Effect of using drip irrigation on the growth, yield and its components of soybean grown in a low rainfall region in Japan

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ABSTRACT
Field experiments were carried out for 3 years to assess the effect of using drip irrigation on the growth and yield of two Japanese soybean cultivars in Kagawa Prefecture, which has the second-lowest rainfall in Japan. The treatments were drip irrigation (Drip) and rainfed (Rainfed) from the blooming stage to the full-seed stage. The largest seed yield was achieved in 2017, followed by 2018 and 2016. This order corresponded to the total water input (TWI, the sum of effective rainfall and irrigation) throughout cultivation. TWI was the main factor affecting the variation of yield and its components among years. Similarly, the higher TWI in Drip than in Rainfed contributed to the higher yield in Drip than in Rainfed within each year. ANOVA detected a significant effect of drip irrigation on total seed yield, above-ground dry matter (AGDM) at maturity, and numbers of branches, nodes, and fertile pods. AGDM had a significant correlation with the mean crop growth rate (CGR) during the treatment, and CGR was closely correlated with the mean net assimilation rate (NAR). Significant positive correlation among NAR, radiation use efficiency, and leaf water potential suggested that drip irrigation prevented the decrease of plant water status that contributed to maintain dry matter production. The advantage of using drip irrigation for soybean cultivation at the experiment site would be suppressing the decrease in yield in years with low rainfall rather than achieving higher yield than standard in years with normal or high rainfall.

Abbreviations: AGDM, aboveground dry matter; CGR, crop growth rate; CISR, cumulative intercepted solar radiation; DAS, days after sowing; ETa, actual evapotranspiration; ETr, reference evapotranspiration; FIPAR, fraction of intercepted photosynthetically active radiation; LAI, leaf area index; ΨL, leaf water potential; mLAI, mean leaf area index; NAR, net assimilation rate; PAR, photosynthetically active radiation; PCC, percent of canopy coverage; RUE, radiation use efficiency; TWI, total water input

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1. Introduction

Recent soybean yield in Japan has been 1636 kg ha\(^{-1}\) (average of 5 years from 2014 to 2018, MAFF, 2020), which is about half of that in the USA (3326 kg ha\(^{-1}\), average during the same period, USDA National Agricultural Statistics Service, 2020). A rainy season at the time of sowing, drought stress after the rainy season, and typhoons are disadvantageous climate constraints for soybean cultivation in Japan (Fatichin et al., 2013; Matsuo et al., 2016). Drought stress in soybean cultivation in southern Japan frequently decreases the germination rate and vegetative growth, increases flower and pod abortion, and reduces seed weight (Takeda & Sasaki, 2013). Severe water deficit is rare, but drought stress after the rainy season reduces soybean yield in Japan (Fatichin et al., 2013; Matsuo et al., 2016). Therefore, complementary irrigation in soybean cultivation should alleviate drought stress, even for a short period, and contribute to increasing yield.

Among various irrigation methods, drip irrigation enables a more effective water supply to plants compared with other irrigation systems because of the ease of controlling watering (Dasberg & Or, 1999). Drip technology improves irrigation efficiency by reducing evaporation from the soil surface, reducing, or eliminating runoff and deep percolation, and enabling even application of water in fields (Skaggs, 2001). Isoda et al. (2006) demonstrated a very high soybean yield with frequent and sufficient irrigation using a drip system in an arid area of central Asia.

The standard spatial arrangement of soybean plants in Japan is 10 to 20 plants m\(^{-2}\), with 0.60 to 0.80 m between rows, 0.07 to 0.20 m between plants within the rows, and tilling between the rows. Recently, narrow-row planting (ca. 0.3 m between rows) in soybean cultivation has become common in Japan, and high soybean yield using narrow-row planting has been reported (Maitree & Toyota, 2017). Furrow irrigation is common for the standard spatial arrangement of soybeans. In contrast, drip irrigation is well suited for narrow-row planting, which does not require tilling between rows during cultivation.

Irrigation in areas where water is deficient has been reported to significantly increase seed yield in soybean (Garcia Y Garcia et al., 2010; Heatherly, 1983; Kadhem et al., 1985; Karam et al., 2005; Korte et al., 1983). However, the effect of irrigation on soybean cultivation in Japan is uncertain because Japan has 1700 mm of annual average precipitation, which is approximately twice the average world precipitation. In addition, Scott et al. (1987) showed that soybean yield from irrigation was greater than the yield from rainfed cultivation in a year receiving low precipitation, but not in a year receiving high precipitation in a mid-south region in the USA. Accordingly, soybean yield under rainfed cultivation decreases in a year with low precipitation, and the effect of irrigation on soybean production appears more clearly in a region with low precipitation. Kagawa Prefecture has the second-lowest precipitation in Japan (average 1082 mm per year from 1981 to 2010, Statistics Bureau of Japan, 2020). Hence, the prefecture is suitable for experiments to investigate the effect of complementary irrigation on the growth and yield of soybean.

Consequently, this study aimed to assess whether using drip irrigation on soybean cultivation in Kagawa Prefecture increases the growth and yield of the Japanese determinate soybean cultivars Hatusuyaka and Sachiyutaka. Because drought stress during the reproductive stages severely decreases the yield of soybean (Çigdem et al., 2010; Cox & Jolliff, 1986; Karam et al., 2005; Westgate & Peterson, 1993), irrigation treatment is set from the blooming stage to the full-seed stage (R1–R6 stages, classification by Fehr & Caviness, 1977).

