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Responses of leaf gas exchange rate to acute soil drying in *Jatropha curcas* L

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ABSTRACT

Development of a water-efficient irrigation system is essential in order to cultivate *Jatropha curcas*, a bioenergy crop, in semiarid regions. In the present study, to determine the useful parameter for the efficient irrigation system, *Jatropha* plants were grown in pots and root boxes under greenhouse conditions in Okinawa, Japan. At 6 to 9 months after transplanting, we measured the gas exchange rate during ongoing soil water deficits and attempted to identify the threshold of soil matrix potential (pF) with changes of both intrinsic water use efficiency (*A/g_s*) as a stomatal-based index and carboxylation efficiency (*A/C_i*) as an index of non-stomatal limitation. When irrigation was stopped, *A* and *g_s* began to decline, with each parameter reaching zero at pF 4.0. However, *g_s* was more sensitive to soil water deficits than *A*. After re-watering, stomatal openings and carbon uptake recovered to the value observed before water stress treatment. At pF 3.5 and higher values (representing more severe water stress), *A/g_s* intended to increase and *A/C_i* sharply decreased. These results suggested that approximately pF 3.5 in the soil was the threshold of non-stomatal limitations to photosynthetic rate and should be useful to the re-watering point for the water-efficient irrigation system in *Jatropha*.

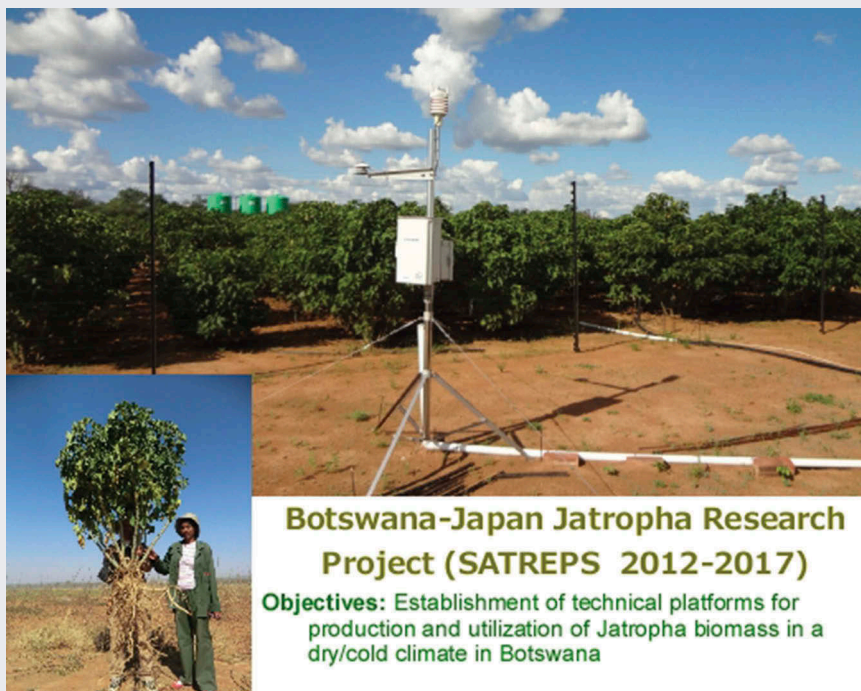
Abbreviations: *A*: CO₂ assimilation rate; *C_i*: intercellular CO₂ concentration; *E*: transpiration rate; *g_s*: stomatal conductance; pF: soil matrix potential; PFD: photon flux density; TDR: time domain reflectometry; VPD: leaf-to-air vapor pressure difference; VWC: volumetric water content.

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Gas exchange rate; internal CO₂ concentration; *Jatropha curcas*; pF; stomata; water stress



Introduction

Use of edible oils for biodiesel production in developing countries can cause global food shortages and escalation of food prices. This necessitates non-edible oil crops that are productive in harsh environments where other crops are difficult to grow. *Jatropha* (*Jatropha curcas* L.), a member of the Euphorbiaceae, is a potential candidate for the non-edible oil crop in subtropics or tropics. *Jatropha* seeds contain as much as 25–30% of seed weight in the form of oil. Optimum growing conditions for *jatropha* are found in areas with an annual rainfall of 1000–1500 mm and with temperatures of 20–28°C (Brittaine & Lutaladio, 2010; Kumar & Sharma, 2005). Economic yield starts after 3 years, when the plants are sufficiently mature to produce fruits, and *jatropha* bushes continue to produce seeds for a period up to 50 years (Kumar & Sharma, 2005). The commercial seed yield from *jatropha* plantations is about 2.5 tons ha⁻¹ year⁻¹, although the observed yield varies largely with the type of planting model (Inafuku-Teramoto et al., 2013; Ishimoto et al., 2017, 2018). However, lack of agronomical knowledge of this undomesticated plant, including how agronomic practices can contribute to the yield in unfavorable environments, is a major barrier to its commercial production.

