

# Effect of early sowing on growth and yield of determinate and indeterminate soybean (*Glycine max* (L.) Merr.) cultivars in a cool region of northern Japan

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## Abstract

To increase Japanese soybean yield, both practical and genetic improvements are needed. Early sowing improved yields in the United States, where indeterminate cultivars may produce better yields than determinate cultivars, particularly with early sowing. However, the mechanism for the yield response is not fully understood. Furthermore, whether these results are valid for cool regions in northern Japan is unknown. This study tested the effects of early sowing on yield and its attributes in three years using a Japanese determinate cultivar (Ryuhou) and three indeterminate cultivars (Karikei-881 and Karikei-879, recombinant inbred lines from determinate  $\times$  indeterminate crosses; UA4910, a high-yield American cultivar). The effects of early sowing varied among the years. Early sowing significantly increased pod number and seed yield by increasing cumulative intercepted solar radiation (thus, growth during early reproductive stages) in years with high precipitation during mid-season (July and August). High precipitation potentially inhibited canopy development from vegetative to early reproductive stages more strongly in normally sown plants than in early sown plants. In contrast, no significant effect of early sowing was observed in a year with low precipitation during mid-season, when low precipitation during vegetative stage promoted canopy development in both sowing dates. Therefore, early sowing could escape from excess water stress and ameliorate canopy development and thereby seed yield in this region. There was no significant sowing date  $\times$  cultivar interaction for seed yield in any year; thus, the indeterminate cultivars did not benefit more from early sowing in northern Japan. UA4910 had greater seed yield than Ryuhou owing to its longer growth duration and greater aboveground biomass and pod number; Karikei-881, Karikei-879, and Ryuhou showed similar seed yields. Thus, indeterminate cultivars did not necessarily have higher yields in northern Japan. These results suggest that early sowing could be effective for increasing soybean yield in northern Japan.

**Key words:** Cumulative intercepted radiation, Seed yield, Soil moisture, Sowing date, Stem determinacy

## 1. Introduction

Soybean (*Glycine max* [L.] Merr.) is a major source of plant protein and oil, and a major contributor to the world's food supply. In the United States, soybean productivity has been increasing for many years. The mean soybean yield increased from 2.0 t ha<sup>-1</sup> to 3.0 t ha<sup>-1</sup> in the past 30 years (USDA-NASS, 2016). On the other hand, the yield in Japan is much lower and has changed little over time; the 30-year average (1982–2012) soybean 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). Thus, a strategy to boost Japanese soybean yield is needed based on both agronomic practices and genetics.

Optimization of sowing dates is the most important and least expensive agronomic practice that affects soybean yield (Robinson *et al.*, 2009). Some researchers have suggested that earlier sowing dates contributed to recent soybean yield gains in the United States. For example, sowing soybeans in late April and early May is currently recommended in the Midwestern

United States to achieve the maximum seed yield (Bastidas *et al.*, 2008; De Bruin and Pedersen, 2008; Robinson *et al.*, 2009). Sowing before the optimal date is restricted mainly by cool soil temperatures (Hu and Wiatrak, 2012). Cool and wet soils during early sowing may delay germination and seedling emergence (Andales *et al.*, 2000) and may reduce canopy development, thereby decreasing seed yield (Kane *et al.*, 1997). Moreover, the effects of sowing date on soybean yield depend on meteorological conditions and location, especially in terms of the amount and pattern of precipitation (Egli and Cornelius, 2009). Because the beneficial effects of early sowing could be negated under suboptimal soil water conditions (Heatherly and Elmore, 1983; Kane *et al.*, 1997; Robinson *et al.*, 2009), efforts to determine the optimal sowing date should account for regional variations in the amount and pattern of precipitation.

In Tohoku, a cool region of northern Japan (from 36 to 43°N) at about the same latitude as the Midwestern United States (from 36 to 49°N), the currently recommended sowing date for soybeans is late May to early June. In this region, rice transplant monoculture is the dominant crop system, and the optimal transplanting time for paddy rice is mid-May to late May. If the optimal soybean sowing date is earlier than the current recommendation, soybean sowing and rice transplanting would overlap. Therefore, early sowing of soybeans would be difficult

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for farmers. In addition, the spring is cooler in this region than in the Midwestern United States: May and June temperatures are 2 to 5°C lower in Tohoku (Supplemental Table S1). Furthermore, there is a risk that earlier-sown soybean may be exposed to extremely low temperatures and late-spring frosts, which can also inhibit germination and emergence and early canopy development. The complexity of these climatic factors has prevented researchers from verifying the effect of early sowing on soybean yield, and it remains unclear how much yield could be improved by earlier sowing.

Waterlogging is one of the major problems in the production of soybean in converted paddy fields, which sometimes have poor drainage and a high-water table in Japan (Bajgain *et al.*, 2015). This circumstance is common in East Asia (Kokubun, 2013). The rainy season lasts from June to July in northern Japan, so waterlogging occurs during the early vegetative stage. Excess soil water during the vegetative stage inhibits root growth, and soybean with poor root systems suffers from water deficits in the summer (Hirasawa *et al.*, 1998). Since soybean production in northern Japan depends on the amount and pattern of precipitation, which vary between years and locations, the effect of early sowing on seed yield will also depend on these precipitation characteristics.

In addition to climatic factors, genetic factors affect soybean growth and yield. Stem determinacy is an important trait that affects seed yield. Soybean cultivars can be classified into indeterminate, semi-determinate, or determinate, depending on how strongly their growth responds to environmental cues. Stem growth and leaf formation and expansion in indeterminate accessions continue for a long period after flowering. In determinate accessions, growth of the stem node ends when flowering begins or soon afterward. Most Japanese cultivars are determinate, but indeterminate cultivars are the dominant form in the Midwestern United States. Several American researchers have reported the effects of stem growth habit on soybean seed yield; some reported a higher seed yield for indeterminate cultivars (Parvez *et al.*, 1989; Robinson and Wilcox, 1998), whereas others have found a higher yield for determinate cultivars (Weaver *et al.*, 1991). The yield components of these growth types also respond differently to sowing date. Beaver and Johnson (1981) reported that seed yield of indeterminate cultivars in Illinois increased steadily with an increasing shift from early-June sowing to early-May sowing, whereas the yield of determinate cultivars did not. Wilcox and Frankenberger (1987) compared the two types using three to five sowing dates from early May to late June in Indiana. They reported that determinate cultivars had the highest yield when sown in late May or early June, whereas indeterminate cultivars produced their maximum yield when sown from early to late May. Robinson and Wilcox (1998) also reported that the magnitude of the yield increase caused by shifting from July to May sowing was greater in indeterminate lines than determinate lines. Although a superior yield increase by indeterminate cultivars in response to early sowing was reported in the United States, to my knowledge, there has been no attempt to clarify the underlying mechanisms that determine these responses. Furthermore, there have been no studies that compared determinate and indetermi-

nate cultivars in cool regions of northern Japan.