2. Materials and methods

2.1. Experiment design and plant material

Three field experiments were conducted at the Faculty of Agriculture, Kagawa University (34°16’N, 134°7’E), in 2016, 2017, and 2018. The experimental field had a well-drained loam soil. Four ridges (1.8 m by 20 m) in 2016 and three ridges (1.8 m by 21 m) in 2017 and 2018 were established with 1 m distance between ridges. The experiment was arranged in a split-plot design with irrigation treatments as the main plot and cultivars as the subplot with four replications in 2016 and three replications in 2017 and 2018. The plot area was 1.8 × 5.0 m (2016) and 1.8 × 5.5 m (2017 and 2018) with six rows separated by 0.3 m running from north to south. Determinate type soybean cultivars [Glycine max (L.) Merr., cv. Hatusuyaka and Sachiyutaka] were sown on 12 July 2016, 11 July 2017, and 10 July 2018 at 0.09 m between seeds using a hand-powered sowing machine (HS-120; Mukai Kogyo, Inc., Osaka, Japan). Seedlings were thinned at the first-node stage (V1 stage) to a spacing of 0.18 m between plants (18.5 plants m\(^{-2}\)). Compound fertilizer was applied before sowing at 20, 56, and 56 kg ha\(^{-1}\) of N, P\(_2\)O\(_5\), and K\(_2\)O, respectively, for all 3 years. An herbicide mixture of benthiocarb, pendimethalin, and linuron (Cleartum; Kumiai Chemical Industry Co.,
2.2. Irrigation management and evapotranspiration measurement

Treatments were drip irrigation (hereafter, Drip) and rainfed (hereafter, Rainfed) during stages R1 (14 August for all years) to R6 (27 September 2016, 19 September 2017 and 2018). Three drip tubes with emitters every 0.3 m (Dripnet PC 12, Netafim Japan Ltd, Tokyo, Japan) were installed between the rows at 0.6 m intervals for each ridge. The daily irrigation amount was determined based on the daily reference evapotranspiration (ETr) and rainfall over the past few days. Irrigation was suspended on rainy days and for several days after heavy rain. Actual evapotranspiration (ETA) was estimated as the product of ETr and a crop coefficient (Kc). ETr was measured using an atmometer (ETgage, model A, ETgage Company, Colorado, USA) installed in the experimental field. The ETgage with No. 54 canvas cover estimates the evapotranspiration of green well-irrigated alfalfa with 75% canopy coverage. We determined Kc from emergence to R1 stage based on the percent of canopy coverage (PCC) according to the ETgage product manual (Complete Model A manual, www.etgage.com), i.e. Kc = 0.3 if PCC<10%; Kc = 0.5 if 10%≤PCC<50%; Kc = 0.8 if 50%≤PCC<75%, Kc = 1.0 if 75%≤PCC. The closed linear relationship between PCC and the fraction of photosynthetically active radiation intercepted by the plant canopy (FIPAR) was used to convert FIPAR to PCC. The measurement of FIPAR will be described later. The linear regression equation (PCC = −7.36 + 1.08x FIPAR) was derived from our previous data set of soybean Sachiyutaka canopy coverage estimated by digital image as Purcell (2000) and FIPAR (unpublished). As PCC during treatment was always higher than 75%, Kc during treatment is 1.0. For the Kc at the late maturity stage, we assumed that Kc gradually decreased from 1.0 on 30 days before maturity to 0.5 at maturity.

2.3. Measurements

Daily maximum and minimum air temperature, daily total solar radiation and daily precipitation were measured at the meteorological station of the Faculty of Agriculture, Kagawa University, which is located adjacent to the experimental field. We assumed effective rainfall (effective rainfall ≥ 5 mm d⁻¹) to be that according to the Ministry of Agriculture, Forestry and Fisheries (1982). Because there were many missing values in air temperature from September to November in 2016 due to mechanical trouble, air temperature at the Takamatsu meteorological observatory, which is located 8 km northwest of the experimental field, was substituted for the corresponding periods.

Leaf water potential (Ψw) and leaf greenness (SPAD values) of the topmost fully expanded leaves from three plants of each plot was measured using a pressure chamber (Model 600; PMS Instrument Company, Albany, USA), and a SPAD meter (SPAD-502; Konica Minolta Inc., Tokyo, Japan). We measured Ψw and SPAD seven times each year at weekly or biweekly intervals, depending on weather condition. The Ψw at 57 DAS in 2018 were missing because of difficulty to decide the timing of water appearance at the cut surface of the petiole for unknown reason.

Above-ground material from four plants (before the start of irrigation treatment) or three plants (after the start of irrigation treatment) per plot were collected at 2-week intervals: at 15, 29, 44, 58, 72, and 86 days after sowing (DAS) in 2016, at 15, 29, 43, 57, 72, and 86 DAS in 2017 and at 15, 29, 48, 63, 77 and 91 DAS in 2018.

Table 1. Mean monthly maximum and minimum temperature, solar radiation, and rainfall measured at the meteorological station of the Faculty of Agriculture, Kagawa University.