In previous studies, we attempted to identify the best management practices for *jatropha* cultivation under semi-arid environments in the Republic of Botswana (Inafuku-Teramoto et al., 2013; Ishimoto et al., 2017, 2018; Tominaga, Inafuku, Coetzee & Kawamitsu, 2014). In Botswana, with an annual precipitation of less than 500 mm, drought is a common event and considered the major limitation of the *jatropha* production. Photosynthesis is the primary source of plant production and is also among the primary processes to be affected by drought (Chaves, Flexas & Pinheiro, 2009; Kramer & Boyer, 1995). Stomatal closure may be the first event restricting photosynthesis via limiting CO₂ supply at mild to moderate water stress. Following the reduction of CO₂ supply, a decrease in the activities of enzymes involved in photosynthesis (a non-stomatal limitation) may also occur (Chaves et al., 2009; Du et al., 1996; Lawlor, 2002). Strict stomatal regulation in *jatropha* plants under water deficit has been identified in diurnal gas-exchange measurements under the field conditions (Tominaga et al., 2014). In general, stomatal limitation increases water use efficiency of photosynthesis whereas non-stomatal limitation decreases the efficiency. Therefore, in order to maximize the *jatropha* production while minimizing water use, plants need to be watered before the non-stomatal limitation prevails.

For irrigation control, soil pF can be used as an index of soil water status. As it determines the tension of water held by soils, it is directly associated with available water for

plants. Because soil pF is a universal measure of soil moisture regardless of soil types, it could be used to indicate water and/or physiological status of the plant (e.g., wilting). Caveat, however, may be that a plant may respond to a soil pF differently with special and temporal dynamics (e.g., drying vs wetting). In addition to this physiological status, stem diameter is known to respond sensitively to soil moisture conditions and may be one of the useful parameters for water-saving irrigation (Nortes, Pérez-Pastor, Egea, Conejero & Domingo, 2005).

The objectives of the present study were (1) to determine the threshold of the soil pF that causes the non-stomatal limitation of photosynthesis during acute drought and re-watering treatment in *jatropha*; and (2) to find a more direct index in gas-exchange parameters that reflect physiological responses to the threshold of the soil pF. We used **A/g_s** as a stomatal-based index, **A/C_i** as an index of non-stomatal limitation and stem diameter as an index of water stress.

Materials and methods

Pot experiment

A cultivar (IP-2P) of *Jatropha curcas* L. was obtained from Indonesia. The plants were grown from March to December in 2016 under glasshouse conditions at the University of the Ryukyus, Okinawa, Japan (26°25'N, 127°45'E, 126 m above mean sea level). Similar-sized seeds were selected and germinated in vermiculite. After the second leaves had expanded, healthy seedlings were transplanted on May 10 to plastic pots (1/5000a Wagner pot, 2 L) containing a red soil–sand–peat moss mixture (1:1:1, v v⁻¹). In each pot, a pF sensor (MPS-6; METER) was installed at 15 cm depth. Plants were watered every morning with 500-mL tap water and also fertilized weekly with 500-mL modified Hoagland's nutrient solution (6 mM Ca (NO₃)·4H₂O, 4 mM KNO₃, 2 mM KH₂PO₄, 2 mM MgSO₄·7H₂O, 25 μM H₃BO₃, 10 μM MnSO₄·5H₂O, 2 μM ZnSO₄·7H₂O, 0.5 μM CuSO₄·5H₂O, 0.5 μM H₂MoO₄, 0.1 μM FeC₆H₅O₇).

Root box experiment

In the pot experiment, root system may be limited and the relationship with soil pF may not be evaluated correctly. Therefore, the same experiment was performed on root box which does not prevent root growth. Two root boxes (72.5 × 9 × 86 cm, 52 L, Figure 1) were filled to 80 cm in depth with a soil mixture (red soil–sand–peat moss, 1:1:1, v v⁻¹) passed through a 2-mm sieve. In each root box, a pF sensor was installed at 25 cm depth. Healthy seedlings were transplanted on May 10, and

then water was irrigated onto the topsoil using an automated irrigation system. The plants were fertilized weekly by replacing irrigation water with 500 mL of the modified Hoagland's nutrient solution.

Gas exchange measurement system and data collection

Weather parameters were recorded at 10-min intervals using weather stations (Harusa-View; ADS Co. Ltd.) which were installed beside and inside the glasshouse. Soil pH and wind speed (DS-2; METER) were recorded by a data logger (Em50; METER). The potted plants were brought into the laboratory and acclimatized under fluorescent light (PFD = 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$). Gas exchange rates were measured for two pots, during August 19 to September 8 (Part 1, Figure 1(a) and October 13 to 19 (Part 2, Figure 1(b)). In Part 1, the plant height, leaf number and total leaf area were 80 cm, 25 and 2573 cm^2 for pot 1, respectively, and 81 cm, 25 and 2711 cm^2 for pot 2, respectively. In Part 2, the plant height, leaf number and total leaf area were 110 cm, 44 and 4731 cm^2 for pot 1, respectively, and 112 cm, 42 and 4591 cm^2 for pot 2, respectively. Single leaf area (y) was measured with the equation using leaf width \times length (x) ($y = 0.696x + 4.92$, $r = 0.99$). Photosynthetic rates were measured in the morning (10:00 to 12:00) for two leaves (leaf positions 18th and 22nd in Part 1, 32nd and 33rd in Part 2) according to Kawamitsu, Agata and Miura (1987, 1993) and Fukuzawa et al. (2012). CO_2 and H_2O vapor concentrations were monitored in the air using an infrared gas analyzer

(LI-840A; LI-Cor). The gas exchange rates were measured at PFD 1700 $\mu\text{mol m}^{-2} \text{s}^{-1}$, $30 \pm 0.2^\circ\text{C}$ leaf temperature, 2.0 ± 0.2 kPa VPD, and 400 ± 5 $\mu\text{mol mol}^{-1}$ of CO_2 . Flow rates were set at 8 to 12 L min^{-1} according to the leaf area or photosynthetic capacity. The gas exchange parameters were calculated according to von Caemmerer and Farquhar (1981). Subsequently, soil pH, chlorophyll content (SPAD-502; Minolta), and pot weight (BX12K; Shimadzu) were recorded.

