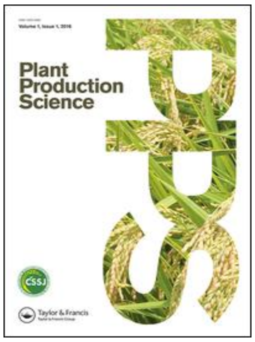

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Effect of plant density on growth and yield of new soybean genotypes grown under early planting condition in southwestern Japan

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ABSTRACT
Soybeans planted in early to mid-June (early) are less affected by rainfall during rainy season than those conventionally planted in early to mid-July in southwestern Japan. Also, narrow row cultivation is expected to increase soybean yield and save labor for inter-tillage and ridging. Field experiments were performed in 2014 and 2015 to test the effect of plant density (high, middle, and low) under early planting condition on growth, yield, and several agronomical traits of Sachiyutaka A1 and three new genotypes (Sakukei 155, Kanto 127 and Shikoku 15). Early planting was performed in mid- to late June, even though rainy season started in early June. Higher plant densities produced 13% greater yield than low plant density through an increase in biomass accumulation, especially at R5. Among yield components, only pods m^{-2} was significantly and positively correlated with yield, indicating that an increase in pods m^{-2} led to a greater yield with higher plant densities. The yields of Sachiyutaka A1 were relatively stable for two years, but the lodging resistance should be further improved. Shikoku 15 had greater yield potential and lodging resistance, but its resistance to damping-off disease should be improved. Sakukei 155 with medium plant density produced relatively high and stable yield with less lodging. Although the yield of Kanto 127 fluctuated between experimental years, this genotype showed higher yield potential in higher plant densities with less lodging in 2015. Thus, Sakukei 155 and Kanto 127 with high or medium density may be suitable for early planting in this region.

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Introduction

Soybean (*Glycine max.* (L.) Merr.) is one of the most important legume crops around the world because it contains abundant protein and oil contents and functional components, such as isoflavone. In Japan, soybean has long been used to process traditional foods such as tofu, miso, natto, and soy source. The soybean yield per unit area has been steadily increasing from 2.3 t ha^{-1} in 1985 to 3.2 t ha^{-1} in 2014 in the United States and from 1.8 t ha^{-1} in 1984 to 2.9 t ha^{-1} in 2014 in Brazil (FAOSTAT, 1985–2014). In Japan, however, soybean production has not increased in the past 30 years; the average soybean yield during this period was 1.65 t ha^{-1} (FAOSTAT, 1985–2014).

In southwestern Japan, where double-cropping systems and crop rotation systems are widely dominant, rice (*Oryza sativa* L.) or soybean is cultivated in summer and wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), or vegetables, such as onions (*Allium cepa* L.), are alternately cultivated in winter in the same fields. A conventional double-cropping system in southwestern Japan is carried out as follows: winter crops are harvested until early June, rice seedlings are transplanted in mid- to late June, and soybeans are then planted in early to mid-July. Summer crops are harvested until mid-November, and then winter crops are planted or transplanted.

A leading soybean cultivar, 'Fukuyutaka', has been widely cultivated for more than 30 years in southwestern Japan. The optimum planting date of this cultivar in this region is thought to be around 10 July (Ohga et al., 1985; Uchikawa, Fukushima, & Matsue, 2003), when southwestern Japan is in the middle of or at the end of the rainy season, and thus heavy rainfall frequently occurs. Therefore, planting dates are often affected by the rainfall amount or the timing when the rainy season ends and are sometimes delayed into late July to early August (late planting). Furthermore, more than 90% of soybeans in southwestern Japan are cultivated in upland fields converted from rice paddies (MAFF,

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2015). Although the duration after conversion varies depending on cropping systems and regions, drainage in these fields is generally poor. The poor drainage of these paddy fields also delays the planting dates for soybeans because agricultural machinery cannot be used for several days after heavy rainfall due to excessive soil moisture. In general, late planting decreases the soybean yield in southwestern Japan (Fatichin et al., 2013; Uchikawa, Fukushima, & Matsue, 2004; Uchikawa, Tanaka, Miyazaki, & Matsue, 2009). Therefore, the unstable planting date is one of the reasons for the unstable soybean yield in southwestern Japan.

The rainfall amount at the beginning of the rainy season (early to mid-June) in southwestern Japan is usually much less than the amount in the middle of or at the end of the rainy season (early to mid-July). Therefore, planting can be performed consistently in early to mid-June (early planting) regardless of the cultivation year. Previous researchers have studied the effect of early planting on growth, yield, and several agronomical traits, such as lodging for Fukuyutaka. Ohga et al. (1985) reported that early planting decreased the seed yield by approximately 10% over conventional mid-July planting in two of three years. Furthermore, early planting exacerbated lodging due to rank growth. Therefore, early planting with Fukuyutaka has not become widespread. Ohga et al. (1988) revealed that cultivars that mature earlier than Fukuyutaka showed greater seed yields with early planting than with conventional mid-July planting, indicating that early maturing cultivars might be adaptable to early planting.

