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Article

Physiological Properties of Two *Japonica* Rice (*Oryza sativa* L.) Cultivars: Odae and Ilpum

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The properties of two cultivars of *japonica* rice, Odae (early ripening variety) and Ilpum (late ripening variety), were compared. They grew on MS (Murashige and Skoog) medium but the growth of both cultivars was strongly retarded by 50 mM or more salt. There was no clear difference between the growths of seedlings of the two cultivars for the first 24 h after germination. The amylopectin chain-length profiles of the two cultivars did not differ significantly, and amylopectin content was estimated at 16.0 ± 0.4% in cv. Odae and 16.4 ± 0.4% in cv. Ilpum. A total of 114 RAPD (randomly amplified polymorphic DNA) fragments ranging from 0.4 to 2.5 kb were isolated from the two cultivars, 61 from cv. Odae and 53 from cv. Ilpum, indicating that there is little genetic variation between them.

Key words: *amylopectin, early ripening variety, germination, japonica rice, late ripening variety, morphology, salt concentration*

Rice can respond to a variety of stresses such as salinity, light, drought, and low and high temperature that affect its germination, growth and development. Salinity in particular completely inhibits germination at high levels and induces a state of dormancy at lower levels [Khan *et al.*, 1997]. Dormancy alters enzymatic activities, protein metabolism, and the synthesis of plant growth regulators [Khan *et al.*, 1994], and ions such as calcium, potassium and magnesium have been reported to stimulate seed germination [Noble *et al.*, 1992]. Although these phenomena have been studied in relation to signals and factors in plants [Li *et al.*, 2000], the mechanisms involved remain unclear.

RAPD (randomly amplified polymorphic DNA) analysis is a PCR (polymerase chain reaction)-based procedure that involves amplifying segments of target DNA with random primers. This technique has been extensively used to examine rice strains that are regarded as different cultivars on the basis of morphological, physiological and enzymatic markers [Maab *et al.*, 1995], and to assess the genetic diversity of various plant species [Virk *et al.*, 1995]. Rice cultivars have been divided into two subspecies, *japonica* and *indica*, on physiological and morphological grounds [Oka *et al.*, 1997]. The amylose and amylopectin content of starch, a major constituent of rice endosperm, also differs between these subspecies. Generally, the rice starch of *indica* subspecies has a higher amylose to amylopectin ratio [Umemoto *et al.*, 2002]. Genes affecting starch granules have been located on chromosome 6 and one with a major effect has been mapped to the *alk* locus [Goff *et al.*, 2002; He *et al.*, 1999].

Here we describe differences between two cultivars of *japonica* rice, differed in ripening period, cv. Odae and Ilpum, based on their physiological and biochemical properties, and on RAPD analysis.

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Abbreviations: HPAEC-PAD, High performance anion exchange chromatography equipped with pulsed amperometric detector; HPLC, High performance liquid chromatography; MS, Murashige and Skoog; PCR, Polymerase chain reaction; RAPD, Randomly amplified polymorphic DNA; SEM, scanning electron microscopy; TAE, Tris-acetate.

Materials and Methods

Plant materials. Seeds of *japonica* rice (*Oryza sativa* L.) cv. Odae (early ripening variety) and Ilpum (late ripening) were obtained from the National Crop Experiment Station (Suwon, Korea). They were soaked in distilled

water and kept at 25°C in the dark for 2 days Imbibed seeds were germinated in a growth chamber at 25°C with a photoperiod of 16 h light/ 8 h dark.

Physiological morphological analysis. To investigate physiological effects, twenty of seeds were treated with 2% sodium hypochlorite for 15 min, thoroughly washed and inoculated in MS medium (Murashige & Skoog, 1962), respectively. Salt concentrations were 10, 50, 100, 250 and 500 mM and the seeded plates were incubated at 25°C in 16 h light/8 h dark cycle for 10 days.