Gas exchange rates for plants grown in root boxes were measured from November 3 to December 9 under glasshouse conditions (Part 3, Figure 1(c)). The plant height, leaf number and total leaf area were 121 cm, 42 and 5605 cm^2 for RB 1, respectively, and 126 cm, 42 and 5789 cm^2 for RB 2, respectively. Two leaves from each plant (30th and 34th from base) were selected for the measurement of gas exchange rates between 10:00 and 14:00 using a portable photosynthesis system (LI-6400XT; LI-Cor). PFD, leaf temperature, CO_2 concentration and flow rate were set at 2000 $\mu\text{mol m}^{-2} \text{s}^{-1}$, $33 \pm 2^\circ\text{C}$, 420 $\mu\text{mol mol}^{-1}$ and 200–400 $\mu\text{mol s}^{-1}$, respectively. To avoid increases in chamber temperature, the leaf chamber and console were placed under a beach parasol. SPAD (index of chlorophyll content) values (SPAD502, Minolta) were also recorded on the same leaf. Stem diameters were measured using a stem gauge sensor (SE-SD6M; SENECOM) at the base of the stem (25 cm from the soil surface). When all measurements were completed, the root boxes were disassembled, and the root system was determined. When jatropha seeds were germinated and cultivated in the root box, three thick roots extended down to the deeper soil layer, whereas the fine and intermediate roots were evenly spread laterally. The

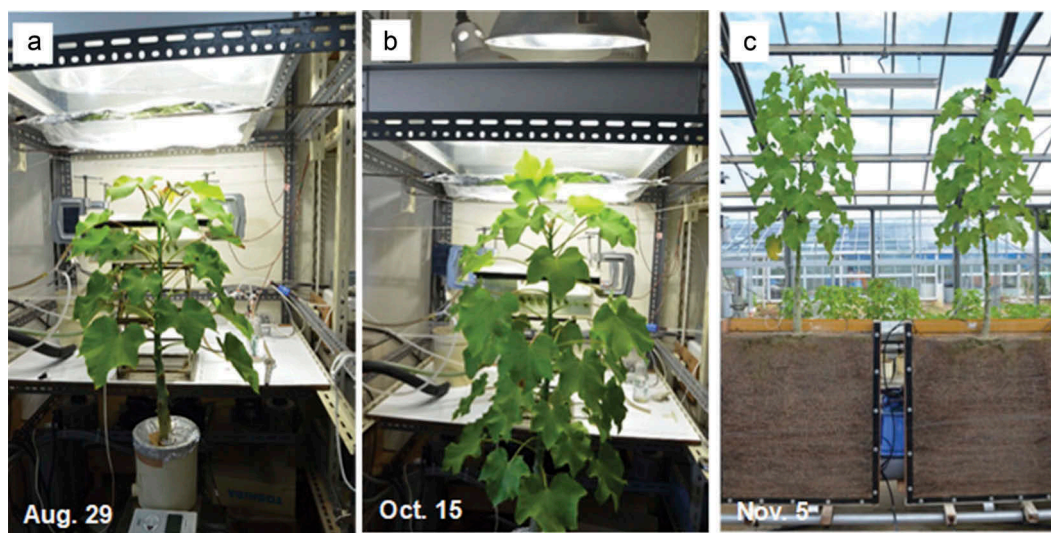


Figure 1. Gas exchange measurements were conducted by the homemade photosynthesis device from August 29 to September 8 (a: Part 1) and from October 11 to October 19 (b: Part 2) in the laboratory and by the LI-6400 XT from November 3 to December 9 (c: Part 3) under glasshouse conditions.

