Growth promoting effect of L-β-phenyllactic acid on rice (Oryza sativa L.) seedling grown under polyethylene glycol (PEG)-induced water deficit condition

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Abstract: Water stress is a major limiting factor for plant growth and development. In this study, we investigated the effects of L-β-phenyllactic acid (LPA) on growth of rice (Oryza sativa L.) seedlings under polyethylene glycol (PEG)-induced water deficit conditions. Seedlings were cultured at 30°C for 14 days in growth pouches supplemented with 1/100-strength Murashige and Skoog (MS) medium and PEG in the presence or absence of 100 mg L⁻¹ LPA. As evidenced by plant height, LPA application enhanced seedling growth under PEG-induced water deficit by 13%. The shoot dry weight was slightly increased, whereas that of roots was markedly enhanced during LPA treatment by 26% under water-deficit conditions. No difference was observed among treatments in the number of roots per seedling. The ratio of shoot dry weight to shoot length (RWL) was constant regardless of treatment, indicating that LPA does not cause spindly shoot growth. The total length, surface area, and volume of fine roots were increased by LPA under PEG-induced water deficit conditions. Plant height was significantly correlated with total root surface area and volume. The results imply that PEG-induced water deficit in rice seedlings can be alleviated by LPA application. This alleviative effect is partially attributable to alterations in root system developmental patterns, with increases in fine root total length, surface, and volume accelerating water and nutrient acquisition from the culture medium.

Keywords: L-β-phenyllactic acid, plant growth substance, rice (Oryza sativa L.), root, root elongation-promoting substances, water stress

Abbreviations: LPA, L-β-phenyllactic acid; PEG, polyethylene glycol; MS, Murashige-Skoog; REPS, root elongation-promoting substances; RWL, ratio of shoot dry weight to shoot length

Introduction

Growth and yield of rice (Oryza sativa L.), a staple food crop, is strongly dependent on environmental conditions. For example, rice plants are adversely affected by drought stress, which occurs when water supply to roots is limited or leaf transpiration rates become very high. Increasing drought tolerance is thus crucial to rice production.

Growth and development of roots are necessary for rice adaptation to soil water stress conditions (Fukai and Cooper 1995, Ming et al. 2011, Wade et al. 2000, Wang and Yamauchi 2006). In addition, root system architecture is an important functionality during taking up water and nutrition (Lynch 1995).

With respect to chemical regulation for crop production, one focus of research concerns the effect of plant growth regulator (PGR) application on the ability of roots to maintain nutrient and water uptake required for plant sustainability (Wittwer 1978, Field and Whitford 1982). The PGRs, including plant hormones, also mediate biochemical changes that play a vital role during water deficit (Thakur et al. Adachi Y, Kimura K, Saigusa M, Ohyama T, Takahashi Y, Watanabe H 2014 Growth promoting effect of L-β-phenyllactic acid on rice (Oryza sativa L.) seedling grown under polyethylene glycol (PEG)-induced water deficit condition. Plant Root 8: 64-71. doi:10.3117/plantroot.8.64

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1998). Little work has been done with rice in relation to PGRs and drought resistance (Fukai and Cooper 1995), however various attempts have been made to alleviate water stress by applying PGRs in several plant species (Wittwer 1978, Nickell 1982). For instance, brassinosteroids have been found to restore germination and growth of sorghum seeds inhibited by polyethylene glycol (PEG)-induced deficit (Vardhini and Rao 2003), while ABA application maintains root growth in water-stressed rice and Arabidopsis plants by regulating auxin transport (Xu et al. 2012). Methyl jasmonate has been found to eliminate the effect of water stress in strawberry plants, by altering the structure and composition of lipid membrane (Wang 1999). Finally, benzyladenine enhances photosynthetic rate, chlorophyll content, and nitrate reductase activity and ameliorates the negative effects of water stress in *Cassia angustifolia* (Singh et al. 2001). These studies suggest that PGR application can alleviate or counteract water stress in many plant species by altering physiological or morphological traits.