Given this background, the soybean cultivar 'Sachiyutaka', which matures earlier than Fukuyutaka and has lodging resistance due to its short main stem length, was developed in 2003 (Takahashi et al., 2004). Uchikawa et al. (2004) demonstrated that the seed yield of Sachiyutaka with early (mid-June) planting was similar to that of Fukuyutaka planted in early to mid-July (conventional cultivation method). Furthermore, Furuhata et al. (2008) reported that the seed yield of Sachiyutaka increased as the row width narrowed from 80 to 60, 40 and 30 cm (the hill space was 20 cm for all treatments), indicating the adaptability of Sachiyutaka to higher plant density. It has been reported that narrow-row cultivation not only increased the seed yield but also reduced the weed density due to more rapid canopy closure (Harder et al., 2007; Saitoh et al., 2007). Therefore, it was expected that Sachiyutaka would replace Fukuyutaka in this region. However, Sachiyutaka lacked shattering resistance, causing seeds to naturally drop onto the soil surface before harvest and leading to head loss during machine harvest. These adverse agronomical traits inhibited the spread of Sachiyutaka.

In 2012, a new soybean cultivar, 'Sachiyutaka A1', was developed by the National Institute of Crop Science, Tsukuba, Japan (Yamada et al., 2013). In this cultivar, shattering resistance was only introduced into Sachiyutaka by a pinpoint improvement method with marker-assisted selection. Thus, the agronomical traits of Sachiyutaka A1 other than shattering resistance were similar to those of its recurrent parent cultivar, Sachiyutaka (Yamada et al., 2013). Matsuo et al. (2015) reported that the combination of high or medium plant density (20- or 35-cm row × 20 cm hill) and early (around 10 June) planting of Sachiyutaka A1 produced greater seed yields than did low plant density (75-cm row × 20 cm hill) and conventional (around 20 July) planting. Their results suggested the possibility that cultivating Sachiyutaka A1 with denser plant density and early planting might be adaptable for soybean production in southwestern Japan. In their study, they tested the adaptability to denser plant density and early planting only for Sachiyutaka A1, although soybean breeders have developed new soybean genotypes which are thought to be suitable for early planting and denser plant density. Little information is available for the adaptability of these new soybean genotypes to early planting and denser plant density.

The objective of the present study was to test the effect of plant density (adjusted by row width) on growth, yield, and several agronomical traits for three newly developed soybean genotypes and Sachiyutaka A1 under early planting conditions. This information will provide growers with new cultivation options which will lead to more stable and higher soybean yields than those obtained using conventional cultivation methods.

Materials and methods

Site description and plant materials

Field experiments were conducted in 2014 and 2015 at the Kyushu Okinawa Agricultural Research Center (KARC), Chikugo, Fukuoka, Japan (33°12′ N, 130°30′ E, 10 m elevation). The soil was lowland paddy soil (Typic Endoaquept). Because crop rotation practices were used at the KARC, the fields used in this study differed between the experimental years, and the details of the soil properties are listed in Table 1. The previous crops for the 2014 and 2015 growing seasons were barley and rice, respectively. The soybean genotypes used were Sachiyutaka A1, Sakukei 155, Kanto 127, and Shikoku 15. Sachiyutaka A1, Sakukei 155, and Kanto 127 were bred at the National Institute of Crop Science, Ibaraki, Japan, and Shikoku 15 was bred at the West Region Agricultural Research Center, Kagawa, Japan. The breeding method, background, and agronomic characteristics of each genotype are shown in Table 2.

Crop management

The planting dates were June 11 and June 23 in 2014 and 2015, respectively (Table 3) and three seeds per hill were planted by hand at 20, 35, and 70 cm row width for high, middle, and low plant densities, respectively. The hill space was 20 cm for all plant densities. Thus, the target plant density was 25.0, 14.3, and 7.1 plants m^{-2} for high, medium, and low plant densities, respectively. The area of the subplot was 11.5, 12.6, and 12.6 m^2 for high, middle, and low plant densities, respectively. After seedling establishment, the plants were thinned to one plant per hill. A pre-emergence herbicide, which contained 8% thiobencarb, .8% pendimethalin, and 1.2% linuron, was applied at a rate of 5 g m^{-2} just after planting. A conventional cultivar, 'Fukuyutaka', was also planted in the same experimental field at a conventional row width of 70 cm on 8 July 2014 and 27 July 2015 with three replications (Table 3). The planting of Fukuyutaka was carried out with a seeding machinery (ADRG-U, AGRITECNO YAZAKI CO., Ltd., Hyogo, Japan) attached with a rotary of 1.5 m width (RSP15, Kubota Co., Osaka, Japan), and the planting density was a 14.3 plants m^{-2} (two seeds per hill).

Measurements

The air temperature, sunshine hour, solar radiation, and rainfall amount were measured at the meteorological station of the KARC, located about 100 m away from the experimental field. At the R2 and R5 stages (according to Fehr, Caviness, Burmood, & Pennington, 1971), the aboveground parts growing in a .8-m^2 area for high plant density and in a .84-m^2 area for middle and low plant densities were collected. The dates of R2 and R5 for each year and genotype are shown in Table 3. At each sampling, the leaves were separated from whole plants, and the leaf area was determined with a leaf area meter (Li-3000C, Li-COR, Lincoln, NE). The leaf area index (LAI; m m^{-2}) was calculated by dividing the measured leaf area by the sampling area. The aboveground parts were then dried at 80 °C in a ventilated oven for at least 72 h to determine the shoot dry weight (SDW; g m^{-2}). The crop growth rate (CGR; g m^{-2} d^{-1}) during the R2 to R5 stages was calculated by subtracting the SDW at the R2 stage from the SDW at the R5 stage and dividing this value by the number of days from R2 to R5.