| Year | July | August | September | October | November |
|------|------|--------|-----------|---------|----------|
|      | 2016 | 2017   | 2018      | 2017    | 2018     | 2017     | 2018     | 2017    | 2018     | 2017    | 2018     | 2017    | 2018     | 2017    | 2018     |
| Temp. Max (°C) | 31.8 (0.5) | 32.4 (1.1) | 32.8 (0.4) | 27.5 (1.1) | 20.9 (−2.1) | 16.3 (−0.8) | 17.5 (−0.1) | 17.5 (−0.1) | 17.5 (−0.1) | 16.3 (−0.8) | 17.5 (−0.1) | 17.5 (−0.1) | 17.5 (−0.1) | 17.5 (−0.1) | 17.5 (−0.1) |
| Temp. Min (°C) | 24.3 (0.4) | 24.5 (0.6) | 24.6 (0.8) | 19.0 (−0.8) | 14.0 (0.4) | 6.2 (−1.3) | 6.2 (−1.3) | 6.2 (−1.3) | 6.2 (−1.3) | 6.2 (−1.3) | 6.2 (−1.3) | 6.2 (−1.3) | 6.2 (−1.3) | 6.2 (−1.3) | 6.2 (−1.3) |
| Radiation (MJ m⁻² d⁻¹) | 20.7 (1.3) | 22.3 (2.3) | 22.3 (2.3) | 12.2 (−3.7) | 11.0 (−1.8) | 8.7 (−0.5) | 8.7 (−0.5) | 8.7 (−0.5) | 8.7 (−0.5) | 8.7 (−0.5) | 8.7 (−0.5) | 8.7 (−0.5) | 8.7 (−0.5) | 8.7 (−0.5) | 8.7 (−0.5) |
| Rainfall (mm) | 21.3 (1.9) | 22.0 (1.9) | 11.6 (−4.4) | 12.8 (0.0) | 10.2 (1.0) | 10.2 (1.0) | 10.2 (1.0) | 10.2 (1.0) | 10.2 (1.0) | 10.2 (1.0) | 10.2 (1.0) | 10.2 (1.0) | 10.2 (1.0) | 10.2 (1.0) | 10.2 (1.0) |

Numbers in the parenthesis are the difference from the 15-year mean (2001 – 2015)
* Maximum and minimum temperature of Takamatsu meteorological observatory were substituted for the Faculty data from September to November in 2016 due to mechanical trouble.
(Supplemental Table 1). The event of plant correction was named as ‘1st sampling’, ‘3rd sampling’ according to the chronological order in each year. We did not correct plants in the border rows to avoid edge effects. We counted the number of branches (including sub-branches (second- and third-order racemes with compound leaves)), nodes and pods per plant. After that, all leaf blades were removed and spread in a single layer on a large copy stand, except for dead and yellowed parts, and images were taken with a digital camera. The leaf area per plot was measured from the digital images using image analysis software (LIA32; http://www.agr.nago.yau.ac.jp/~shinkan/LIA32/). The dry weight of the plant parts was determined after oven-drying at 80°C for more than 72 h. The above-ground dry matter (AGDM, g m⁻²) and leaf area index (LAI, m² m⁻²) were determined from the dry weight, leaf area, and plant density. The changes in AGDM (W₂ and W₁) and LAI (L₂ and L₁) at two sampling times (t₂ and t₁) were used to calculate crop growth rate (CGR), net assimilation rate (NAR), and mean leaf area index (mLAI) as follows:

\[
CGR = \frac{(W_2 - W_1)}{(t_2 - t_1)}
\]

\[
NAR = \frac{CGR}{mLAI}
\]

\[
mLAI = \frac{(L_2 - L_1)}{(lnL_2 - lnL_1)}
\]

The fraction of photosynthetically active radiation (PAR) intercepted by the plant canopy (FIPAR) in each plot was measured weekly using the SunScan Canopy Analysis System (Delta-T Devices, Cambridge, UK). PAR above the canopy was measured using a sunshine sensor placed at the center of the experimental field at a point higher than the canopy, and PAR below the canopy was measured using a 1-m probe inserted into the canopy at ground level, perpendicular to the row direction. The mean of five measurements per plot was used to calculate FIPAR according to Purcell et al. (2002):

\[
FIPAR = [1 - (\text{PAR below the canopy}) \times (\text{PAR above the canopy})]^{-1}
\]

Daily total intercepted solar radiation was calculated based on the daily total solar radiation measured at the meteorological station multiplied by the FIPAR measured with the SunScan instrument. FIPAR for days on which no measurements were taken was estimated through linear interpolation between the two closest measurements. Cumulative intercepted solar radiation (CISR, MJ m⁻²) was calculated by summing the daily intercepted solar radiation after emergence of the seedlings in each plot. Radiation use efficiency (RUE) was determined as the slope of a simple linear regression between AGDM and CISR for each plot using all of the destructive sample data (i.e. six times) during the periods from 27 July (15 DAS) to 6 October (86 DAS) in 2016, 26 July (15 DAS) to 5 October (86 DAS) in 2017, and 25 July (15 DAS) to 9 October (91 DAS) in 2018.

At maturity, 20 plants from each plot were sampled on 26 November (137 DAS) in 2016, 2 November (114 DAS) in 2017, and 31 October (114 DAS) in 2018. Yield (‘fine’ seed weight, adjusted to a 15% moisture content) and the yield components were obtained from the number and dry weight of the main stems, branches (including sub-branches), nodes, pods (total and fertile), and seeds. Seeds were classified into fine and nonconforming (which includes seeds that were damaged by pests and diseases, that split, or that showed other obvious defects) using a 7.3-mm sieve, and weighed. The number of fine seeds was calculated from the fine seed weight divided by the 100-seed weight. The 100-seed weight doesn’t include the nonconforming seeds.

2.4. Statistical analyses

Data were analyzed by means of three-way ANOVA using the model for a split-plot design with four (2016) or three (2017 and 2018) replications to evaluate the effects of irrigation, cultivars, years, and their interaction on all measured variables except Ψₛ and SPAD value. We performed two-way ANOVA to evaluate the effects of irrigation, cultivar, and their interaction on Ψₛ and SPAD value for each measurement date and year. Regression analysis between AGDM and CISR was carried out to determine RUE. Correlation analysis among yield, growth, and physiological variables was performed for pooled data of two cultivars to analyze the relationships between those variables. All statistical analyses were performed using JMP statistical software (SAS Institute Japan, Tokyo, Japan).