Among such PGRs, root elongation-promoting substances (REPSs), such as L-β-phenyllactic acid (LPA), particularly enhance root elongation (Takanaka 1993). LPA was isolated from culture filtrates of *Exobasidium symploci-japonicae* (Tamura and Chang 1965), and have been widely designated as REPS (Takanaka 1993). The rice seminal roots elongated around twice as compared with those of control plants by 100 mg L⁻¹ LPA treatment (Tamura and Chang 1965).

Total root length, surface area, and volume are important traits related to water and nutrient uptake (Yamauchi et al. 1996, Wang et al. 2006, Gowda et al. 2011). Our previous report presented that LPA enhance the root traits including, seminal roots length, total length and surface area of fine roots indispensable for water and nutrient uptake from soil (Adachi et al. 2013). LPA was also suggested to promote shoot growth by increasing nutrient and water assimilation or kernel endosperm use efficacy (Adachi et al. 2013).

Until now, no studies have currently focused on LPA’s growth-promoting effects in rice seedlings grown under PEG-induced water deficit conditions. In addition, very little published information related to chemical regulation of water stress tolerance is available regarding alleviation of PEG-induced water deficit in rice seedlings by LPA treatment. In this study, we therefore investigated the effects of LPA on rice seedling growth, with a focus on shoot and root growth under water-deficit conditions induced by PEG osmotic stress.

### Materials and Methods

**Plant materials and culture conditions**

Koshihikari rice seeds were surface sterilized for 24 h using in a solution of 0.1% benomyl (Sumitomo Chemical Company, Tokyo, Japan). Seed were soaked in water at 10°C for 10 days, and then germinated in darkness at 30°C.

Three treatments were set up using different nutrient solutions (control, PEG and PEG+LPA treatments): 1/100-strength Murashige and Skoog (MS) nutrient solution (control), 1/100-strength MS nutrient solution with PEG 6000 (Wako Pure Chemical Industries Ltd., Osaka, Japan) to induce water deficit (PEG), and 1/100-strength MS nutrient solution and PEG supplemented with LPA (Sigma, St. Louis, MO, USA) at 100 mg L⁻¹ (PEG+LPA). Following Takahashi and Kaufman (1992) dealing with relation between water deficit and rice seedling growth, water deficit treatments were initiated by addition of PEG to culture medium to achieve a 17.2% final concentration.

Growth pouches, consisting of blotting paper covered with plastic film, are excellent for high-throughput root system phenotyping, and facilitate non-destructive scanning of root traits followed by digital image analyses (Hund et al. 2009, Trachsel et al. 2010). We used this system to apply PEG-induced water deficit to the root systems of rice seedlings as was described in our previous study (Adachi et al. 2013) with slight modification. Seed growth pouches (Daiki Rika Kogyo, Saitama, Japan) were used as growth pouches. The growth pouches vertically divided into two parts by cutting down the center of packs and sealing the cut edge with plastic tape. Then, the bottoms of seed pack growth pouches were cut off and paper towels (Kimwipe, Nippon Paper Crecia, Tokyo, Japan) were used to supply growth culture medium to the seeds.

Two sterilized pregerminated seeds were transferred to the moistened each seed pack, and then the pack was covered with aluminum foil to prevent exposing roots to the light. For each treatment, the seed packs were placed in a plastic box (8.7 × 11.1 × 10.5 cm) in an upright position containing 300 mL solution culture medium. All plastic boxes were placed in a clear acrylic case (40.0 × 32.0 × 35.0 cm) and covered with a clear acrylic lid to prevent evaporation of culture medium. Seedlings were allowed to grow at 25°C for 14 days with a 12-h photoperiod, in a growth chamber (EZ-022, Nippon medical chemical instruments, Osaka, Japan). Fluorescent lamps supplied a photosynthetic photon flux of 120 μmol m⁻² s⁻¹. Detail preparation of growth pouches and culture conditions were as described in...
our previous paper (Adachi et al. 2013) unless otherwise stated.