Several researchers recently suggested that genetic improvement has contributed to the rapid soybean yield gain in the United States (Koester *et al.*, 2014; Rincker *et al.*, 2014; Rowntree *et al.*, 2014; Specht *et al.*, 2014). More recently, Kawasaki *et al.* (2016) reported that American cultivars had better seed yield than Japanese cultivars in a warm region of Japan. Matsuo *et al.* (2016) also compared the yields with both early (late May) and normal (late June) sowing between American and Japanese cultivars in a warm region of southwestern Japan, and found a greater yield increase with early sowing in the American cultivars.

Based on this review of the literature, I hypothesized that in the cool climate of northern Japan, early sowing would increase soybean yield, and that indeterminate soybean cultivars would have higher seed yield and a greater yield increase than determinate cultivars with early sowing. To test these hypotheses, I compared the changes in yield and yield components between determinate and indeterminate cultivars with early sowing (mid-May) and normal sowing (late May) in three consecutive years. Although the yield responses to early planting were well documented in previous studies, those studies provided limited information about the plant growth parameters responsible for these responses. The present experiments were conducted to quantify the increases in crop growth and yield, cumulative intercepted radiation, and radiation-use efficiency in determinate and indeterminate cultivars sown earlier than normal, which the literature suggested would increase soybean yield.

## 2. Materials and Methods

The experiments were conducted at the NARO Tohoku Agricultural Research Center in Morioka, Iwate Prefecture, Japan (39°44'N, 141°7'E), in 2013, 2014, and 2015. Different converted paddy fields were used in each of the three years. Supplemental Table S2 summarizes the soil characteristics and cultivation conditions. The soil in each field was an Andosol. Each field received 200 g m<sup>-2</sup> of fused phosphate fertilizer, and 100 g m<sup>-2</sup> of magnesium lime approximately 1 month before sowing in each year. In addition, the fields received 3 g m<sup>-2</sup> of N, 12.5 g m<sup>-2</sup> of P (P<sub>2</sub>O<sub>5</sub> equivalent), and 5 g m<sup>-2</sup> of K (K<sub>2</sub>O equivalent) in the form of a compound fertilizer 1 day before sowing in each year. Fertilizers were applied and incorporated to a depth of approximately 15 cm using rotary tillers.

In 2013, I grew two cultivars: a determinate Japanese cultivar (Ryuhou) and an indeterminate line (Karikei-881). In 2014, I grew four cultivars: Ryuhou and three indeterminate cultivars (Karikei-881 and Karikei-879 from Japan, and UA4910 from the United States). In 2015, I grew three cultivars: Ryuhou, Karikei-879, and UA4910. Ryuhou is a leading cultivar from maturity group (MG) IV in the Tohoku region (Kumagai and Sameshima, 2014). Karikei-881 and Karikei-879 are recombinant inbred lines derived from crosses between the determinate Japanese cultivar Ohsuzu and the indeterminate American cultivar Athow (Kato *et al.*, 2015). Karikei-881 and Karikei-879 are late- and early-maturing lines, respectively, and the latter's growth duration is similar to that of Ryuhou. UA4910 is an indeterminate American cultivar from MG IV, with a high yield

(Chen *et al.*, 2011). It had greater seed yield than commonly grown Japanese cultivars in southwestern Japan (Matsuo *et al.*, 2016).

These cultivars were grown to maturity (September–October). Seeds were treated with a combined insecticide and fungicide (CruiserMaxx, Syngenta Co., Tokyo, Japan) at the dosage recommended by the manufacturer and then sown by hand at a density of three seeds per hill in mid-May (the early-sowing [ES] treatment) and late May (the normal-sowing [NS] treatment), with a difference of 14 to 15 days between the two dates (Supplemental Table S2). The sowing density was 9.5 hills (plants) m<sup>-2</sup>, at 0.15 m between hills × 0.70 m between rows, in 2013 and 2015, and 8.9 hills m<sup>-2</sup>, at 0.15 m between hills × 0.75 m between rows, in 2014. This corresponds to the recommended local planting densities, which range from 7 to 15 hills m<sup>-2</sup>. Plants were thinned to one per hill after establishment. A pre-emergence herbicide (Ecotop, Maruwa Biochemical Co., Tokyo, Japan), which contained 1.0% Dimethenamide-p and 1.4% linuron, was applied just after sowing, at the dosage recommended by the manufacturer, for weed control.

Plants were not irrigated, and relied entirely on precipitation for their water. A month after sowing, tilling was performed between the rows to a depth of 15 cm, and ridging was performed to control weeds and lodging. The pesticides and insecticides were reapplied to maximize the yield and seed quality. In each year, the experiment design was a split-plot arrangement; the main plot was sowing date (NS and ES) and the subplot was cultivar, with three replicates. The subplot consisted of 7 rows × 4 m long in 2013, 7 rows × 3 m long in 2014, and 6 rows × 3.3 m long in 2015.

For both NS and ES, I monitored the phenology of 10 plants in the center row of each plot at intervals of 2 or 3 days and recorded the dates of each growth stage based on the staging system of Fehr and Caviness (1977): VE, emergence; R1, the beginning of flowering; R2, full flowering; R5, the beginning of seed filling; and R7, the beginning of maturity.