At harvest (dates of harvest for each year and genotype are shown in Table 3), the lodging angle of the main stem was determined according to Matsuo et al. (2015) for 10 plants per subsample plot. Briefly, the lodging angle was measured with a clear acryl plate of 15 cm × 15 cm on which straight lines were drawn with a marker at 10°, 20°, 40°, and 60° to the upright. If the lodging angle was between 0°–10°, 10°–20°, 20°–40°, 40°–60° and more than 60°, the lodging score was defined as 0, 1, 2, 3, and 4, respectively. After the lodging score measurement, the plants in a 2.4-m^2 area for high plant density and a 2.52-m^2 area for middle and low plant densities were collected to determine the final plant density, the plant height, the number of main stem nodes, the lowest node height which had pods (LNHP), the yield and the yield components (i.e. the number of pods per m^{-2} (pods m^{-2}), the number of seeds pod^{-1} (seeds pod^{-1}), and the 100-seed weight). Because agricultural machines harvest seeds above approximately 10 cm from the soil surface on average, we also estimated yield loss, as previously reported by Matsuo et al. (2015). Briefly, before harvest, the plants were marked with a spray at a position of 10 cm above the ground surface with the aid of a metal plate (10 cm high × 20 cm long). Then, pods above 10 cm were separated from those below 10 cm. The yield, pods m^{-2}, seeds pod^{-1}, and weight of seeds more than 10 cm above the soil surface were measured, but the yield components below 10 cm above the soil surface were not measured (only the seed weight was determined). The seed yields were adjusted to 130 g kg^{-1} moisture. The crude protein and oil content of the seeds were determined by near-infrared spectroscopic analysis (Infratec™ 1241 Seed Analyzer, Foss Tecator AB, Höganäs, Sweden). The conversion factor for the calculation of the

Table 1. Soil characteristics of the experimental sites in 2014 and 2015.

	2014	2015
Soil type	Light clay	Heavy clay
Sand (%)	25.8	14.0
Silt (%)	43.8	40.1
Clay (%)	30.5	46.0
Soil pH (1:1)	6.6	6.6
Available P (mg kg^{-1})	207	207
Exchangeable K (mg kg^{-1})	429	301
Exchangeable Mg (mg kg^{-1})	502	464
Exchangeable Ca (mg kg^{-1})	6259	4678
CEC (molc kg^{-1})	32.1	26.7

Table 2. Breeding methods, background, and agronomic characteristics of each genotype.

Genotype	Breeding method	Back ground	Agronomic characteristics
Sachiyutaka A1	Backcross method	Sachiyutaka	Shattering resistance
Sakukei 155	Backcross method	Sachiyutaka	Shattering resistance
Kanto 127	Backcross method	Fukuyutaka	Shattering resistance, earler matureing than Fukuyutaka
Shikoku 15	Pedigree method	Sachiyutaka/Tanyo	Shattering resistance, lodging resistance

protein content was 6.25. For Fukuyutaka, the plants in a 2.8-m^2 area were collected at harvest (Table 3), and the yield and yield components were determined.

Experimental design and statistical analysis

The experimental design was a split plot on a randomized complete block design with three replications. The main plot was the genotype and the subplot was the plant density. Statistical analysis was carried out using SPSS v. 23 (SPSS, Inc., Chicago, IL), and a linear mixed model was used in this analysis. Genotype, plant density, experimental year, and their interactions were considered fixed effects, and replication (nested within year) was considered a random effect. Analysis of variance (ANOVA) was conducted to test the effect of genotype, plant density, year, and their interactions on the shoot growth parameters (SDW, LAI, CGR) and agronomical traits at harvest (final plant density, plant height, the number of main stem nodes, LNHP, yield, yield components, yield loss, lodging score, and seed components). Means were separated using Fisher's protected least significant difference (LSD) if the F test of ANOVA exceeded the .05 probability level. Simple linear regression analyses were computed to evaluate the relationships between seed yield and the agronomical traits measured. For the statistical analysis, the yield and yield components data of Fukuyutaka were not used.

Table 3. Planting dates and days to reach specific reproductive stages in 2014 and 2015.

Year	Genotype	Planting date	R2	R5	R8
2014	Sachiyutaka A1	6/11	7/31	8/31	10/23
	Sakukei 155	6/11	7/31	8/27	10/20
	Kanto 127	6/11	7/30	8/26	10/20
	Shikoku 15	6/11	7/29	8/29	10/25
	Fukuyutaka	7/8			11/6
2015	Sachiyutaka A1	6/23	8/8	9/4	11/2
	Sakukei 155	6/23	8/6	9/1	10/30
	Kanto 127	6/23	8/3	9/3	10/31
	Shikoku 15	6/23	8/4	9/2	11/2
	Fukuyutaka	7/27			11/16

Results

Weather

The monthly mean air temperature, sunshine hour, solar radiation, and rainfall amount in 2014 and 2015 and the 30-yr average (1983–2013) values are presented in Table 4. The characteristics of weather in 2014 were that cloudy and rainy conditions caused cooler air temperatures, fewer sunshine hour, and lower solar radiation in August. In 2015, the mean air temperature was lower than the 30-yr average throughout the crop season and the mean sunshine hour and solar radiation in 2015 were both lower than the 30-yr averages in summer season. A typhoon directly hit the experimental field on 25 August 2015 and caused heavy rainfall.

