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# Simplified estimation of branching plasticity of soybean cultivars in relation to planting density by branch development in the row with the gradient of distance between plants and after pinching

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## ABSTRACT

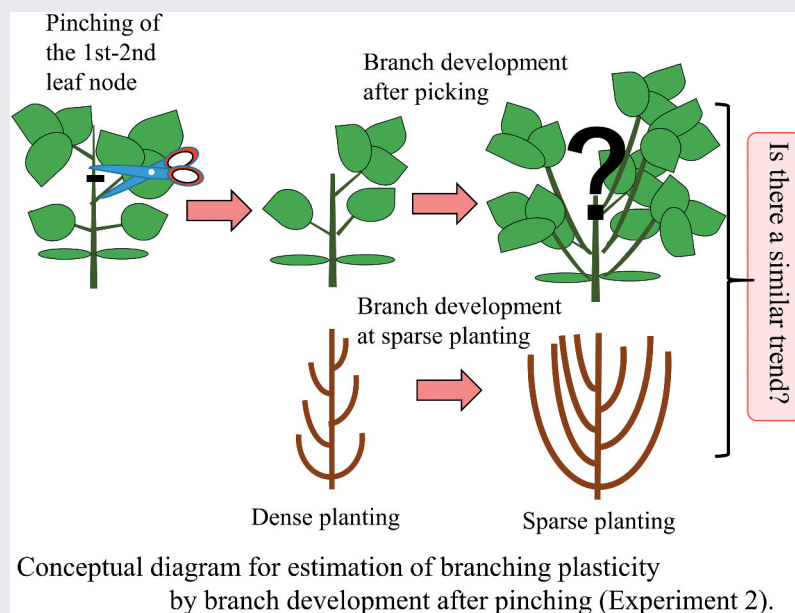
The branching plasticity of soybean, i.e. its ability to adjust branch development to planting density, differs among cultivars. Field experiments are required to measure the degree of branching plasticity, but such experiments require a great deal of time and labor, and it is difficult to analyze and compare a large number of cultivars. A simple evaluation method needs to be established to investigate branching plasticity for a wide range of materials. Therefore, we conducted two methods to estimate this value. In the first method, we investigated the relationship between the number of branching nodes and intra-row planting distance with a gradient of distance between plants (5, 7.5, 10, 15, 20, 25, and 30 cm). The slope of the regression line between these two factors revealed significant differences among soybean cultivars, and was correlated with the measured values of branching plasticity determined in field experiments in 2015 and 2017. In the second method, the top of the stem was pinched out between the first and second leaf nodes at the V4 stage, and then the number of branch nodes was counted at maturity. There were differences in the number of branching nodes among cultivars, and a significant positive correlation between these values and the branching plasticity values measured in the 2015 and 2017 field experiments. Considering the time and effort required for field management and morphological surveys, the pinching method is considered to be an effective and simple method to evaluate branching plasticity.

## ARTICLE HISTORY

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## Introduction

Since 1950, Japanese soybean yields have shown little improvement, averaging only  $1.7 \text{ t ha}^{-1}$  nationwide, while the average yield of U.S. soybean cultivars has continued to increase over the past 50 years. In northern production areas, soybean yields have exceeded  $3.0 \text{ t ha}^{-1}$ , and the gap between U.S. and Japanese soybean yields continues to widen (ARS, 1997; Katsura et al., 2011). According to Yoshihira et al. (2009), the yield differences between Japanese and U.S. soybean cultivars in Hokkaido are greater under sparse planting. This is because the U.S. cultivars branch vigorously under sparse planting, leading to increased numbers of branch nodes and increased branch yield, which stabilizes the total yield because it compensates for the decrease in main stem yield under sparse planting. In contrast, Hokkaido cultivars show smaller increases in the number of branch nodes under sparse planting, resulting in a reduction in total yield.

Agudamu et al. (2016) defined branching plasticity as the ability of soybean to exhibit a density response that maintains stable yield by adjusting or suppressing branch growth under dense planting, while vigorously branching under sparse planting with increased branch yield compensating for the reduced main stem yield. The degree of branching plasticity is quantified as the ratio of the number of branch nodes under sparse planting to that under dense planting. Furthermore, according to Yoshihira et al. (2020), branching plasticity values were correlated between years and found to be positively and significantly correlated, with varietal differences in branching plasticity values being stable between years, although they varied with annual weather conditions.

Soybean cultivars with high branching plasticity have higher ability to compensate for seed yield in an environment where the number of nodes per area are insufficient, such as in the case of missing plants (Ozaki et al., 2015), late sowing due to long rains, uneven planting density due to poor machine sowing accuracy, and reduced branching due to growth failure cold injury (Yamamoto & Narukawa, 1972) in northern Japan. It is also considered to be yield compensator under sparse planting conditions when high potting soil is required to prevent moisture damage and wide row spacing (Kitayoshi et al., 2015) is necessary, or when seeding quantity is reduced to lower seed cost.

Branching plasticity is strongly related to the maturity group (MG) and stem growth habit (i.e. determinate or indeterminate) of soybean. Branching plasticity tends to be higher in late-maturing cultivars than in early ones; and higher in indeterminate cultivars than in determinate ones (Yoshihira et al., 2020). Whether branching plasticity differs among cultivars with the same stem

growth habit and in the same MG has not yet been fully investigated.

Field experiments with three or more levels of planting density are essential to investigate branching plasticity. It is very difficult to investigate varietal differences in branching plasticity among many cultivars simultaneously, because field experiments require a large area and a great deal of labor and time. Therefore, it is necessary to develop a simple survey method to evaluate the degree of branching plasticity and to link this to future breeding objectives.

Previous studies have focused on differences in the planting density (Wilcox, 1977; Miura & Gemma, 1986; Nakaseko & Gotoh, 1981) and the planting pattern (Ikeda, 1992; Miura et al., 1987; Moore, 1991; Tobitani et al., 2022) response of soybean cultivars. They found that the yield responses were greater when the distance between plants (planting density) was changed than when row width or row direction was changed. Therefore, if a large number of soybean cultivars and lines can be grown without missing plants in rows with a gradient of spacing between plants, but a constant row width, it is possible to evaluate the planting density response (i.e. number of branch nodes) of soybean cultivars, and then compare this parameter among multiple cultivars.

Ozaki et al. (2013) used two determinate and two indeterminate cultivars planted in rows with a gradient of distance between plants. They detected varietal differences in soybean branch development with respect to planting distance. These differences were reflected by the slopes of the regression lines between planting distance and branch length, number of branch nodes, and number of branch pods. The degree of branching plasticity in a field planting density experiment can be estimated using that method. However, to our knowledge, no previous studies have examined the relationship between branching plasticity measured in a planting density field experiment and branching plasticity estimated on the basis of plants grown in rows with a gradient distance between plants, taking into account year-to-year differences, and comparing many cultivars simultaneously.

Many studies have reported varietal differences of determinate cultivars (Hayashi et al., 2008, 2015; K. Watanabe et al., 2014; Kikuya et al., 2013; Nakamura & Yokoh, 1985) and indeterminate cultivar (Conley et al., 2009; Nakade et al., 2018) in the yield response to pinching. Considering the need to allow sufficient space for branches to develop and for enough light to reach the leaf axils of the lower nodes, pinching at the early growth stage is common under sparse planting.