I determined dry weight of aboveground parts per m<sup>2</sup> by means of periodic sampling two or three times during the growing stage of each year. Aboveground parts of center three rows (0.63 m<sup>2</sup> and 0.68 m<sup>2</sup> in 2013 and 2014, respectively) or center four rows (0.84 m<sup>2</sup> in 2015) were harvested. The dry weight of aboveground part was measured after oven-drying at 80°C for 3 days. Sampling dates (day of year, DOY) are listed in Supplemental Table S3. In 2013, the sampling dates were 4 July (late vegetative stage), 24 July (0 to 20 days after R1; this number depended on the sowing date and cultivar), and 22 August (0 to 15 days after R5; this number depended on the sowing date and cultivar). In 2014, sampling was performed at R1 and 30 days after R1. In 2015, sampling was performed at R2 and the beginning of seed filling (R5).

During vegetative stage (from VE to 4 July, R1 and R2 in 2013, 2014 and 2015, respectively), the crop growth rate per unit area (CGR; g m<sup>-2</sup> day<sup>-1</sup>) was calculated as the aboveground dry weights on the first sampling date, divided by the number of days from VE to the first sampling. Because plants were not sampled at VE, the aboveground dry weights at VE were assumed to be zero. During reproductive stage (from 4 July to 22 August, from

R1 to 30 days after R1, from R2 to R5 in 2013, 2014 and 2015, respectively), in 2013, the CGR was determined from the slope of the linear regression of aboveground dry weight against DOY from three sampling dates in two cultivars. In 2014 and 2015, CGR was calculated as the difference between the aboveground dry weights on the two sampling dates, divided by the number of days between the two measurements.

The fraction of intercepted solar radiation ( $F$ ) was estimated by measuring solar radiation below the canopy ( $I_o$ ) and incident radiation above the canopy ( $I$ ) using integrated solarimeter films (color acetate films; Opto Leaf Y1-W; Taisei Chemical Industries, Tokyo, Japan). In 2013, the intercepted solar radiation was calculated as follows (Shiraiwa *et al.*, 2011):

$$F = 1 - (I_o / I) \quad (1)$$

$I_o$  and  $I$  were estimated by installing several 1-m-long bars holding seven squares of film (each 2 cm × 1.5 cm at 15-cm intervals), between the rows, below and above the canopy. The color acetate films faded as they absorbed solar radiation, and the degree of fading of each film was determined using a portable photometer (D-Meter RYO-470, Taisei Chemical Industries, Ltd., Tokyo, Japan). This degree was assumed to be negatively and nonlinearly correlated with the amount of solar radiation following an empirical equation derived by the manufacturer. The measurements were collected for 5 to 7 days, and  $I_o$  and  $I$  were subsequently determined. These measurements were conducted repeatedly between the first and the last sampling dates.

In 2014 and 2015, I estimated the intercepted solar radiation by using digital imaging techniques (GACS1; Kimura Ouyou-Kougei Co. Ltd., Saitama, Japan). The fractional canopy cover ( $C_f$ ) was determined by using digital images taken above the canopy at 1-week intervals from the early vegetative to late reproductive stages. In each year, total daily solar radiation (MJ m<sup>-2</sup> day<sup>-1</sup>) was recorded at a weather station near the study field, and daily incident radiation above the canopy (DIR) was computed as the product of daily solar radiation and  $C_f$ . Cumulative intercepted radiation (CumIR) from VE to the last sampling was computed by summing the DIR values. The mean radiation-use efficiency (RUE) for biomass production was determined from two or three samples as the slope of the linear regression between the dry weight of the aboveground parts and CumIR (Monteith, 1994). Although I used two different methods to estimate intercepted solar radiation, a previous study of soybean showed a significant correlation in the estimated values between the methods (Shiraiwa *et al.*, 2011).

In 2014 and 2015, I sampled soil between the plants to a depth of 30 cm in each plot using a hand soil sampler (30 cm in length and 5 cm in inner diameter; HS-30S; Fujiwara Scientific, Co., Ltd., Tokyo, Japan) at the final sampling dates to determine the root length density (RLD; cm cm<sup>-3</sup>). I divided the samples into depths of 0 to 15 cm and 15 to 30 cm, and then carefully removed the soil from the root samples with tap water. I then completely removed the root nodules before measuring RLD with a WinRHIZO system (Regent Instruments, Montreal, PQ, Canada).

At maturity, I manually harvested the aboveground parts from

3.36 m<sup>2</sup> of each plot in 2013, 3.38 m<sup>2</sup> in 2014, and 4.20 m<sup>2</sup> in 2015. After completely air-drying the plants, I removed the few remaining leaves and petioles from the aboveground parts, and then weighed the remaining parts. Aboveground biomass equaled the sum of the weights of the stems, pod shells, and seeds. Filled pods and the number of mature seeds were counted and the mature seeds were weighed. Seed yield per m<sup>2</sup> was adjusted to a 15% moisture content, and the 100-seed weight was determined as (the seed yield / the number of seeds) × 100.

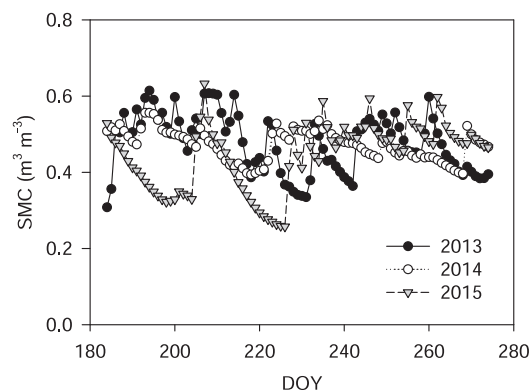
In addition to solar radiation, daily means of temperature and precipitation during the growing season were recorded at a weather station near the field. The soil volumetric moisture content (SMC, m m<sup>-3</sup>) was monitored with soil moisture sensors (EC5; Decagon Devices Inc., Pullman, WA, USA) at 7.5 cm below the soil surface (the midpoint of the plow layer) of the NS and ES plots. SMC was measured and recorded every 30 min using a datalogger (CR10X; Campbell Scientific Inc., Logan, UT, USA).

