Rice coleoptile growth under water and in air - Possible effect of buoyancy on growth and cell walls

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Abstract

Maximum growth was achieved in rice coleoptiles (Oryza sativa L. cv. Sasanishiki) grown under water; they reached maximum length of 81.2 mm on day 5. The maximum length of coleoptiles grown in air or under water with air bubbling was 12.4 mm and 23.5 mm in day 5, respectively. Differences in coleoptile growth between air bubbling and air conditions, namely approximately 11 mm at day 5, could be due to buoyancy effect under water. Promoted growth under water was due to a decrease in cell wall extensibility. The decrease in cell wall extensibility could be related to the inhibition of the formation of diferulic acid-bridges among arabinoxylans in cell walls under water.

Introduction

Water immersion method has been used to simulate microgravity conditions in medical sciences. Usually, most of land plants are not able to grow under water. Rice is an exceptional plant. Rice coleoptiles grow faster under water than in air (Wada 1961). This growth promotion is due to promoted cell elongation under submerged condition of a limited oxygen supply (Ohwaki 1967). Air bubbling under water suppressed growth, but the degree of inhibition was much small as compared with that of coleoptiles grown in air (Zarra and Masuda 1979). The differences in coleoptile growth between air and air bubbling conditions could be due to buoyancy effect under water.

Cell wall extensibility, a major parameter which limits the growth rate of plant cells, is determined by physicochemical properties of molecules constructing cell walls (Masuda 1978). Major constituents of growing plant cell walls are cellulosic polymers and matrix polymers such as pectic and hemicellulosic polysaccharides (McNeil et al. 1984). The formation of cross-links among cell wall polymers can modify the mechanical properties of cell walls (Fry 1986).

The primary cell wall of Poaceae contains a significant amount of monophenols such as ferulic acid and coumaric acid which are ester-linked to wall matrix polysaccharides (Harris and Hartley 1976, Smith and O'Brien 1979, Shibuya 1984). Ferulic acid bound to cell walls is considered to be subjected to a coupling reaction by peroxidase, to produce diferulic acid which cross-links matrix polysaccharides, resulting in a decrease in cell wall extensibility (Fry 1979). In fact, an increase in the amount of diferulic acid in cell walls of oat coleoptiles correlated with a decrease in cell wall extensibility (Kamisaka et al. 1990).

In the present study, the possible effects of buoyancy on rice coleoptiles grown under water was examined, paying special attention to the physicochemical properties of cell walls.

Materials and methods

Plant material

Seeds of rice (Oryza sativa L. cv. Sasanishiki) were sterilized in 4% sodium hypochlorite for 1 h, and soaked for two days in water at 30°C in the dark. Next, they were germinated and grown in the dark at 30°C under three different conditions: under sterilized water of 10 cm depth in a polyvinyl cylinder (diameter:11.5 cm, height:15 cm) (water type), under sterilized water with constant air bubbling (bubbling type), and on gauze moistened with sterilized water (air type). Everyday, from the 2nd through 5th day after germination, the coleoptile length was measured, and the coleoptiles were excised. In some experiments, the coleoptiles were subdivided from the tip to the base uniformly for the three growth types, 7 mm from the tip and 10 mm subsequently.