As mentioned above, cultivars with high branching plasticity in relation to planting density may have higher numbers of branch nodes per plant as a result of

vigorous branching under sparse planting. Thus, they may show a higher degree of branching after pinching compared with cultivars with low branching plasticity. According to Suzuki et al. (2014), pinching at an early growth stage under standard planting density ( $60 \times 10$  cm) breaks the apical bud dominance and promotes branch elongation. Thus, the degree of branching plasticity can be estimated in a field planting density experiment on the basis of the total branch length and number of branch nodes after pinching. To our knowledge, there are no previous reports on the relationship between branching plasticity measured in field planting density experiments and branching plasticity estimated using the pinching method, including year-to-year differences, determined for many cultivars simultaneously.

The aim of this study was to determine whether branching plasticity measured in field-based planting density experiments could be estimated accurately using a simpler method, to allow for simpler comparisons among multiple cultivars. We conducted two methods; first, we determined varietal differences in the number of branch nodes in plants grown in rows with a gradient of distance between plants (Experiment 1); and second, we determined varietal differences in the number of branch nodes at maturity after a pinching treatment (Experiment 2). The overall aim of these experiments was to develop a simplified evaluation method to investigate differences in branching plasticity among many cultivars without the need for costly and time-consuming planting density experiments.

## Materials and methods

### Cultivars and lines

The field experiments were completed in 2015 and 2017 at The Field Education and Research Station of Rakuno Gakuen University ( $43^{\circ}04'N$ ,  $141^{\circ}30'E$ ; 42.0 m above mean sea level), Ebetsu, Japan. Three Hokkaido soybean cultivars ('Toyomusume', 'Toyoharuka', and 'Yuzuru', all determinate types) and three northern US soybean cultivars ('Jack', 'Athow', and 'LD00-3309', all indeterminate types) were analyzed in both experiments. Additionally, the following three pairs of near-isogenic lines (NILs) related to stem growth habit were tested in both experiments: 'Harosoy-dt1' and 'Harosoy-Dt1' (Canadian cultivars), 'Williams-dt1' and 'Williams-Dt1' (US cultivars), and 'ST-dt1' and 'ST-Dt1' [crosses between the US and Japanese cultivars 'Stressland'  $\times$  'Tachinagaha' (ST)]. The notations '-dt1' and '-Dt1' indicate determinate and indeterminate types, respectively.

'Toyomusume' is known for its stable and high yields, whereas 'Toyoharuka' is cultivated in Hokkaido because

of its lodging resistance. Both of these cultivars have similar maturity dates (MG I). 'Yuzuru' is cultivated in southern Hokkaido and produces large seeds. 'Jack' is a soybean cultivar traditionally cultivated in the midwestern states of the USA. 'Yuzuru' and 'Jack' were selected because of their similar maturity (MG II). 'Athow' and 'LD00-3309' are new high-yielding cultivars grown in the midwestern states of the USA (MG III and IV, respectively).

### *Simplified evaluation of branching plasticity in rows with a gradient of distance between plants (Experiment 1)*

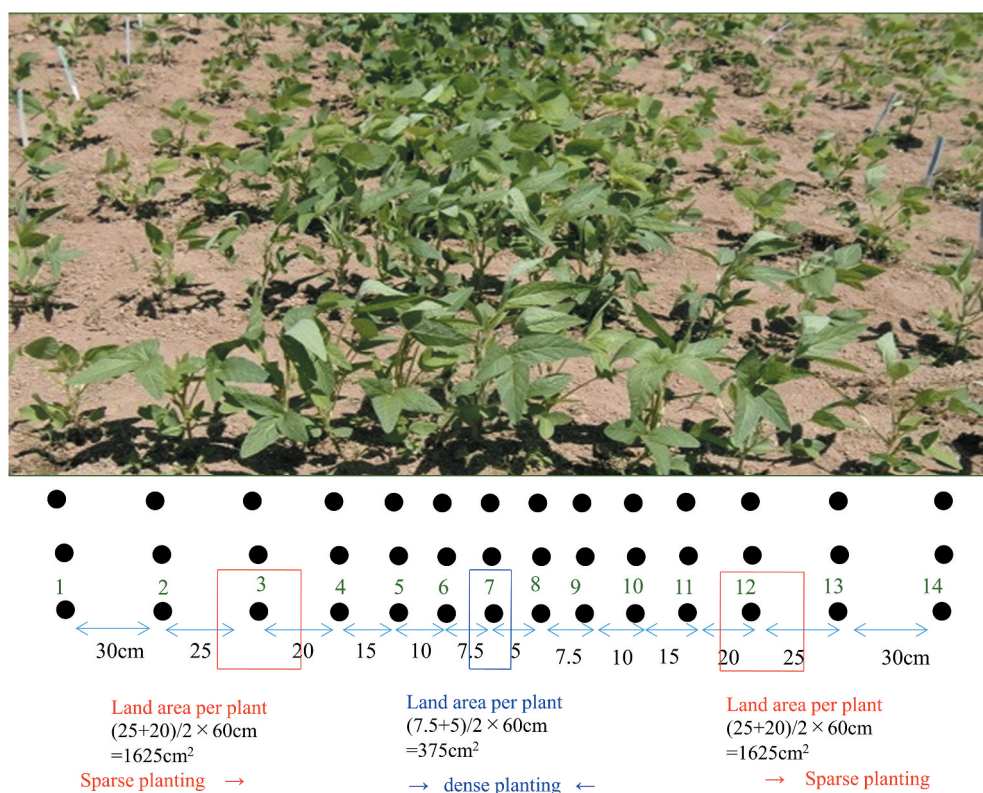
In 2015 and 2017, plants were grown in rows with a gradient of distance between plants (intra-row spacing). Within each row, the plants were symmetrically arranged with the distance between plants set at 30, 25, 20, 10, 7.5, 5, 7.5, 10, 20, 25, and 30 cm. The distance between rows was 60 cm (Figure 1). Three such rows were planted for each cultivar and line.

The fertilization schedule was as follows: calcium carbonate ( $100 \text{ g m}^{-2}$ ) was applied as a soil amendment before basal fertilizer in both years. Nitrogen (N), phosphate ( $P_2O_5$ ), and potassium oxide ( $K_2O$ ) were applied according to the Hokkaido fertilizer standard: nitrogen as ammonium sulfate at  $2 \text{ g m}^{-2}$ , phosphate as lime superphosphate at  $14 \text{ g m}^{-2}$ , and potassium as potassium sulfate at  $8 \text{ g m}^{-2}$ . In 2015 and 2017, calcium carbonate was applied on April 15<sup>th</sup> and 18<sup>th</sup>, respectively; nitrogen, phosphate, and potassium were applied on May 8<sup>th</sup> and 7<sup>th</sup>, respectively; and seeds were sown on May 8<sup>th</sup>. In both years, two seeds were sown per hill.

When 1st trifoliate leaves emerged at the V1 stage (Fehr & Caviness, 1977), the seedlings were thinned to one plant per hill. Seedlings were transplanted from adjacent hills to vacant hills to ensure that there were no missing plants on May 28<sup>th</sup> in 2015 and on May 31<sup>st</sup> in 2017. Weeds were removed manually up until the flowering stage. Seeds were treated with thiamethoxam to protect plants from pests (e.g. seedcorn flies, aphids, and cutworms). Metalaxyl and fludioxonil emulsions were applied in late July and early August on an as-required basis to protect the plants from Japanese beetles and aphids.