For morphological characterization, seeds were grown on MS medium at 25°C for 72 h and germinated seeds were fixed at 12 h intervals in formalin : acetic acid: 70% ethanol (5 : 5 : 90). Fixed seedlings were dehydrated through a t-butyl alcohol series and embedded in paraplast with a melting point of 56-58°C. Sectioning was done with a rotary microtome at 6-8 μm thickness, and the sections were stained successively with Heidenhain's haematoxylin (0.5%) for 20 min, Safranin (1%) for 2 days and Fastgreen (1%) for 30 sec. Stained sections were viewed with a microscope and photographed.

Analysis of amylopectin chain-length profiles. After debranching the starch fraction with *Pseudomonas amyloderamosa* isoamylase, the amylopectin chain length distribution was determined by HPAEC-PAD (high performance anion exchange chromatography equipped with pulsed amperometric detector) [Umemoto *et al.*, 1999]. Preparation of the debranched samples was scaled down to 1 mg of rice grain powder as starting material, and elution was with a liner gradient of sodium-acetate (0 to 450 mM) in 100 mM NaOH for 85 min at a flow rate of 1 mL/min.

Analysis of amylose content. Ten mg of powdered endosperm was suspended in 600 μL of 100% methanol and boiled at 100°C for 10 min. After centrifugation, the pellet was washed twice with 1 mL of distilled water and boiled at 100°C for 60 min to gelatinize the starch. The gelatinized sample was added to 50 μL of 600 mM Na-acetate (pH 4.6) and 10 μL of 2% NaN_3, and 10 μL of *Pseudomonas amyloderamosa* isoamylase (1,420 units, Hayashibara, Okayama, Japan) at 37°C for 24 h. The samples were vacuum dried after boiling at 100°C for 2 min. Separation and detection of the amylose and amylopectin fractions was carried out by HPLC (Waters 2695 Separation Module) with a refractive index detector (Waters 2414) and a TSK gel G3000PWXL column (7.8 mm × 30 cm, TOSOH K. K., Osaka, Japan). The dried materials were gelatinized by adding 200 μL of 1 N NaOH and incubated at 30°C for 1 h, then added to 800 μL of distilled water. Insoluble materials were removed by centrifugation, and the supernatant was filtered (Cellulose Acetate 0.45 μm, ADVANTEC, Tokyo, Japan). Sample of 100 μL were applied to the column pre-equilibrated with eluent composed of 100 mM Na_2HPO_4, 50 mM NaH_2PO_4, and 0.02% NaN_3, and eluted with the same solution at a flow rate of 0.5 mL/min. The first peak corresponded to amylose and the second and third peaks were malto-oligosaccharides derived from amylopectin. Percentage of amylose content was calculated from the ratio of carbohydrate in the first peak to the total carbohydrate in the three peaks.

RAPD analysis. Genomic DNA was isolated according to Sambrook *et al.* (1989) with some modifications. Plant leaves were ground in liquid nitrogen with a mortar and pestle. The powder was extracted with extraction buffer [0.1 M Tris-HCl (pH 8.0), 0.05 M EDTA (pH 8.0), 0.5 M NaCl, 1.25% SDS] and kept on ice for 10 min. Phenol : chloroform (1 : 1) extraction was performed twice, and the DNA was precipitated by the addition of an equal volume of iso-propanol, recovered by centrifugation at 10,000 g for 10 min and dissolved in TE.

RAPD was carried out using 18 random primers (Operon, California, USA) and the 50 μL reaction mixtures contained 20 ng of template DNA, 20 mM dNTP mix, 10 pM primer and 2 units of *Taq* DNA polymerase. A drop of mineral oil was added to each tube to prevent evaporation. PCR was carried out in a DNA thermal cycler at 92°C for 5 min followed by 42 cycles of 92°C for 1 min, 34°C for 1 min, 72°C for 1.5 min and a final extension at 72°C for 7 min. The amplification products were separated electrophoretically on a 1% agarose gel using TAE buffer. The experiments were repeated three times and gave reproducible patterns. Individual RAPD products were scored as present or absent and fragment sizes were determined by comparison with the 1 kb ladder included in each gel as a size standard.

