Prohydrojasmon prevents spindly growth and induces the expression of an abiotic and biotic stress marker gene, PBZ1p::sGFP, in rice

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ABSTRACT

Prohydrojasmon (PDJ) is a synthetic derivative of the plant hormone jasmonic acid (JA), which is used in apples, grapes, and mandarin oranges as a plant growth regulator. Here, we show that irrigation of PDJ could prevent spindly growth of rice seedlings in both indica and japonica cultivars. PDJ also inhibited root elongation in hydroponically growing seedlings in various cultivars. The higher dry root weight following PDJ application than the controls was observed in two japonica cultivars. Furthermore, its reduction was slight in comparison with the inhibition of root elongation. Starch degradation in the endosperm of seedlings grown in PDJ solution was slower than those grown in the control. The inhibition of root elongation could be mediated by the combined application of PDJ and appropriate concentrations of a synthetic auxin, NAA (1-Naphthaleneacetic acid). Additionally, we generated transgenic rice carrying sGFP driven by the PBZ1 promoter, a marker gene for abiotic and biotic stress responses. The expression of PBZ1p::sGFP was induced by PDJ treatment in the roots and seeds. PDJ could be used as a plant growth regulator to prevent spindly growth and to induce stress responses in rice seedlings.

Introduction

Climate change, which is attributed to global warming has raised serious concerns for agricultural production and food supply, considering the associated plant growth defects and decreased yields (De Vries, 2000). In rice, decreasing grain quality is a major problem attributed to warming during seed maturation. Therefore, cultivars with resistance to warming during seed maturation are under development (Sakai et al., 2007; Tanamachia et al., 2016). Numerous cultivars that have been developed to be resistant to warming during seed maturation show a tendency for exhibiting spindly growth in seedlings (Iwabuchi, 2010). Whereas, it is recommended to delay transplanting onto paddy fields to avoid warming during seed maturation. However, delayed transplanting implies that seedlings grow during conditions that are warmer than their conventional transplanting conditions, causing to spindly seedlings (Arai, 1990). Therefore, excess elongation of seedlings should be repressed because spindly growth causes smaller grain yield due to growth defects after transplantation (Arai, 1990; Ishibashi et al., 2015; Yamaguchi & Inoue, 2005). A potential solution for spindly growth in seedlings is to use a plant growth regulator to inhibit excess elongation. Plant growth regulators are naturally or chemically synthesized substances that have roles in growth and development of plants. Plant hormones are naturally synthesized plant growth regulators by plants to adapt to various environmental conditions, and the use of derivatives and inhibitors in agricultural practices has long been explored (Nakayama et al., 1990; Rodríguez-Furlán et al., 2016). Gibberellins (GA) and their inhibitors are popular growth regulators used to enhance or inhibit plant stem elongation (Rademacher, 2000). In rice, inhibitors of GA synthesis are generally used to inhibit stem elongation in mature plants and nursery plants (Izumi et al., 1984). However, variation of effective chemical that can be used as a plant growth regulator to adapt to climate change is limited in rice.

Over the past decade, the molecular mechanisms of GA function in plant growth responses have been elucidated (Binenbaum et al., 2018; Davière & Achard, 2013). Briefly, GA bind to GID1, a GA receptor, which stimulate the degradation of the DELLA subfamily of plant regulatory proteins, major repressors of the GA signaling pathway, in a ubiquitin and proteasome-dependent manner, followed by the repression of the transcription of GA-responsive genes involved in plant growth by the function of DELLA (Fu et al., 2002; Li et al., 2016; Wang & Deng, 2011). In the case of JA signaling pathway, the jasmonate ZIM-domain (JAM) proteins function as...
negative regulators of the JA signaling pathway, which repress the transcription of JA-responsive genes to bind to their transcription factors. When JA receptor, Coi1 (Coronatine Insensitive 1), percepJA, Coi1, and JA complex bind to JAZs, they cause the degradation of JAZs in a ubiquitin and proteasome-dependent manner, similar to DELLAs does (Browse, 2009; Chini et al., 2007). Recently, it has been reported that JA mediates plant growth, which is antagonistic to GAs in a dose-dependent manner, through direct interactions among DELLAs and JAZs, or interactions between either DELLAs or JAZs and a transcriptional complex, to regulate trichome development in Arabidopsis (Qi et al., 2014). Such reports suggest that JA signaling regulates plant growth in shoots and roots by antagonizing the GA signaling. Additionally, JA signal attenuates auxin synthesis and local auxin distribution via anthranilate synthase α1 (ASA1), and, MYC2, a major transcription factor of the JA signaling pathway, inhibits PLT1 (PLETHORA1) and PLT2 (PLETHORA2), playing key roles in the direct maintenance of root meristematic cells (Chen et al., 2011; Wasternack & Hause, 2013).

