Inhibition of transpiration in rice by prohydrojasmon compared with that by commercially available microcrystalline and paraffin wax agents

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ABSTRACT
Prohydrojasmon (PDJ), a synthetic derivative of the plant hormone jasmonic acid, has been identified as a plant growth regulator used in various fruit and rice seedlings. In this study, we demonstrate the inhibition of transpiration by PDJ in the indica rice cultivar cv. Takanari compared with commercially available microcrystalline and paraffin wax agents in a greenhouse. Microcrystalline and paraffin wax agents inhibited approximately 30%–50% transpiration 3 h after application, and the inhibition lasted for seven days after treatment, depending on the concentration and the agent used. In contrast, PDJ inhibited approximately 40%–80% transpiration compared with control 0.5 h after treatment, and the effect lasted for one day. However, the effect almost disappeared 7 days after treatment. Thus, PDJ affects transpiration differently than commercially available products and could be used to lower excess transpiration in rice, specifically when an early, transient, and reversible inhibition is needed.

Introduction
Global warming is a serious concern for water deficits in agricultural production and food supply (Bita & Gerats, 2013; Ge., 2000). A water deficit would impact crop production in Europe, India, South America, and the United States (Zhang & Cai, 2013). Rising temperatures induce increased transpiration in plants owing to the upregulation of stomatal conductance and decreases in leaf water potential (Urban et al., 2017). A possible solution to improve tolerance to water deficit in rice is to lower excess transpiration using a transpiration inhibitor. Commercially available microcrystalline and paraffin wax agents are currently used for this purpose. These agents can physically cover leaf surfaces to reduce water loss (Kamuro, 1995). A paraffin wax can be used to inhibit uptake of cadmium, a toxic metal, by preventing transpiration in tobacco (Liu et al., 2016) and to moderate white ears caused by the foehn phenomena during seed maturation in rice (Chiba, 2020).

A second solution is using naturally or artificially synthesized plant hormones and their derivatives that promote adaptation to abiotic and biotic stresses, including heat and water deficit. Abscisic acid (ABA), a plant hormone, induces stomatal closure and decreases transpiration rate, resulting in an increase in leaf temperature under heat stress (Li et al., 2020), which might be used to prevent excess transpiration. Jasmonic acid (JA) was also found to induce stomatal closure. This plant hormone can function together with ABA (Förster et al., 2019; Saito et al., 2008).

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Supplemental data for this article can be accessed here

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PDJ is a synthetically produced JA derivative used as a plant growth regulator. The compound has been identified to affect various biological processes similar to JAs (Huang et al., 2017). PDJ enhances the accumulation of anthocyanins in grapes and apples and improves hand-picking efficiency for mandarin oranges (Ersin, 2015; Sato & Ikoma, 2016). PDJ also induces defense responses against common armyworm in corn (Mandour et al., 2013). Effects of PDJ on phenolic compounds, anthocyanin accumulation, and antioxidant activity in komatsuna and lettuce were reported (Azis et al., 2020). We previously reported that PDJ is a growth regulator that prevents shoot and root growth in both indica and japonica rice cultivars (Chiba & Morino, 2018; Morino et al., 2020). This study investigated the effect of PDJ on transpiration compared with commercially available microcrystalline and paraffin wax agents. We describe PDJ activity for preventing transpiration in rice using the high-yield indica cultivar Takanari, which shows higher transpiration than general japonica cultivars (Fukuda et al., 2018). Our results showed that PDJ functions differently from the commercially available agents and reduces excess transpiration in rice.

Results and discussion

First, we examined experimental conditions using a microcrystalline wax agent, which is a commonly used agent for inhibiting transpiration in various plants, including rice. Stomatal conductance was measured using a porometer on expanded leaves of indica rice cv. Takanari (Inbe et al., 2004), which were sprayed with the microcrystalline wax.

Application of a 5- or 10-fold dilution of the microcrystalline wax agent inhibited transpiration rate by approximately 50% 3 h after spraying. The effect continued for 2 days, regardless of the agent’s concentration. The effect was observable 7 days after treatment (Table 1). Inhibition of transpiration in leaves can also be monitored by canopy temperature (Fukuda et al., 2018; Iseki & Olaleye, 2020); therefore, thermal images were collected using a thermal camera. Canopy temperature of control plants was also determined to be lower than that of the plants sprayed with the microcrystalline wax agent 2 days after treatment; a difference among treatment and control could still be observed 7 days after treatment.