Results and Discussion

Physiological morphological analysis. The effect of calcium chloride, sodium chloride, magnesium chloride and potassium chloride was determined by measuring seedling shoot lengths. Shoot length decreased rapidly as the concentration of each salts was increased (Fig. 1). Occasionally the two rice cultivars grew even in 250 mM NaCl and KCl but beyond this salt concentration no seed germination was observed. Progressive reduction in seedling development with increasing salinity has been reported [Munns, 2002]. In earlier studies, physiological mechanisms that underlie traits for salt tolerance could be used to identify new genetic sources of salt tolerance [Munns *et al.*, 2006].

Longitudinal sectioning of embryos of cv. Odae and Ilpum after germination was used to evaluate morphological

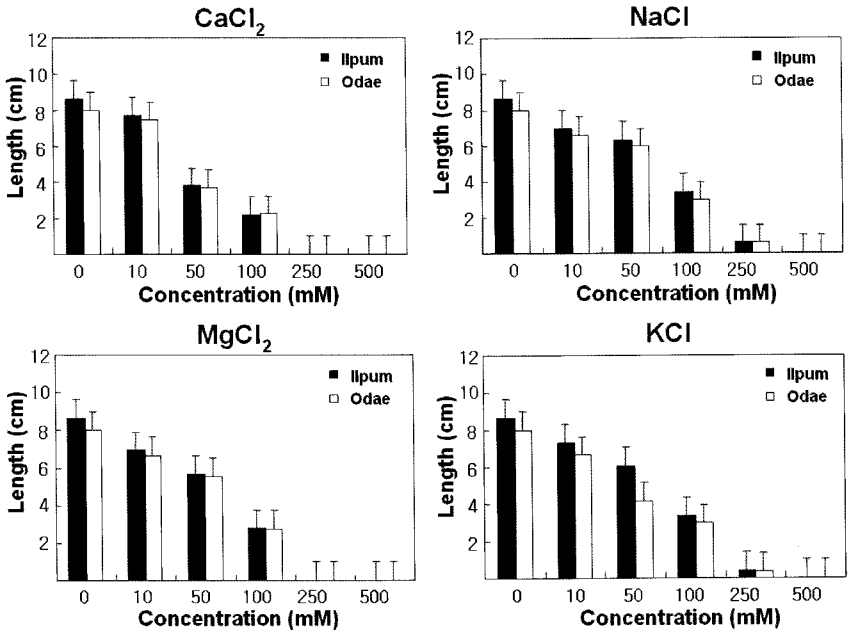

Fig. 1. Salt responses of *japonia* cultivars Odae and Ilpum. Seeds of the cultivars were germinated in MS medium, supplemented with various concentrations of $CaCl_2$, NaCl, $MgCl_2$, or KCl for 10 days at 25°C. Bars represent mean ± SEM (scanning electron microscopy) 10 seedlings were used for each measurements.