PDJ is a synthetically produced plant growth regulator, which is expected to show activity resembling the function of jasmonates, which are involved in various biological processes, including the inhibition of normal growth in plants (H. Huang et al., 2017). Therefore, PDJ could function as a plant growth regulator for the control of shoot and root growth. PDJ has already been used for the color improvement of fruit by enhancing the accumulation of anthocyanins in grapes and apples, in addition to the improvement of handpicking efficiency in mandarin oranges (Atay, 2015; Sato & Ikoma, 2016). Recently, the application of PDJ to induce defense responses against common armyworm in corn was reported (Mandour et al., 2013). However, the application of PDJ as a plant growth regulator or an inducer of defense responses in rice has not been reported. While Methyl jasmonate (MeJA) is a methyl ester of JA and is used to study the JA signaling pathway as a plant growth regulator (Fahad et al., 2016; Gan et al., 2015; Reyes-Díaz et al., 2016). Therefore, PDJ possibly functions as a plant growth regulator for the control of shoot and root growth as well.

Here, we describe a PDJ function for preventing spindly growth during high-temperature conditions in rice seedlings. PDJ could prevent spindly growth in rice seedlings regardless of whether a cultivar is an indica or a japonica cultivar. The inhibitory effect of PDJ on shoot elongation is lower than that of MeJA. PDJ also inhibits root elongation in various cultivars; however, in two japonica cultivars, the root dry weight in seedlings growing in PDJ solution was higher than that in the controls. The reduction in root dry weight was low in contradiction to the inhibition of root elongation. Starch degradation in seeds was delayed in seedlings. Inhibition of root elongation could also be mediated by an appropriate concentration of 1-naphthaleneacetic acid (NAA), a synthetic auxin application. A study of the expression of sGFP fusion driven by PBZ1 promoter, a marker gene associated with abiotic and biotic stress responses (L. F. Huang et al., 2016; Midoh & lwata, 1996), revealed that the expression of sGFP markers was induced following treatment of roots and seeds with PDJ. The results indicated that PDJ prevented spindly growth in the course of shoot and root elongation. However, the reduction in root weight was not considerable. Therefore, PDJ could be applied as spindly growth inhibitor in rice seedlings, which can be attenuated by NAA application during root growth. Moreover, an elicitation of both abiotic and biotic stress responses by application of PDJ would be expected from a result of the PBZ1 promoter::sGFP gene expression induction.

**Results**

To examine the effect of PDJ on shoot growth and to compare it with that of MeJA, which is a generally used chemical for the analysis of JA-related signaling pathway, we used 3-day pre-cultured seedlings in indica cv. Kasalath, and common japonica cultivar cv. Koshihikari, cv. Nipponbare, and cv. Hinohikari, growing in nursery soil irrigated with of 2,000-fold diluted PDJ solution (98.3 μM) or corresponding concentrations of MeJA for 3 days at 27.5°C, which was a higher temperature than that used in conventional growth conditions. Shoot elongation was significantly inhibited; however, the effect of PDJ on shoots was lower than that of MeJA in all the tested cultivars (Table 1). Subsequently, to evaluate the effects of PDJ on root growth, plants were grown hydroponically in 20,000-fold diluted PDJ solution, and the concentrations were determined to not demonstrate strong defects of growth but show a PDJ effect using preliminary experiments (Supplementary Figure 1). Maximum root lengths, root

| Table 1. PDJ solution and MeJA prevent spindly growth in seedlings. |
|-----------------------------|----------------|----------------|----------------|
| Cultivar | Control (cm) | PDJ (cm) | MeJA (cm) |
| Kasalath | 13.60 | 12.90 | ** 7.43 | *** |
| Koshihikari | 15.67 | 14.55 | *** 8.47 | *** |
| Nipponbare | 13.24 | 12.27 | *** 7.82 | *** |
| Hinohikari | 13.36 | 11.67 | *** 5.39 | *** |
| Cultivar | *** | | |
| Treatment | *** | | |
| Cultivar * Treatment | *** | | |