To test for significant differences between the two sowing dates and between the cultivars in each year, and for significant date × cultivar interactions, I used two-way ANOVA performed by the General Linear Model procedure of SPSS 22.0 for Windows (IBM, Tokyo, Japan). Sowing date, cultivar, and their interaction were each considered as fixed effects, and replication (block) was considered as a random effect. When the ANOVA produced a significant result, Fisher's LSD test for significant differences between means was performed. For  $C_f$ , I used Student's *t*-test to detect a significant difference between the two sowing dates in each cultivar on a given date (DOY) in 2014 and 2015. I performed a curvilinear regression for the relationship between mean precipitation and mean  $C_f$  during the vegetative stage.

### 3. Results

#### 3.1 Weather conditions and SMC

Table 1 summarizes the weather conditions during the three growing seasons. The 6-month (May to October) mean temperature in the three years was 0.6 to 0.9°C higher than the 30-year mean. The large difference was observed in June 2013 and 2014 (1.8 and 2.4°C, respectively, above the mean). In 2015,



**Fig. 1.** Changes in the soil volumetric moisture content (SMC) from July to September during the growing season in the three years of the study. DOY, day of year.

mean temperatures in May and July were 2.5 and 2.1°C higher than the 30-year mean. The 6-month mean daily solar radiation in 2013 was similar to the 30-year mean, but those in 2014 and 2015 were 1.7 and 2.0 MJ m<sup>-2</sup> d<sup>-1</sup> higher than the mean. The 6-month mean precipitation in 2014 was close to the 30-year mean (143.8 vs. 139.4 mm, respectively), but 2013 was wetter than normal (by 70.4 mm). 2013 and 2014 had higher-than-normal mid-season (July to August) precipitation, whereas 2015 had lower-than-normal one. In 2015, monthly total precipitation in July and August was 87.9 and 36.4 mm lower than the 30-year mean, respectively.

Soil moisture contents (SMC) in 2013 and 2014 were high and stable throughout the growing season, whereas that in 2015 decreased to low levels during July and mid-August (Fig. 1).

#### 3.2 Growth duration, aboveground biomass, seed yield, pod number and 100-seed weight

In all three years, early sowing significantly extended days from VE to R1 and R1 to R7 (by 3 to 5 days in both parameters) when averaged across the cultivars ( $P < 0.001$ ; Table 2). These parameters differed significantly among the cultivars: Days from VE to R1 were longest in Karikei-881, followed by Ryuhou, UA4910 and Karikei-879. Days from R1 to R7 of indeterminate

**Table 1.** Monthly mean air temperature, solar radiation, and total precipitation during the 2013, 2014, and 2015 growing seasons, and comparison with the 30-year mean values.

	Year	May	June	July	Aug.	Sept.	Oct.	Mean
Temperature (°C)	2013	13.1	19.6	21.8	23.5	19.1	13.3	18.4
	2014	14.9	20.2	22.7	22.7	17.1	11.1	18.1
	2015	15.9	18.5	23.4	22.7	17.9	10.8	18.2
	30-year mean	13.4	17.8	21.3	22.9	18.2	11.5	17.5
Solar radiation (MJ m <sup>-2</sup> d <sup>-1</sup> )	2013	17.2	20.4	12.0	16.6	12.2	8.3	14.5
	2014	20.4	18.6	18.4	12.2	15.3	11.6	16.1
	2015	21.3	19.2	17.8	15.6	13.2	11.1	16.4
	30-year mean	17.4	17.1	15.1	15.1	11.9	9.9	14.4
Precipitation (mm)	2013	49.5	37.5	503.5	202.5	210.0	255.5	209.8
	2014	69.0	71.5	261.5	236.0	95.0	130.0	143.8
	2015	87.0	123.5	108.5	145.5	104.5	124.0	115.5
	30-year mean	101.4	108.1	196.4	181.9	155.9	92.5	139.4

**Table 2.** Days from emergence (VE) to beginning flowering (R1) and from R1 to beginning maturity (R7), aboveground biomass, seed yield and yield components in the soybean cultivars with normal and early sowing dates (NS and ES, respectively) in 2013, 2014, and 2015.

Year	Sowing date	Cultivar	Days from VE to R1 (days)	Days from R1 to R7 (days)	Aboveground biomass (g m <sup>-2</sup> )	Seed yield (g m <sup>-2</sup> )	Pod number (m <sup>-2</sup> )	100-seed weight (g)
2013	NS		52	69	781	356	775	33.3
		ES	57	73	899	414	862	33.6
	ANOVA	Ryuhou	52	63	755	392	809	33.2
		Karikei-881	56	78	925	378	828	33.7
		Sowing date (S)	***	***	*	*	*	ns
		Cultivar (C)	***	***	***	ns	ns	ns
		S × C	**	ns	ns	ns	ns	ns
2014	NS		44	78	730	398	827	26.8
	ES		48	82	876	470	908	28.5
		Ryuhou	47 b	71 d	665 c	394 b	687 c	34.7 a
		Karikei-881	52 a	83 b	862 ab	391 b	780 bc	31.4 b
		Karikei-879	41 c	77 c	760 bc	435 b	843 ab	25.4 c
	ANOVA	UA4910	44 d	89 a	928 a	517 a	1158 a	19.0 d
		S	***	***	**	**	*	***
		C	***	***	**	**	***	***
		S × C	***	***	ns	ns	ns	ns
		2015	NS		49	71	689	398
ES			52	76	710	401	813	26.8
	Ryuhou		54 a	66 c	649 b	387 b	668 a	34.9 a
	Karikei-879		48 b	73 b	645 b	371 b	743 b	26.5 b
	UA4910		50 c	83 a	804 a	439 a	993 b	19.6 c
ANOVA	S		***	***	ns	ns	ns	ns
	C		**	***	***	*	***	***
	S × C		*	***	†	ns	ns	ns

Values of a parameter followed by the same letter did not differ significantly between cultivars (ANOVA followed by Fisher's LSD test,  $P < 0.05$ ). ANOVA results: \*\*\* $P < 0.001$ ; \*\* $P < 0.01$ ; \* $P < 0.05$ ; † $P < 0.10$ ; ns, not significant.