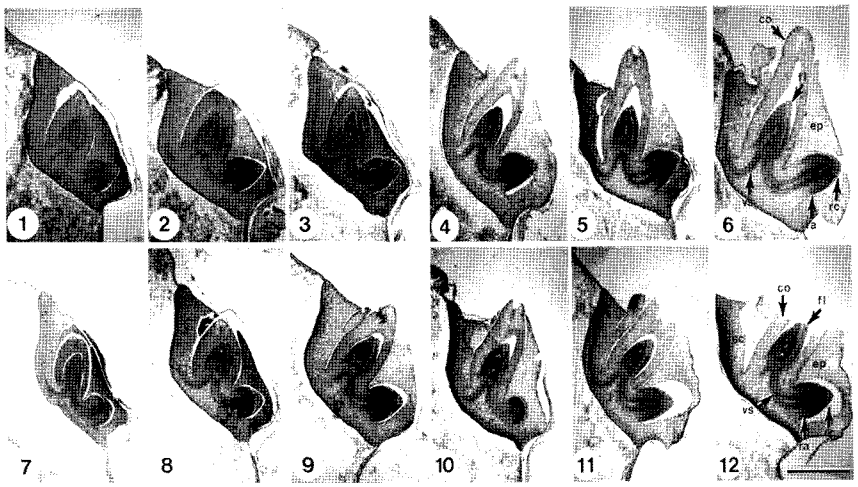

Fig. 2. Comparison of the germination patterns of Odae and Ilpum. Longitudinal sections of Odae (1-6) and Ilpum (7-12) were compared with a light microscope after germination for various times. Sections were 6-8 μm thick. Co, coleoptile; ep, epiblast; fl, first leaf; ra, radicle; rc, root cap; sc, scutellum; vs, vascular bundle. Scale bar is 10 μm.

changes, specifically plumule and radicle length with respect to time (Fig. 2). There was no significant difference between the seedlings of the two cultivars in the first 24 h of germination. However both plumule and radicle were produced earlier in cv. Odae.

Analysis of amylose content and amylopectin chain-length profiles. The starch composition and structure of the starches of the two cultivars were compared. There was no significant difference in the amylose content of the starch. cv. Odae contained 16.0 ± 0.4% and cv. Ilpum contained 16.4 ± 0.4% amylose. These amylose contents suggest that both cultivars possess the Wx^b allele that is predominant among *japonica* varieties. The *Wx* gene codes for granule-bound starch synthase, which is responsible for amylose synthesis [Okagaki *et al.*, 1988].

The structure of the amylopectin was assessed from the chain-length distribution. There was no appreciable difference between cv. Odae and Ilpum in amylopectin structure as determined by this method (Fig. 3). It is a known that the structure and crystalline organization of starch granules, and the molecular structure of amylose and amylopectin, differ between plant species and tissues [Oka *et al.*, 1997]. However, no significant differences in chain length of amylopectin have been reported between

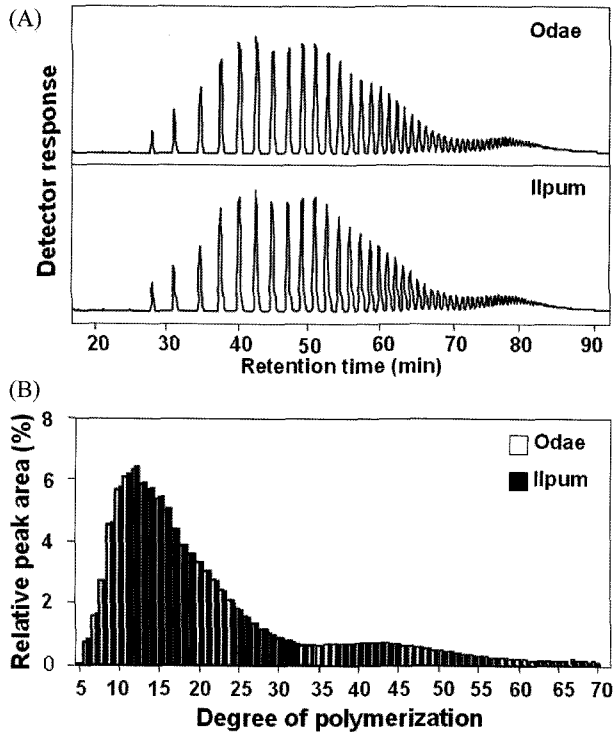

Fig. 3. Comparison of the chain-length profiles of the endosperm amylopectin of Odae and Ilpum. A, Amylopectin from Odae and Ilpum analyzed by HPACE-PAD. B, Chain-length distribution of amylopectin.

japonica rice varieties bred in Japan, such as Nipponbare and Kinmaze [Umemoto *et al.*, 2002]. Evidently the Korean rice varieties, cv. Odae and Ilpum, both classified as *japonica* subspecies, also have similar amylopectin chain distributions.