3. Results

3.1. Meteorological conditions and irrigation

Table 1 shows the mean monthly maximum and minimum temperature, solar radiation, and rainfall. In all years, the maximum and minimum temperatures and solar radiation in July and August were higher than the 15-year mean. The maximum temperatures and solar radiation in September of all years and October in 2016 and 2017 were lower than the 15-year mean. Rainfall in July in 2016 was lower than the 15-year mean, and those in 2017 and 2018 were higher than the 15-year mean. But the rainfall in July after sowing was 18, 60, and 28 mm for 2016, 2017 and 2018, respectively. After August, rainfall was lower than the 15-year mean in all
months except September in 2016 and 2018, and higher than the 15-year mean in all months except November in 2017.

Table 2 shows total rainfall, amount of irrigation, ETr, ETa, and days of rainfall and irrigation during and before or after the irrigation treatment period. Before treatment, there was no irrigation event in 2017 due to high effective rainfall. Conversely, the amount of irrigation before treatment was 180 mm (2016) and 177 mm (2018) due to low effective rainfall. Effective rainfall during treatment in 2016 and 2017 was 23% and 34% lower than that in 2018. The amount of irrigation during treatment was the highest in 2016 (115 mm), followed by 2017 (99 mm) and 2018 (42 mm). After the treatment, little effective rainfall continued in 2016, whereas there was much effective rainfall in 2017 and 2018. The ETr and ETa before the treatment were the highest in 2018, followed by 2016 and 2017. However, the difference in those values between years was small. Since the PCC during the treatment period was over 75%, ETr and ETa values during treatment are the same, and there was no clear difference between ETr and ETa after the treatment. ETr and ETa after treatment was the highest in 2016, followed by 2018 and 2017. The total effective rainfall throughout cultivation was the highest in 2017 (875 mm), followed by 2018 (679 mm) and 2016 (395 mm). The rank order of the total ETa and irrigation throughout cultivation was the opposite of the order of total rainfall. Total water input throughout cultivation (TWI, the sum of effective rainfall and irrigation) in Drip were 690, 974, and 897 mm, and those in Rainfed were 575, 875, and 856 mm for 2016, 2017, 2018, respectively.

Figure 1 shows accumulated ETa, irrigation, and rainfall after sowing. Accumulated rainfall in 2016 was low until late August (47 DAS), and it increased higher than the accumulated ETa in late September (70 DAS). The increase in accumulated rainfall after late September was small, so the total effective rainfall throughout cultivation in 2016 was the lowest among the 3 years. The change in accumulated rainfall in 2018 was similar to that in 2016, but the accumulated rainfall in 2018 exceeded accumulated ETa after early September (56

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**Table 2.** Mean, total values and days of rainfall and irrigation, reference evapotranspiration (ETr) and estimated actual evapotranspiration (ETa) during and before or after the irrigation treatment period.

| Period | Year | Rainfall (mm) | Irrigation (mm) | ETr (mm) | ETa (mm) |
|--------|------|---------------|----------------|----------|----------|
|        |      | No. days | Total | Daily mean<sup>a</sup> | Total | Daily mean | Total |        |
| Before | 2016 | 4 (3)     | 36 (36) | 19 | 9.5 | 180 | 6.1 | 200 | 122 |
|        | 2017 | 5 (3)     | 244 (239) | 0 | 0 | 0 | 5.6 | 183 | 112 |
|        | 2018 | 3 (2)     | 32 (32) | 22 | 8.0 | 177 | 7.0 | 246 | 134 |
| During | 2016 | 20 (11)   | 318 (302) | 16 | 7.2 | 115 | 4.3 | 188 | 188 |
|        | 2017 | 8 (6)     | 262 (259) | 17 | 5.8 | 99 | 4.6 | 170 | 170 |
|        | 2018 | 16 (10)   | 399 (391) | 6 | 7.0 | 42 | 4.6 | 165 | 165 |
| After  | 2016 | 24 (6)    | 87 (57) | 0 | 0 | 0 | 2.3 | 136 | 121 |
|        | 2017 | 21 (13)   | 390 (377) | 0 | 0 | 0 | 2.2 | 80 | 73 |
|        | 2018 | 15 (7)    | 274 (257) | 0 | 0 | 0 | 3.0 | 112 | 95 |
| Mean or Total<sup>b</sup> | 2016 | 48 (20)   | 441 (395) | 35 (19) | 8.4 (9.5) | 295 (180) | 4.2 | 524 | 431 |
|        | 2017 | 34 (22)   | 895 (875) | 17 (0) | 5.8 (0) | 99 (0) | 4.1 | 433 | 355 |
|        | 2018 | 34 (19)   | 705 (679) | 28 (22) | 7.8 (8.0) | 218 (177) | 4.8 | 523 | 394 |

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<sup>a</sup>Numbers within parenthesis in rainfall are values of effective rainfall (rainfall ≥ 5 mm d<sup>−1</sup>)

<sup>b</sup>Numbers within parenthesis in irrigation are values of the rainfed treatment.

<sup>c</sup>Daily means of irrigation did not include days with no case.

<sup>d</sup>Mean or total throughout the cultivation period.

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Figure 1. Accumulated actual evapotranspiration (ETa), irrigation, effective rainfall and daily effective rainfall after sowing in 2016 (a), 2017 (b), and 2018 (c). There was no irrigation event throughout cultivation for Rainfed in 2017. The gray bar on X axis indicates the period of irrigation treatment.
3.2. Growth stages

Table 3 shows the mean date and DAS of cultivars and treatments of reproductive stages for each year. ANOVA detected significant effects of year and cultivar in all stages except the effect of cultivar at R8, but the effect of irrigation was not significant in any stage (Supplemental Table 2). The mean DAS of cultivars and treatments up to R5 in 2016 slightly advanced than in 2017 and 2018. But the mean DAS after R6 in 2016 was delayed up to 6 days (R6) and 24 days (R8) than in 2017 and 2018. The date to reach each stage in Sachiyutaka advanced approximately one day than in Hatsusayaka.