Ozaki et al. (2014) analyzed branching plasticity in four soybean cultivars, and determined the slope of the regression between branch traits at maturity and the distance between plants in rows with a gradient of intra-row spacing. Across the four cultivars, the correlation coefficients were significantly higher between the field-measured branching plasticity values and total branch length and number of branch nodes than between field-measured





**Figure 1.** Plant placement of soybean in the row with gradient of the distance between plants at the V3 stage (Experiment 1). ● indicates a soybean plant.

branching plasticity values and other branch traits. Therefore, in this study, as not to underestimate the degree of branching plasticity in central Hokkaido, either in late-maturing cultivars that do not mature or when lodging occurs, we followed another previous reports (Agudamu et al., 2016; Yoshihira et al., 2020) and focused on the number of branch nodes.

Each plant was harvested at the R8 stage (Fehr & Caviness, 1977). We measured the number of branch nodes, and determined the regression coefficient between the number of branch nodes and the distance between plants to estimate the branching plasticity value. We examined the correlation between this value and field-measured branching plasticity value to verify the accuracy of the estimates (Figure 4).

### ***Simplified evaluation of branching plasticity by the pinching method (Experiment 2)***

Suzuki et al. (2014) grew two determinate and two indeterminate cultivars at 60-cm row width and 10 cm intra-row spacing, and pinched out the tip of the plant between the first and second, second and third, or third and fourth leaf nodes at the V3, V4, and V5 stages (Fehr & Caviness, 1977) to encourage branch development. The branch length, number of branch nodes, and branch weight were

measured in each pinching treatment, and these values were used to calculate the branching plasticity value.

In this study, two plot types were established in Experiment 2. Pinching was conducted between the first and second leaf nodes in the pinching plot (P) and plants were not pinched in the control plot (C). Similar to Experiment 1, we focused on the number of branch nodes, which is easy to discuss in relation to yield. We attempted to determine whether branching plasticity can be estimated on the basis of the number of branch nodes in C, and/or in P, and/or the ratio between these values (P/C).

Pinching was conducted at the V3 stage (Fehr & Caviness, 1977) in both 2015 and 2017. To prevent the spread of viruses, pruning shears were sprayed with an 8% v/v solution of sodium hypochlorite between plots. The experiment arrangement was a split-plot method with three replications, with cultivar as the main plot and the pinching treatment as the subplot.

All other cultivation methods in Experiment 2 were the same as in Experiment 1. We recorded the number of branch nodes by node position, by main stem and branching at R8 stage, as well as seed yield and yield components. After the air-dried weight of the seeds was measured, the moisture content was measured using a grain moisture meter (PM480, Mettler Toledo, Switzerland) and corrected to 15% moisture.

We counted the number of branch nodes at the R8 stage in the C and P plots, calculated the ratio of the number of branch nodes between the C and P plots, and determined the correlation between these values and the branching plasticity values measured in the field planting density experiment to verify the accuracy of the estimates (Figure 6).

Data were subjected to analysis of variance (ANOVA) and Tukey's multiple comparison test using statistical free software (STAR). The meteorological data were obtained using a meteorological observation device from Rakuno Gakuen University.

### ***Varietal differences in branching plasticity determined in field planting density experiments in previous studies***

In 2012 and 2013, six cultivars and six NILs for stem growth habit were sown at three planting densities ( $60 \times 7.5$ , 10, and 20 cm) in the field (Yoshihira et al., 2020). The degree of branching plasticity in the field planting density experiments (field branching plasticity value) was measured as the ratio of the number of branch nodes under sparse planting ( $60 \times 20$  cm) to that under dense planting ( $60 \times 5$  cm) according to the method of Agudamu et al. (2016). The field branching plasticity values are shown in Table 2 (Yoshihira et al., 2020). We then determined the correlations between these field-measured branching plasticity values and those estimated based on the simplified evaluation in the row with a gradient of distance between plants (Experiment 1) and after pinching (Experiment 2) to assess the suitability of each simplified method.

## **Results**

### ***Varietal differences in branching plasticity determined in field planting density tests in previous studies and meteorological conditions during field experiments***

Details of the temperature, precipitation, and solar radiation during the 2015 and 2017 field experiments are

shown in Table 1. The precipitation and solar radiation throughout the growing season were higher in 2017 than in 2015. Compared with other months, July had higher temperatures, slightly more rainfall, and more sunshine. These meteorological conditions were conducive to branch development, which was particularly vigorous during July.

Table 2 shows the branching plasticity values obtained in our previous field planting density study (Yoshihira et al., 2020) in 2012 and 2013, averaged over the 2 years. The field branching plasticity values in those 2 years were higher for the indeterminate cultivars than for the determinate cultivars. For the NILs with different stem growth habits, the branching plasticity values were higher for the indeterminate lines than for the determinate lines.

The branching plasticity of 'Yuzuru' was higher than that of 'Toyomusume' and 'Toyoharuka' among the determinate cultivars; and the branching plasticity of 'LD3309' was higher than that of 'Jack' and 'Athrow' among the indeterminate cultivars. Comparing the NILs, the branching plasticity of 'Harosoy-Dt1' was higher than that of the other indeterminate lines; and that of 'ST-dt1' was higher than that of the other determinate lines.

### ***Number of branch nodes on plants within a row with a gradient of distance between plants (Experiment 1)***

Figure 2 shows the varietal differences in branch development in relation to land area per plant in rows with gradient of distance between plants (Experiment 1). The increase in the number of branches, branch length, and branch pods with increasing land area per plant was greater for the indeterminate cultivars 'Athrow' and 'Jack' than for the determinate cultivars, and greater for the determinate cultivar 'Yuzuru' than for the other determinate cultivars 'Toyomusume', 'Toyoharuka', and 'Harosoy-dt1'.

The positive and significant correlations between land area per plant and number of branch nodes (Figure 3(a,b))

**Table 1.** Temperature, Precipitation, and Solar Radiation (2015, 2017).

Month	Air temperature		Precipitation		Solar radiation	
	2015	2017	2015	2017	2015	2017
	(°C)		(mm)		(MJ m <sup>-2</sup> )	
May	12.7	12.5	27	58	695	629
June	15.2	14.4	100	175	555	604
July	17.6	20.9	19	103	287	608
August	20.5	19.5	95	107	541	539
September	16.7	16.1	184	191	400	447
October	9.4	10.2	79	84	326	314
Total	15.4	15.6	503	717	2803	3141

**Table 2.** Field branching plasticity values and the estimated branching plasticity by the row with the gradient distance between plants (RGTP, Experiment 1) and the pinching method (Experiment 2).

Cultivar	Stem growth habit	Field branching plasticity	Estimated branching plasticity <sup>b</sup>	
			RGTP <sup>c</sup>	Pinching method <sup>d</sup>
Average of the year <sup>a</sup>				
Toyoharuka	D	0.76 d	13.62 g	274 f
Toyomusume	D	0.79 d	14.70 fg	293 ef
Yuzuru	D	1.24 cd	25.99 d	495 c
Athow	I	1.76 b	37.90 bc	547 b
Jack	I	1.88 b	41.02 b	586 b
LD00–3309	I	2.20 a	50.00 a	775 a
Harosoy-dt1	D	0.95 d	19.21 f	330 de
Harosoy-Dt1	I	1.66 b	34.41 c	573 b
Williams-dt1	D	0.89 d	16.04 fg	363 d
Williams-Dt1	I	1.35 c	32.59 cd	582 b
ST-dt1	D	1.00 cd	23.34 de	345 de
ST-Dt1	I	1.39 c	32.50 cd	580 b
Mean of cultivars				
First year		1.24 b	25.5 b	471 b
Seconde year		1.50 a	29.9 a	521 a
Year		*	ns	†
Cultivar		***	***	***
Year×Cultivar		***	**	**

a: Field branching plasticity values are were taken from Yoshihira et al. (2020) as averages of 2012 and 2013.

b: Branching plasticity estimates are averages of 2015 and 2017.