cultivars were longer than that of Ryuhou. ANOVA showed no significant sowing date × cultivar interaction for aboveground biomass, seed yield, pod number or 100-seed weight in all three years (Table 2). The effect of early sowing on aboveground biomass, seed yield and pod number differed among the years. When averaged across the cultivars in 2013 and 2014, early sowing significantly increased aboveground biomass (by 15% and 20%, respectively), seed yield (by 16% and 18%, respectively) and pod number (by 11% and 10%, respectively). In 2015, in contrast, sowing date had no significant effect on these parameters. ANOVA showed a significant cultivar effect on aboveground biomass in 2013: Karikei-881 had 23% higher aboveground biomass than Ryuhou. The 2014 and 2015 experiments both showed significant cultivar effects on aboveground biomass, seed yield and pod number: When averaged between the two sowing dates in 2014, aboveground biomass was greatest in UA4910, followed by Karikei-881, Karikei-879, and Ryuhou. Moreover, UA4910 showed significant 31% greater seed yield and 68% greater pod number than Ryuhou. In 2015, aboveground biomass, seed yield and pod number in UA4910 were significantly higher than that in Ryuhou. Averaged across the cultivars, the 100-seed weight was

6% greater in ES than in NS in 2014; there was no significant difference between sowing dates in the other years. In 2014 and 2015, there were significant cultivar effects on the 100-seed weight: that was greatest in Ryuhou, followed by Karikei-879 and UA4910.

### 3.3 $C_f$ , CGR, RUE, and CumIR

$C_f$  was significantly higher in ES than in NS for most of the growing period (DOY 170 to 220) in all cultivars in 2014 (Supplemental Fig. S1). In 2015,  $C_f$  in ES plants were also higher until around DOY 200 in all three cultivars but there was no difference between two sowing dates during the most of reproductive stage after R1. This reason was that NS plants showed more rapid increase of  $C_f$  during early stage in 2015 than in 2014.

Tables 3 and 4 summarizes the growth and light-use parameters. As shown in Table 3, in 2013 and 2014, early sowing significantly increased CGR during vegetative stage (by 50% and 31%, respectively) when averaged across the cultivars. Early sowing also increased significantly CumIR and mean  $C_f$  during early stage by 31% and 14%, respectively in 2014. In 2015, in contrast, early sowing did not increase these parameters, and

**Table 3.** Crop growth rate (CGR), radiation-use efficiency for dry matter production (RUE), cumulative intercepted irradiation (CumIR) and mean fraction of canopy cover (mean  $C_f$ ) during vegetative stage of the soybean cultivars with normal and early sowing dates (NS and ES, respectively) in 2013, 2014, and 2015.

Year	Sowing date	Cultivar	CGR ( $\text{g m}^{-2} \text{d}^{-1}$ )	RUE ( $\text{g MJ}^{-1}$ )	CumIR ( $\text{MJ m}^{-2}$ )	Mean $C_f$ (%)
2013 <sup>a</sup>	NS		0.91	-	-	-
		ES	1.37	-	-	-
	ANOVA	Ryuhou	1.18	-	-	-
		Karikei-881	1.11	-	-	-
		Sowing date (S)	*	-	-	-
		Cultivar (C)	ns	-	-	-
	S × C	ns	-	-	-	
2014 <sup>b</sup>	NS		1.56	0.63	114	14
	ES		2.05	0.63	150	16
		Ryuhou	2.03 b	0.62 b	155 b	17 b
		Karikei-881	2.96 a	0.77 a	201 a	21 a
		Karikei-879	1.21 c	0.58 b	91 c	11 c
		UA4910	1.01 c	0.55 b	81 c	10 d
	ANOVA	S	*	ns	*	*
		C	***	**	***	***
		S × C	ns	ns	***	***
	2015 <sup>c</sup>	NS		2.55	0.64	195
ES			2.00	0.52	208	19
		Ryuhou	3.01 a	0.65	255 a	25 a
		Karikei-879	2.14 b	0.57	184 b	19 b
		UA4910	1.66 c	0.52	165 c	17 c
ANOVA		S	†	ns	†	*
		C	**	ns	***	***
		S × C	ns	ns	ns	ns

<sup>a</sup> CGR, RUE and Mean  $C_f$  were determined on two sampling dates (VE and day of year [DOY] 187). CumIR was determined from VE to DOY 187.

<sup>b</sup> CGR, RUE and Mean  $C_f$  were determined on two sampling dates (VE and R1). CumIR was determined from VE to R1.

<sup>c</sup> CGR, RUE and Mean  $C_f$  were determined on two sampling dates (VE and R2). CumIR was determined from VE to R2.

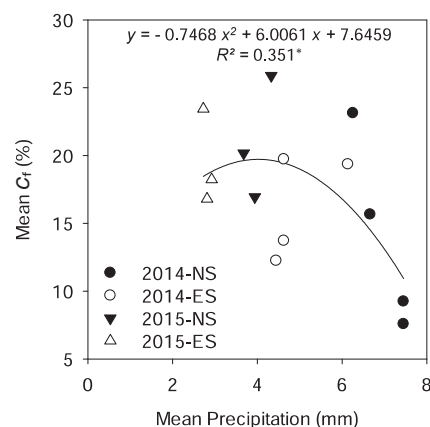
Values followed by the same letter did not differ significantly between cultivars (ANOVA followed by Fisher's LSD test,  $P < 0.05$ ). ANOVA results: \*\*\* $P < 0.001$ ; \*\* $P < 0.01$ ; \* $P < 0.05$ ; † $P < 0.10$ ; ns, not significant. -, not determined.

these values were higher in 2015 than in 2014. Significant effects of sowing date on RUE were not observed in 2014 and 2015. Significant effects of cultivar on CGR, CumIR and mean  $C_f$  was observed in 2014 and 2015.

