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Assessment of Antioxidative Capacity in Relation to Seed Traits of Rice Varieties

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ABSTRACT: In order to assess antioxidant capacity in relation to seed traits of rice (*Oryza sativa* L.), ninety-six varieties were examined for antioxidative activity of brown rice grain using superoxide dismutase (SOD), 1,1-diphenyl-2-picrylhydrazyl (DPPH), and thiobarbituric acid (TBA) assays. Overall, average total activities measured by the three methods were of very wide range between 64% and 13%. Significant differences were noted depending on the variety and evaluation method. Rice varieties with foreign origin, middle maturity, colored hulls, and colorless awn exhibited statistically significant higher total activity. As for the measurements, total activity was significantly correlated with SOD (r=0.29*), DPPH (r=0.82***) and TBA (r=0.76***). Between the three activities, SOD was not positively correlated with DPPH (r=-0.15*), while TBA was significantly correlated with DPPH value (r=0.51***). DPPH (55.20%) and TBA (50.36%) were significantly higher in foreign rice, while SOD activity (44.29%) was significantly higher in domestic rice. However, an average total activity was significantly higher in foreign rice (47.31%) than in domestic rice (35.92%). SOD, DPPH and TBA activities of middle maturity in maturity time were the highest total activity (44.96%) and significantly differed from the other two groups. Total activity was significantly higher in rice with a colorless awn (42.18%) than with a colored awn (35.87%).**

Keywords: antioxidative activity; rice, SOD, DPPH; TBA

Rice (*Oryza sativa* L.) has been cultivated worldwide and as a staple food crop in Asian countries. The nourishment of world's population would be improved by the development of better rice varieties and the technology for better rice production and utilization. The nutritional quality of harvested grain can be depraved by oxidation during the storage that may affect biochemical properties of rice grain and the palatability of cooked rice. There are some reports of antioxidative compounds being present in rice. For example, gramma-oryzanol, which is often blended with ferulate esters of triterpene alcohol, is found in rice bran (Xu *et al.*, 2001). Cyanidin-3-glucoside is most abundantly present in black pericarp rice (Osawa, 1999). In addition, rice seeds contain flavonoid, isovitexin, cyanide, oryzanol, α-tocopherol, and phytic acid, which all exhibit strong antioxidant properties (Ramarathnam *et al.*, 1986, 1989; Wu *et al.*, 1994; Osawa *et al.*, 1985). Especially, isovitexin and phytic acid isolated from rice hulls were found to be very strong antioxidants (Ramarathnam *et al.*, 1989). Antioxidant compounds are commercially used in manufacturing of lipid or lipid-containing foods since they inhibit lipid peroxidation that is known to be associated with aging, carcinogenesis, mutagenesis and atherosclerosis (Culter, 1984; Yagi, 1987). Thus, rice hulls have received much attention as an attractive source of natural antioxidants. At present, rice seeds are usually stored before consumption. During a prolonged storage, metabolic activities can be resumed depending on the moisture and temperature, and freshly-harvested rice seeds may break dormancy after short-term storage (Baber, 1972; Navasero *et al.*, 1975).

A free radical is simply defined as any atom or molecule that possesses an unpaired electron (Punchard and Kelly, 1996). The major free radical compounds of biological importance are those of oxygen called reactive oxygen species (ROS). Examples are superoxide radical (O_2^-), hydrogen peroxide (H_2O_2), and hydroxy radical (HO^-) (Sies, 1993). ROS is often generated by a variety of environmental stresses such as drought, cold, heat, and pollutants, leading to a number of physiological disorders. So, plants have evolved a wide range of enzymatic and non-enzymatic mechanisms to cope with the ROS-mediated oxidative stress.

A major enzymatic antioxidant system includes superoxide dismutase (SOD, EC 1.15.1.1), catalases (EC 1.11.1.6), peroxidases (POD, EC 1.11.1.7). SOD reacts with superoxide radicals to produce hydrogen peroxide ($2O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$), and its activity determines with the concentrations of O_2^- and H_2O_2 and increases in response to a variety of environmental and chemical stimuli (McCord and Fridovich, 1969; Fridovich, 1986; Perl-Treves and Galun, 1991). Being present in almost all organisms, this enzyme exists in three