Seedling growth

On day 14, plant height, seminal root length, and number of roots (seminal and nodal roots) were determined. In addition, six seedlings were taken at this time from one replicate per treatment. Shoots and roots were separated from seedlings. After oven drying at 70°C for 72 h to a constant weight, the dry weight of shoot and root of seedlings were measured.

Root imaging and quantification

Measuring root traits of rice seedling by digital image analysis was carried out by the method as was described before (Kimura et al. 1999, Adachi et al. 2013) unless otherwise stated. After sampling of rice seedlings from each treatment, roots were collected and then fixed in 50% (v/v) ethanol. To analyze total length, surface area and volume of roots, the ethanol-fixed roots were washed in water and gently shaken in methyl violet B solution for 15 min. The stained roots were floated on water in an acrylic tray (20.0 × 26.0 × 2.0 cm) placed on a flatbed scanner (GT-X970, Seiko Epson Inc, Nagano, Japan) connected to a computer, and scanned (300 dpi as 16-bit grayscale image) using a film scan unit. The total length, surface area and volume of roots were estimated using the macro programs developed for Scion Image™ ver. 4.0.3.2 (Scion Corporation, Frederick, MD, USA). Six seedlings per treatment were used for root imaging analysis. The analyses were performed with three replications.

Experimental design and statistics

The experimental design was a randomized complete block with three replications. Statistical analysis was conducted using JMP 4.0 (SAS Institute, Cary, NC, USA). The data was performed analysis of variance (ANOVA), and when statistically significant, means were separated by Fisher’s protected least significant difference (PLSD) test at the 5% level. A correlation analysis was also performed.

Results

Rice seedling growth

The effect of LPA treatment on shoot growth was visually evident in seedlings subjected to PEG-induced water deficit (Fig. 1). Polyethylene glycol significantly inhibited rice seedling growth in terms of plant height; however, this inhibitory effect of PEG

Table 1. Differences in growth parameters of rice seedlings with or without L-ß-phenyllactic acid (LPA) under water stress conditions

<table>
<thead>
<tr>
<th>Character</th>
<th>Control</th>
<th>PEG</th>
<th>PEG+LPA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plant height (cm)</td>
<td>15.2 ± 0.3 a</td>
<td>13.5 ± 0.3 b</td>
<td>15.3 ± 0.1 a</td>
</tr>
<tr>
<td>Shoot DW (mg plant⁻¹)</td>
<td>18.0 ± 1.1 ns</td>
<td>16.8 ± 1.2</td>
<td>18.5 ± 1.6</td>
</tr>
<tr>
<td>RWL (mg cm⁻¹)</td>
<td>1.1 ± 0.0 ns</td>
<td>1.2 ± 0.1</td>
<td>1.2 ± 0.1</td>
</tr>
<tr>
<td>Seminal root length (cm)</td>
<td>24.6 ± 0.2 a</td>
<td>17.9 ± 0.9 b</td>
<td>19.0 ± 0.5 b</td>
</tr>
<tr>
<td>Root DW (mg plant⁻¹)</td>
<td>8.5 ± 0.9 a</td>
<td>6.4 ± 0.3 b</td>
<td>8.6 ± 0.2 a</td>
</tr>
<tr>
<td>No. of root (no. plant⁻¹)</td>
<td>7.6 ± 0.4 ns</td>
<td>7.3 ± 0.4</td>
<td>8.5 ± 0.0</td>
</tr>
<tr>
<td>Root to shoot ratio (mg mg⁻¹)</td>
<td>0.5 ± 0.0 ns</td>
<td>0.4 ± 0.0</td>
<td>0.5 ± 0.0</td>
</tr>
</tbody>
</table>

Seedlings were cultured at 30°C for 14 days. Values are means ± standard error (n=3). Different letters indicate significant differences among treatments at the 5% level based on Fisher’s protected least significant difference test after significant ANOVA. ns: no significant difference.
was eliminated by LPA treatment (Fig. 1 and Table 1). No significant difference was observed among treatments in shoot dry weight. When LPA's effects on shoot length and dry weight were considered together, RWL, which refers to ratio of shoot dry weight to shoot length was similar among treatments (Table 1).

