Okushirome in 2016. This difference was due mainly to the response of total node number to narrow intra-row spacing, the increase in which was due to higher ΔAGB and CumLR, due in turn to greater canopy development during vegetative and reproductive stages. The lack of seed yield increase due to narrow intra-row spacing of Okushirome in 2016 presumably resulted from a larger LAI and a higher lodging score. These results suggest that high plant density by narrow intra-row spacing is an effective option for increasing soybean yields in the Tohoku region, although agronomic traits such as LAI and susceptibility to lodging should be considered.

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## Disclosure statement

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