The 2013 and 2014 experiments both showed that early sowing significantly increased CGR (by 23% in 2013 and 18% in 2014), CumIR (by 8% in 2013 and 26% in 2014) and mean  $C_f$  (by 10% in 2013 and 13% in 2014) during reproductive stage when averaged across the cultivars (Table 4). In 2015, there were no significant effects of sowing date on CGR and CumIR. Mean  $C_f$  was significantly higher in NS plants than in ES plants. The effect of sowing date on RUE differed between the years: ES plants showed greater RUE than NS plants in 2013, but there was no significant difference between ES and NS in 2014 or 2015. A significant effect of cultivar on CGR and RUE was observed in 2013 and 2014: However, there were no significant differences in CGR or RUE between the cultivars in 2015. CumIR differed significantly among the cultivars in 2014 and 2015.

When data were combined across all cultivars grown under two sowing dates in 2014 and 2015, a relationship between mean precipitation and mean  $C_f$  during the vegetative stage was

curvilinear with a significant regression coefficient ( $R^2 = 0.351$ ,  $P < 0.05$ , Fig.2).



**Fig. 2.** A curvilinear regression for the relationship between the mean precipitation from emergence to the first sampling and the mean  $C_f$  during the stage for all cultivars combined between 2014 and 2015. All data were combined. \* $P < 0.05$ .

**Table 4.** Crop growth rate (CGR), radiation-use efficiency for dry matter production (RUE), cumulative intercepted irradiation (CumIR) and mean fraction of canopy cover (mean  $C_f$ ) during reproductive stage of the soybean cultivars with normal and early sowing dates (NS and ES, respectively) in 2013, 2014, and 2015.

Year	Sowing date	Cultivar	CGR ( $\text{g m}^{-2} \text{d}^{-1}$ )	RUE ( $\text{g MJ}^{-1}$ )	CumIR ( $\text{MJ m}^{-2}$ )	Mean $C_f$ (%)	
2013 <sup>a</sup>	NS		10.1	1.13	436	61	
			12.4	1.28	470	67	
	ES	Ryuhou	12.0	1.28	446	65	
		Karikei-881	10.5	1.12	460	64	
		ANOVA	Sowing date (S)	**	*	***	*
			Cultivar (C)	*	*	ns	ns
	S × C	ns	ns	***	**		
2014 <sup>b</sup>	NS		9.6	0.85	336	79	
			11.3	0.79	423	89	
	ES	Ryuhou	10.9 b	0.83 b	396 b	88 b	
		Karikei-881	12.0 a	0.92 a	405 a	96 a	
		Karikei-879	10.0 b	0.80 b	382 b	78 c	
		UA4910	8.7 c	0.73 b	335 c	72 d	
	ANOVA	S	*	ns	***	*	
		C	**	*	***	***	
S × C		*	ns	***	***		
2015 <sup>c</sup>	NS		19.5	1.15	479	95	
			18.9	1.17	494	93	
	ES	Ryuhou	21.9	1.23	368 b	95 a	
		Karikei-879	18.6	1.16	542 a	95 a	
		UA4910	17.1	1.10	564 a	93 b	
		ANOVA	S	ns	ns	ns	*
		C	†	ns	***	*	
		S × C	ns	†	ns	ns	

<sup>a</sup> CGR, RUE and Mean  $C_f$  (=  $F$ ) were determined on three sampling dates (day of year [DOY] 187, 207, and 235). CumIR was determined from DOY 187 to DOY 235.

<sup>b</sup> CGR, RUE and Mean  $C_f$  were determined on two sampling dates (R1 and R1 + 30 days). CumIR was determined from R1 to R1 + 30 days.

<sup>c</sup> CGR, RUE and Mean  $C_f$  were determined on two sampling dates (R2 and R5). CumIR was determined from R2 to R5.

Values followed by the same letter did not differ significantly between cultivars (ANOVA followed by Fisher's LSD test,  $P < 0.05$ ). ANOVA results: \*\*\* $P < 0.001$ ; \*\* $P < 0.01$ ; \* $P < 0.05$ ; † $P < 0.10$ ; ns, not significant.

### 3.4 RLD

Table 5 summarizes the RLD results in 2014 and 2015. Early sowing significantly increased RLD at a depth of 0 to 15 cm in 2014 but not in 2015. RLD at a depth of 15 to 30 cm, averaged across the cultivars, was significantly higher in NS than in ES in 2015, but there was no difference between the sowing dates in 2014. The 2014 experiment showed marginally significant and significant effects of cultivar on RLD at depths of 0 to 15 cm and 15 to 30 cm, respectively. In 2014, Karikei-881 tended to produce greater RLD than the other cultivars in both depth ranges and for both sowing dates. In 2015, there was no significant difference in RLD between the cultivars in the upper soil depth and only a marginally significant effect on RLD in the lower soil depth. RLD of UA4910 and Karikei-879 tended to be higher than that in Ryuhou. RLD of the NS plants at both soil depths was higher in 2015 than in 2014, presumably because of the lower SMC in 2015.

## 4. Discussion

The objective of this study was to test the hypotheses that early sowing would increase soybean yield and that the indeterminate

cultivars would show greater yield and a greater yield response to early sowing in the cool climate of northern Japan. The first hypothesis was partially validated, but the second hypothesis was rejected.

### 4.1 Early sowing ameliorated seed yield by increasing $C_f$ , CumIR and CGR from vegetative to reproductive stage in years with high precipitation, but not in a year with low precipitation

Some researchers previously reported that the beneficial effects of early sowing on soybean yield were affected by precipitation (Heatherly and Elmore, 1983, Kane *et al.*, 1997, Robinson *et al.*, 2009). The present study provides the first data on the causes of the yield gain due to early sowing and differences in yield responses between years in terms of CumIR and RUE and root growth. The effect of early sowing on yield varied substantially among the three years: When averaged across the cultivars, early sowing significantly increased seed yield in 2013 and 2014 with high precipitation during mid-season (July and August) but not in 2015 with low precipitation (Table 2). This discrepancy resulted from the

**Table 5.** Root length density (RLD) at depths of 0 to 15 cm and 15 to 30 cm for the soybean cultivars with normal and early sowing dates (NS and ES, respectively) in 2014 and 2015.