With respect to root traits, seminal root lengths of PEG- and PEG+LPA-treated seedlings were significantly reduced; compared with controls, they were 27.2% and 22.8% shorter, respectively. Root dry weights were also decreased by PEG treatment, however the inhibition effect of PEG was eliminated by LPA treatment (Table 1). The number of root (seminal and nodal roots) in rice seedlings was similar among treatments. Root to shoot ratio, which is a measure of the allocation of dry matters between shoot and root, was not changed among the treatments (Table 1).

**Total length, surface area, and volume of rice seedling roots**

The effects of LPA on total root traits, such as length, surface area, and volume, of rice seedlings under PEG-induced water deficit conditions are shown in Fig. 2. Total root length of PEG-induced water deficit seedlings (PEG in Fig. 2A) was 19.3% less than the controls, a significant reduction. This PEG-induced inhibition was partially attenuated by LPA treatment. Furthermore, LPA completely overcame PEG inhibition with respect to root total surface area and volume.

The rice root system can be divided into three components, i.e., seminal, nodal, and lateral roots. Individual root has distinct morpho-physiological characters with respect to thickness. We thus compared differences in total root length, surface area, and volume of rice seedling roots among treatments in relation to root thickness. The effects of LPA on various total root traits of rice seedlings in different diameter classes under PEG-induced water deficit conditions are shown in Fig. 3. The criteria concerning the root diameter classes were followed as mentioned in our previous report (Adachi et al. 2013). Compared with controls, PEG significantly decreased total root length of fine roots (<0.757 mm in diameter) (Fig. 3A): in the presence of PEG, total lengths were 17.9% and 62.5% shorter in class A (<0.379 mm) and B (0.379-0.757 mm), respectively. In contrast, LPA modestly increased the total length of fine roots (Fig. 3A). In class C (0.757-1.29 mm), no significant difference in total length was observed between controls and PEG-treated plants, whereas LPA markedly increased total root length.

Furthermore, PEG decreased total surface area and volume of roots smaller than 0.757 mm in diameter (Fig. 3B and C); total surface area and volume, respectively, of class A (<0.379 mm) PEG-treated roots were 19.2% and 21.5% less than those of controls. In addition, PEG reduced total surface area and volume by 61.9% and 61.1%, respectively, in class B (0.379-0.757 mm) roots. The inhibitory effects of PEG on total surface area and volume of class A and B roots were erased by LPA application. In the thicker root class (class C), no significant difference in total surface was observed among treatments, but LPA markedly increased root volume (Fig. 3B and C).

**Relationship between total root traits and plant height**

To examine morphological root traits associated with differences in shoot growth, a correlation analysis was
performed. Fig. 4 shows the relationship between several total root traits and plant height, a function of selected shoot traits, in rice under PEG-induced water deficit conditions. Total root surface area and volume were found to be positively correlated with plant height. In contrast, plant height did not show a significant correlation with total root length. Total root length, surface area, and volume are important indicators of water and nutrient uptake potential. We investigated the relationship between plant height and
total root length, surface area, and volume in detail with reference to root diameter class (Fig. 5). Total root length did not correlated with plant height regardless of root diameter classes. Plant height was significantly correlated with total root surface and volume in diameter class A, but not in classes B and C.

Discussion

The result presented here showed that the PEG induced water-deficit conditions have a significant influence in both the growth of shoots and roots in rice seedlings. There have been several reports on the alleviative effect of PGRs under water deficit conditions. The ameliorative influence of brassinosteroids against water deficit is associated with enhanced soluble protein and free proline content and increased catalase activity (Vardhini and Rao 2003). Benzyladenine has been found to enhance photosynthetic rate, chlorophyll content, and nitrate reductase activity and to amend the negative effect of water stress (Singh et al. 2001). The results of those studies, combined with ours, suggest the possibility that LPA may alter the morphological and physiological traits largely responsible for growth enhancement of rice seedlings under PEG-induced water deficit conditions. Radiclonic acid (Sassa et al. 1973) and Capillarol (Ueda et al. 1986) -REPSs like LPA - promote root growth in rice. Although LPA has been found previously to promote both shoot and root growth in rice seedlings (Adachi et al. 2013), our study is the first to report an REPS, i.e., LPA, with growth-promoting effects on rice seedlings under PEG-induced water deficit conditions. In terms of differences in dry matter production of shoot, RWL were not different by LPA treatment regardless of

