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Growth Promotion of Rice (*Oryza sativa* L.) Seedlings by Application of L-β-phenyllactic Acid

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Abstract: Fine branching of lateral roots and the framework of seminal or nodal roots play an important role in performing function of a root system. The effects of L-β-phenyllactic acid (LPA) on growth of rice (*Oryza sativa* L.) seedlings have not been fully elucidated. Therefore, growth promotive effects of LPA on roots and shoots in rice seedlings were investigated. Rice seedlings were cultured in seed packs™ containing 1/100-strength Murashige and Skoog (MS) medium with or without 100 mg L^{-1} LPA at 30°C for 14 days. The total root length and total root surface area were calculated by digital image analysis. LPA-treated seedlings showed a 24% increase in plant height and a 26% increase in seminal root length compared with those of the control. The dry weight of shoots was markedly increased and that of roots was slightly increased by LPA. LPA treatment had no effect on the number of seminal and nodal roots per seedling. Digital image analysis revealed that LPA significantly increased the total length and surface area of fine roots (less than 0.757 mm in diameter). In addition, LPA did not affect the distribution of total length or surface area of rice roots in any diameter classes compared with that of control. The findings suggest that the growth-promoting effects of LPA on rice shoots can be attributed to increased water and nutrient absorption and/or the seed endosperm utilization efficiency, as a result of increased total length and surface area of roots.

Key words: L-β-phenyllactic acid, plant growth substance, rice seedlings, root, root elongation promoting substances

INTRODUCTION

Plant hormones play crucial roles in controlling plant growth and development and affect root growth in many species (Goodwin, 1978; Tanimoto, 1988). Plant roots elongate in response to low concentrations of auxins (Thimann, 1937) and auxins interact with zinc to promote rhizogenesis in rice (Yokoyama *et al*., 2004). Salicylic acid is another substance that affects elongation of plant roots (Coronado *et al*., 1998). Among these plant growth substances, some compounds known as root elongation promoting substances (REPS) particularly enhance root elongation (Takenaka, 1993). Typical examples of REPS include helminthosporol (Sakurai and Tamura, 1965), L-β-phenyllactic acid (LPA) (Tamura and Chang, 1965), radiclonic acid (Sassa *et al*., 1973) and capirarol (Ueda *et al*., 1986). Capirarol, radiclonic acid and LPA have growth-promoting effects on the seminal root of rice seedlings. Sakurai and Tamura (1965) found that capirarol and its related compounds elongated roots of rice and lettuce seedlings. Radiclonic acid showed root growth-promoting activities in Chinese cabbage and rice (Sassa *et al*., 1973, 1975). Tamura and Chang (1965) isolated LPA as a REPS for rice and lettuce seedlings from culture filtrates of *Exobasidium symploci-japonicae*, a pathogenic fungus for *Symplocos japonica*. LPA showed maximum activity at a concentration of approx. 100 mg L^{-1}. When rice seedlings were treated with 100 mg L^{-1} LPA, the seminal roots were more than twice as long as those of the control (Tamura and Chang, 1965). However, the effects of LPA on shoot and root growth of rice seedlings have not been fully elucidated. In addition, there is very little published data on this subject. Therefore, the effects of LPA on rice growth with special reference to growth-promoting effects on both roots and shoots were reinvestigated.

MATERIALS AND METHODS

Plant materials and culture conditions: The rice (*Oryza sativa* L.) cultivar Koshihikari was used in these experiments. Uniform rice seeds were sterilized with 0.1%

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benomyl solution (Sumitomo Chemical Co., Tokyo, Japan) for 24 h according to the manufacturer's instructions and then immersed in water for 24 h. Seeds were imbibed in water for 10 days at 10°C and then allowed to germinate at 30°C in the dark. 1/100-strength Murashige and Skoog (MS) medium was used as the control and the same medium containing LPA at 100 mg L^{-1} as the LPA treatment. LPA was purchased from Sigma Chemical Company (St. Louis, MO, USA). Seed pack growth pouches™ (Daiki Rika Kogyo, Saitama, Japan) were 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 inserted to supply growth culture medium to the seeds. Two seeds were sown in each seed pack and then the pack was covered with aluminum foil to prevent exposing roots to the light. For each treatment, six seed packs were placed in a plastic box (8.7×11.1×10.5 cm) in an upright position filled with 300 mL solution culture medium. All plastic cases 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 incubated at 30°C in the light for 14 days in a growth chamber (EZ-022, Nippon medical chemical instruments, Osaka, Japan). Fluorescent lamps supplied a photosynthetic photon flux of 120 μmol m^{-2} sec^{-1}.