Second, we also examined commonly used paraffin wax agent for inhibiting transpiration. Application of 100- or 200-fold dilutions of the paraffin wax agent lowered transpiration rate by approximately 35%–50% 3 h after spraying regardless of the agent’s concentrations. These effects continued for 2 days and could still be detected 7 days after treatment (Table 2). However, canopy temperature in the thermal images 2 and 7 days after treatment was not different from that in the control images (Figure 2). The transpiration was inhibited in plants sprayed with paraffin wax agent, which could not be monitored using thermal images. However, it was unclear why the canopy temperature could not reflect the paraffin agent’s inhibitory effect, which might be due to the presence of a detergent in the solvent.

These agents cover leaf surfaces physically, and their effects could not be measured before they dried to a waxy coating. Early responses were thus not detected using a porometer. Therefore, thermal images of plants sprayed with microcrystalline or paraffin wax agents after 0.5 h were captured, which showed no distinct differences from plants sprayed with these agents initially (Supplementary Figures 1, 2). Although the difference between the microcrystalline and paraffin wax agents was not clarified in this study, both agents could inhibit transpiration in a greenhouse under natural light conditions.

The effects of PDJ on transpiration inhibition were measured on expanded leaves sprayed with 2,000- or 20,000-fold dilutions of the PDJ solution using the same protocols used for microcrystalline and paraffin wax agents. PDJ treatment lowered transpiration by approximately 40%–80% 0.5 h after spraying regardless of the concentration. At 1 day after spraying, PDJ inhibition was observed, which disappeared 2 and 7 days after spraying with the 20,000-fold dilution. The disappearance of inhibitory effects observed 1 day after spraying with the 2,000-fold dilution was earlier than that of the control plants sprayed with the microcrystalline wax agent 2 days after treatment; a difference among treatment and control could still be observed 7 days after treatment.

Table 1. A microcrystalline wax agent (Greener) prevents transpiration in rice leaves.

| Treatment | 3 hr Mean of transpiration (mmol m⁻² s⁻¹) | 1 day Mean of transpiration (mmol m⁻² s⁻¹) | 2 day Mean of transpiration (mmol m⁻² s⁻¹) | 7 day Mean of transpiration (mmol m⁻² s⁻¹) |
|-----------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
| control   | 9.86                            | 5.03                            | 10.8                            | 6.16                            |
| 5 fold    | 4.44**                          | 4.52**                          | 6.71**                          | 4.64**                          |
| 10 fold   | 4.33**                          | 4.31**                          | 7.32**                          | 4.44**                          |

N = 20, **p < 0.01, *p < 0.05 vs. control. Statistical differences were determined by Dunnett comparison test.
observed with 20,000-fold dilution (Table 3). Similar results were reproduced in two duplicate experiments independently. This result indicated that the concentration of the 20,000-fold dilution is enough to inhibit transpiration, and the higher concentration of the 2000-fold dilution might induce a negative feedback response earlier than the 20,000-fold dilution. Therefore, the concentration of PDJ should be selected according to its intended use. The canopy temperature was monitored 0.5 and 3 h and two and 7 days after PDJ treatment, and temperature was measured at 10 randomly selected points from thermal images. Although the difference between the sprayed PDJ concentrations was unclear, the canopy temperature of control plants was lower than that of plants sprayed with PDJ 0.5 h after treatment. The effects of PDJ were not observed 2 and 7 days after treatment, according to the data on stomatal conductance measurements (Figure 3, Supplementary Table 1). Cumulatively, PDJ inhibited transpiration in rice early and transiently compared with other agents.

The present study suggests that PDJ functions to inhibit transpiration in a manner that differs from physical inhibition induced by commercially available microcrystalline and paraffin wax agents. These agents seal stomata or leaf surface physically, and their effects were observed 3 h after treatment and were apparent up to 7 days after application.

PDJ is a derivative of JA. Thus, it acts similar to its parent compound. Classically, a methyl ester of JA (MeJA) elicits water stress tolerance in several plant species, including soybean and barley (Horton, 1991; Wang et al., 2020). The precursor of JA, 12-oxo-phytodienoic acid (12-OPDA), functions as a regulator of stomatal closure along with ABA in Arabidopsis (Savchenko et al., 2014). Considering these reports, JA, its derivative, and precursor play a role in adaptation to drought stress through stomatal closure. However,
the effective concentration and timing are altered depending on the compound and plant species. PDJ acts quickly and is active in relatively small amounts. The effect was detected 0.5 h after spraying with a 20,000-fold dilution of PDJ, which was observed 1 day after spraying, but disappeared 2 days after spraying (Table 3, Figure 3). Understandably, a hormonal response is reversible, and various signals such as light, humidity, and sugar degradation regulate stomatal closure (Daszkowska-Golec & Szarejko, 2013; Lawson & Matthews, 2020). Therefore, any long-term inhibitory effect of exogenous application of PDJ would be unstable, and a negative feedback regulation might be induced depending on the PDJ concentration.