RGTP: Row with gradient distance between plants.

c: Slope of the regression line of the number of branch nodes with respect to planting density in the row with the gradient distance between plants in Experiment 1.

d: Number of branch nodes in the plots in Experiment 2.

D: Determinate type, I: Indeterminate type.

†, \*, \*\*, \*\*\* indicate significant differences at 10, 5, 1, 0.1% level, respectively.

Different alphabets indicate that there is a significant difference at the 5% significance level between cultivars.

were obtained for all cultivars in both 2015 and 2017. The slope of the regression line between land area per plants and the number of branch nodes was greater for the indeterminate cultivars ('Jack', 'Athow', and 'LD3309') than for the determinate cultivars ('Toyomusume', 'Toyoharuka', and 'Yuzuru'); and greater for 'Yuzuru' in MGII than for 'Toyoharuka' and 'Toyomusume' in MGI.

The distance between plants was positively and significantly correlated with the number of branch nodes (Figure 3(c,d)) in all lines in both 2015 and 2017. We calculated the slope of the regression line between land area per plant and number of branch nodes in the NILs. The slope of this regression line was greater in the indeterminate lines than in the determinate lines 'Harosoy', 'Williams', and 'ST'. Thus, the estimates of branching plasticity values from Experiment 1, i.e. the slope of the regression between the number of branching nodes and the distance between plants in the row, was greater for the indeterminate US cultivars than for the Hokkaido determinate cultivars.

The estimated branching plasticity values were higher for 'LD3309' than for 'Athow' and 'Jack' among the indeterminate cultivars, and higher for 'Yuzuru' than for 'Toyomusume' and 'Toyoharuka' among the determinate cultivars (Table 2). Comparing the NILs with different stem

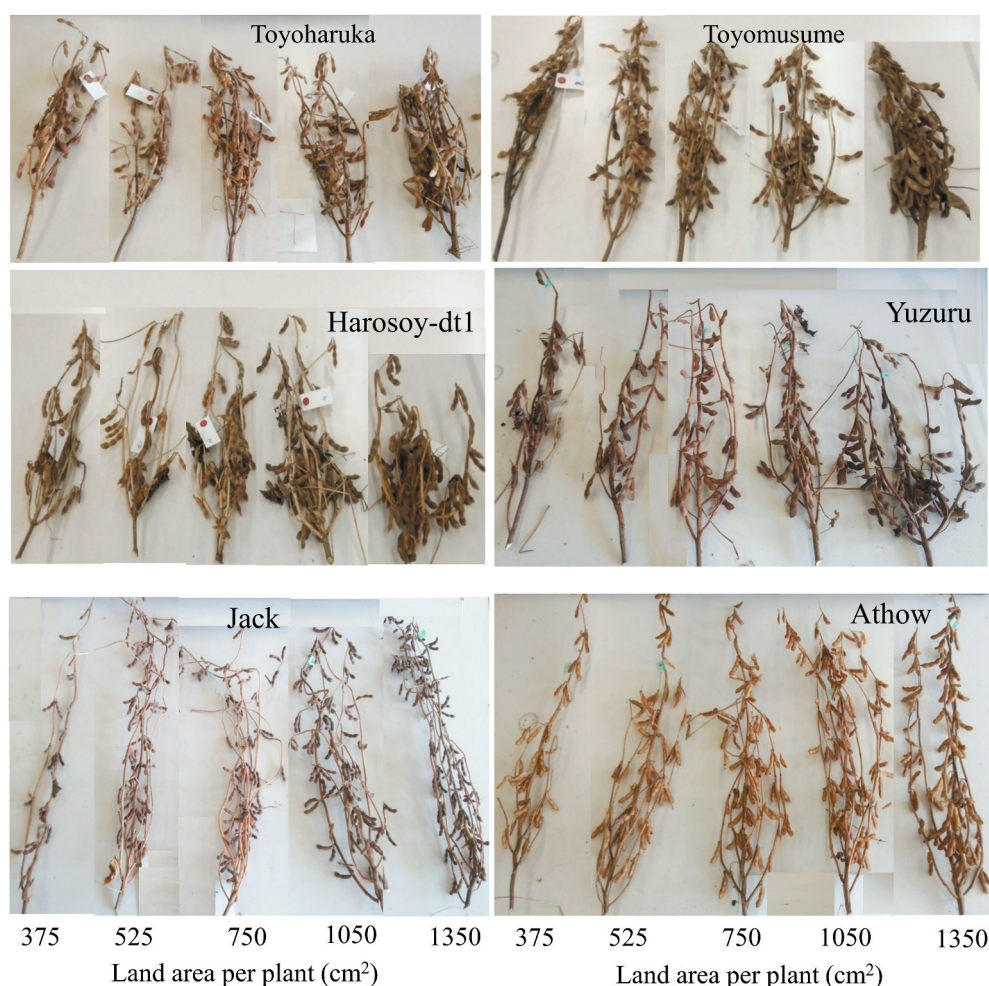
growth habit, the indeterminate lines had higher estimated branching plasticity values, and the determine lines ('Harosoy' and 'Williams') had lower ones. However, the differences between indeterminate and determinate lines was small. The analysis of variance revealed significant differences in estimated branching plasticity values among cultivars and a significant year × cultivar interaction.

### **Relationship between measured branching plasticity values and those estimated from plants grown in rows with a gradient of intra-row spacing**

Figure 4(a,b) show the relationships between the branching plasticity estimated in Experiment 1 (by growing plants with variable intra-row spacing) and the branching plasticity measured in the field experiment. We detected significant positive correlations between the estimated and measured values in both 2015 ( $r = 0.97^{***}$ ) and 2017 ( $r = 0.97^{***}$ ).

Significant positive correlations between these two factors were also found separately for determinate and indeterminate cultivars in both 2015 ( $r = 0.83^*$  and  $0.92^{**}$ , respectively) and 2017 ( $r = 0.85^*$  and  $0.94^{***}$ ,





**Figure 2.** Varietal differences in branch development in relation to land area per plant in rows with gradient of distance between plants (Experiment 1, 2015).

respectively). Significant positive correlations ( $r = 0.98^{***}$ ) were also found between the estimates of branching plasticity in the row with the in the distance between plants in 2015 and 2017 (Figure 4(c)). Furthermore, a positive and significant correlation ( $r = 0.94^{**}$ ,  $0.93^{**}$ ) was also found between them separately for determinate and indeterminate cultivars.