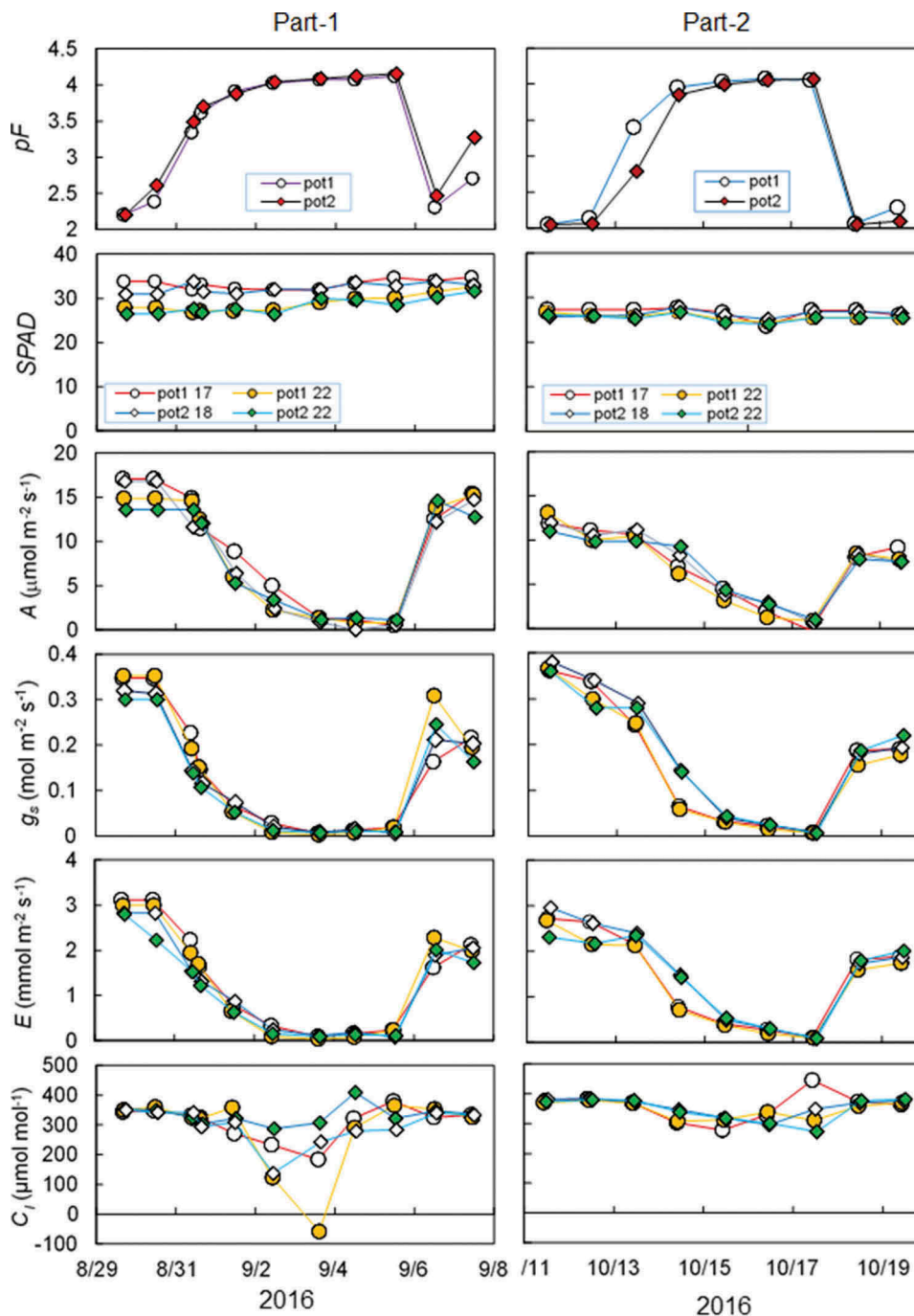


Figure 2. Time changes in soil pF, SPAD, A , g_s , E , and C_i after stopping or re-commencing irrigation. Measurements were carried out twice from August 29 to September 8 (Part-1) and October 11 to October 19 (Part-2) using two pots and two leaves per plant. Pot number and leaf position are indicated in the figure. After measuring photosynthesis on August 29 (Part-1) and October 11 (Part-2), we stopped and re-started watering on September 5 (Part-1) and October 18 (Part-2), respectively.

total root dry weight in RB 1 was 180.8 g, and the cumulative distribution was 39%, 54.6%, 67.3% and 78.2% at 10, 20, 30 and 40 cm depth from the soil surface, respectively.

Water stress treatment

The irrigation stop and re-irrigation date were on August 29 and September 5 in Part-1, October 11 and 18

in Part-2, and November 4 and 25 (RB-1) or 28 (RB-2) in Part 3, respectively. In the current experiments, control plants (fully watered plants) were not included. As a result of preliminary experiments under greenhouse conditions, it had been observed that the gas exchange rate of the control plants did not change for about 10 days. Therefore, the value shortly after withholding water was regarded as the control.