In summary, PDJ can be employed as an inhibitor of excess transpiration in rice, specifically for early, transient, and reversible inhibition. These characteristics are essential for adaptation to transient heat stress and water deficit, such as the foehn phenomena, and early recovery from inhibited transpiration is required for normal growth and harvest. However, MeJA inhibits stomatal development in Arabidopsis (Deng et al., 2020). PDJ might also inhibit stomatal development, which would then longitudinally reduce transpiration. Therefore, further investigation on the longer term inhibitory effects of PDJ on transpiration is needed.

### Materials and methods

#### Plant growth conditions

Rice seeds of cv. Takanari were sterilized with 5% ipconazole and 4.6% copper hydroxide by soaking in a 1/10 MS medium containing MS vitamins in a growth chamber with continuous white fluorescent light at 25.0°C. Germinated seeds were then precultured in nursery soil containing nitrogen, phosphorus, and potassium.

### Table 3. PDJ solution prevents transpiration in rice leaves.

| Treatment  | Mean of transpiration (mmol m-2 s-1) 0.5 hr | 1 day  | 2 day  | 7 day  |
|------------|------------------------------------------|-------|-------|-------|
| control    | 5.27                                     | 1.28  | 1.4   | 2.01  |
| 2000 fold  | 3.2**                                    | 1.02  | 1.07  | 1.28  |
| 20,000 fold| 1.15**                                   | 0.59**| 1.04  | 1.75  |

N = 20, **p < 0.01, *p < 0.05 vs. control.
Statistical differences were determined by Dunnett comparison test.

Figure 2. A paraffin wax agent inhibits transpiration in rice. Thermal image (left) and corresponding visible image (right) of plants sprayed with 100- or 200-fold dilutions of the paraffin wax agent after 2 days (a, b) and 7 days (c, d).
potassium under the above conditions for 10–14 days. Five seedlings were transferred to a pot (150 mm × 150 mm) containing 350 g soil; next, they were grown in a greenhouse under natural light conditions at 25°C/20°C (day/night) 5–6 weeks which is a nearly maximum tilling stage.

Application of agents
Twenty plants were sprayed with 100 ml of a 2,000- or 20,000-fold dilution of PDJ (5% PDJ, Meiji Seika Pharma Co. Ltd., JP), a 5- or 10-fold dilution of the microcrystalline wax agent (Greener; 10% microcrystalline wax, Greener Co. Ltd. JP), or a 100- or 200-fold dilution of the paraffin wax agent (Abion C; 36% paraffin wax included, Abion Co. Ltd. JP), following the manufacturer’s instructions for each agent used with plastic spraying bottles to assess the inhibition of transpiration.

Measurement of transpiration rate
Transpiration rate was measured using a porometer (AP4, Delta–T, UK) at 0.5 h, 1 day, 2 days, and 7 days after treatment with PDJ and at 3 h, 2 days, and 7 days after treatment with paraffin wax and microcrystalline wax agents as described in the above section. Concomitant thermal images were collected using a thermal camera (Thermal Shot F30S, NEC/Avio) at 0 h, 0.5 hours, 2 days, and 7 days after spraying. The images show the inhibition of transpiration by PDJ at different time points.

Figure 3. PDJ inhibits transpiration in rice. Thermal image (left) and corresponding visible image (right) of plants before spraying (a, b) and 0.5 h (c, d), 2 days (e, f), and 7 days (g, h) after spraying with 2,000- or 20,000-fold dilutions of PDJ.
0.5 h, 2 days, and 7 days after treatment with PDJ and 2 and 7 days after treatment with microcrystalline wax and paraffin wax agents. The thermal images were analyzed using the image analysis software (NS9500LT, Avio, JP). Two duplicate experiments were independently performed.

**Statistical analysis**

Differences among chemical treatments were analyzed using Dunnet comparison tests. The scatter plot matrix analysis was performed using statistical software (Bellcurve for Excel, Bellcurve, JP).

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**Disclosure statement**

No potential conflict of interest was reported by the author(s).

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