Fig. 5. Relationship between plant height and total root traits of rice seedlings in different diameter classes under water stress conditions. Seedlings were cultured at 30°C for 14 days. ** and * indicate significant correlations at 1% and 5% levels, respectively, and ns indicates no significant correlation at the 5% level.
PEG application, suggesting that LPA did not cause spindly shoot growth (Table 1). The root to shoot ratio was not change among the treatments (Table 1), indicating that LPA did not affect dry matter allocation of rice seedling with or without PEG. In addition, LPA had no observable growth-promoting influence on seminal root length or number of roots in rice seedling treated with PEG (Table 1). This growth inhibition of seminal and nodal root might be due to water deficit induced by PEG treatment, which indicates that LPA did not completely reverse the inhibitory effects of PEG on these two organs.

In the correlation analysis of total root traits and plant height, total root surface area and volume were found to be positively correlated with plant height, but not shown in total root length (Fig. 4). Also, plant height was significantly correlated with total root surface area and volume in smaller root diameter classes (Fig. 5). These results indicate that total surface area and volume might contribute more to plant height than does total root length of 14 days old rice seedling grown in growth pouch placed in a growth chamber which can control growth environment.

Root system architecture is a well-known functionality for uptake of soil resources (Lynch 1995). For nutrient and water uptake to occur the root surface must first be in contact with soil minerals and water (Wang et al. 2006); if this prerequisite is met, a large root system is useful for relatively high total uptake of water and nutrients. In addition, the fine lateral roots are responsible for root system enlargement and soil water acquisition (Hoshikawa 1989, Yamauchi et al. 1987). The total length, surface area, and volume of fine roots were increased by LPA under PEG-induced water deficit conditions (Fig. 3). These results imply that PEG-induced water deficit in rice seedlings can be alleviated by LPA application. This alleviative effect is partially attributable to alterations in root system developmental patterns, with increases in fine root total length, surface, and volume accelerating water and nutrient acquisition from the culture medium.

Another contributing factor of root system architecture is phenotypic plasticity, the ability of an organ to vary its phenotype as different environmental conditions (O’Toole and Bland 1987). Plastic root system development has been found to be functionally effective for the acquisition of water and nutrient uptake under water stress (Kano-Nakata et al. 2011). We observed root thickness-related differences in total root length, surface area, and volume in PEG-induced water deficit rice seedlings treated with LPA (Fig. 3), suggesting that LPA may change the morphology of rice root systems under PEG-induced water deficit conditions. Our results also suggest that LPA can enhance rice root system phenotypic plasticity by increasing length, surface area, and volume of fine lateral roots under PEG induced water-deficit conditions.

In summary, LPA treatment increased total length, surface, and volume of fine roots of rice seedling grown under growth-chamber conditions, and alleviated the negative effects of PEG-induced deficit in the early growth stage. With respect to future perspectives, the manner in which LPA regulates osmolyte metabolism in rice seedling under PEG-induced water deficit at physiological and biochemical levels would be worthy of investigation. In addition, our study showed that LPA had a profound effect of rice seedlings grown under PEG-induced water deficit conditions (Fig. 1 and Table 1). The mechanism underlying this effect was unknown, but must involve alterations in processes controlling cell enlargement and division in root tissue. Even under PEG-induced water deficit conditions, LPA may increase water and minerals uptake or grain endosperm use efficacy as described previously (Adachi et al. 2013). Although the present experiment was carried out in the growth pouches containing culture medium at early growth stages, the study’s implications may be of particular importance in situations where rice seedling roots must penetrate multiple soil layers to acquire water or nutrients under water-deficit conditions.

References


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