3.3. Yield and yield components

Table 4 shows fine and total seed yield, 100-seed weight, AGDM, and harvest index. Fine seed yield ranged from 217 to 518 g m⁻² across cultivars, irrigations, and years. ANOVA detected significant effect of irrigation on total seed yield and AGDM, and significant effect of cultivar and year on all variables except for effect of cultivar on AGDM. The mean of total seed yield and AGDM across years and cultivars in Drip was about 14% higher than in Rainfed. The mean of all variables across years and irrigations in Sachiyutaka were higher than those in Hatsusayaka. The mean of fine and total seed yield and AGDM across cultivars and irrigations were the highest in 2017, and those of 100-seed weight and harvest index were the lowest in 2017.

Table 5 shows number of fine seeds and its components associated with the numbers (i.e., numbers of seeds, pods, nodes, and branches). The number of fine seeds ranged from 573 to 1427 seed m⁻² across cultivars, irrigations, and years. ANOVA detected the significant effect of irrigation on the numbers of branches, nodes, and fertile pods, and significant effect of cultivar and year on all variables except for the numbers of total and fertile pods. The mean of the numbers of branches, nodes, and
Table 5. Number of branches, nodes, total pods, fertile pods, seeds per square meter, and number of seeds per pod at maturity of soybean grown under drip irrigation and a rainfed condition during the reproductive stages (from R1 to R6) in 2016, 2017, and 2018.

| Year | Cultivar | Year (Y) | C (Cult.) | L (Irrig.) | Y × C | ANOVA |
|------|----------|----------|-----------|-----------|-------|-------|
|      |          | Mean     | ANOVA     | ANOVA     | ANOVA | ANOVA |
|      |          |          |           |           |       |       |
| 2016 | Hats     | 2016     | ***       | ***       | ***   | *     |
|      | Sach     | 2017     | **        | **        | **    | ***   |
|      | Sach     | 2018     | ***       | ***       | ***   | *     |
| 2017 | Hats     | 2016     | ***       | ***       | ***   | *     |
|      | Sach     | 2017     | **        | **        | **    | ***   |
| 2018 | Hats     | 2016     | ***       | ***       | ***   | *     |
|      | Sach     | 2017     | **        | **        | **    | ***   |

Values are means of four replication plots (2016) and three replication plots (2017, 2018).
*p < 0.05, ** p < 0.01, *** p < 0.001, ns: not significant.

fertile pods across years and cultivars in Drip were larger than in Rainfed. The mean of the numbers of branches, nodes, total and fertile pods across years and irrigations in Sachiyutaka were higher than those in Hatsusayaka, while those of the number of seed per m² and per pod were smaller in Sachiyutaka than in Hatsusayaka. The mean of the number of total and fertile pods and seed per m² across cultivars and irrigations were the highest in 2017. The year × irrigation interaction was significant on number of seed per pod: number of seed per pod in Drip were higher in Drip than in Rainfed in 2016, but the relation was opposite in 2017, and it varied by cultivar and irrigation in 2018.

Figure 2 shows the changes in the number of nodes, branches, and pods. ANOVA detected a significant effect of irrigation on all variables, in some sampling mainly in the last half of growth (Supplemental Table 3). The number of those variables in Drip tended to be larger than in Rainfed.

3.4. AGDM and LAI and growth analysis

Figure 3 shows the changes of AGDM and LAI. AGDM and LAI in Drip tended to be higher than in Rainfed throughout cultivation for both cultivars for all years. ANOVA detected a significant effect of irrigation on AGDM and LAI from 3rd to 6th sampling (Supplemental Table 4). Significant effect of cultivar and year were detected more in LAI compared to those in AGDM. The significant year × irrigation interaction at 4th sampling in both AGDM and LAI was owing to that AGDM and LAI in Drip were obviously higher than in Rainfed in 2016 and 2017, while those difference between irrigation was not clear in 2018.

Figure 4 shows the changes in CGR, NAR, and mLAI. The maximum CGR across cultivars, irrigations, and years ranged from 24.9 to 45.5 g m⁻² d⁻¹. ANOVA detected a significant effect of irrigation in 2nd period on CGR and NAR, and all periods except 1st period on mLAI (Supplemental Table 5). Significant effects of cultivar and year were detected more in mLAI compared to those in CGR and NAR. The significant year × irrigation interaction at 5th period in both CGR and NAR was due to higher CGR and NAR in Drip than in Rainfed in 2017 and 2018, but the relation was opposite in 2016.

There were highly significant correlation between CGR and NAR in any period, whereas correlation between CGR and mLAI were significant in 1st and 4th period (Supplemental Table 6).

3.5. Radiation use efficiency

Table 6 shows the RUE and CISR calculated from the dataset during the 1st sampling to the sampling on 86 DAS in 2016 and 2017, and 91 DAS in 2018. RUE across cultivars, irrigations, and years ranged from 1.01 to 1.96 g MJ⁻¹. ANOVA detected a significant effect of year and irrigation on RUE and significant effect
The mean RUE across years and cultivars in Drip was 28.3% higher than in Rainfed. The highest RUE and CISR was achieved in 2017.

### 3.6. $\Psi_L$ and SPAD

Figure 5 shows the changes of $\Psi_L$ and SPAD value. In 2016 and 2018, $\Psi_L$ in the first half of growth in Drip tended to be higher than in Rainfed, whereas the trend was opposite in the latter half of growth. $\Psi_L$ in 2017 tended to be higher in Drip than in Rainfed throughout cultivation.