#### **Estimation of branching plasticity based on total branch length and number of branch nodes by node position after pinching (Experiment 2)**

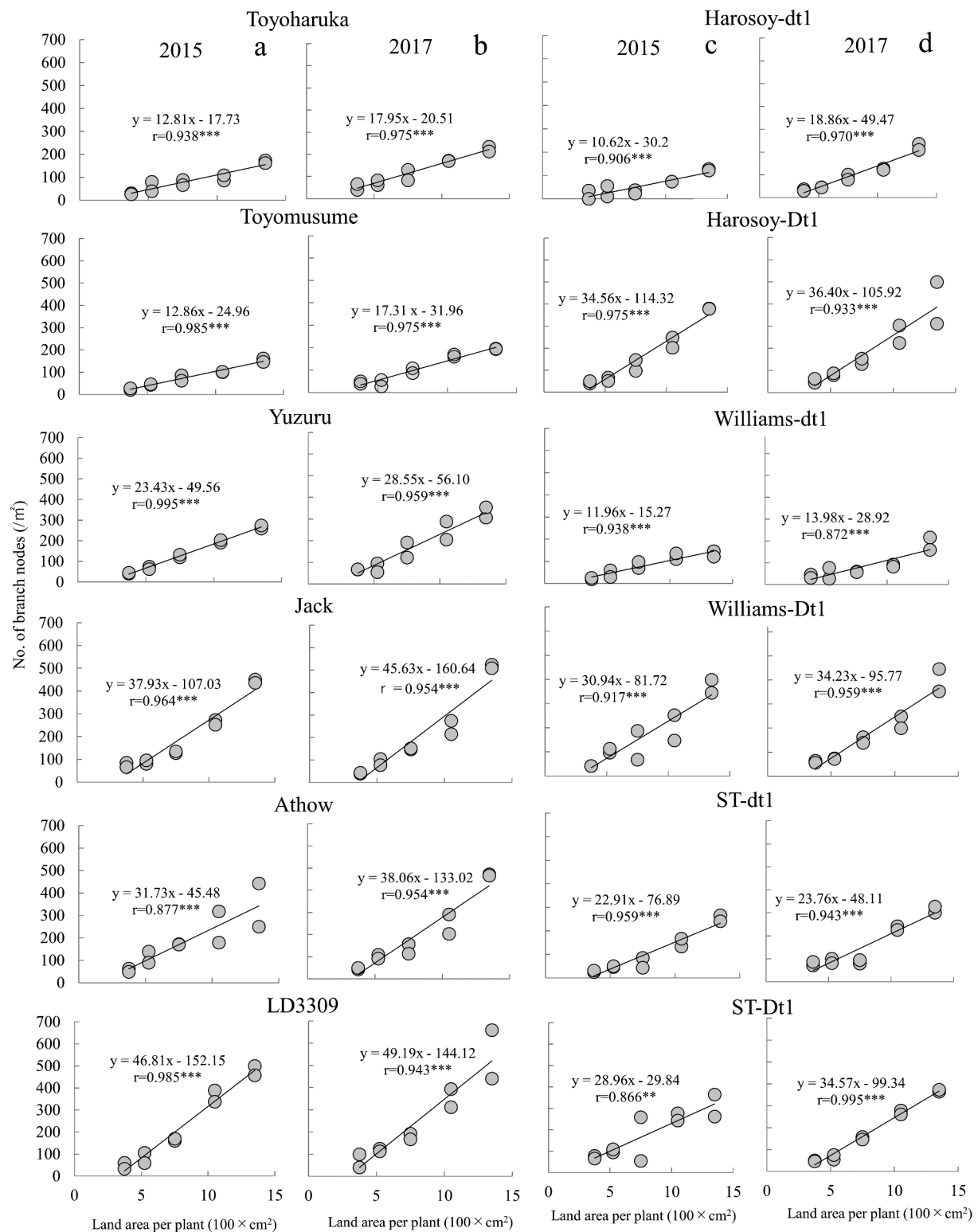
Figure 5 shows the differences among cultivars in branch development at maturity after pinching at the V3 stage. In the P treatment, the total branch length was longer in the indeterminate cultivars than in the determinate cultivars. Total branch length was increased in all indeterminate cultivars, but did not change significantly in determinate cultivars, compared with total stem length (sum of main stem length and branch length) in

C. Pinching resulted in decreased total branch length in 'Toyoharuka' and 'Toyomusume'.

#### **Seed yield and total number of nodes after pinching (Experiment 2)**

Table 3 summarizes the seed yields and number of nodes in each cultivar in C and P, as determined in Experiment 2. Yields did not differ between C and P for the US-bred indeterminate cultivars. In contrast, the seed yields were significantly higher in C than in P for the Hokkaido-bred determinate cultivars and the determinate NILs. In both C and P, the seed yields were higher for indeterminate cultivars than for determinate cultivars.

Among the determinate cultivars, 'Yuzuru' had the highest seed yield and 'Toyoharuka' had the lowest. Among the indeterminate cultivars, 'LD3309' had a higher seed yield than those of 'Athow' and 'Jack'. Comparing the three NILs with different stem growth



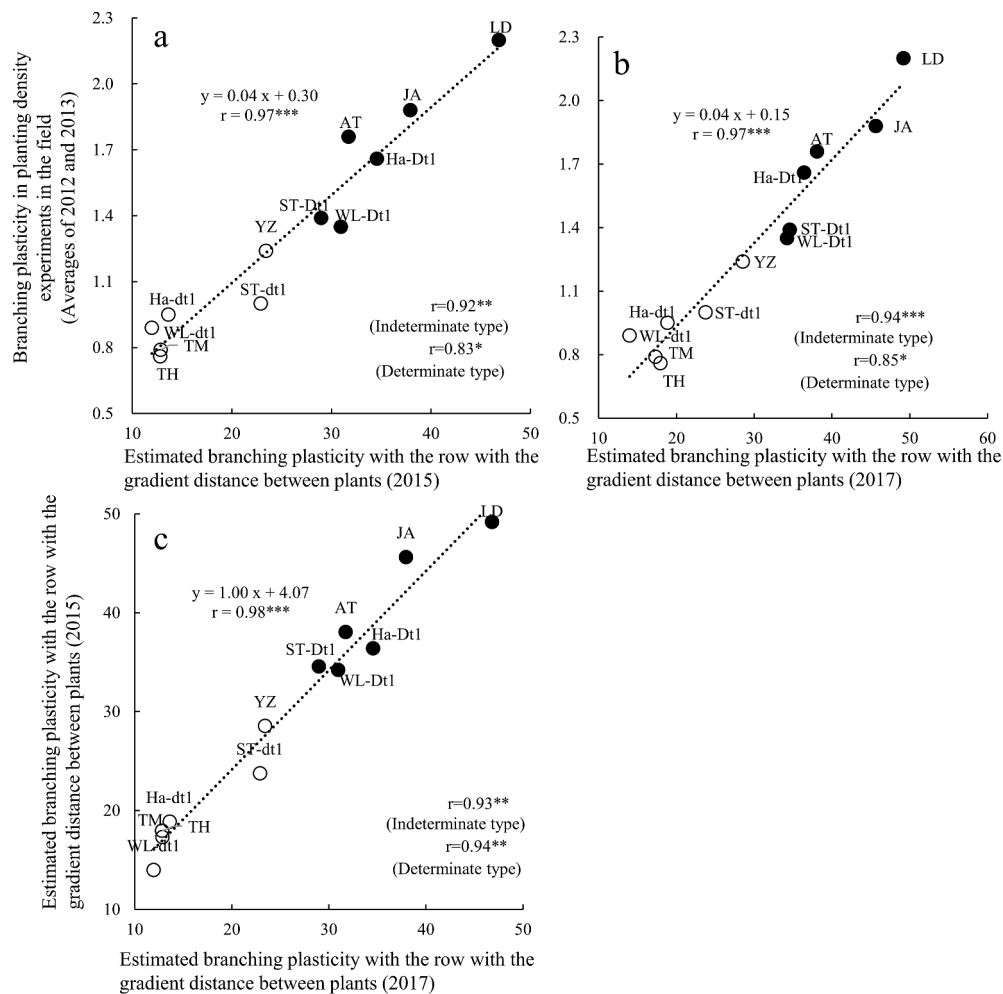
**Figure 3.** Relationship between land area per plant and the number of branch nodes in the row with the gradient distance between plants (Experiment 1). \*\*\* indicate significant differences at 0.1% level.

habit, the indeterminate lines had higher seed yields than did the determine lines in both C and P.