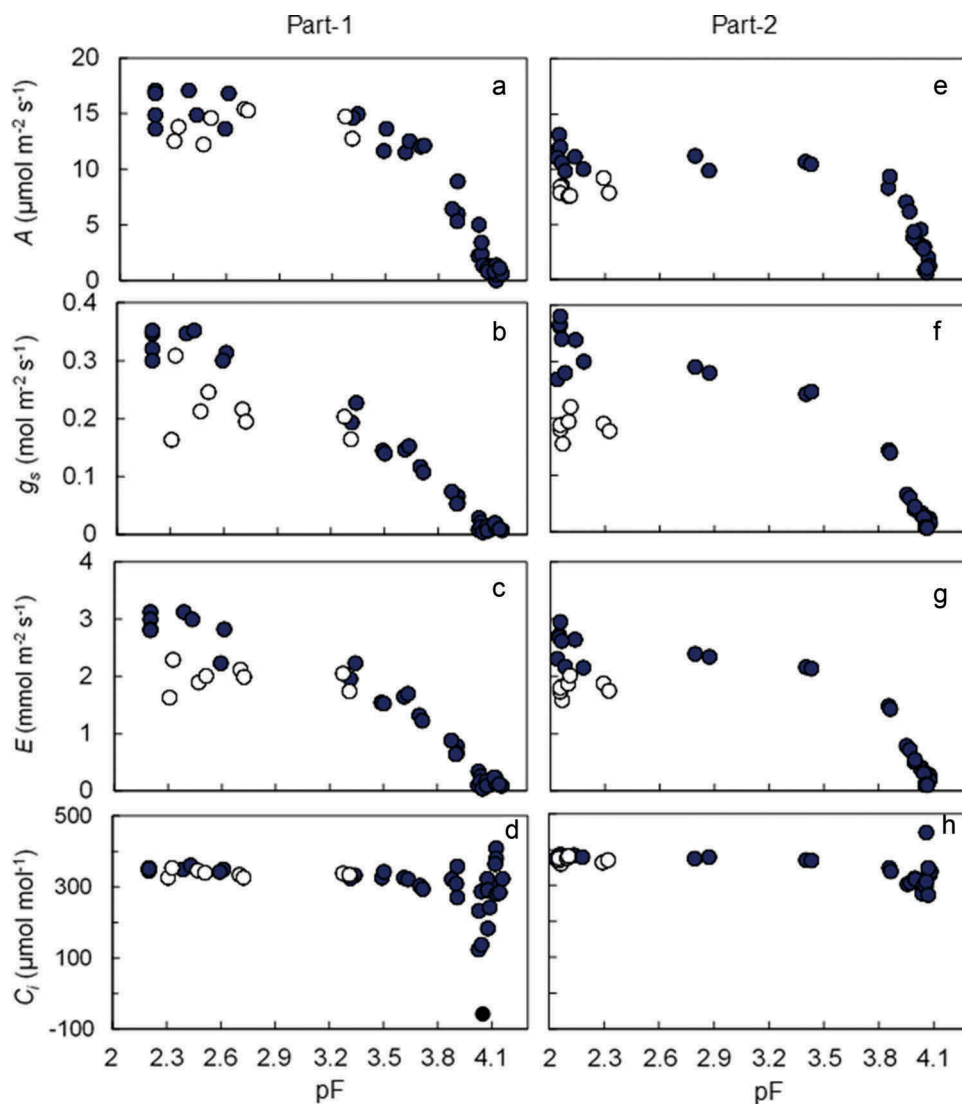


Figure 3. Relationship between soil pF and CO₂ assimilation rate (a, e), stomatal conductance (b, f), transpiration rate (c, g), and intercellular CO₂ concentration (d, h) after withholding water (●) and re-watering (○).

Results

Pot experiment

When the irrigation was stopped on August 29 in Part 1, soil pF reached 3.5 on day 2 and 4.0 on day 3 after withholding water (Figure 2, left). In Part 2, although there were small differences between replicate pots, pF reached 4.0 on day 3 after withholding water (Figure 2, right). Subsequently, pF was maintained at 4.0–4.2, and when irrigation began again on September 5 and October 18, it declined to 2.0 but started to rise again the next day. In spite of the marked decrease in soil moisture, SPAD was stable even after re-watering and tended to rise slightly to the right overall. In Part 1, the SPAD values in the 17th and 18th leaves were higher than those of the 22nd leaves. The *A*, *gs*, and *E* values

shortly after withholding water were $15 \mu\text{mol m}^{-2} \text{s}^{-1}$, $0.32 \text{ mol m}^{-2} \text{s}^{-1}$ and $3.0 \text{ mmol m}^{-2} \text{s}^{-1}$, respectively. These gas exchange rates declined rapidly as pF increased, with *gs* and *E* reaching zero 1 day earlier than did *A*. After re-irrigation, the gas exchange rates recovered to the values observed shortly after withholding water. On the other hand, fluctuations in *C_i* increased after September 2 (Part 1) and October 16 (Part 2), when *gs* reached zero; a negative value was also observed. After re-watering, *C_i* recovered almost to the value at the beginning of the treatment, and the fluctuations also disappeared.

To clarify the effects of soil moisture stress, the relationships between pF and gas exchange rate were plotted (Figure 3). The *A* value decreased from around pF 3.5, whereas *gs* and *E* gradually decreased from around pF