Canopy development

The LAI and SDW at the R2 stage were affected by the main effects of year, genotype, and plant density (Table 5). The LAI and SDW at the R2 stage in 2014 were greater than those in 2015 and Sachiyutaka A1 and Sakukei 155 had greater values than Kanto 127 and Shikoku 15 for all plant densities in both years. The LAI and SDW at the R2 stage increased as plant density increased for all genotypes. The LAI at the R2 stage was influenced by an year × genotype interaction and the SDW at the R2 stage was influenced by year × genotype and year × plant density interactions.

There were significant main effects of genotype and plant density on the LAI and SDW at the R5 stage, although there were no effects of any interaction (year, genotype, and plant density) on them (Table 5). High and medium plant densities had at least .6 larger LAI values than low plant density, and the SDW increased as plant density increased, except for the SDW of Shikoku 15 in 2015. The LAI and SDW of Shikoku 15 at the R5 stage were lower than those of the other genotypes for all plant densities in both years. There were no effects of any interaction (year, genotype, or row width) on the CGR during R2 to R5 stage and a significant main effect on the CGR was found only for genotype (Table 5). The CGR of Shikoku 15 was lower than those of the other genotypes in all plant densities,

Table 4. Monthly mean air temperature, sunshine hours, solar radiation, and rainfall in 2014 and 2015 and the 30-yr averages.

	Air temperature (°C)			Sunshine hours (h)			Solar radiation (MJ m^{-2})			Rainfall (mm)		
Month	2014	2015	30-yr	2014	2015	30-yr	2014	2015	30-yr	2014	2015	30-yr
June	22.8	22.5	23.3	3.5	2.6	4.6	15.5	12.8	15.3	138	320	352
July	26.7	26.3	27.0	4.5	4.4	6.1	16.1	16.0	17.7	334	233	340
August	26.6	27.4	27.8	2.9	6.0	6.9	13.2	17.8	18.2	313	281	200
September	24.1	23.3	24.2	5.2	5.5	6.1	15.1	15.8	15.2	139	150	157
October	19.2	18.2	18.5	6.2	7.6	6.1	13.0	14.9	12.7	79	69	70

especially in high and low plant densities in 2014 and in medium plant density in 2015.

Final plant density, plant height, and LNHP at harvest

Significant main effects of genotype and plant density on final plant density were found (Table 6). For all genotypes, final plant density increased as plant density increased. The final plant density of Shikoku 15 was lower than those of the other genotypes for all plant densities. For all genotypes other than Shikoku 15, final plant density was over 90% of the target plant density. For Shikoku 15, however, the final plant density in high, medium, and low plant densities was 20.0, 14.8, and 30.9% lower in 2014 and 24.4, 35.2, and 15.3% lower in 2015 than target plant density.

There were significant main effects of genotype and row width on plant height (Table 6). Shikoku 15 had the highest plant height, followed by Kanto 127, Sachiyutaka A1 and Sakukei 155 in all plant densities, and the plant height increased as plant density increased.

There was a significant year × genotype interaction on the LNHP (Table 6). The LNHPs of Kanto 127 and Shikoku 15 in 2014 were shorter than those in 2015, while the LNHPs of Sachiyutaka A1 and Sakukei 155 did not differ significantly between experimental years. In 2014, no significant difference in the LNHP was found among genotypes, while Kanto 127 and Shikoku 15 had approximately 2.0 cm higher LNHPs than Sachiyutaka A1 and Sakukei 155 for all plant densities in 2015. The LNHP increased as plant density increased for all genotypes in both years, except for low plant density of Kanto 127 in 2015 (Table 6).

Yield and yield components

There was a significant year × genotype × row width interaction on pods m^{-2} (Table 6). This interaction might be generated by the variability in the pods m^{-2} of Shikoku 15 with experimental years and plant densities. For Shikoku 15 with medium plant density, the pods m^{-2} in 2014 was significantly greater than that in 2015 (1014 vs. 552 pods m^{-2}), while the pods m^{-2} with other combinations of plant density and genotype did not differ between experimental years. For Shikoku 15, the pods m^{-2} in high and medium plant densities (821 and 1014 pods m^{-2}, respectively) was greater than that in low plant density (623 pods m^{-2}) in

Table 5. Leaf area index (LAI), shoot dry weight (SDW) at R2 and R5, and crop growth rate (CGR) during R2 to R5 of four genotypes in response to plant density in 2014 and 2015.