For seed yield, the ratio between P and C was higher for the indeterminate cultivars than for determinate

cultivars. Among the NILs, the indeterminate line of 'Williams' had a higher seed yield than that of the determinate line, but this difference was not detected in the other pairs of NILs.





**Figure 4.** Relationship between the estimated branching plasticity with the gradient distance between plants and branching plasticity values in the field planting density experiment (2015, 2017) and the relationship between the estimated branching plasticity in both years (Experiment 1). ○:Determinate type, ●: Indeterminate type TM,TH, YZ:Toyomusume, Toyoharuka, Yuzuru (Hokkaidou cultivars) AT, JA, LD:Athow, Jack, LD3309 (US cultivars) Ha-dt1, Ha-Dt1: Harosoy-dt1, Harosoy-Dt1, WL-dt1, WL-Dt1: Williams-dt1, Williams-Dt1 ST-dt1, ST-Dt1: Stressland×Tachinagaha-dt1, Stressland×Tachinagaha-Dt1 (Near isogenic lines related determinate and indeterminate type) \*, \*\*, \*\*\* indicate significant at the 5, 1, 0.1% levels, respectively.

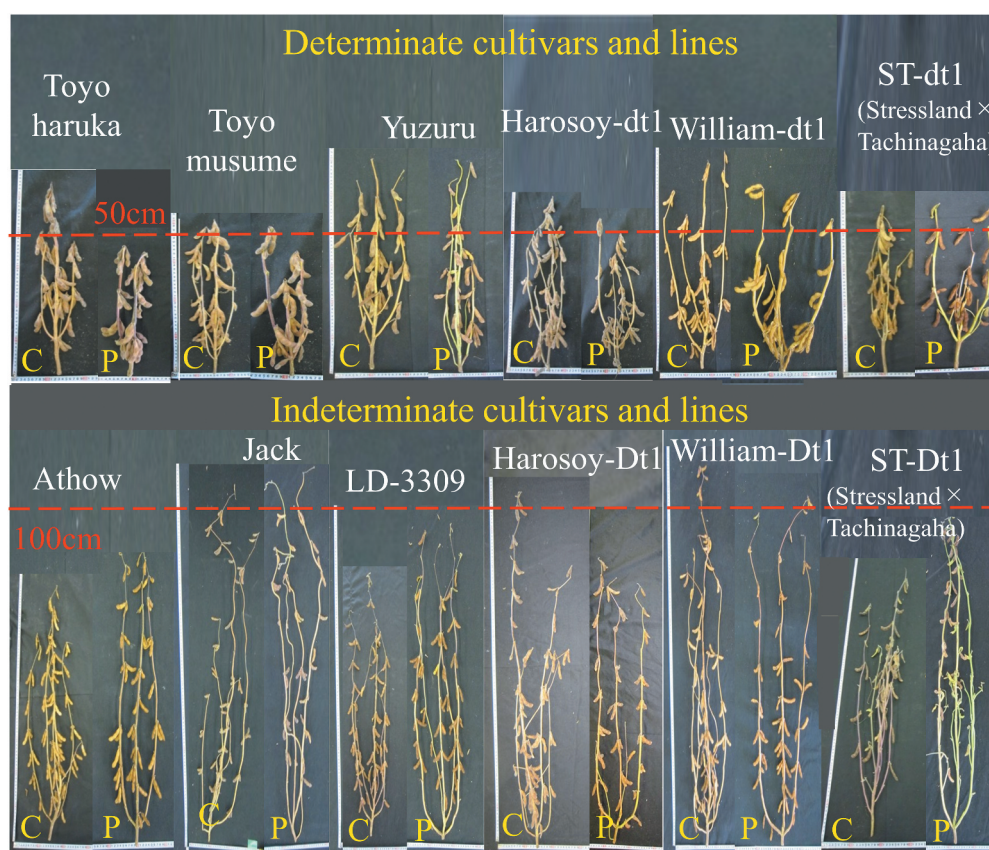
The number of nodes per land area was significantly higher in C than in P for all cultivars and lines. In both C and P, the number of nodes was higher in the high-yielding US-bred indeterminate cultivars than in the Hokkaido-bred determinate cultivars. The number of nodes per land area was higher in the indeterminate lines than in the determinate lines, as was the yield in all the NILs. However, there was no consistent trend in the number of nodes between P and C in relation to stem growth habit.

Two-way analysis of variance revealed significance differences among cultivars in the number of nodes in both C and P; and in P/C. We detected significant year × cultivar interactions in these analyses. The number of nodes in C differed significantly between 2015 and 2017, the P/C differed between 2015 and 2017, but the number of nodes in P did not differ significantly between 2015 and 2017.

#### **Relationship between branching plasticity estimated using pinching method and branching plasticity values measured in field planting density experiment (Experiment 2)**

Figure 6 shows the relationships between the measured field branching plasticity values and the number of branch nodes in P, the number of branch nodes in C, and P/C.

We detected positive and significant correlations ( $r = 0.95^{***}$  and  $0.79^{**}$ ) between the number of branch nodes in P and C, respectively, and measured field branching plasticity in 2015 (Figure 6(a,b)). We also detected significant positive trends between the measured branching plasticity values and the number of branch nodes in P for both determinate and indeterminate cultivars ( $r = 0.78^{\dagger}$  and  $0.83^{*}$ , respectively). There



**Figure 5.** Varietal differences in branching development to maturity by pinching treatment at the V3 stage (Experiment 2, 2015). C: Control, P: Pinching

was no correlation between measured field branching plasticity values and P/C (Figure 6(c)).

The measured field branching plasticity was positively and significantly correlated with the number of branch nodes in P and C ( $r = 0.88^{***}$  and  $0.79^{**}$ , respectively) in 2017 (Figure 6(d,e)). Significant positive trends ( $r = 0.75^{+}$  and  $0.73^{+}$ ) were also found between the measured field branching plasticity and number of branch nodes in P for determinate and indeterminate cultivars, respectively. However, no correlation was found between measured field branching plasticity and P/C in 2017 (Figure 6(f)), as was the case in 2015.

In both 2015 and 2017, the measured branching plasticity values showed the strongest correlations with the number of branch nodes in P ( $r = 0.96^{***}$  and  $0.99^{***}$ , respectively) (Figure 6(g,h)). The differences in the number of branch nodes among cultivars were detectable in both years in both P and C.

We detected a significant positive correlation ( $r = 0.70^{*}$ ) between P/C and the number of branch nodes across both years (Figure 6(i)). However, the P/C values were less stable among years than were the numbers of branch nodes in P and C.