Year	Sowing date	Cultivar	RLD (cm cm <sup>-3</sup> )	
			0–15 cm	15–30 cm
2014	NS		3.89	2.01
		ES	4.55	2.29
	ANOVA	Ryuhou	4.44	1.77 b
		Karikei-881	4.88	3.02 a
		Karikei-879	3.81	2.12 b
		UA4910	3.76	1.68 b
		Sowing date (S)	*	ns
		Cultivar (C)	†	*
	S × C	ns	ns	
	2015	NS		4.72
ES			5.12	1.99
ANOVA		Ryuhou	4.82	1.89
		Karikei-879	5.00	2.64
		UA4910	4.93	3.22
		S	ns	*
		C	ns	†
		S × C	ns	ns

Values followed by the same letter did not differ significantly between cultivars (ANOVA followed by Fisher's LSD test,  $P < 0.05$ ). ANOVA results: \* $P < 0.05$ ; † $P < 0.10$ ; ns, not significant.

difference in CGR, CumIR and  $C_f$  from VE to the early reproductive stage (Tables 3 and 4) that might be associated with the differences in precipitation (Table 1) and in SMC (Fig. 1). In general, seedling establishment and early canopy development were important to achieve high yield of soybean. It is widely accepted that early leaf expansion and canopy development are both inhibited strongly by excess water stress (e.g., Bajgain *et al.*, 2015). The present study showed that the curvilinear relationship existed between mean  $C_f$  and mean precipitation during vegetative stage when combined across two sowing dates in 2014 and 2015 (Fig. 2): In 2014, NS plants (especially Karikei-879 and UA4910) showed lower  $C_f$  under higher precipitation condition than ES plants. In contrast, both NS and ES plants showed higher mean  $C_f$  under low precipitation during vegetative stage in 2015. The differences of  $C_f$  between two sowing dates were also observed during early reproductive stage in the two years. These results raised the possibility that early sowing would escape from the excess water stress and ameliorate canopy development and thereby yield in years with higher-than normal precipitation of this region.

Generally, CGR, CumIR and RUE during reproductive stage are significantly positively correlated with soybean yield (e.g., Kawasaki *et al.*, 2016). In the present study, CGR, CumIR and RUE were measured from the different stages in each year (from late vegetative stage to around R5 in 2013, from R1 to 30 days after R1 in 2014, and from R2 to R5 in 2015, respectively). Although CGR cannot be accurately compared among the years in this study because the values were strongly depended on the growth stages (e.g., Van Roekel and Purcell, 2014), this value was higher in 2015 than in 2013 and 2014 (Table 4). The increases of CGR and CumIR caused by early sowing appear to be the main contributors to the increased yield in the two years. On the other hand, low precipitation and SMC promoted more

strongly  $C_f$ , CumIR and CGR in NS than in ES in 2015. These differences of parameters between sowing dates were probably owing to those in root development as discussed later. Thus, early sowing did not increase mean  $C_f$ , CumIR, CGR and thereby aboveground biomass and seed yield in 2015 (Tables 2, 4).

It was reported that the longer growth duration was involved with yield increase (Rowntree *et al.*, 2014; Matsuo *et al.*, 2016). The present study revealed that early sowing extended the periods from VE to R1 and R1 to R7 regardless of the cultivars in the three years (Table 2), but the longer growth duration by early sowing did not always resulted in yield increase.

Robinson *et al.* (2009) reported that the difference in yield between sowing dates was strongly explained by pod number per unit area. Dry matter production during the early reproductive stage is essential for determining the pod and seed number in soybean (Egli, 2010; Shiraiwa *et al.*, 2004). The present results confirm that the increased pod number due to early sowing was most likely derived from the increased CGR, which in turn resulted from the increased CumIR from the flowering stage (or late vegetative stage) to the middle reproductive stages. The 100-seed weight was significantly increased by early sowing in 2014 (Table 2). However, the increase of the 100-seed weight was not consistent throughout the experiments. Several researchers reported no effect of sowing date on the 100-seed weight (Bastidas *et al.*, 2008; De Bruin and Pedersen, 2008; Rowntree *et al.*, 2014), which agrees well with the present results.

Early sowing significantly increased RLD in the upper soil in 2014, when there was excess soil water (Table 5). This agrees with the result of Turman *et al.* (1995), who found that soybean sown in mid-May in Missouri had a larger root system 30 days after emergence than plants sown in mid-June. In contrast, in 2015 with a low SMC, NS plants had significantly higher RLD



than ES plants in the deep soil layer (3.18 vs 1.99 cm cm<sup>-3</sup>, respectively), although there was no difference in RLD between the two dates in the upper soil. Water deficiency stimulates root growth of soybean (Hoogenboom *et al.*, 1987). In Japan, soybean grown under conditions with less-than-normal SMCs before flowering had a larger RLD (Hirasawa *et al.*, 1998). This agrees with the present result: the RLD in 2015 was tended to be higher than that in 2014 for both sowing dates and both depths. ES plants in 2015 gradually restricted their canopy development due to low SMC and RLD, whereas NS plants could absorb water from the deeper soil and develop their canopy better. Although I did not explore possible causes for the difference in RLD in the deeper soil between the two dates, one possible explanation was the effect of the timing of water deficiency on root development. Hoogenboom *et al.* (1987) observed a large increase in root growth when water deficits occurred during later stages of vegetative growth or early stages of reproductive development (from R1 to R2). Root growth was less affected by drought after plants had reached the pod development stage (R4) and finally ceased during R5. On the other hand, roots continued to extend linearly into deep and barrier-free soils from the early vegetative stage to R5 (Mayaki *et al.*, 1976; Mitchell and Russell, 1971), but the pattern of root growth could be site- and cultivar-specific. In 2015, roots of NS plants might have been stimulated by a low SMC during the most developmental stages and then grow more in the deeper soil. Further investigation of root dynamics during the growing season in the cool Tohoku region of northern Japan will be needed in future studies.

The magnitude of the relative seed yield increase due to early sowing (16% in 2013 and 18% in 2014, on average, across the cultivars) was similar to previous results obtained in the Midwestern United States. Bastidas *et al.* (2008) reported higher yields from late-April and early-May sowings in both years of an irrigated experiment in Nebraska, but the increase was only 8% in the first year and 16% in the second year. De Bruin and Pedersen (2008) evaluated the response to sowing date in two years in Iowa, and found that late-April and early-May sowings produced higher yields (by an average of 8%) than late-May plantings.