Growth characteristics of rice plants: After 14 days of incubation, the plant height, length of seminal roots and number of roots (seminal and nodal roots) were measured. Fourteen days after sowing, six rice seedlings showing average growth were sampled from each replicate in each treatment and were separated into shoots and roots. The dry weight of shoots and roots was determined after oven-drying at 70°C for 72 h to a constant weight. The shoots contained the seeds in the experiments.

Image analysis of rice roots: Total root length and total root surface area were analyzed as described previously by Kimura *et al.* (1999) and Kimura and Yamasaki (2001, 2003) with minor modifications. After sampling of LPA-treated or control rice seedlings, roots were collected and then fixed in 50% (v/v) ethanol. To analyze total root length and surface area, the ethanol-fixed samples 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 root length and total root surface area were estimated using the macro programs developed for Scion Image™ ver. 4.0.3.2 (Scion Corporation, Frederick, MD, USA). Six seedlings per treatment (in triplicate) were analyzed.

Statistical analysis: The experiments were arranged in a randomized design with three replications per treatment. All experiments were repeated twice or more with similar results. One set of data is presented. The mean values were compared by Student's t-test (n = 3) at the 5 and 1% levels. Statistical analysis was carried out using JMP 4.0 (SAS Institute Inc., Cary, NC, USA).

RESULTS AND DISCUSSION

Growth characteristics of rice plants: The plant height of LPA-treated rice seedlings was 24% greater than that of the control (Table 1, Fig. 1). Although the number of roots (seminal and nodal roots) was not affected by LPA treatments, the seminal root length of LPA-treated seedlings was 26% greater than that of the control (Table 1, Fig. 2). Tamura and Chang (1965) reported that LPA increased the length of rice seminal root by 70% more than control, whereas the growth of seminal roots of LPA-treated plants was increased by 26% in the experiment. The differences of growth response of rice seminal root to LPA might be due to experimental system including, the differences in media and growth period. In

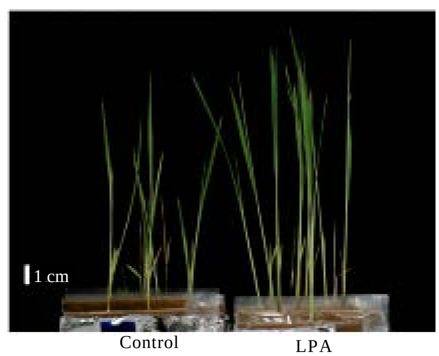

Fig. 1: Effects of LPA on shoot growth of rice plants

Table 1: Differences in growth parameters of rice seedlings with or without LPA

Character	Control	LPA
Plant height (cm)	12.0±0.4	14.9±0.4**
Shoot DW (mg $plant^{-1}$)	16.3±0.4	19.9±0.5**
RWL (mg cm^{-1})	1.4±0.1	1.3±0.0ns
Seminal root length (cm)	18.1±1.4	22.8±0.9*
Root DW (mg $plant^{-1}$)	5.3±0.1	6.8±0.8ns
No. of root (No. $plant^{-1}$)	5.9±0.4	6.5±0.7ns

Seedlings were cultured at 30°C for 14 days. Values show Mean±SE (n = 3). *,** Indicate significant differences between control and LPA treatment at 1 and 5% level, respectively. ns: Not significant difference at 5% level, LPA: L-β-phenyllactic acid, DW: Dry weight, RWL: Ratio of shoot dry weight to shoot length