N = 60, **p < 0.01, *** p < 0.001 vs. control. Statistical differences were determined by Dunnett’s multiple comparison test.
numbers, and the shoot lengths were measured using five cultivars; indica cv. Kasalath, japonica cv. Koshihikari, cv. Nipponbare, cv. Hinohikari and cv. Koinoyokan. Cv. Koinoyokan is one of the cultivars showing strong resistance to warming. Similarly, the effects of uniconazole-P (UCZ, Sumitomo chemical, Japan) on seedlings were measured, since UCZ, a plant growth regulator in diverse plants, is a GA synthesis inhibitor (Rademacher, 2000; Sasaki et al., 2013). Shoot elongation was inhibited in all the examined cultivars, which grew in both PDJ and UCZ solutions. Although root length decreased in all the seedlings grown in PDJ solution, UCZ did not inhibit root elongation except for Koinoyokan (Table 2, Figure 1). Although statistically significant differences could not be observed, the root number increase in several cultivars (Table 2).

Furthermore, to determine the effect of PDJ on plant filling, 3-day pre-cultured germinated seeds were grown in PDJ solution for 7 days (the age of 10 days) with common japonica cultivars; cv. Koshihikari, cv. Nipponbare, and cv. Hinohikari. First, the shoot length, root length, and root

![Figure 1](image.png)

**Figure 1.** The difference in the PDJ effects among cultivar in hydroponics.

Notes: Seedlings which exhibited representative effects of PDJ or UCZ were shown in cv. Kasalath (a), cv. Koshihikari (b), cv. Nipponbare (c), cv. Hinohikari (d), and cv. Koinoyokan (e).

| Cultivar   | Shoot length (cm) | Root length (cm) | Root number |
|------------|-------------------|------------------|-------------|
|            | Control | PDJ   | UCZ   | Control | PDJ   | UCZ   | Control | PDJ   | UCZ   |
| Kasalath   | 6.28    | 5.05  | ***   | 3.68   | ***   |       | 12.14   | 6.12  | ***   | 11.04   | 5.58  | 6.09  | 5.11    |
| Koshihikari| 9.57    | 7.26  | ***   | 4.54   | ***   |       | 9.75    | 5.32  | ***   | 10.27   | 6.27  | 5.87  | 5.87    |
| Nipponbare | 8.71    | 6.25  | ***   | 4.30   | ***   |       | 9.88    | 4.51  | ***   | 10.66   | 6.53  | 6.64  | 6.00    |
| Hinohikari | 7.70    | 4.01  | ***   | 3.13   | ***   |       | 10.36   | 5.04  | ***   | 10.74   | 5.51  | 5.62  | 5.40    |
| Koinoyokan | 9.84    | 5.15  | ***   | 2.86   | ***   |       | 12.48   | 4.07  | ***   | 9.86    | 5.49  | 5.44  | 6.02    |
| Cultivar   | Treatment| ***  |       |       |       |       | ***     |       |       |         |       |       |         |
| Treatment  | Cultivar * Treatment| ***  |       | ***   |       |       |       |       |       |         |       |       |         |

N = 45, *p < 0.05, **p < 0.01, ***p < 0.001 vs. control.

Statistical differences were determined by Dunnet’s multiple comparison test.
numbers of plants were measured and then the shoot, root, and seed dry weight were measured after 7 days of drying.

The average shoot length in all cultivars was approximately 80–90% of the growth observed compared with that in the controls. While shoot weight was approximately reduced by 16% in cv. Koshihikari, other cultivars show a small difference compared with the controls (Table 3, Supplementary Figure 2). The average root length of seedlings growing in PDJ solution was approximately 65–80% of the growth observed compared with that of the controls. While root weights in cv. Koshihikari showed 15% reduction compared with the controls, cv. Nipponbare and cv. Hinohikari showed approximately 10% higher weight than the controls. Root numbers show small differences compared to the controls in 10-day seedlings. Additionally, the average dry seed weight of seedlings growing in PDJ solution was approximately 10–20% higher than that of the controls (Table 3, Supplementary Figure 2). Therefore, we examined seeds after growth in PDJ solution, which revealed that the breakdown of starch was inhibited in endosperms (Figure 2).