Although the present study suggests that early sowing is an effective management strategy to increase soybean yield in the cool Tohoku region of northern Japan, several points must be considered. First, as discussed above, the beneficial effect of early sowing was affected by the amounts and patterns of precipitation during growing season. Therefore, the combination of both early sowing and water management such as irrigation and drainage will be required to increase soybean yield in this region. Second, the present results were derived from field data in years with unusually warm weather early in the growing season (May in all years except 2013 and June in all years). The inhibition of seedling emergence and therefore of canopy development that occurs at low temperatures was not observed in any year, even in 2013, when the May weather was slightly colder than the long-term average. As Horai *et al.* (2013) noted, the mean air temperatures in May and June have been increasing significantly in Iwate Prefecture since the 1920s, but there has been high fluctuation between years. Further experiments during

years with a cooler May and June might have produced different results. Previous growth chamber experiments by my research group showed that future increasing temperatures and atmospheric CO<sub>2</sub> concentrations will increase the seed yield with a normal sowing date (Kumagai *et al.* 2012, Kumagai and Sameshima, 2014; Kumagai *et al.*, 2015). I hypothesize that early sowing may be more successful in the future and that soybean production will therefore increase if temperatures continue to increase in this region owing to global warming.

A final problem is that the early sowing dates tested in this study overlap the currently recommended period for rice transplanting in Iwate Prefecture (Shimono *et al.*, 2010). Field experiments by Horai *et al.* (2013) revealed that transplanting 10 days earlier did not increase the grain yield of the current rice cultivars that are grown widely in this region. To develop an agricultural system that will increase the production of both rice and soybean in this region, the positive effect of ultra-early sowing (in early May, which does not overlap the paddy rice transplanting period) on soybean growth and yield should be tested in future studies.

#### 4.2 Indeterminate cultivars did not always surpass determinate Ryuhou in a cool region

The present study revealed that early sowing significantly increased the seed yield, with no sowing date × cultivar interaction, in the two years with adequate soil moisture (Table 2), indicating that the magnitude of the yield increase in response to early sowing did not differ among the cultivars tested. However, it is possible that using only a single determinate cultivar (Ryuhou) prevented the present study from detecting a difference between determinate and indeterminate cultivars. Further investigation of the response to early sowing dates using many cultivars will be needed to select materials with the strongest response to ES in northern Japan.

A significant effect of cultivar on seed yield was observed in 2014 and 2015, but not in 2013 (Table 2). However, the indeterminate cultivars did not always surpass the determinate cultivar Ryuhou. Kato *et al.* (2015) compared the yield of indeterminate and determinate lines among Ohsuzu × Athow recombinant inbred lines with a normal sowing date at Akita, in the Tohoku region, and showed that indeterminate lines with early maturity had significantly higher yield than determinate lines with early maturity. Similarly, the present study showed that early-maturing Karikei-879 had a growth duration (days from VE to R7) similar to that of Ryuhou when averaged between the sowing dates in two years, but that the aboveground biomass and seed yield of this cultivar were tended to be higher than those of Ryuhou in 2014 (a year with high precipitation and high SMC), but not in 2015, the year with low SMC (Table 2). On the other hand, late-maturing Karikei-881 had a longer growth duration and heavier aboveground biomass than Ryuhou, but a similar pod number and yield, when averaged between the two dates in 2013 and 2014 (Table 2). In both 2014 and 2015, UA4910 had a longer growth duration, and greater pod number per m<sup>2</sup> than Ryuhou, and thereby had a higher seed yield. A recent yield comparison between Japanese and American cultivars by Matsuo *et al.* (2016) showed that this cultivar had a

higher pod number and seed yield than several Japanese cultivars in southwestern Japan. Kawasaki *et al.* (2016) also reported that the American cultivars showed superior seed yield to Japanese cultivars due to their high biomass production capacity and high RUE during the seed-filling stage in a warmer region of Japan. In the present study, CGR and RUE were calculated during the 30 days after R1 in 2014 and from R2 to R5 in 2015, which is earlier than the stages studied by Kawasaki *et al.* (2016). Therefore, further analysis of these parameters, with a focus on the whole growing season, but especially on the late seed-filling stage, will be needed to elucidate the factors responsible for superior seed yield.

An interesting finding was the plasticity of RLD in UA4910 in 2014 and 2015: RLD values of this cultivar in both the shallow and deep soil layers were similar to those of Ryuhou in 2014, the year with high SMC, whereas there was a tendency toward higher RLD in the deep soil layer in UA4910 in 2015, the year with low SMC. This characteristic probably contributed to the cultivar's high yield even in 2015 with low SMCs (Table 2). Karikei-881 had higher RLD in both soil layers than the other three cultivars in 2014. There has been limited information obtained on the effect of stem determinacy on root characteristics so far. An early report by Allmaras *et al.* (1975) in southwestern Minnesota showed that the total root dry weight per unit of shoot dry matter in an indeterminate isolate of Harosoy was generally greater than that in a determinate isolate, but that more root dry matter was concentrated in the upper 30 cm of the soil. However, since these differences are presumably site- and cultivar-specific, it will be essential to compare many accessions in different years and at different locations. Further investigations of the physiological bases of yield differences between Japanese and American soybean cultivars will be needed to guide breeding for high-yielding Japanese cultivars in future studies.

## 5. Conclusions

Three years of field experiments in northern Japan showed that early sowing increased soybean yield in years with warm temperatures and with high precipitation and SMC. The yield increase was owing to greater pod number and higher CGR and CumIR due to a greater canopy development during vegetative and reproductive stage, which might be associated with the decreased precipitation and SMCs by early sowing. These results suggest that early sowing is an effective option for reducing the risk of excess water stress in vegetative stage and increasing yield of soybean in this region. In both the indeterminate and determinate cultivars, seed yield was increased by earlier sowing, but without a cultivar  $\times$  sowing date interaction, suggesting that the indeterminate soybean cultivars did not benefit more than the determinate soybean from early sowing in northern Japan.

## Supplemental information

Supplemental information for this paper is available at <http://doi.org/10.2480/agrmet.D-17-00009>.

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