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types, copper/zinc (Cu/Zn-SOD), manganese (Mn-SOD) and iron (Fe-SOD) according to the binding of metal cofactors to the active site. In higher plants, the most prominent SOD is Cu/Zn isozymes located in the cytosol and plastids (Bannister *et al.*, 1987; Sakamoto *et al.*, 1992). Hydrogen peroxide produced by SOD is disposed by the action of catalases (EC 1.11.1.6) and peroxidases (POD, EC 1.11.1.7), which are also indispensable for protection from cellular damages caused by reactive oxygen molecules. Chung *et al.* (2000d, 2001) reported that SOD and POD activities tend to increase when rice varieties are exposed to heavy metal ions and that O_3-resistant rice has significantly higher activities of SOD and POD. Catalase is found predominantly in peroxisomes where it functions to remove H_2O_2 formed during photorespiration (Tolbert, 1981; Lazarow and Fujiki, 1985). Although SOD in rice has been purified and characterized (Kanematsu and Asada, 1989, 1990; Pan and Yau, 1991), there have been few reports on SOD activity in rice grain of different varieties.

Non-enzymatic antioxidant system includes water- and lipid-soluble compounds. For instance, lipid-soluble are vitamin E (tocopherol) and carotenoids (including beta-carotene), and water-soluble are vitamin C, anthocyanin, uric acid, thiols, and glutathione. The bioactivity of these compounds can be recorded using various methods such as flow injection-chemiluminescence (FI-CL), thiobarbituric acid (TBA), 1,1-diphenyl-2-picrylhydrazyl (DPPH), electron spin resonance (ESR) spectroscopy, pulse radiolysis technique, redox potential measurement, and chemiluminescent assays (Mitsuta *et al.*, 1990; Van Acker *et al.*, 1996; Choi *et al.*, 2000). Among these methods, DPPH and TBA were used in the present study. DPPH method is based on the reduction of colored free radical DPPH by free radical scavenger. TBA method is most frequently used for measuring the lipid oxidation (Park, 1995). It measures the amount of a secondary lipid oxidation product, malonaldehyde (MA), which forms a complex with TBA and produces a red chromogen with a maximum absorbance at 532 nm.

Our study is focused on the radical-scavenging properties of rice grain because their potential is known to foster health protection against oxidative damage that has been attributed to more than 100 diseases (Halliwell, 1992). To date little information is available on the antioxidant potential of brown rice grain from different varieties. A successful breeding of rice variety with high antioxidative capacity requires an efficient screening method. The objectives of this study were to evaluate antioxidative activities of different rice germplasms using SOD, DPPH and TBA methods, and to assess antioxidant capacity in relation to genetic and phenological traits of seed. Here, we report a positive correlation between antioxidant capacity and the three traits of regional origin, maturity and awn color with a significant difference.

MATERIALS AND METHODS

Seed sample preparations

Ninety-six rice (*Oryza sativa* L.) varieties were cultivated at the experimental farm of Konkuk University at Seoul City, Korea during summer of 2002. They were initially grown in the seedling boxes for 45 days, then were transplanted to the field in 30 cm rows with 15 cm spacing (30 cm × 15 cm per 3.3 m^2) on 30 May, 2002. Fertilizer was applied at the rate of 110 (N) -70 (P_2O_5) -80 (K_2O) kg ha^{-1}. Other management practices were conducted according to the conventional methods in Korea. Plants were harvested and separated in October 2002. Harvested unhulled seeds were dried at room temperature (24°C) and were hulled to brown rice using a milling machine. Extract was prepared from brown rice grain within one month after harvest and were stored at -35°C under vacuum until analysis.

Measurement of superoxide dismutase (SOD) activity

Enzyme extraction

For each sample, brown rice grain (0.2 g) was pulverized with mortar and pestle, mixed with 0.4 g polyvinylpolypyrrolidone (PVP), and was suspended in 2 ml extraction buffer (pH 7.0, 100 mM potassium phosphate, 10 mM sodium ascorbate and 5 mM EDTA). The homogenate was centrifuged at 15,000 rpm for 20 min, the resulting upper phase (1 ml) was applied to PD-10 column (1.6 x 10cm) of Sephadex G-25, and the flow-through fraction (1 ml), was used to measure SOD activity.