RAPD analysis. As shown in Fig. 4, primers (OPAG-08; OPAG-09; OPAG-10; OPAG-11, OPAG-13) yielded different RAPD patterns from the two cultivars, whereas the other primers yielded similar pattern. A total of 114 fragments were generated from the DNA of the two cultivars, 61 from Odae and 53 from Ilpum. Clearly there is not much difference between these two cultivars at the genomic level (common band/total band = 104/114). RAPD has been used to demonstrate polymorphism and clarify phylogenetic relationship in rice [Bao *et al.*, 2002] and *Pisum* and to study intraspecific differentiation in garlic (*Allium sativum* L.) [Maab *et al.*, 1995].

In the present study we have examined possible differences between two important *japonica* rice cultivars in Korea. We could not find any significant difference in abiotic stress tolerance, biochemical property endosperm and DNA levels.

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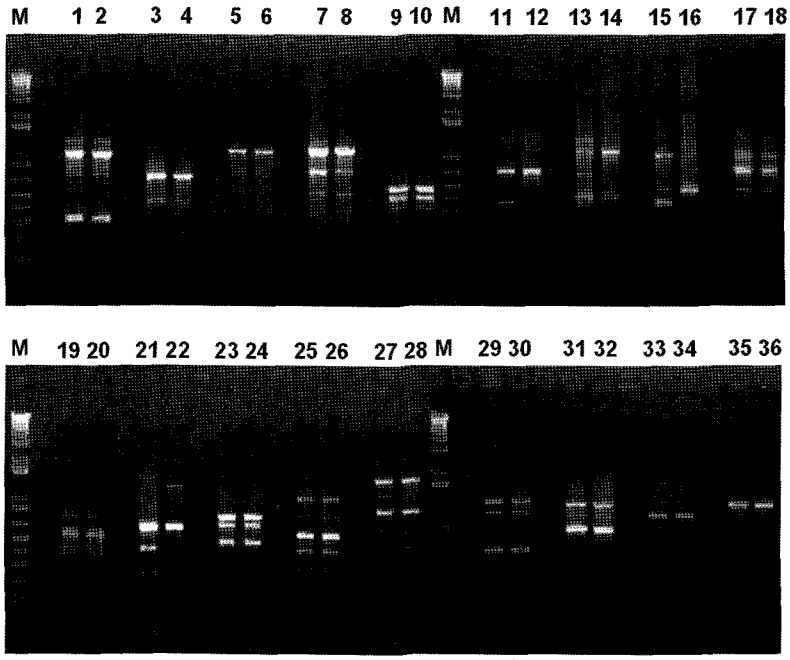

Fig. 4. Gel electrophoretic RAPD profiles obtained with Odae and Ilpum genomic DNA and 18 different primers. RAPD fragments ranged from 0.4-2.5 kb. Lane M is a 1 kb ladder in the first lane of each gel. Lanes 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35 are derived from Odae, while lanes 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36 are from Ilpum. OPAG-02 (lanes 1, 2); OPAG-03 (3, 4); OPAG-04 (5, 6); OPAG-06 (7, 8); OPAG-07 (9, 10); OPAG-08 (11, 12); OPAG-09 (13, 14); OPAG-10 (15, 16); OPAG-11 (17, 18); OPAG-12 (19, 20); OPAG-13 (21, 22); OPAG-14 (23, 24); OPAG-15 (25, 26); OPAG-16 (27, 28); OPAG-17 (29, 30); OPAG-18 (31, 32); OPAG-19 (33, 34); OPAG-20 (35, 36).

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