A root growth attenuation effect of an auxin in plants growing in PDJ solution was also examined. It has been reported that 40 nM NAA induced a 50% inhibition in root elongation (Chhun et al., 2003). Root elongation was measured in cv. Nipponbare plants, grown hydroponically in 20,000-fold diluted PDJ solution with 1, 10, or 100 nM NAA. The 1 nM and 100 nM NAA treatments on plants growing in PDJ solution additively inhibited root elongation, while treatment with 10 nM NAA reduced the PDJ inhibitory effect on root elongation (Figure 3(a)). To test the effect of the treatment with 10 nm NAA in plants growing in PDJ solution was approximately 10–20% higher than that of the controls (Table 3, Supplementary Figure 2). Therefore, we examined seeds after growth in PDJ solution, which revealed that the breakdown of starch was inhibited in endosperms (Figure 2).

| Table 3. The dry weight of shoots, roots, and seeds from seedlings hydroponically growing in PDJ solution (10 days). |
|---|---|---|---|---|---|---|---|---|
| Cultivar | Shoot length (cm) | Root length (cm) | Root number | Shoot weight (g) | Root weight (g) | Seed weight (g) |
| | Control | PDJ | Control | PDJ | Control | PDJ | Control | PDJ | Control | PDJ |
| Koshihikari | 12.24 | 11.29 *** | 15.73 | 11.42 *** | 6.43 | 6.55 | 6.57 | 5.55 | 4.35 | 3.47 *** | 7.38 | 8.72 ** |
| Nipponbare | 7.87 | 7.40 ** | 13.99 | 8.97 *** | 7.10 | 7.00 | 4.88 | 4.77 | 4.50 | 5.10 * | 7.70 | 8.75 |
| Hinohikari | 7.61 | 6.17 *** | 13.12 | 10.46 *** | 6.68 | 6.08 ** | 4.05 | 4.15 | 4.15 | 4.67 | 8.20 | 8.97 |
| Treatment | *** | *** | *** | *** | *** | *** | *** |
| Cultivar*Treatment | * | * | * | * | * | * | * | * | * | * | * | * |

*N = 40, *p < 0.05, **p < 0.01, ***p < 0.001 vs. control.
Statistical differences were determined by Dunnet’s multiple comparison test.

Figure 2. The PDJ effects on inhibition of starch degradation.
Notes: Photographs show the rice seedlings and magnified their seeds that were grown on 1/2 MS medium containing final concentration of 2000-fold diluted PDJ with agar.
growing in PDJ solution, root and shoot length, and root number were evaluated in cv. Koshihikari, and cv. Hinohikari. The 10 nM NAA did not have a considerable effect on shoot elongation (Figure 3(b)). However, it complemented the effect of PDJ on root elongation in cv. Hinohikari as well as in cv. Nipponbare. However,
reproducible significant attenuation effects of NAA were not observed in cv. Koshihikari (Figure 3(c)). Additionally, we did not observe any effect of NAA on root number could in all the tested cultivars (Supplementary Figure 3). To observe the effect of PDJ on a stress response visually, the transgenic rice carrying sGFP driven by the PBZ1 promoter was grown on 1/2 strength Murashige and Skoog (MS) medium with or without PDJ. PDJ treated plants exhibited greater fluorescence in seeds and roots 3–7 days after treatment with PDJ (Figure 4).