Fig. 2: Typical effects of LPA on morphology of seminal root in rice plants

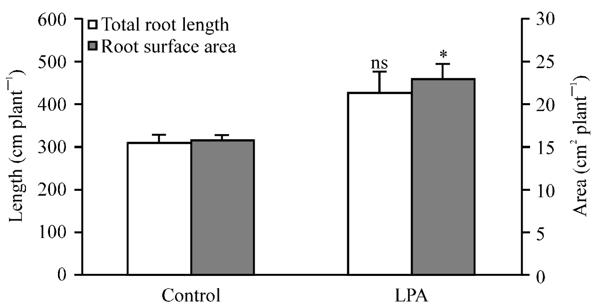

Fig. 3: Differences in total root length and total root surface area of rice seedlings with or without LPA, *Indicates significant differences between control and LPA treatment at 5% level, ns: No significant difference at 5% level

LPA-treated plants, the dry weight of shoots was markedly increased, while that of roots was slightly increased, compared with those of the control (Table 1). There was no difference in the ratio of shoot dry weight to shoot length (RWL) between the LPA treatment and the control (Table 1). These results confirm that LPA does not result in spindly growth of the shoot, because the increase in shoot growth was accompanied by increased dry weight (Table 1).

Root length and surface area: Figure 3 shows the effects of LPA on the total root length and total root surface area of rice seedlings. Compared with the control, the total roots length of LPA-treated seedlings increased slightly, but the total surface area of roots increased markedly by 26%.

Table 2: Distribution of total length and surface area of rice roots in different diameter classes with or without LPA

Diameter (mm)		Percentage frequency (%)			
		Length		Surface area	
From	To	Control	LPA	Control	LPA
0	0.379	94.3±0.8	94.0±0.5ns	80.9±2.6	81.8±1.0ns
0.379	0.757	5.2±0.7	5.9±0.4ns	16.1±2.2	17.6±0.9ns
0.757	1.185	0.5±0.3	0.1±0.0ns	2.5±1.4	0.6±0.0ns
1.185	1.982	0.1±0.0	0.0±0.0ns	0.5±0.3	0.1±0.0ns

Seedlings were cultured at 30°C for 14 days. Values show Mean±SE (n = 3). ns: Not significant differences between control and LPA treatment at 5% level, LPA: L-β-phenyllactic acid

The root system of rice plants is composed of several different kinds of roots including the seminal root, the nodal root and lateral roots (Hoshikawa, 1989; Yamauchi *et al*., 1987). Each root type has different morphological traits in terms of length and thickness. The fine branching lateral roots play an important role in enlarging the root system (Hoshikawa, 1989; Yamauchi *et al*., 1987). Therefore, based on the present results and those of previous reports, the distribution of the total length and total surface area of roots in different diameter classes were investigated.

Table 2 shows the distribution of total length and surface area of rice roots in different diameter classes with or without LPA. Analyses of roots showed that more than 99.5% of the total root length lay in the diameter range of less than 0.757 mm in both control and LPA treatment. In LPA-treated plants, fine roots (diameter range of less than 0.757 mm) accounted for 99.4% of the root surface area, similar to the trend observed for control-plants; 97.0% of the root surface area in control-plants lay in diameter range of less than 0.757 mm. In addition, it should be noted that LPA did not affect the distribution of total length or surface area of rice roots in any diameter classes compared with that of control (Table 2). Figure 4 shows the effects of LPA on total root length of rice seedlings in different diameter classes. Although LPA-treated plants showed a trend towards increased total root length, LPA significantly increased the total root length of fine roots (less than 0.757 mm in diameter), compared with the control (Fig. 4). The effects of LPA on the root surface area of rice seedlings are shown in Fig. 5. LPA significantly increased the total area of root of seedlings by increasing the area of the thinner lateral roots (less than 0.757 mm in diameter) but not thicker ones (Fig. 5).