Measurement of SOD activity

SOD activity was measured using the Nitro Blue Tetrazolium (NBT) reduction method (Beyer and Fridovich, 1987). Reaction mixture contained 60 μl of crude enzyme and 30 μl of riboflavin (3 μM) in 3 ml of assay buffer (pH 7.0, 50 mM potassium phosphate, 0.1 mM EDTA, 30 mM L-methionine, 0.625 mM NBT-2HCl and 1% Triton X-100), and the reaction was initiated by illuminating for 7 min at 25°C in an aluminum foil-lined box containing two 20-W Slyvania Groiux fluorescent lamps. The absorbance of the reaction solution was measured at 560 nm. Enzyme activity was calculated as follows:

$$\text{SOD activity (\%)} = (1 - \text{absorbance of sample/absorbance of blank}) \times 100$$

Measurement of radical scavenging activity

Sample preparation

Brown rice grain (5 g) was pulverized and sonicated in 100 ml of 80% methyl alcohol for 24 h at room temperature. The sonicated extract was filtered through Whatman No. 4 paper. The filtrate was dried in a rotary vacuum evaporator at below 30°C and was freeze-dried at -40°C. The dried filtrate was redissolved to 1% (w/v) in 80% methyl alcohol and was used for DPPH and TBA reactions.

DPPH assay

Radical scavenging activity was measured following the method of Yoshida *et al.* (1989). Reaction mixture contained 0.25 ml of 1% sample and 2.5 ml of 0.35 mM DPPH (dissolved in 50% ethyl alcohol). The mixture was incubated for 10 min at room temperature and was measured at 517 nm to detect any changes in DPPH absorbance. The activity was calculated against 80% methyl alcohol as a blank control as follows:

Inhibition (%) = (1 - absorbance of sample/absorbance of blank) × 100

Measurement of lipid peroxidation

Preparation of substrate solution of TBA

TBA reaction solution was prepared as previously described (Wong *et al.*, 1981). Linoleic-acid (30 mM) was added to the solution of 99% ethanol 100 mM potassium phosphate buffer. This solution was mixed with 1% sample, and the mixture was incubated at 40°C for 24 h in a shaking water bath before reaction with TBA.

Measurement of TBA inhibition

Two milliliters of TBA reaction solution were mixed with 1 ml of 35% trichloroacetic acid (TCA) and 2 ml of 0.75% TBA and were incubated at 95°C for 40 min. The mixed solution was cooled down to room temperature and was centrifuged at 3,000 rpm for 5 min. To the clarified supernatant, 1 ml acetic acid and 2 ml chloroform were added. The absorbance of the reaction supernatant was measured at 532 nm against a blank solution without TBA. The activity was calculated as follows:

Inhibition (%) = (1 - absorbance of sample/absorbance of blank) ×100

Statistical analysis

In all experiments, triplicate measurements were made with four independent samples using a randomized complete block design. Analysis of variance (ANOVA) was performed using the general linear model procedure (GLM) of SAS program. The pooled mean values were separated based on the least significant differences (LSD) at the 0.05 probability level. Correlations among the SOD activity, DPPH radical scavenging activity, and TBA measurement of lipid peroxidation inhibition were also measured using the SAS program (SAS Institute, Inc., 2000).

Table 1. Seed traits and antioxidative activities of 96 rice varieties.

Variety	Varieties characteristics							SOD	DPPH	TBA	Total†
	Ori	M	HC	A	AC	P	G				
									----- Inhibition (%) -----		
143 (PI 274471)	D	EM	HC-	A+	AC+	W	1.80	44.2	4.7	41.3	30.1
AC1423	F	MM	HC+	A+	AC-	Br	2.22	42.3	90.1	54	62.1
AGUDO	D	MM	HC-	A+	AC+	Br	1.89	28.8	9.3	43.9	27.3
ARONGBYEO	D	MM	HC-	A+	AC+	W	1.76	34.3	4.6	21.6	20.2
B1293B-PN-24-2-1	F	LM	HC-	A-	AC-	W	3.15	11.4	59.5	33.1	34.7
BADOLBYEO	D	MM	HC-	A+	AC-	W	1.70	54.7	69.5	45.7	56.6
BAEKGWANGOK	D	LM	HC-	A+	AC+	W	1.65	64.4	64	55	61.1
BAEKJO	D	MM	HC-	A-	AC-	W	1.54	62.6	9.4	26.9	33
BAEKKYEONGJO	D	MM	HC-	A+	AC-	W	1.76	67	48.9	55.6	57.2
BAEKMANGJO	D	EM	HC-	A-	AC-	W	1.60	38.5	53.1	45.2	45.6
BANCHONJO	D	MM	HC-	A+	AC-	Br	1.73	43	53.6	50.8	49.1
BARAMDUNGKURI	D	LM	HC+	A-	AC-	W	1.79	56	60.4	52	56.1
BASMATI	F	MM	HC-	A+	AC-	G	3.58	67	54.9	25.5	49.1
BORIBYEO	D	LM	HC-	A-	AC-	W	1.85	58.3	41.1	54.2	51.2

Table 1. Continued-1.