**Discussion**

PDJ is a synthetically produced compound, expected to exhibit biological activity resembling the function of plant hormone JA. In the present study, we examined whether PDJ could be applied as a plant growth regulator to prevent spindly growth during seedling development and to induce stress responses in rice. The irradiation of PDJ solution showed an inhibitory effect on shoot elongation in both indica cultivar cv. Kasalath and common japonica cultivars; cv. Koshihikari, cv. Nipponbare, cv. Hinohikari (Table 1). Since PDJ is a derivative of JA, the effects of PDJ were compared with MeJA, which is commonly used for the inducer of JA signaling pathway. The inhibitory effect of PDJ on shoot growth was relatively low rather than that of MeJA (Table 1). Since the plants were grown hydroponically in 20,000-fold diluted PDJ solutions, root and shoot elongation were inhibited in all the tested cultivars, including warming resistant cultivar; Koinoyokan, which shows spindly growth in seedlings (Table 2, Figure 1). Compared to the 3500-fold UCZ treatment, the inhibition of shoot elongation by PDJ was lower than that by UCZ. However, the inhibition of root elongation by PDJ was much greater than that by UCZ. It is well known that UCZ inhibits the synthesis of GAs, which plays a role in cell elongation. It reported that the inhibitory effects of JA on root elongation occurred through the inhibition of both cell proliferation and cell elongation in Arabidopsis (Chen et al., 2011). However, JA exhibited negative effect on leaf expansion by inhibiting cell division in Arabidopsis (H. Huang et al., 2017). MeJA impaired normal cell cycle by preventing G1/S transition in Taxus cells (Patil et al., 2014). Considering these reports and our results, PDJ might have a repressive effect on plant growth by affecting both cell elongation and cell division. Therefore, PDJ could be used as a plant growth regulator with an additive or different function from UCZ.

Interestingly, root elongation in cv. Koinoyokan was uniquely inhibited by UCZ treatment, indicating that cv. Koinoyokan is more sensitive to GA inhibitor than other cultivars. Although it is highly predictive, warming resistance is possibly correlated with GA signaling pathway.

The measurement of dry weight of plants growing in PDJ solution to examine the effect of PDJ on plant filling revealed that the dry weights of roots in cv. Nipponbare and cv. Hinohikari were higher than those of the control. The reduction of dry weight of shoots in cv. Koshihikari and cv. Hinohikari and of roots in cv. Koshihikari was observed; however, the reduction was relatively small in proportion to the decrease in shoot and root length (Table 3, Supplementary Figure 2). These results indicate that PDJ prevents both shoot and root elongation but
has a small effect on plant filling. Although we observed a significant increase in root numbers after 4 days of growing (the age of 7 days) in PDJ solution, we could not observe that in the plant growing for 7 days in PDJ solution at the age of 10 days (Table 3, Supplementary Table 1). Therefore, it was not clear in our experimental condition whether PDJ may have a function in increasing root numbers. We reported that the strength of roots in the PDJ-irradiated rice seedlings is sufficient for practical use (Chiba & Morino, 2018). Therefore, the effect of PDJ on the inhibition of root elongation is not a matter to use for PDJ as a plant growth regulator. Since crosstalk between the JA signaling pathway and auxin signaling pathway has been reported, an auxin flow which plays a key role in root development both of crown root and lateral root (Chen et al., 2011; Coudert et al., 2010; Meng et al., 2019). However, high concentrations of NAA inhibit root elongation in plants (Rahman et al., 2001). We observed that treatment with 10 nM NAA reduced the inhibitory effect of PDJ on root elongation in both cv. Nipponbare and cv. Hinohikari. However, both lower and higher concentrations of NAA exhibited additive inhibitory effects. Therefore, the ratio of PDJ and NAA could be applied in the mediation of root growth for practical applications and to understand the detailed mechanism of the effect of PDJ on root growth, which requires further study. Conversely, the inhibition of starch degradation could be observed in endosperms after the growth of germinating seeds in PDJ solution (Table 3, Figure 2). Yang et al. reported that MeJA repressed α-amylase induction by GA using the embryoless seeds and RNAi seeds of JA receptor gene, showing higher levels of α-amylase activity than in wild-type cv. Nipponbare seeds (Yang et al., 2012). Therefore, PDJ might have an inhibitory effect on α-amylase activity, leading to the delayed starch degradation in seeds.