Year	Genotype	Plant density	LAI (m^2 m^{-2}) R2	LAI (m^2 m^{-2}) R5	SDW (g m^{-2}) R2	SDW (g m^{-2}) R5	CGR during R2-R5 (g m^{-2} d^{-1})
2014	Sachiyutaka A1	High	4.7	6.0	359	739	12.3
		Medium	4.3	5.8	288	687	12.9
		Low	2.6	5.2	174	576	13.0
	Sakukei 155	High	5.0	5.7	353	658	11.3
		Medium	4.5	6.2	286	660	13.9
		Low	2.6	5.1	178	553	13.9
	Kanto 127	High	4.6	5.9	342	661	11.8
		Medium	3.7	6.0	246	625	14.0
		Low	2.0	3.9	134	427	10.9
	Shikoku 15	High	4.7	4.0	325	628	9.8
		Medium	3.3	4.6	224	577	11.4
		Low	1.8	3.4	131	406	8.9
2015	Sachiyutaka A1	High	5.1	6.3	350	715	13.5
		Medium	4.1	6.2	265	638	13.8
		Low	2.2	4.9	151	517	13.5
	Sakukei 155	High	4.3	6.7	274	679	15.6
		Medium	3.5	6.6	228	647	16.1
		Low	2.2	5.1	146	490	13.2
	Kanto 127	High	3.8	5.6	249	663	13.4
		Medium	2.4	6.1	158	644	15.7
		Low	1.2	5.0	80	517	14.1
	Shikoku 15	High	3.3	5.1	237	612	12.9
		Medium	2.5	3.7	176	401	7.8
		Low	1.6	3.9	109	444	11.6
	LSD (0.05)						
	Year (A)		0.4	ns	22	ns	ns
	Genotype (B)		0.3	0.7	13	67	2.2
	Plant density (C)		0.2	0.6	12	58	ns
	A × B		0.5	ns	28	ns	ns
	A × C		ns	ns	13	ns	ns
	B × C		ns	ns	ns	ns	ns
	A × B × C		ns	ns	ns	ns	ns

Table 6. Agronomical traits at harvest, yield components, yield, yield loss, lodging score, and protein and oil content of four genotypes in response to plant density in 2014 and 2015.

Year	Genotype	Plant density	Final plant density (no. m^{-2})	Plant height (cm)	LNHP[a] (cm)	Pods m^{-2} (no. m^{-2})	Seeds pod^{-1} (no. pod^{-1})	100-seed weight (g)	Yield (g m^{-2})	Yield loss (g m^{-2})	Lodging score (0-4 scale)	Protein (%)	Oil (%)
2014	Sachiyutaka A1	High	24.7	57.9	12.6	682	1.63	29.1	324	15	1.4	46.1	19.4
		Medium	13.9	48.9	10.0	659	1.69	30.3	336	11	1.6	45.8	19.6
		Low	6.7	43.5	8.0	566	1.79	32.3	327	21	1.9	45.9	19.8
	Sakukei 155	High	24.7	56.1	11.5	716	1.68	28.8	345	12	1.3	46.6	19.3
		Medium	14.3	44.7	9.1	717	1.69	30.4	368	12	1.3	46.4	19.4
		Low	6.7	39.9	8.6	568	1.75	31.6	316	22	1.7	47.0	19.3
	Kanto1 27	High	24.0	62.9	10.7	750	1.57	25.6	302	18	1.5	43.0	21.7
		Medium	14.0	51.0	10.4	686	1.66	25.8	290	26	1.3	43.0	21.5
		Low	6.5	43.6	8.7	625	1.61	26.3	264	26	1.4	43.3	21.7
	Shikoku 15	High	20.0	64.5	12.1	821	1.74	22.5	321	3	2.2	44.4	20.3
		Medium	12.2	59.1	10.0	1014	1.67	22.6	383	10	1.1	44.4	20.4
		Low	4.9	48.0	9.1	623	1.70	24.3	252	18	1.6	45.3	20.4
2015	Sachiyutaka A1	High	24.9	56.2	12.0	609	1.64	35.4	356	15	2.6	46.9	19.0
		Medium	14.0	48.7	10.8	633	1.63	36.6	378	16	2.6	46.9	19.0
		Low	6.7	42.1	10.4	527	1.67	37.8	333	27	2.8	47.6	18.6
	Sakukei 155	High	24.3	52.7	12.1	563	1.49	37.4	312	24	2.4	48.3	18.3
		Medium	14.2	46.1	10.0	629	1.53	37.6	361	11	2.1	48.6	18.3
		Low	6.5	41.1	9.4	537	1.55	38.5	322	17	2.4	49.1	18.1
	Kanto 127	High	24.9	57.0	13.7	805	1.56	31.4	393	7	2.1	44.2	20.4
		Medium	14.0	53.6	12.8	766	1.60	32.2	393	3	2.0	44.7	20.5
		Low	6.7	44.7	14.1	671	1.67	32.7	366	12	2.0	44.7	20.0
	Shikoku 15	High	18.9	61.4	14.3	901	1.70	27.6	421	0	1.9	48.8	18.9
		Medium	9.3	51.9	13.4	552	1.72	27.5	266	2	1.5	48.0	19.3
		Low	6.0	47.1	12.0	569	1.76	26.8	267	4	0.8	47.6	19.7
	LSD (0.05)												
	Year (A)		ns	ns	0.2	ns	0.03	0.9	ns	ns	0.3	0.3	0.2
	Genotype (B)		1.4	2.2	0.3	80	0.05	0.9	ns	8	0.3	0.4	0.2
	Plantdensity (C)		1.2	1.9	0.3	69	0.04	0.8	33	ns	ns	ns	ns
	A×B		ns	ns	0.5	ns	0.07	1.3	53	11	0.5	0.6	ns
	A×C		ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	B×C		ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	A×B×C	Year[b]				198							
		Genotype[c]	ns	ns	ns	196	ns	ns	ns	ns	ns	ns	ns
		Plant density[d]				196							

[a]LNHP: lowest node height which had pods.
[b]LSD among years for given genotype × row width.
[c]LSD among genotypes for given year × row width.
[d]LSD among row widths for given year × genotype.