Fractionation of wall polysaccharides

Coleoptiles were boiled for 5 min in methanol, washed several times with methanol, then stored in methanol until use. Rehydrated coleoptiles were treated with 5 ml of 200 mg l^{-1} pronase (Type V, Sigma Chemical Co., St. Louis, MO, USA) dissolved in 0.05 M potassium-phosphate (pH 7.0) at 37°C for 18 h, then with 5 ml of 2 units ml^{-1} pancreatic amylase (Sigma) dissolved in 0.1 M sodium acetate (pH 6.5) containing 3 mM $CaCl_2$ at 37°C for 3 h. Coleoptiles thus treated were homogenized with mortar and pestle, and cell wall polysaccharides were fractionated by partly modifying the method of Nishitani and Masuda (1979). Cell wall material was extracted twice with 20 mM ammonium oxalate (pH 4.0) at 70°C for 1 h to obtain the ammonium oxalate fraction. Methanol was added to the ammonium oxalate-soluble fraction in order to fractionate 35% methanol-insoluble and -soluble material. Cell wall material was further extracted with 0.1 M NaOH at room temperature under nitrogen atmosphere for 24 h. Residual cell wall material was extracted with 17.5% (w/v) NaOH solution at 24 h under nitrogen atmosphere to obtain the alkaline fraction. The alkaline insoluble fraction was washed with water, 1 mM acetic acid and ethanol, and dried at 37°C (alpha-cellulose). The amount of total sugars in each fraction was determined by the phenol-sulphuric acid method (Dubois et al. 1956). The amount of sugars was expressed as the mean of triplicate samples in each experiment.

Determination of diferulic acids

Phenolic acids liberated from cell wall material by 0.1 M NaOH treatment were extracted with ethyl acetate after acidifying the fraction to ca. pH 3 with HCl. The ethyl acetate fraction was air-dried, then stored in the dark. Diferulic acid was analyzed according to the method of Shibuya (1984) as previously reported by Kamisaka et al. (1990). The amount of phenolic acids was determined with a digital integrator using trans-ferulic and trans, trans-diferulic acids as standards. Trans, trans-diferulic acid was synthesized by the method of Richtzenhain (1949). The amount of diferulic acids was expressed as the mean of triplicate samples in each experiment.

Determination of cell wall mechanical properties

The mechanical properties of coleoptile cell walls were determined with a tensile tester (RTM-25, Toyo Baldwin Co., Tokyo, Japan) connected to a computer (PC-9801, NEC, Tokyo, Japan). Methanol-killed coleoptiles were rehydrated, then fixed between the upper and lower movable clamps (the distance between clamps was 3 mm) of the tensile tester, and stretched by lowering the clamp at 20 mm min^{-1} to produce a stress of 10 g (for the air and bubbling type coleoptiles), or of 2 g (water type coleoptiles). Cell wall extensibility (mm g^{-1}) was determined and recorded by the computer.

Results

Growth

As shown in Fig. 1, maximum growth was achieved by the coleoptiles growing under

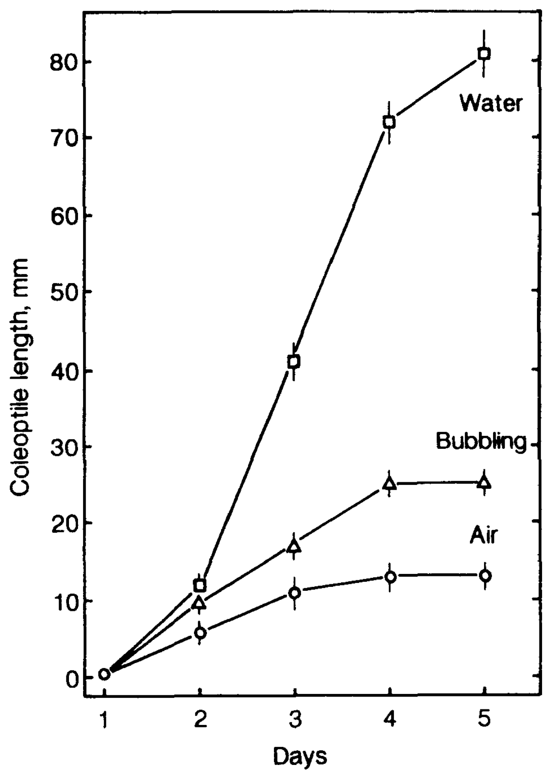

Fig. 1.
Kinetic changes in rice coleoptile length. Seeds were germinated for 2 days, then the seedlings were sown in the dark at 30°C. Bars represent SE (n = 30).