The change of SPAD values was similar in the first half of growth in all 3 years, whereas SPAD values at the last sampling in 2016 were about 23 and 20% higher than in 2017 and 2018, respectively. The higher SPAD value in 2016 may be due to the occurrence of delayed leaf senescence. Two-way ANOVA detected significant effect of cultivars on SPAD values in many measurement, whereas the effect of irrigation was only significant at 4th measurement in 2017. Hatsusayaka had higher SPAD values than in Sachiyutaka.

### 3.7. Relations among yield and its component, growth variables, and TWI

We performed correlation analysis to identify the factors responsible for the variation in yield and its components among years, cultivars and irrigation treatments. Total water input had significant positive correlations with total seed yield, AGDM, harvest index, numbers of fine seeds, fertile pods, and branch pod ratio (Figure 6).

Table 7 shows correlation coefficients among total seed yield, its components, RUE, CISR, the mean of growth parameters (CGR, NAR, and mLAI) during treatment, and the mean of $\Psi_L$ and SPAD value during
treatment. AGDM had a significant positive correlation with the means of CGR, NAR, mLAI, $\Psi_l$ during treatment period, and RUE. All correlation coefficients among CGR, NAR, mLAI, $\Psi_l$, and RUE were all significant. SPAD didn’t have a significant correlation with any variables.

Next, we performed correlation analysis among fine seed yield and its components (Table 8). Fine seed yield was closely correlated with number of seeds but not with 100-seed weight. Number of fine seed had significant correlations with number of fertile pod and branch pod ratio, but not with numbers of branches and pods.

4. Discussion

4.1. ETa, irrigation, rainfall and yield

Accumulated ETa of soybean in Drip in this study ranged 355 to 431 mm, which is lower than the values under various irrigation regimes (452 to 601 mm) in Nebraska (Irmaek et al., 2014), and close to the values under various irrigation regimes (388 to 541 mm) and in rainfed (262 mm) also in Nebraska (Payero et al., 2005). Daily mean ETr during treatment observed in this study (4.3 to 4.6 mm) agreed with the monthly average actual evapotranspiration of soybean in August (4.5 mm) measured by Bowen ratio energy balance technique in Tokyo (Attarod et al., 2009).

Because seasonal rainfall deficits and their occurrence times during the growing season account for much of the annual variation in soybean yield (Heatherly, 1983), not only the total amount of rainfall throughout cultivation but also the rainfall by period would make a difference in seed yield. Hence, we compare the changes in accumulated effective rainfall and accumulated ETa (Figure 1) to estimate the plant water status of soybean in each year. Accumulated effective rainfall was lower than accumulated ETa before R1 in 2016 and 2018, but that in 2017 was much higher than accumulated ETa. Because low effective rainfall before R1 in 2016 or 2018 was compensated by irrigation, there would be no water stress before R1 in any year. Accumulated rainfall in 2017 always exceeded accumulated ETa, but that during treatment was the lowest in 2017 among 3 years. Accumulated rainfall greatly exceeded accumulated ETa during the latter half of cultivation in 2017 and 2018, whereas that in the same period in 2016 were very closed to accumulated ETa. Although effective rainfall during treatment in 2016 and 2017 was 23% and 34% lower than in 2018, the amount of rainfall and the occurrence of rain in this study varied by growth period and year. Accordingly, we compare total water input (sum of effective rainfall and irrigation) to identify the factors responsible for the variation in yield and its components among years, cultivars and
irrigation treatments. As a result, we found many significant correlations between total water input and seed yield or its components (Figure 6). These results indicated that total water input was the main factor affecting the variation of seed yield and its components among years. Similarly, the higher total water input in Drip than in Rainfed contributed to higher yield and yield components in Drip than in Rainfed within each year.

4.2. The effect of drip irrigation on yield and its determination

ANOVA revealed a significant effect of drip irrigation on total seed yield, AGDM at maturity, RUE, and numbers of branches, nodes, and fertile pods (Table 4, 5, 6). Because seed yield is the product of AGDM at maturity and harvest index, the more closed linear relationship between total seed yield and AGDM rather than between total seed yield and harvest index indicated the greater contribution of AGDM to seed yield (Table 7). AGDM has a significant correlation with the means of growth parameters (CGR, NAR, and mLAI) during the treatment period, and CGR correlated with NAR more closely than with mLAI. Highly significant correlation coefficients among NAR, RUE, and ΨL suggested that maintaining plant water status higher by drip irrigation substantially increased the efficiency of dry matter production per unit leaf area during the R1 to R3 stage (period 2) and per intercepted solar radiation throughout whole growth period until just before the maturity.

On the other hand, the variation of fine seed yield were analyzed by correlation among yield components associated with the numbers (i.e. numbers of seeds, pods, nodes, and branches) (Table 8). The results revealed a significant correlation among fine seed...
yield, number of fine seeds, fertile pods and branch pod ratio. Although ANOVA detected a significant effect of drip irrigation on the number of branches, nodes, and fertile pods, only the number of fertile pods had a significant correlation with the number of fine seed. The significant increase of the number of branch and node by drip irrigation would affect the number of fine seed indirectly through the increase in branch pod ratio. Namely, the significant correlations between branch pod ratio and numbers of fine seeds and fertile pods suggested that an increase of branch growth by drip irrigation contributed to an increase in the number of pod in branch, which increased number of fine seed in Drip. This result consistent with Frederick et al. (2001), who reported that drought stress during R1 to R5 decreased total seed yield primarily by reducing branch vegetative growth, which reduce branch seed number and branch seed yield, without reducing main stem seed yield.