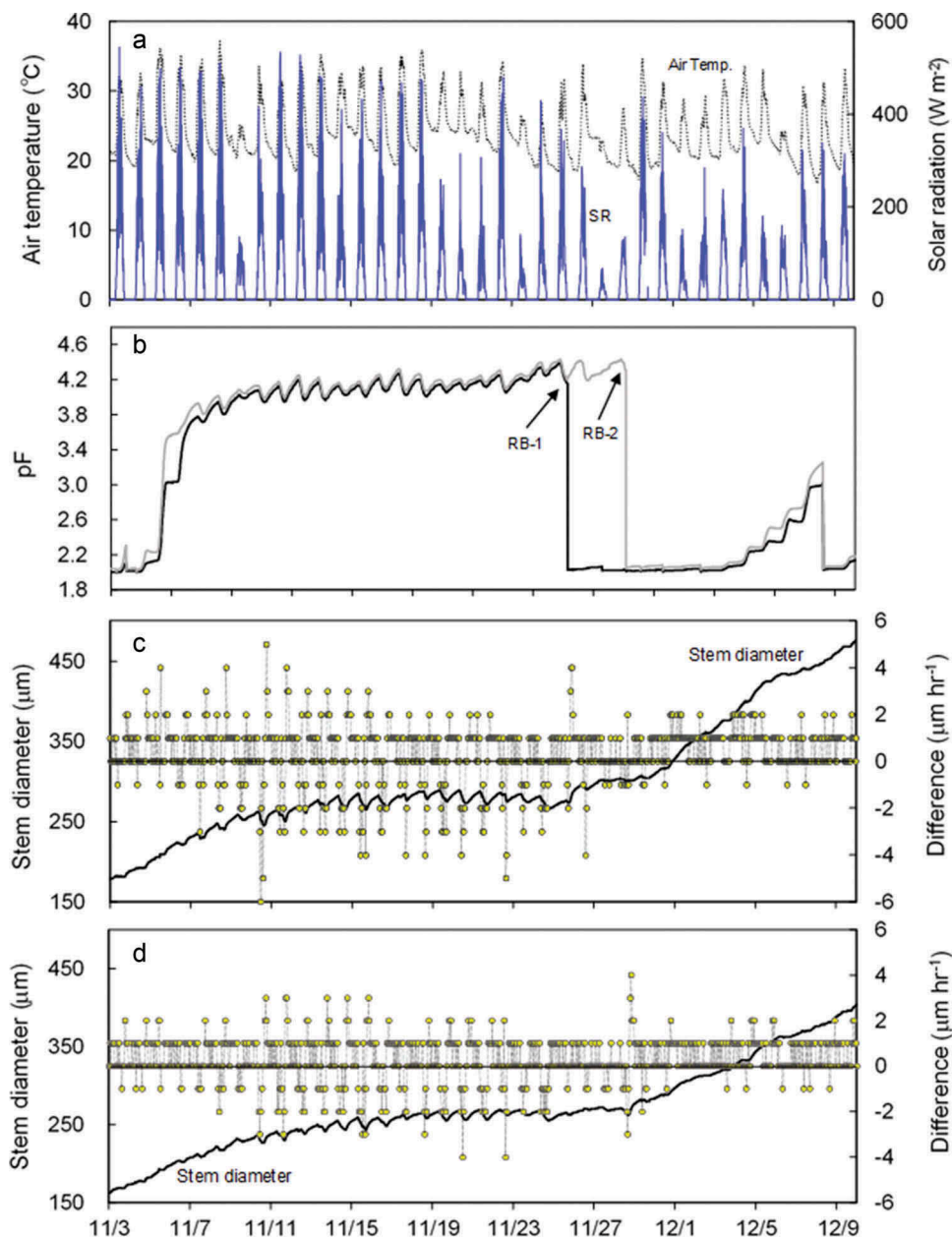


Figure 4. Time changes in air temperature and solar radiation (a), soil matric potential (pF) (b), stem diameter, and its differences in 60 min (C: RB 1, D: RB 2) after stopping or re-starting irrigation. See Figure 4B for the day when watering was re-started.

2.5, reaching zero values near pF 4.0. On the other hand, C_i increased beyond pF 3.8, and a negative value was also observed in Part 1, which was unstable under extreme water stress. After re-irrigation, the A and C_i values recovered almost to the values before the water stress treatment was imposed.

Root box experiment

During the gas exchange measurements in Part 3, air temperature changed daily within the range of 20–35°C, whereas the average temperature was 24.3°C

(Figure 4(a)). Solar radiation under glasshouse conditions varied between 100 and 500 $W m^{-2}$. When irrigation was stopped on November 4, pF for both root boxes rapidly increased from 2.0 to 3.8 (Figure 4(b)). There were no significant differences in pF between the root boxes. When the root boxes were re-watered on November 25 for RB 1 and on November 28 for RB 2, pF decreased from 4.2 to 2.0. When irrigation was stopped on December 2, pF increased again. Up to pF 3.8, pF increased during daytime and remained stable at night; however, the opposite was true at pF greater than 4.0.