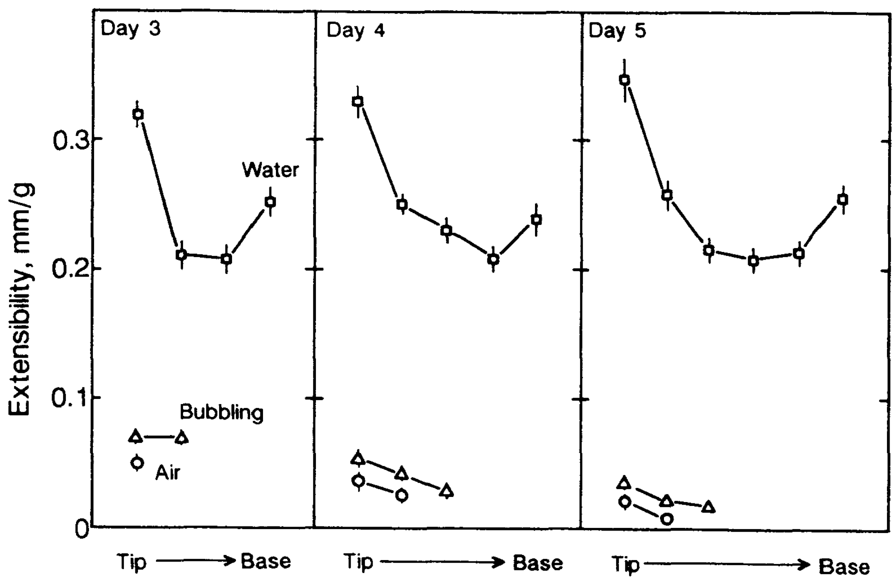

Fig. 2.
Extensibility of cell walls of rice coleoptiles that had been subdivided into shorter sections, 7 mm at the tip and 10 mm for the subsequent zones. Cell walls of 3-, 4- and 5-day-old coleoptiles were used. Bars represent SE (n = 30).

water which reached a maximum length of 81.2 mm on day 5. Growth of the bubbling and air type coleoptiles was lesser, the maximum length being 23.5 mm in the former and 12.4 mm in the latter at days 4 and 5, respectively. Growth of the water and bubbling type coleoptiles was rapid between days 2 and 4, but the growth rate decreased after day 4. The growth rate of the air type coleoptiles was relatively steady and slow.

Mechanical properties of cell walls

Rice coleoptiles were subdivided from the tip to the base, and the cell wall extensibility of different coleoptile zones were determined (Fig. 2). In general, the extensibility of the air and bubbling type coleoptiles was lower than that of the water type. In air and bubbling type coleoptiles, the extensibility decreased towards the base at days 4 and 5. In water type coleoptiles, on the other hand, the extensibility was consistently lowest at the middle zones but was highest at the tip. The differences in cell wall extensibility between air and bubbling type coleoptiles existed, as is the case for coleoptile growth.