The expression of a marker gene for abiotic and biotic stress responses, PBZ1, is induced following treatment with JA (Lee et al., 2001). PBZ1p::GFP plants, grown hydroponically in 20,000-fold diluted PDJ, exhibited higher fluorescence in seeds and roots 3–7 days after transfer into PDJ solution. PBZ1 was originally isolated as a highly expressed gene, in response to a disease resistance signaling pathway (Iwai et al., 2007; Nakashita et al., 2001), and recently, reported to exhibit RNase activity, function in various stress responses (L. F. Huang et al., 2016; Kim et al., 2011). JA functions in various stress responses, including disease and insects resistance (Ahmad et al., 2016; De Vleesschauwer et al., 2013), while PDJ functions induce resistance responses against armyworm in corn were recently reported (Mandour et al., 2013). Cumulatively, PDJ could function as a plant growth regulator and an inducer of abiotic and biotic stress responses in rice seeds and roots. Overall, the results of the present study suggest that PDJ could be used to inhibit excess elongation of rice seedlings in both japonica and indica rice cultivars. PDJ application may inhibit root elongation, which could be mediated through the combined use of appropriate auxin concentration. Our results were obtained from plants growing under controlled incubators and greenhouse conditions. The reproducibility of the findings in natural condition should be needed.

Materials and methods

Plant growth conditions, application of chemicals, and growth measurement

For seed germination, sterilized rice seeds with 5% ipconazole and 4.6% copper hydroxide were soaked in 1/10 MS medium containing MS vitamin in a growth chamber with continuous white fluorescent light at 27.5°C. A PDJ (PDJ 5%, Meiji Seika pharma, Japan) solvent was used for the controls. For comparing the effect of PDJ and MeJA on shoot growth, germinated seeds were pre-cultured in nursery soil containing nitrogen, phosphorus, and potassium under the above-mentioned conditions for 3 days, which were irrigated with 2000-fold diluted PDJ solution or MeJA solution at a corresponding concentration of PDJ solution. Shoot lengths were measured from the soil surface to the highest leaf tip after 3 days irradiation with PDJ. Unless otherwise indicated, two independent replicates were examined and all examined samples were statistically analysed collectively throughout the course of this study. To measure root and shoot growth in the hydroponic systems, germinated seeds were pre-cultured in 1/10 MS medium for 3 days, which were transferred to 20,000-fold-diluted PDJ solution or 3500-fold-diluted UCZ solution (UDZ 0.025%, Sumitomo chemical, Japan), and grown for 5 days. Three independent replicates were examined. To measure shoot, root, and seeds dry weights, seedlings pre-cultured for 3 days were grown in PDJ solution for 4 or 7 days, which were washed with sterilized water and dried for 7 days. Plants were cut into shoots, roots, and seeds and were subsequently weighted. For observation of starch degradation in germinating seeds, the seeds of cv. Nipponbare were grown on 1/2 MS medium containing final concentration of 2000-fold diluted PDJ with agar under the above conditions for 20 days.

To assess the combined effects of PDJ and NAA, 3-day pre-cultured seedlings were transferred into 20,000-fold diluted PDJ solution with final concentrations of 1, 10, or
100 nM NAA, and grown under the above conditions. Shoot and root lengths from seed to shoot tip or root tip and root numbers were measured 7 days after transfer into appropriate solutions.

Statistical analysis

The differences among chemical treatments or among cultivars were analysed using Tukey’s or Dunnet’s multiple comparison tests.

Production of transgenic plants and observation of sGFP expression

To generate transgenic rice carrying the promoter – sGFP fusion, the promoter region of PBZ1 gene was amplified using the primer pair; 5′- cggcgagggtcagctctga – 3′ and 5′- cctactagctgttgcc – 3′. The amplified fragment was fused to the sGFP reporter gene (Niwa et al., 1999) in the binary vector pTN1 (Fukuoka et al., 2000). The construct was introduced into the rice cv. Nipponbare using Agrobacterium-mediated transformation using a G418-resistance marker driven by the NCR promoter as a selection marker. All transgenic plants were grown in a growth chamber or a greenhouse. For sGFP observation, homozygous seeds were germinated on 1/2 MS liquid medium containing 1% sucrose and MS vitamin, and incubated at 27.5°C for 3 days, and, then, transferred to 20,000-fold diluted PDJ solution. Excitation light (450 nm, wavelength) and a 450 nm band-pass filter (Fuji Film BP45, Japan) were used to perform visualization of sGFP expression. sGFP fluorescence was photographed using a digital camera (Canon, PowerShot G11, Japan) and a filter that cuts out light with wavelengths shorter than 500 nm (Kodak, Watten Filter No12, Japan).

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Disclosure statement

No potential conflict of interest was reported by the authors.

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