4.3. AGDM, LAI and dry matter production

AGDM and LAI in Drip tended to be higher than in Rainfed throughout cultivation for both cultivars for all years. Although considerably high AGDM and LAI were observed in Drip in 2017, AGDM and LAI in 2016 and 2018, and those in Rainfed in 2017 were within the range of our previous study (Maitree & Toyota, 2017).

ANOVA showed the significant effect of irrigation on CGR and NAR in the 2nd period (Supplemental Table 5).

Table 6. Radiation use efficiency (RUE) and cumulative intercepted solar radiation (CISR) of soybean grown under drip irrigation and a rainfed condition during the reproductive stages (from R1 to R6) in 2016, 2017, and 2018. RUE and CISR were calculated from the dataset during the 1st sampling to the sampling on 86 DAS in 2016 and 2017, and 91 DAS in 2018.

| Year | Cultivar | RUE (g MJ⁻¹) | CISR (MJ m⁻²) |
|------|----------|--------------|---------------|
|      |          | Drip | Rainfed | Drip | Rainfed | Drip | Rainfed | Drip | Rainfed | Drip | Rainfed | Drip | Rainfed |
| 2016 | Hatsusayaka | 1.39 | 1.01 | 1083 | 1067 | Sachiyutaka | 1.34 | 1.13 | 1102 | 1087 |
| 2017 | Hatsusayaka | 1.96 | 1.35 | 1124 | 1116 | Sachiyutaka | 1.56 | 1.18 | 1120 | 1122 |
| 2018 | Hatsusayaka | 1.42 | 1.10 | 1145 | 1098 | Sachiyutaka | 1.19 | 1.13 | 1114 | 1116 |
| Mean |          | 1.22 | 1.14 | 1085 | 1098 | Sachiyutaka | 1.35 | 1.18 | 1103 | 1108 |

ANOVA Year (Y) *** Cult. (C) ns Img. (I) *** Y × 1 ns Y × I ns C × I ns Y × C × I ns

Values are means of four replication plots (2016) and three replication plots (2017, 2018).

*** p < 0.001, ns: not significant.

Figure 5. Changes in $\Psi_l$ (a, b, c), and SPAD values (d, e, f) of the two soybean cultivars Hatsusayaka (H) and Sachiyutaka (S) grown under drip irrigation (D) and a rainfed (R) condition in 2016, 2017, and 2018. ‘C’ and ‘I’: Significant effect of cultivar and irrigation, respectively by two-way ANOVA. Values are means ± S.E. ($n = 4$, 2016; $n = 3$, 2017 and 2018). The gray bar on X axis indicates the period of irrigation treatment.
Figure 6. Relationships between total water input and (a) total seed yield, (b) AGDM, (c) harvest index, (d) number of fertile seed, (e) number of fertile pods, and (f) branch pod ratio of the two soybean cultivars Hatsusayaka and Sachiyutaka grown under drip irrigation and a rainfed condition in 2016, 2017, and 2018. Values are means ± S.E. (n = 4, 2016; n = 3, 2017 and 2018). r: correlation coefficient, *p < 0.05, **p < 0.01.

Table 7. Correlation coefficients among total seed yield and its components, growth parameters and physiological variables associated with dry matter production of soybean grown under drip irrigation and a rainfed condition during the reproductive stages (from R1 to R6) in 2016, 2017, and 2018. Correlation analysis was performed for pooled data of two cultivars.

| Variables                  | AGDM   | HI     | CGR<sup>a</sup> | NAR<sup>a</sup> | mLAI<sup>a</sup> | Ψ<sub>L</sub><sup>a</sup> | SPAD<sup>a</sup> | RUE<sup>a</sup> | CSR<sup>a</sup> |
|----------------------------|--------|--------|-----------------|-----------------|-----------------|-----------------|----------------|----------------|---------------|
| Total seed yield           | 0.888*** | 0.768** | 0.745**         | 0.687**         | 0.616*          | 0.806**         | 0.236          | 0.649*         | 0.659*        |
| AGDM                      | 0.392  | 0.916*** | 0.824***        | 0.725**         | 0.776**         | 0.209           | 0.836***       | 0.542          |               |
| HI                        | 0.228  | 0.958*** | 0.779**         | 0.809**         | −0.030          | 0.868***        | 0.477          |               |               |
| CGR<sup>a</sup>            |        |        | 0.644*          | 0.818**         | −0.038          | 0.711**         | 0.385          |               |               |
| NAR<sup>a</sup>            |        |        | 0.736**         | −0.423          | 0.907***        | 0.696**         | 0.593*         |               |               |
| mLAI<sup>a</sup>           |        |        |                 |                 |                 |                 |                |                |               |
| Ψ<sub>L</sub><sup>a</sup>  |        |        |                 |                 |                 |                 |                |                |               |
| SPAD<sup>a</sup>           |        |        |                 |                 |                 |                 |                |                |               |
| RUE<sup>a</sup>            |        |        |                 |                 |                 |                 |                |                |               |
| CSR<sup>a</sup>            |        |        |                 |                 |                 |                 |                |                |               |

*p < 0.05, **p < 0.01, ***p < 0.001.

<sup>a</sup>means of the measurements performed during 38 – 80 DAS in 2016, 37 – 80 DAS in 2017, and 43 – 84 DAS in 2018.
<sup>b</sup>calculated from the dataset during the 1st sampling to the sampling on 86 DAS in 2016 and 2017, and 91 DAS in 2018.