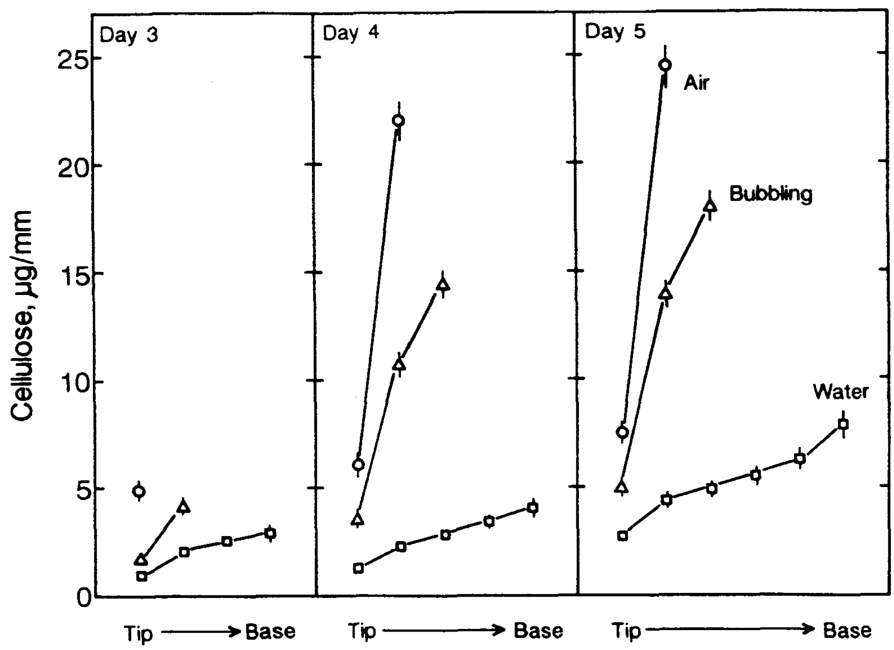

Fig. 3.
alpha-Cellulose content per unit length of rice coleoptiles that had been subdivided into shorter sections. Cell walls of 3-, 4- and 5-day-old seedlings were analyzed. Bars represent SE (n = 30).

Wall polysaccharides

The amounts of both alpha-cellulose (Fig. 3) and hemicellulose (Fig. 4) per unit length were much smaller in water type coleoptiles than in air or bubbling type ones, and both of the polysaccharides increased towards the base of the coleoptile. Aging increased these wall polysaccharide contents in all coleoptile regions. The differences in the content of cellulosic and hemicellulosic polysaccharides between air and bubbling type coleoptiles existed, as is the case for coleoptile growth.

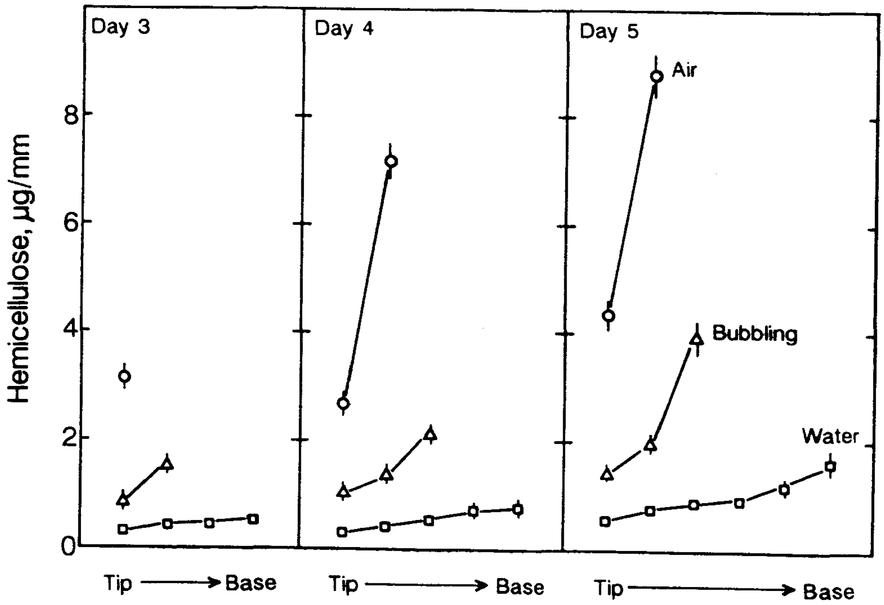

Fig. 4.
Hemicellulose content per unit length in cell walls of rice coleoptiles that had been subdivided into shorter sections. Cell walls of 3-, 4- and 5-day-old coleoptiles were analyzed. Bars represent SE (n = 30).

Diferulic acids

The amounts of diferulic acids per unit length were much smaller in water type coleoptiles than in air or bubbling type ones (Fig. 5). Diferulic acid content increased towards the coleoptile base in air and bubbling type coleoptiles at days 4 and 5, but not in water type coleoptiles; being consistently higher at the middle zones compared to the base and tip. In general, aging increased the diferulic acid content. The differences in diferulic acid content between air and bubbling type coleoptiles existed.