Plant growth substances including plant hormones have roles in root growth and development in several plant species. For typical example, a natural auxin (IAA),

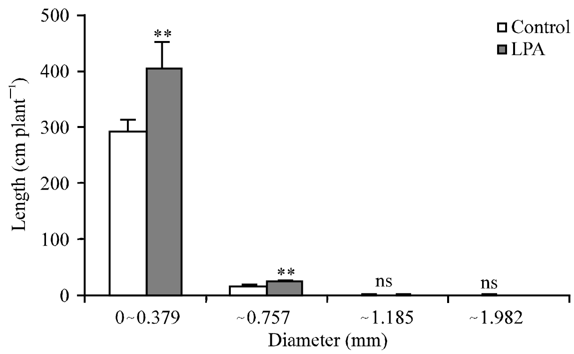

Fig. 4: Effect of LPA on total length of rice roots in different diameter classes, **Indicates significant differences between control and LPA treatment at 1% level, ns: No significant differences at 5% level

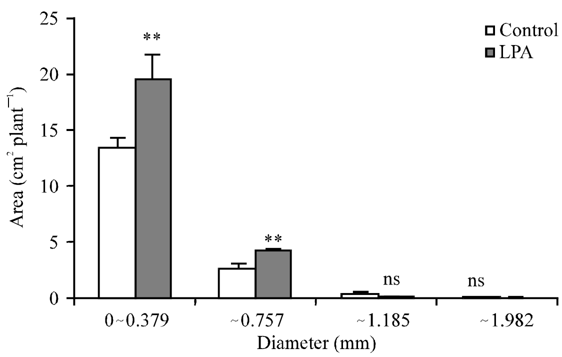

Fig. 5: Effect of LPA on total surface area of rice roots in different diameter classes, **Indicates significant differences between control and LPA treatment at 1% level, ns: No significant differences at 5% level

inhibits root growth, but promotes rooting of grafted plants and cuttings (Masuda, 1994). IAA interacts with zinc to promote rooting (Yokoyama *et al*., 2004) and over expression of an auxin biosynthetic gene caused excess root formation (Zhao *et al*., 2001). In addition, plant growth substances can have positive or negative effects on root growth in plants. Whether they promote or inhibit root growth may depend on the plant species and culture conditions, such as culture medium composition/concentration and/or environmental factors including light, temperature and humidity, etc.

In the present study, LPA treatments promoted growth of seminal roots (Table 1, Fig. 2). It is likely that LPA enhances growth of rice seminal roots both directly and indirectly via inducing or inhibiting the actions of other plant growth substances. In fact, it was observed that synergistic effects between ethylene and gibberellin on the growth of rice seedlings in our previous study (Watanabe *et al*., 2007). Further research is required to clarify interactions between LPA and other plant growth substances in rice. Capirarol (Ueda *et al*., 1986; Ueda, 1989) and radiclonic acid (Sassa *et al*., 1973, 1975) promoted root growth in rice; however these substances did not affect shoot growth. The action of LPA differs from those of capirarol and radiclonic acid, because LPA promotes both root and shoot growth in rice. These differences in action might be because of different physiological effects of these substances on rice growth, and/or differences in the experimental systems used, including the types of media, plant materials (intact plants or excised root segments), temperature and light.

In the present study, LPA treatment increased the total length and total root surface area of fine roots, such as lateral roots (Fig. 4, 5). Because fine roots are responsible for water and nutrient absorption (Kono *et al*., 1972; Yamauchi *et al*., 1987), the growth-promoting effects of LPA on rice shoots may be attributed to increased water and nutrient uptake by the roots. Otherwise, since the seed endosperm have crucial role for growth of rice seedlings, it is possible that LPA affect the seed endosperm utilization efficiency. At present, it is still not clarified the precise mechanism by which shoot and root growth was increased. It is possible that LPA could be involved in the regulation of cell enlargement and division in synergy with other plant growth substances such as auxin, which is recognized to regulate cell enlargement and division during root formation (Arteca, 1996; Davies, 1995). It is clear that more work needs to be done to understand the mechanism by which LPA is increasing root and shoot growth in rice seedling.

CONCLUSION

So far, LPA has been known to increase the growth of only seminal roots of rice and lettuce (Tamura and Chang, 1965). For the first time, this study clarified that LPA did not only elongate seminal root but also increased the total length and surface area of thinner lateral roots which regarded as responsible for water and nutrients absorption. It was also found that LPA promoted growth of both shoot and roots in rice seedlings. These findings suggested that application of LPA can make rice seedlings enlarge root system and it may increase water and nutrients absorption, thereby it can enhance early growth of rice seedlings.

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