Table 8. Correlation coefficients among fine seed yield and its components of soybean grown under drip irrigation and a rainfed condition during the reproductive stages (from R1 to R6) in 2016, 2017, and 2018. Correlation analysis was performed for pooled data of two cultivars.

| Variables                  | Fine seed no. | 100 seed weight | Branch no. | Node no. | Pod no. | Fertile pod no. | Branch pod ratio<sup>a</sup> |
|----------------------------|---------------|-----------------|------------|----------|---------|----------------|-----------------------------|
| Fine seed yield            | 0.947***      | 0.002           | −0.162     | −0.454   | 0.321   | 0.546          | 0.861***                    |
| Fine seed no.              | −0.317        | 0.119           | 0.360      | 0.462    | 0.725** | 0.877***       |                             |
| 100 seed weight            | −0.830***     | −0.178          | 0.411      | 0.558    | 0.064   | −0.205         |                             |
| Branch no.                 | 0.551         | 0.410           | 0.192      | −0.103   |        | 0.645*         |                             |
| Node no.                   |               |                 |            |          |         |                |                             |
| Pod no.                    |               |                 |            |          |         |                |                             |
| Fertile pod no.            |               |                 |            |          |         |                |                             |

*p < 0.05, **p < 0.01, ***p < 0.001.
<sup>a</sup>Branch pod ratio = number of pod in branch (m<sup>-2</sup>)/total pod number (m<sup>-2</sup>).
The highly significant correlation between CGR and NAR in the 2nd period (Supplemental Table 6) suggested that large contribution of NAR to CGR. Although ANOVA showed significant effect of irrigation on mLAI from 2nd to 5th period (Supplemental Table 5), correlation between CGR and mLAI were only significant in 1st and 4th period. The significant correlation between $\Psi_1$ and growth parameters suggested that using drip irrigation should avoid drought stress and maintaining plant water status which contributes to NAR.

Previous studies have reported that a decrease in RUE during drought was caused by a decline in canopy photosynthesis as a consequence of senescence due to water stress (Jefferies & Mackerron, 1989; Kiniry et al., 1989; Li et al., 2008). In the present study, no severe water stress was observed that caused the leaf senescence. According to ANOVA, the effect of irrigation on RUE was highly significant (Table 6), suggesting that using drip irrigation should increase RUE of soybean in areas where there is no severe water deficit, such as our experiment site.

4.4. Cultivar difference on the effect of irrigation

Garcia Y Garcia et al. (2010) showed that response to yield and water use efficiency by drip irrigation in soybean was different among genotypes. In the present study, the mean numbers of branches and nodes across years and irrigations for Hatsusayaka were larger than for Sachiyutaka (Figure 2, Table 5), whereas the mean of fine and total seed yield (Table 4), and number of seed (Table 5) across years and irrigations for Sachiyutaka were larger than for Hatsusayaka. These morphological characteristics in Hatsusayaka and Sachiyutaka in this study are consistent with our previous study (Chomsang et al., 2020; Maitree & Toyota, 2017). The larger number of branches, nodes, and pods for Hatsusayaka support the ability to produce many branches of this cultivar compared with Sachiyutaka (Toyota et al., 2017). Probably due to the limitation of assimilate supply, however, a large proportion of pods and seeds were aborted or grew insufficiently in Hatsusayaka. The lower harvest index for Hatsusayaka than for Sachiyutaka (Table 4) could be attributed to the larger aborted pod and non-conforming seed in this cultivar.

4.5. Amount of rainfall and the effect of irrigation

Total rainfall during the same growth season with this study (sown on July 10 and harvested on November 10) at the Faculty of Agriculture, Kagawa University from 2001 to 2019 were varied between 112 mm (2012) and 903 mm (2017), with a mean of 507 mm. The 95% confidence interval for the 19-year mean was 393 mm (lower) and 622 mm (upper). Total rainfall in 2017 was the highest among 19-year mean and that in 2018 was higher than the upper 95% confidence interval. Total rainfall in 2016 was lower than 19-year mean but within the 95% confidence interval. There were 6 years during 19 years when total rainfall during the soybean cultivation season fell below the 95% confidence interval of 19-year mean.

The results of this study indicated that the use of drip irrigation suppresses the decrease of yield in years with rainfall as low as 2016. Therefore, the probability of avoiding a decrease in soybean yield in a drought year by using drip irrigation will be about once every 3 years. The probability will increase about once every 1.7 years ($\gtrsim 2$ years) if we expect a significant effect of using drip irrigation in a year when rainfall is lower than 19-year mean. More information is needed to estimate the threshold rainfall, which is expected to have a significant effect of using drip irrigation in this region.

5. Conclusion

Total water input was the main factor affecting the variation of yield and its components among years. Similarly, the higher total water input in Drip than in Rainfed contributed to the higher yield in Drip than in Rainfed within each year. ANOVA revealed a significant effect of drip irrigation on total seed yield, AGDM at maturity, RUE, and numbers of branches, nodes, and fertile pods. AGDM has a significant correlation with the means of growth parameters (CGR, NAR, and mLAI) during treatment period, and CGR correlated with NAR more closely than with mLAI. A highly significant correlation among NAR, RUE, and $\Psi_1$ suggested that maintaining plant water status higher by drip irrigation substantially increased the efficiency of dry matter production per unit leaf area and per intercepted solar radiation. The significant increase of the number of branches and nodes by drip irrigation would affect the number of fine seed indirectly through the increase in branch pod ratio. Namely, increase of branch growth by drip irrigation contributed to increase in the number of pod in branch, which increased seed yield in Drip.

From the results, the advantage of using drip irrigation for soybean cultivation at the experiment site would be suppressing the decrease in yield in years with low rainfall rather than achieving higher yield than standard in years with normal or high rainfall. The probability of avoiding a decrease in soybean yield in a drought year by using drip irrigation will be about once every 3 years, or about once every 1.7 years, depending on the level of rainfall judged to be effective. More information is
needed to estimate the threshold rainfall, which is expected to have a significant effect on using drip irrigation in this region.

**Disclosure statement**

No potential conflict of interest was reported by the authors.

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