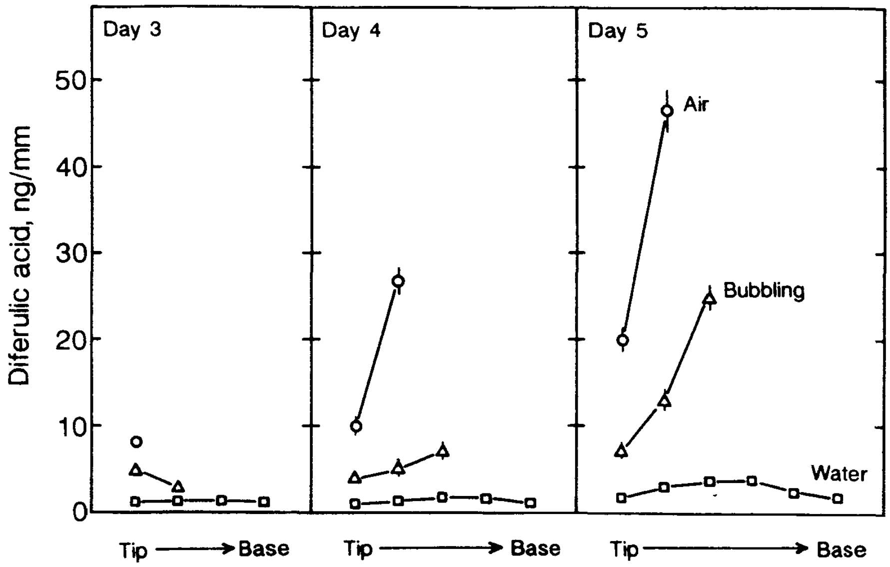

Fig. 5.
The content of diferulic acid per unit length in cell walls of rice coleoptiles that had been subdivided into shorter sections. Cell walls of 3-, 4-, and 5-day-old seedlings were used. Bars represent SE (n = 30).

Discussion

Maximum growth was achieved by rice coleoptiles grown under water, whereas the air and bubbling types were lesser (Fig. 1), as reported by Zarra and Masuda (1979). The extensibility of cell walls in water type coleoptiles was much larger than that in either air or bubbling type ones (Fig. 2). These facts indicate that the growth rate of rice coleoptiles in the present experimental conditions was determined mainly by the extensibility of cell walls.

Tab. 1. Correlation coefficients between the rigidity of rice coleoptile cell walls and the content of hemicellulose and cellulose in cell walls. Coefficients were obtained using data from Figs. 2, 3 and 4. a, Statistically significant at the 0.1% level.

Rigidity of cell walls	Hemicellulose ug mm^{-1}	Cellulose ug mm^{-1}
Extensibility mm g^{-1}	-0.60[a]	-0.62[a]

Tab. 2. Correlation coefficients between the rigidity of rice coleoptile cell walls and the content of diferulic acid in cell walls. Coefficients were obtained using data from Figs. 2 and 5. a, Statistically significant at the 0.1% level.

Rigidity of cell walls	Diferulic acid ng mm^{-1}	ng (ug hemicellulose)$^{-1}$
Extensibility mm g^{-1}	-0.61^a	-0.72^a

The extensibility of rice coleoptile cell walls correlated with the amount of either alpha-cellulose or hemicellulose (Tab. 1). In addition, a decrease in cell wall extensibility correlated with an increase in the amount of diferulic acid per unit length or per hemicellulose correlated with a decrease in the extensibility of cell walls (Tab. 2). Diferulic acid has been considered to be a component which makes cell walls mechanically rigid (Fry 1979). In rice endosperm cell walls, ferulic and diferulic acids were reported to be ester-linked to the arabinose moiety of arabinoxylans (Shibuya 1984). These facts suggest that changes in cell wall properties mentioned above cause a rapid coleoptile growth under water.

The differences in coleoptile growth and physicochemical properties of cell walls existed between air and air bubbling type coleoptiles (Figs. 1 - 5). These differences could be due to buoyancy effect under water. However, further studies are needed under microgravity conditions in space, because the growth of rice coleoptiles is affected by the concentration of oxygen (Wada 1961, Ohwaki 1967) and ethylene (Raskin and Kende 1983, Ishizawa and Esashi 1984) dissolved in water.

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