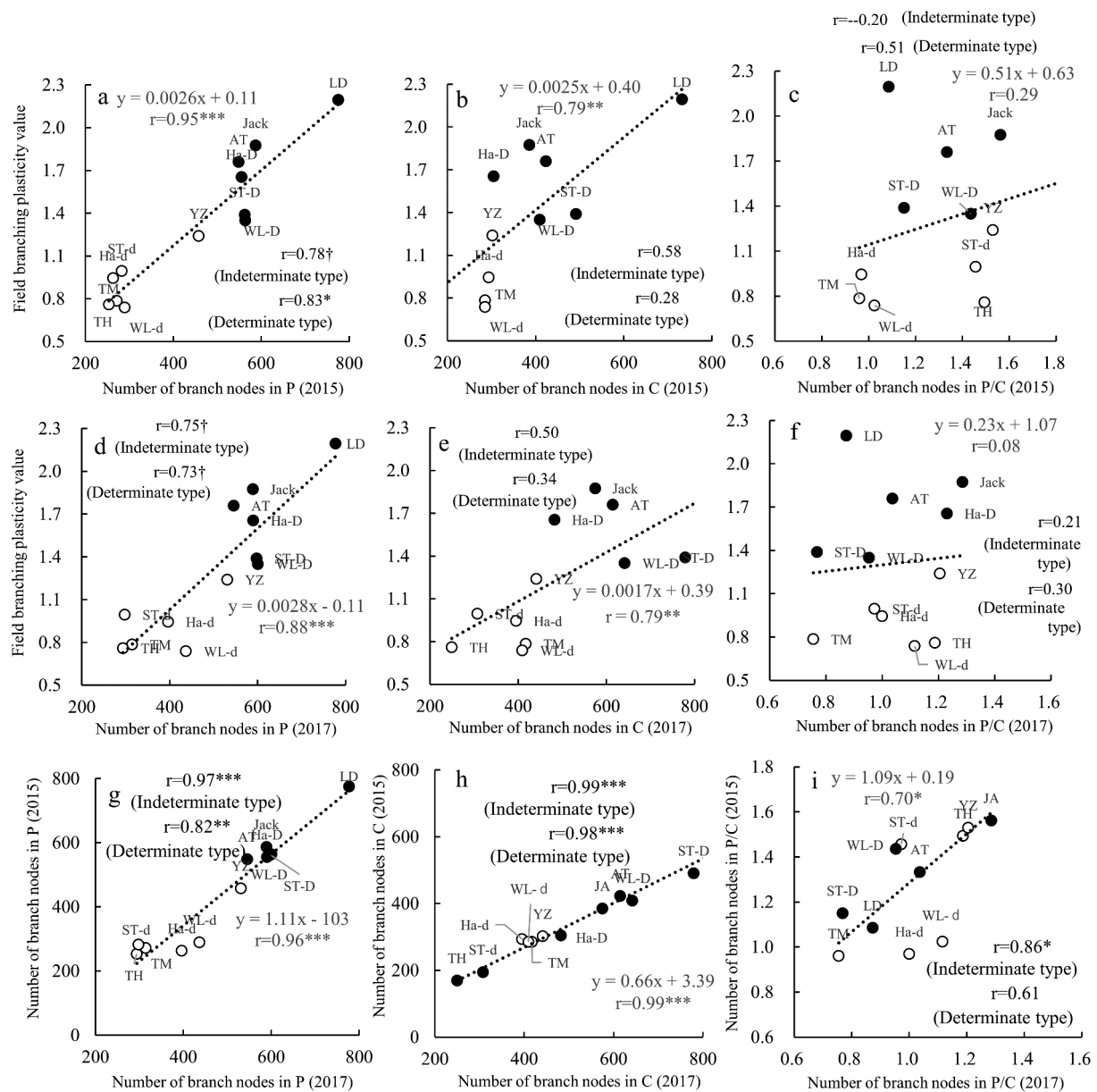
## Discussion

### *Year-to-year differences in branching plasticity estimates using simplified evaluation methods*

Comparing the estimates of branching plasticity in the two years, the slope of the regression line between the number of branch nodes in the row with a gradient distance between plants (Experiment 1) was greater in 2017 than in 2015 for most cultivars (Figures 3). Similarly, in Experiment 2, the total number of branch nodes per plant in P was higher in 2017 than in 2015 for most cultivars. These common year-to-year differences in branching plasticity estimates may be because July in 2017 had higher temperatures, slightly more rainfall, and more sunlight, compared with July in 2015. These climatic conditions were more conducive to branch development in July, the period when branch development was most vigorous.

### *Simplified evaluation of branching plasticity based on plants in rows with a gradient of intra-row spacing (Experiment 1)*

Wilcox (1977) and Moore (1991) analyzed varietal differences in the planting density responses of soybean by



**Figure 6.** Relationship between the measured field branching plasticity values and the number of branch nodes in P (Pinching), the number of branch nodes in C (Control), and P/C (Experiment 2). C: Control, P: Pinching. ○: Determinate type, ●: Indeterminate type. Field branching plasticity value indicates the average of 2012 and 2013 branching plasticity values in field planting density trials in a previous report (Yoshihira et al., 2020). TM, TH, YZ: Toyomusume, Toyoharuka, Yuzuru (Hokkaido cultivars) AT, JA, LD: Athow, Jack, LD3309 (US cultivars) Ha-dt1, Ha-Dt1: Harosoy-dt1, Harosoy-Dt1, WL-dt1, WL-Dt1: Williams-dt1, Williams-Dt1 ST-dt1, ST-Dt1: Stressland × Tachinagaha-dt1, Stressland × Tachinagaha-Dt1. (Near isogenic lines related determinate and indeterminate type) †, \*, \*\*, \*\*\* indicate significant at the 10, 5, 1, 0.1% levels, respectively.

considering it as the response to the distance between plants, without considering hill distance or row spacing. According to Duncan (1986), there are three phases in the planting density response of soybean: phase I, where yield increases in all cultivars when planting density is increased; phase II, when yield increases when planting density is increased, but the magnitude differs among cultivars; and phase III, where yield does not increase or decreases when planting density is increased. According to Lee et al. (2008)

and Parvez et al. (1989), the planting density response of soybean cultivars is greatest at 8 to 25 plants/m<sup>2</sup>, or 400 to 1250 cm<sup>2</sup> land area per plant. In this study, the number of branch nodes varied widely in plants grown with an area of 400 to 1,250 cm<sup>2</sup> (phase II according to Duncan's theory) in rows with a gradient of intra-row spacing. These results show that measuring the number of branch nodes per area in plants grown with a gradient of intra-row spacing could effectively substitute for a full-scale field experiment.

**Table 3.** Comparison of seed yield and number of nodes in control and pinching treatment (Experiment 2).

Table 1. Comparison of seed yield and number of nodes in control and pinching treatment (Experiment 2)										
Cultivar	Growth habit	Seed yield (g/m <sup>2</sup> )				Number of nodes (/m <sup>2</sup> )				
		C	P	Significance between C and P	P/C	C	P	Significance between C and P	P/C	
Average of 2015 and 2017										
Toyoharuka	D	397 d	312 e	**	0.79 ef	411 h	324 g	*	0.79 bc	
Toyomusume	D	412 d	346 d	*	0.84 def	536 g	343 fg	**	0.64 f	
Yuzuru	D	454 bc	348 d	**	0.77 f	668 e	545 d	*	0.82 b	
Athow	I	472 b	460 b	ns	0.98 ab	823 d	597 c	***	0.73 de	
Jack	I	448 c	467 b	ns	1.04 a	798 d	638 b	*	0.80 b	
LD3309	I	562 a	528 a	ns	0.94 bc	1253 a	826 a	***	0.66 ef	
Harosoy-dt1	D	366 e	309 e	**	0.84 de	520 g	380 ef	**	0.73 cd	
Harosoy-Dt1	I	454 bc	400 c	*	0.88 cd	681 e	623 bc	*	0.91 a	
Williams-dt1	D	442 c	344 d	**	0.78 ef	605 f	413 e	**	0.68 def	
Williams-Dt1	I	471 b	419 c	*	0.89 cd	868 c	632 bc	**	0.73 cd	
ST-dt1	D	352 e	307 e	*	0.87 cd	434 h	370 f	*	0.85 ab	
ST-Dt1	I	436 c	399 c	*	0.91 bcd	972 b	630 bc	***	0.65 f	
Average of cultivars										
2015		407 b	364 b		0.89 a	607 b	501 b		0.83 b	
2017		471 a	409 a		0.86 a	821 a	553 a		0.69 a	
Year		***	*		ns	***	ns		**	
Cultivar		***	**		*	**	***		*	
Year×Cultivar		**	***		*	**	*		*	

C: Control, P: Pinching.