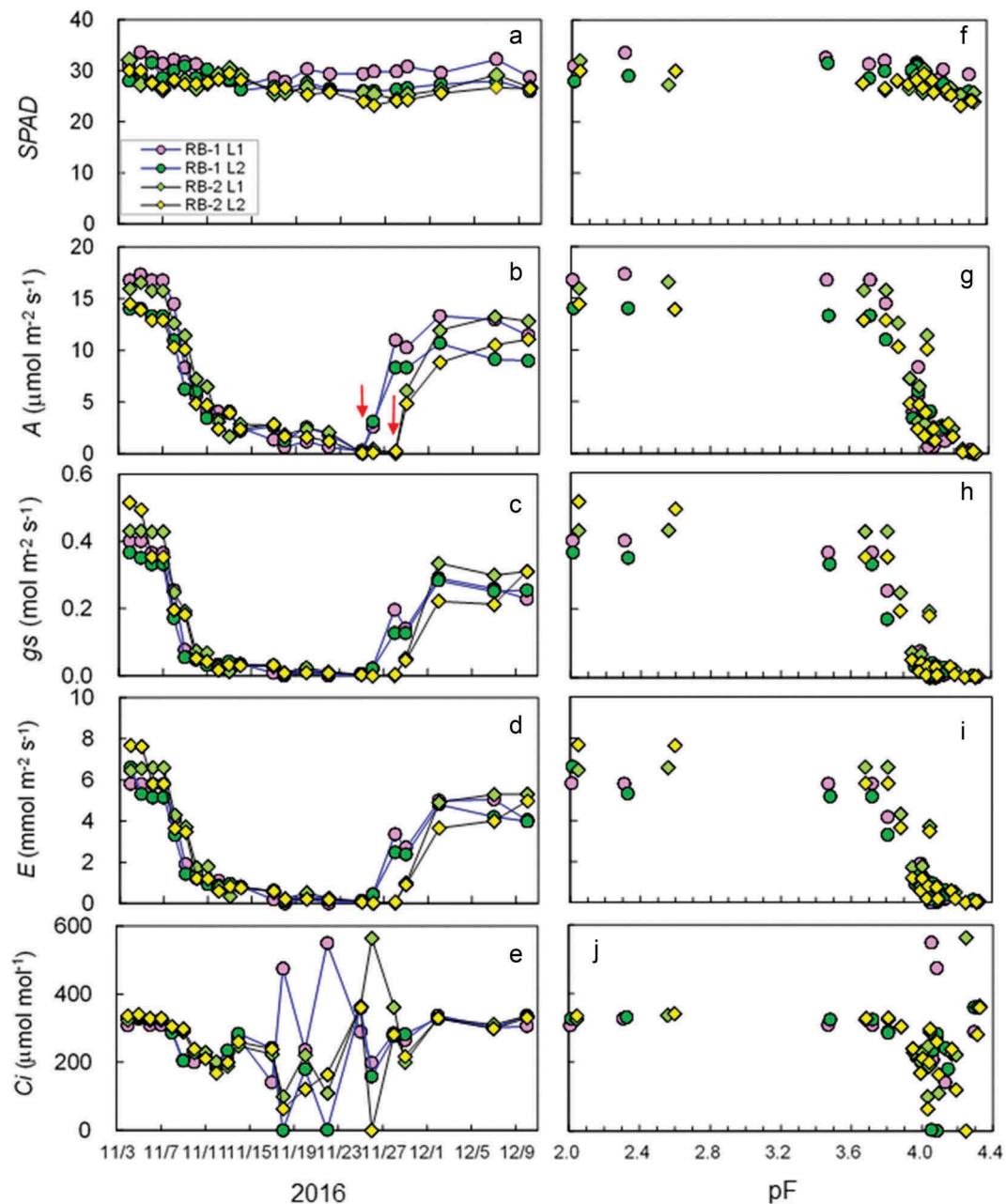


Figure 5. Time changes in SPAD, A , g_s , E , and C_i after stopping or re-starting irrigation (a–e) and their responses to soil pF (f–j). Measurement was carried out for two leaves per plant under glasshouse conditions. The arrow in the figure indicates the re-watering time.

Stem diameters were measured as soil water stress progressed in Part 3 (Figure 4(c, d)). Stem diameter increased until November 11, but when pF reached 4.0, it did not increase, but a diurnal change was observed. To clearly show the change, a 60-min difference was calculated and plotted on a graph (Figure 4(c, d)). At soil pF 4.0 between 11 November and 25 or 28 November, the stem diameter shrank significantly during the day, especially in the afternoon. After re-irrigation, the stem diameter started to expand. Therefore, it was revealed that the stem diameter diurnally changed depending on the water stress conditions.

SPAD did not change despite the marked deterioration of the soil moisture conditions (Figure 5(a)). The gas exchange rate rapidly decreased as the stress increased (Figure 5(b–d)), exhibiting a profile similar to that shown by the potted plants (Figure 2). When the plants were re-irrigated, the gas exchange parameters had almost recovered by day 2. On the other hand, C_i fluctuated from November 15, when g_s reached zero, and then recovered and stabilized following re-irrigation (Figure 5(e)). Based on these data, the response of gas exchange rate to water stress was investigated. Even when pF increased, SPAD was barely affected, decreasing slightly only at pF 4.0 or higher