2014, while the pods m^{-2} in high plant density (901 pods m^{-2}) was significantly greater than the numbers in medium and low plant densities (552 and 569 pods m^{-2}, respectively) in 2015. In 2014, a significant genotypic difference in the pods m^{-2} was found in medium plant density; Shikoku 15 had more pods m^{-2} than the other genotypes. On the other hand, in 2015, significant genotypic differences in the pods m^{-2} were found in high and medium plant densities; in high plant density, Shikoku 15 produced more pods m^{-2} than Sachiyutaka A1 and Sakukei 155, while in medium plant density, the pods m^{-2} of Shikoku 15 was significantly lower than that of Kanto 127 (Table 7).

A significant year × genotype interaction and significant main effects of year, genotype, and plant density were found for seeds pod^{-1} (Table 6). The seeds pod^{-1} of Sakukei 155 in 2015 was significantly smaller than in 2014 for all plant densities, while for the other genotypes, no significant year effect on the seeds pod^{-1} was found. In 2014, Kanto 127 had a significantly lower number of seeds pod^{-1} than the other genotypes for all plant densities, while in 2015, Shikoku 15 had the largest seeds pod^{-1}. The seeds pod^{-1} tended to decrease as plant density increased for all genotypes in both years.

A significant year × genotype interaction and significant main effects of year, genotype, and plant density were observed for the 100-seed weight (Table 6). For all genotypes, the 100-seed weight in 2015 was significantly heavier than that in 2014, and for both experimental years, Sachiyutaka A1 and Sakukei 155 had heavier 100-seed weights, followed by Kanto 127 and Shikoku 15. The 100-seed weight tended to decrease as plant density increased for all genotypes in both years.

Table 7. Correlation coefficients between the seed yield and agronomical traits at harvest, yield components, yield loss, lodging score, and growth parameters.

Traits	*r*	*p*-value
Final plant density	.355	.089
Plant height	.270	.202
LNHP[a]	.358	.086
Pods m^{-2}	.568	.004
Seeds pod^{-1}	−.185	.386
100-seed weight	.306	.145
Yield loss	−.397	.055
Lodging score	.357	.087
LAI at R2	.290	.169
SDW at R2	.244	.25
LAI at R5	.533	.007
SDW at R5	.578	.003
CGR during R2 to R5	.550	.005

Note: Dates for 2 years were used in this analysis (n = 24).
[a]LNHP: lowest node height which had pods.

Differences in the seed yield among cultivars were not consistent across two years as evidenced by a significant interaction between year and cultivar; Sakukei 155 recorded the highest yield in 2014 but Kanto 127 in 2015. A significant difference in seed yield between experimental years was found only for Kanto 127 and the seed yield of this genotype in 2015 was greater than that in 2014 for all plant densities. The top three yields in each year were obtained with Shikoku 15 with medium plant density (383 g m^{-2}), and Sakukei 155 with medium plant density (368 g m^{-2}) and Sakukei 155 with high plant density (345 g m^{-2}) in 2014, and Shikoku 15 (421 g m^{-2}) with high plant density, Kanto 127 with high plant density (393 g m^{-2}), and Kanto 127 with medium plant density (393 g m^{-2}) in 2015. Although the highest seed yield in 2014 and 2015 was observed in Shikoku 15 with medium and high plant densities (383 and 421 g m^{-2}), respectively, the seed yield of Shikoku 15 in response to plant density largely fluctuated between experimental years. For Sachiyutaka A1 and Sakukei 155, medium plant density tended to produce higher seed yield than the other plant densities in both years.

Correlation analysis between yields and agronomic traits indicated that seed yield was positively and significantly correlated with the pods m^{-2}, the LAI and SDW at R5 and the CGR during R2 to R5 stage (Table 7).

The yield and yield components of Fukuyutaka are shown in Table 8. The pods m^{-2} and seeds pod^{-1} in 2014 were greater than those in 2015, while the 100-seed weight was heavier in 2015 than in 2014. Overall, the seed yields of Fukuyutaka planted in July were 315 and 255 g m^{-2} in 2014 and 2015, respectively.

Yield loss and lodging score

There was a significant year × genotype interaction on yield loss (Table 6). A significant difference in yield loss between experimental years was found only for Kanto 127 and the yield loss in 2014 was higher than that in 2015. Shikoku 15 had less yield loss than the other genotypes, especially in 2015. Although the main effect of plant density on yield loss was not significant (p = .087, data not shown), the yield loss in low plant density tended to be

Table 8. Plant height, yield components, and yield of Fukuyutaka planted conventionally in 2014 and 2015.