D: Determinate type, I: Indeterminate type.

\*, \*\*, \*\*\* indicate significant differences at 5, 1, 0.1% level, respectively. NS indicate no significant differences.

Different alphabets indicate that there is a significant difference at the 5% significance level between cultivars.

This simplified evaluation of branching plasticity based on the number of branch nodes in plants grown with a gradient of intra-row spacing accurately estimated varietal differences in branching plasticity, compared with values measured in field planting density experiments in both 2015 and 2017, for both determinate and indeterminate cultivars. In addition, the differences between 2015 and 2017 were relatively small, even though the branch development environment, i.e. the climatic conditions in July, were very different, suggesting that this is a promising and stable simplified evaluation method that is not readily affected by climatic conditions.

To reproduce planting density responses using this method based on a gradient of intra-row spacing, missing plants are not allowed and sowing must be very accurate. In the case of missing plants, replanting must occur promptly, by the 1st trifoliate leaf stage (stage VI, Fehr & Caviness, 1977), so that the replanted plants are sufficiently vigorous. If the gradient of distance between plants cannot be ensured, the continuous change in branch development with respect to the distance between plants cannot be accurately determined.

Therefore, it is essential to carry out careful sowing and supplementary planting. Although the field experiment area can be reduced, a great deal of labor and time is required to ensure proper seedling establishment in the experimental area. In addition, a huge number of individual branching nodes must be surveyed at maturity. Therefore, as in the field-based planting density test,

this method requires a great deal of labor and time, and cannot be considered to be a labor-saving method.

### ***Simplified evaluation of branching plasticity by the pinching method (Experiment 2)***

High branching plasticity means that there is smaller change in the planting density response from dense to sparse planting. Considering the planting density response on the basis of the main stem and branching, it means that the decrease in main stem yield from dense to sparse planting is well compensated by the increase in branch yield, i.e. the strength of the branch development response to the distance between plants. This planting density response, when applied to the pinching method, can be expressed as the magnitude of the difference in branch development between C and P.

As shown in Figure 6, the measured branching plasticity value in the field planting density experiment was more strongly correlated with the number of branch nodes in P than with P/C, or with the number of branch nodes in C, even when the tested cultivars were divided into determinate and indeterminate cultivars.

This may because the number of branch nodes in C tended to be lower in cultivars with lower yields, and the P/C value tended to higher as a result of factors other than branch development caused by pinching, resulting in overestimation. Similarly, US indeterminate cultivars with high yields tended to



have higher numbers of branch nodes in C, and the P/C value tended to be lower because of factors other than branch development after pinching, leading to an underestimation. When comparing the accuracy of field branching plasticity estimates based on differences in branching between P and C, the number of branch nodes in C was significantly and positively correlated with the field-measured branching plasticity value (Figure 6). Thus, cultivars with a higher number of branch nodes under standard planting density tended to have higher branching plasticity.

However, in both 2015 and 2017, the measured field branching plasticity value was more strongly correlated with the number of branch nodes in P than with the number of branch nodes in C. In addition, the branch length was greater and the number of branch nodes at the lower nodes was higher in P than in C. There was no weak branch development from the upper nodes, so branch development was concentrated in the lower nodes and the number of branch nodes was easy to measure. For determinate cultivars, where the variation in branching plasticity among cultivars was relatively small, the P treatment was more informative than C for revealing varietal differences in branch development.

The above results suggest that a simple method for evaluating branching plasticity is to count the number of branch nodes in P. However, although the number of branch nodes in P in 2015 was strongly correlated with that in 2017 (Figure 6), the analysis of variance revealed a significant year  $\times$  cultivar interaction. Thus, the branching plasticity cannot be estimated only by a regression equation using the number of branch nodes in P with a high degree of accuracy. Instead, it would be necessary to investigate the number of branch nodes in P over multiple years to detect differences in branching plasticity among cultivars accurately.

Kakiuchi et al. (2021) detected varietal differences in the response to pinching. They found that the cultivars that did not show decreased yields, or showed increased yields, after pinching were able to secure the number of pods per plant by increasing the number of lower-order branch nodes and the number of pods per node. In this study, some cultivars showed no decrease or only a small decrease in yield in the pinching treatment, and these cultivars had a higher number of branch nodes derived from the primary node and the first leaf node in P than in C. Although the timing and site of the pinching were different, these differences among cultivars showed the same trend as that reported by Kakiuchi et al. (2021).

### ***Relationship between simplified evaluation of branching plasticity based on plants in rows with a gradient of intra-row spacing (Experiment 1) and the pinching method (Experiment 2)***

Estimates of branching plasticity tended to be higher in 2017, when environmental conditions were conducive to branching elongation, than in 2015 (Table 1). Therefore, we should originally test whether we can estimate field branching plasticity by the two methods in the same year.

However, since highly significant positive correlations between the estimates of branching plasticity based on plants in rows with a gradient of intra-row spacing (Experiment 1) and the pinching method (Experiment 2) were found in 2015 and 2017, respectively (Figure 7), that both estimation methods, while showing differences between years, seem to accurately represent genetic differences in field branching plasticity to some extent.

### ***Estimated field branching plasticity values based on a gradient of intra-row spacing (Experiment 1) and the pinching method (Experiment 2), and common problems***

The pinching method is considered to be more effective than the method with the gradient of intra-row spacing as a simplified method to estimate the degree of branching plasticity of multiple cultivars in relation to planting density. This takes into account the labor involved in sowing, the precision of sowing, the precise management of cultivation up to community formation, and the labor required to measure the number of branch nodes per plant at maturity. However, both methods have a certain degree of accuracy in estimating varietal differences within a group of indeterminate cultivars, which show large varietal differences in branching plasticity. Further improvements are needed to accurately estimate small varietal differences in branching plasticity among determinate cultivars in similar MGs (e.g. the Hokkaido-bred cultivars). Such improvements could include measurements of more plants to detect smaller differences among genotypes, and analyses over multiple years.

### ***Further research to develop more efficient and simple evaluation methods***

A later sowing time generally suppresses branch development in soybean, leading to flowering and the transition to reproductive growth when plants are smaller (Koyama et al., 1981; Ohga et al., 1987). Ozaki et al. (2014) conducted late sowing in June, and a simplified



evaluation was conducted for plants growing in rows with a gradient of distance between them. Even though the late sowing resulted in shorter branch lengths and fewer branch nodes, varietal differences could still be determined and measurements could be made efficiently.

If seeds are sown too late, branch development may be insufficient to detect differences in the number of branch nodes among cultivars. Thus, it is important to establish an appropriate sowing time that will allow for accurate detection of varietal differences in the number of branch nodes and surveys of branching, even with the pinching method.

According to Suzuki et al. (2014), branching plasticity in response to planting density can be estimated by branch length and the number of branch nodes derived from the first leaf node in a pinching treatment. Measurement of branch traits limited to the site of branch initiation could potentially improve the efficiency of measurements for estimating branching plasticity.

Deep learning with artificial intelligence can be used to accurately estimate soybean yield and above-ground dry matter weight (T. Watanabe et al., 2018). If the number of branch nodes can be estimated from photographs using this technology, then this will make simplified evaluations of branching plasticity even more efficient. It will be necessary to link such deep learning technology with a simplified evaluation of branching plasticity.

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## Disclosure statement

No potential conflict of interest was reported by the authors.

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