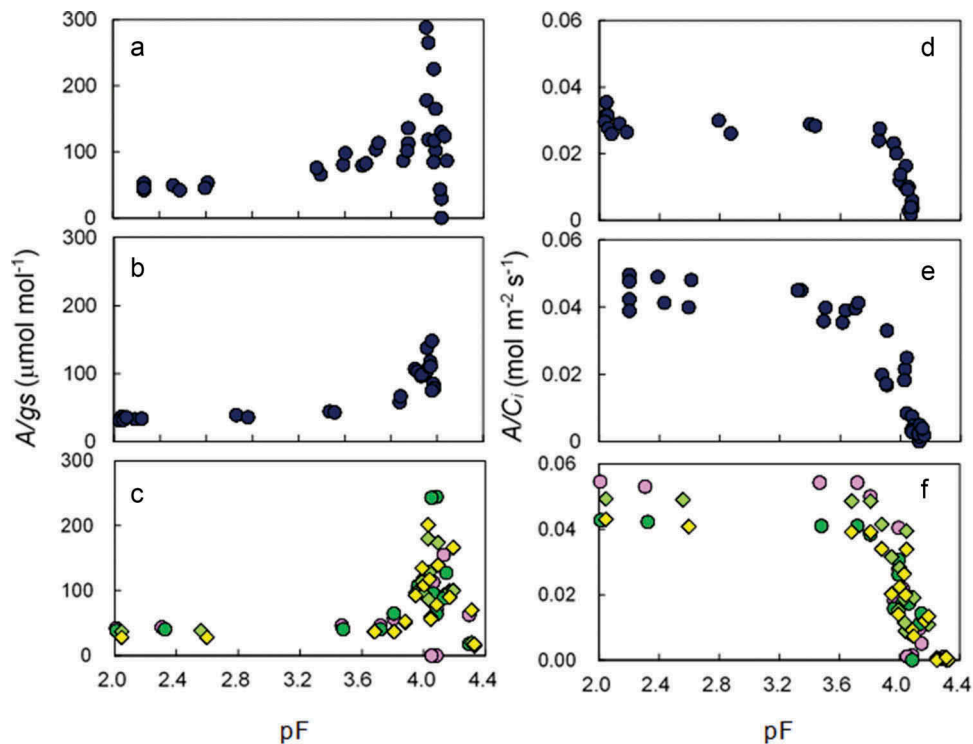


Figure 6. Responses of the intrinsic water use efficiency (A/g_s) and carboxylation efficiency (A/C_i) to acute soil drying in potted plants (a, b, d, e) and root box-grown plants (c, f).

(Figure 5(f)). The A , E and g_s parameters decreased rapidly at pF 3.8 and reached almost zero at pF 4.0 (Figure 5(g–i)). On the other hand, C_i was disturbed at pF 3.8 or higher with values between 0 and $500 \mu\text{mol mol}^{-1}$ (Figure 5(j)).

A/g_s increased at higher pF (>3.5) and fluctuated above pF 4.0 (Figure 6(a–c)). A/C_i dropped sharply at around pF 3.5–3.8 (Figure 6(d–f)).

Discussion

In the present study, we measured gas exchange rates of jatropha plants with a different root system and determined threshold of the soil pF that causes the non-stomatal limitation of photosynthesis during acute drought and re-watering treatments.

When the irrigation was stopped, A decreased at pF 3.5 in pot-grown jatropha plants whereas A in root box-grown plants decreased at severe water stress (pF 3.8) and thereafter, reaching almost zero at pF 4.0 in both experiments (Figures 3 and 5). Under such severe water stress conditions, jatropha plants closed their stomata for the entire day and minimized water loss (Tominaga et al., 2014). Due to these rapid stomatal closures and maintaining the SPAD value, when the plants were re-watered, the gas exchange rate recovered quickly to the value observed before the stress treatment (Figures 3 and 5).

The similar recovery after re-watering was observed by Sapeta, Costa, Lourenco, Linde and Oliveira (2013). Closing stomata to suppress damage inside leaves can be also seen in root box experiments. The re-watering for RB-2 was carried out 2 days later than RB-1, but the stomatal response was almost identical (Figure 5(c and h)). Under such acute soil drying conditions, it is considered that the Jatropha plant has the characteristic of completely closing the stomata, minimizing water loss and waiting for the recovery of rhizosphere water.

We expected that the stem growth would stop during water stress treatment, which could be a target trait for new irrigation system (Nortes et al., 2005; Ortuno et al., 2006). However, diurnal changes of stem diameter (increasing at night and decreasing during daytime) were observed (Figure 4(c, d)). Because the diurnal change masks the stop of stem growth, we concluded that the stem diameter was not appropriate as an index of water stress.

The objective of this study was to determine the threshold of the pF value for the water-efficient irrigation based on the plant-side factors. Initially, we focused on C_i because C_i reflects all of A , g_s , E , CO_2 and VPD parameters (Farquhar, von Caemmerer & Berry, 1980; von Caemmerer & Farquhar, 1981). In other words, C_i is the indicator which represents plant–water relations (Boyer & Kawamitsu, 2011). However, C_i was

significantly disturbed when g_s was suppressed at pF 3.8 or more (Figures 3(d, h and 5j)). The fluctuation in C_i was probably an error problem with the calculation formula (Tominaga & Kawamitsu, 2015a, 2015b; Tominaga, Shimada & Kawamitsu, 2018).

Photosynthetic water use efficiency can usually be obtained by dividing A by E . Since E is strongly affected by VPD, intrinsic water use efficiency is obtained by dividing A by g_s where the VPD effect has been removed. However, the intrinsic water use efficiency fluctuated above pF 3.8 (Figure 6). Based on the relation between soil pF and instantaneous carboxylation efficiency, as estimated by A divided by the calculated C_i , we could identify the thresholds of non-stomatal limitations as pF 3.5. In the present experiments, although the calculated C_i has some uncertainty, the carboxylation efficiency dropped sharply at around pF 3.5–3.8 (Figure 6(d–f)). Therefore, if the watering is done before reaching the pF 3.5, it may be possible to maintain the gas exchange performance without damaging the photosynthetic organ (non-stomatal limitations). However, further studies will be needed to determine the actual pF of various soil types and plant stages.

In conclusion, from the measurements of the gas exchange rates under ongoing water stress, it was revealed that the critical point was at pF 3.5–3.8 in jatropha plants. Therefore, it can be said that it is more efficient to perform irrigation before that with less influence on the photosynthetic organs. However, if C_i were to be measured directly, the A - C_i curve would become smart and a threshold-based thereon could be determined.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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