Year	Planting date	Harvest	Plant height (cm)	Pods m^{-2} (no. m^{-2})	Seeds pod^{-1} (no. pod^{-1})	100-seed weight (g)	Yield (g m^{-2})
2014	July 8	Nov. 6	70.0 ± 2.2	694 ± 42	1.61 ± 0.02	28.3 ± 0.8	315 ± 13
2015	July 27	Nov. 16	54.2 ± 2.0	540 ± 20	1.50 ± 0.02	31.6 ± 0.1	255 ± 9

Mean ± S.E. (n = 3).

larger than those in medium and high plant densities for all genotypes.

A significant effect of year × genotype interaction on lodging score was found (Table 6). For all genotypes, except for Shikoku 15, the lodging score in 2014 was lower than that in 2015. In 2014, no significant difference in lodging score among genotypes was found, although the score of Shikoku 15 with high plant density was relatively large. In 2015, however, Shikoku 15 had the lowest lodging score for all plant densities, followed by Kanto 127, Sakukei 155, and Sachiyutaka A1. The difference in lodging score between experimental years might be caused by the fact that a typhoon hit the experimental field in 2015, but not in 2014. Plant density did not have a statistically significant effect on lodging score.

Seed components

There was a significant year × genotype interaction on protein content (Table 6). For all genotypes, the protein contents in 2015 were higher than those in 2014. In 2014, Sakukei 155 produced the highest protein content, followed by Sachiyutaka A1, Shikoku 15, and Kanto 127, while in 2015, Sakukei 155 and Shikoku 15 had higher protein contents, followed by Sachiyutaka A1 and Kanto 127. The oil contents had a negative correlation ($p < .05$, $r = -.9729$, $n = 4$) with protein contents (i.e. the higher the protein contents were, the lower the oil contents were), regardless of experimental year and genotype. Plant density did not have a statistically significant effect on protein and oil contents.

Discussion

The planting dates in this study for conventional soybean production with Fukuyutaka in 2014 and 2015 were 8 July and 27 July, respectively (Table 3). Although the climatic conditions in 2015 were different from those in 2014 (Table 4), the later planting in 2015 might have resulted in the lower seed yield compared with that in 2014 (255 vs. 315 g m^{-2}) (Table 8). It was reported that late planting (late July to early August) generally caused a yield reduction for Fukuyutaka in southwestern Japan, compared with optimum planting (early to mid-July) (Uchikawa et al., 2004; Fatichin et al., 2013). Therefore, the fluctuation of the planting date from early July to early August causes the variability of the soybean yield in this region. Contrary to July planting, early (June) planting could be carried out until late June in this study, even though the rainy season started at the beginning of June in both experimental years. In addition, the harvest dates were advanced approximately 2 wk in early planting compared to conventional planting (Table 3). This will increase the duration of the land preparation for and planting of the subsequent winter crops in double-cropping systems, which are widely used in southwestern Japan.

The LAI and SDW values at the R2 stage were lower in 2015 than in 2014, except for Sachiyutaka A1 with high plant density (Table 5). Later planting in 2015 shortened the duration from planting to R2 compared with that in 2014 (Table 3). A shorter duration from planting to R2 in 2015 might result in smaller biomass production at R2 in 2015. However, the LAI and SDW values at R5 did not differ between experimental years (Table 5). The correlation analysis showed that the LAI and SDW values at R5 positively and significantly ($p = .007$ and .003, respectively) correlated with seed yield, while those at R2 did not ($p = .169$ and .250, respectively) (Table 8). This result indicates that the biomass production at R5 contributes more to seed yield than that at R2. Board, Harville, and Saxton (1990a, 1990b) reported that greater total dry matter at R5 was related to narrow-row yield increase. Their result supports our results. Because the difference in the planting date of 12 d (11 June 2014 vs. 23 June 2015) had little effect on biomass production at R5, which was positively correlated with the seed yield, the seed yield may not be influenced by the planting date if planting is performed from mid- to late June.

Although there was no significant year × genotype × plant density interaction on the SDW and LAI values at R5 and the CGR during R2 to R5 (Table 5), the response of the SDW and LAI values at R5 and the CGR during R2 to R5 of Shikoku 15 to plant density tended to differ between experimental years. This result was caused by the fact that Shikoku 15 had less resistance to damping-off disease than the other genotypes. Symptoms of damping-off disease were observed at various growth stages (early vegetative to mid-reproductive stages) in Shikoku 15, and this caused several plants to wither, resulting in the reduction of plant density. In fact, the final plant density of Shikoku 15 at harvest was the lowest and the most fluctuated among the genotypes used (Table 6).

It might be possible that a significant effect of year × genotype × row width interaction on the pods m^{-2} (Table 6) was generated by the unstable plant standing of Shikoku 15, as described above. Among the yield components, the seed yield was significantly and positively correlated only with the pods m^{-2} (Table 7), indicating that stable pod production is essential for stable seed yield. The combination of Shikoku 15 and denser plant density produced the highest seed yield over two years, although the best combination differed between the experimental years (Table 6). This result indicates the high yield potential of Shikoku 15 with denser plant density. Therefore, if resistance to damping-off disease is introduced into Shikoku 15, a more stable and higher seed yield can be expected with this genotype.

Although the seed yield of Kanto 127 significantly differed between experimental years (Table 6), the seed yield of Kanto 127 was greater than those of the other genotypes in 2015, indicating high yield potential of this genotype. Because Kanto 127 was developed by back-crossing with Fukuyutaka as a recurrent parent, which is a leading cultivar in southwestern Japan, the agronomic traits of this genotype other than shattering resistance and maturing stage were similar to those of Fukuyutaka. Therefore, this genotype may be more suitable for this region and more acceptable for growers in this region than the other genotypes. Further study is needed to test the yield stability of Kanto 127.

For Sachiyutaka A1 and Sakukei 155, medium plant density tended to produce higher seed yield than the other plant densities in both years (Table 6). Especially for Sakukei 155, the seed yield with medium plant density was relatively high and stable for two years (365 g m^{-2} on average). Therefore, planting Sakukei 155 with medium plant density will lead to high and stable seed yield in this region.

Averaged across genotype and plant density, the seed yield was generally greater in 2015 that in 2014 with some exceptions (for example, Sakukei 155 with high plant density), probably because of significantly heavier 100-seed weight in 2015 than that in 2014. In 2015, the mean air temperature was lower, the sunshine hour was longer, and the solar radiation was larger in September and October (seed filling period) than those in 2014. It was reported that lower air temperature and longer cumulative sunshine hour during seed filling stage increased 100-seed weight (Ikejiri & Takahashi, 2016). Nakamura and Yoko (1987) also showed that longer sunshine hour from pod elongation stage to seed filling stage increased 100-seed weight. Therefore, weather condition during seed filling stage in 2015 was more suitable for seed growth than in 2014. It was reported that 100-seed weight was negatively correlated with seeds m^{-2} or pods m^{-2} (Fatichin et al., 2013). In this study, pods m^{-2} of Sachiyutaka A1 and Sakukei 155 was smaller in 2015 than in 2014. Thus, the increase in 100-seed weight of these cultivars in 2015 might be explained by the compensation between pods m^{-2} and 100-seed weight. For Kanto 127, however, both pods m^{-2} and 100-seed weight larger in 2015 than in 2014, indicating that the weather condition in 2015 was suitable for the pod formation and the seed growth of Kanto 127.

Compared with the seed yield obtained in conventional cultivation (Table 8), the increase in yield induced by early planting was more obvious in 2015 than in 2014; the differences in average seed yields between early (June) planting and conventional (July) planting were 4 and 93 g m^{-2} in 2014 and 2015, respectively. Therefore, the yield-increasing effect of early planting will be more pronounced in seasons when the planting date for conventional cultivation is delayed.

For Kanto 127 and Shikoku 15, the LNHP in 2015 was higher than that in 2014 (Table 6). Although the reason for this was unclear in this study, the increase in the LNHP might contribute to the reduction in yield loss for these genotypes in 2015 compared with that in 2014. Especially for Kanto 127, the reduction in yield loss in 2015 compared to that in 2014 might be one of the reasons for the significantly greater seed yield obtained in 2015 compared to that in 2014.

The LNHP increased as plant density increased and the seed yield in high and medium plant densities tended to be higher than that in low plant density (Table 6). The LNHP was significantly and negatively ($r = -.628$, $p < .01$, $n = 24$) correlated with yield loss (data not shown), and yield loss tended to be negatively ($r = -.397$, $p = .055$) correlated with seed yield (Table 7). Therefore, denser plant density increased the seed yield through increasing the LNHP, which led to the reduction in yield loss. Steele and Grabau (1997) reported a negative relationship between the lowest pod height and harvest loss which was defined as the yield between 0 and 10 cm from the soil surface (similar to yield loss in this study), and their results support our results.

The lodging scores of all genotypes except for Shikoku 15 were higher in 2015 than in 2014 (Table 6). Although, in 2015, a typhoon hit the experimental field directly in late August, the lodging score of Shikoku 15 was not affected by experimental years. For Shikoku 15, the lodging score tended to be higher (less lodging resistance) in high plant density than medium and low plant densities in both years, the yield loss of this genotype was much less than those of the other genotypes. Thus, lodging may not affect yield loss in this genotype. The lodging score of Sachiyutaka A1 was highest among genotypes used in 2015. Thus, lodging resistance of this genotype needs to be further improved. The lodging score of Kanto 127 was relatively lower (more lodging resistance) even in 2015 for all plant densities. The lodging score of Sakukei 155 in 2015 was similar to that of Kanto 127, when it was planted in medium plant density. From the view point of yield potential and lodging resistance, Kanto 127 with high and medium plant densities and Sakukei155 with medium plant density may be suitable for this region. Although there was no significant ($r = .184$, $p = .380$, $n = 24$) correlation between lodging score and yield loss in this study (data not shown), it was reported that if lodging occurred at flowering, pod setting, or seed filling stage, the seed yield was decreased by 9, 34, or 26%, respectively (Saitoh et al., 2012). Therefore, further improvement of lodging resistance will be one of the future breeding objectives.

In Japan, approximately 50% of domestic soybean seeds used for edible products (not for oil and livestock feed) are consumed in the processing of tofu (MAFF, 1997–2014).

It was reported that soybean varieties with higher protein content produced a firmer and spongier tofu texture (Wang et al., 1983). The protein contents of the genotypes used in this study exceeded at least 43% (Table 6), indicating good quality for food use. Further study is needed to test the processability of each genotype for several Japanese traditional foods, such as tofu, miso, natto, and soy sauce.

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Disclosure statement

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