Variety	Varieties characteristics							SOD	DPPH	TBA	Total†
	Ori	M	HC	A	AC	P	G				
								----- Inhibition (%) -----			
BULDO	D	LM	HC+	A+	AC+	W	1.88	42.7	62.6	54.1	53.1
CHANARAK	D	EM	HC-	A-	AC-	W	1.71	44.1	8.4	24.7	25.7
CHEONGGUNBYEO	D	EM	HC+	A+	AC+	W	1.48	39.7	7.9	17.4	21.7
CHEONGSANDO	D	EM	HC+	A+	AC+	W	1.74	39.9	4.2	46.2	30.1
CHINDADACHIKI	D	EM	HC+	A+	AC+	W	1.61	34.4	11	30.2	25.2
CICA-4	F	LM	HC-	A-	AC-	W	3.16	15.1	52.5	36.9	34.8
CUBA 65-V-58	F	LM	HC-	A-	AC-	W	3.16	9.8	56.9	38.8	35.2
DADAJO	D	LM	HC-	A+	AC-	W	1.52	67	31.6	61.7	53.4
DAEGUDO	D	EM	HC-	A+	AC+	W	2.11	24.7	43.1	39.8	35.9
DAMAGUNG	D	MM	HC+	A+	AC-	W	1.58	54.8	57.6	37.5	50
DANGANEUIBANGJU	D	EM	HC-	A-	AC-	W	1.65	0.9	38.8	37.4	25.7
DEOKJEOKJODO	D	EM	HC-	A+	AC+	W	1.68	44.3	57.7	37.9	46.6
DONDUNI KUNLUZ	F	LM	HC+	A-	AC-	W	2.75	14.9	57	38.9	36.9
DONG O BYEO	D	MM	HC+	A+	AC+	W	1.82	30.8	62.9	48.7	47.5
DONGSANJO	D	EM	HC-	A+	AC-	W	1.65	37.8	28.9	18.9	28.5
DONNA	D	LM	HC-	A+	AC+	G	1.75	36	41.4	37.5	38.3
DORAE	D	MM	HC+	A+	AC-	W	1.69	37.6	48.9	35.8	40.8
DUCHUNGJONG	D	MM	HC+	A+	AC+	W	1.57	57.1	51.4	38.6	49
EUMSEON	D	EM	HC-	A-	AC-	W	1.83	47.5	16.5	21.4	28.5
EUNGJO	D	EM	HC+	A-	AC-	W	1.79	57.4	19	33.3	36.6
GANGCHEONGDO	D	MM	HC-	A-	AC-	W	1.67	51.9	39.4	44	45.1
GANGREUNGDO	D	EM	HC-	A-	AC-	W	1.73	46	47.1	46	46.4
GEUMJEOMDO	D	MM	HC-	A+	AC-	W	1.63	40.5	43.5	41.6	41.9
GIN SHUN	F	EM	HC-	A-	AC-	W	2.34	7.4	65.6	45.8	39.6
GPNO 12856	F	EM	HC-	A-	AC-	W	1.90	9.2	67.7	41.7	39.5
GPNO 3005	F	EM	HC-	A+	AC-	W	3.03	12.5	62	44.7	39.7
GUANDO	D	MM	HC+	A+	AC+	W	1.57	48.5	42.3	44.3	45
HAMBUREUBYEO	D	MM	HC+	A+	AC-	W	1.45	33.1	7.1	47.1	29.1
HEUGBAL	D	EM	HC+	A+	AC-	W	1.64	50	16.8	19.7	28.8
HEUGSAEKDO	D	EM	HC-	A+	AC+	W	1.61	49.8	7.8	13.8	23.8
HEUNBE	D	MM	HC-	A-	AC-	W	1.43	44.1	35.1	19.2	32.8
HOCHOKJINDO	D	LM	HC-	A+	AC-	W	1.71	51.7	8.2	56.4	38.8
HONGDODO	D	MM	HC-	A+	AC+	W	1.80	43.3	8	29.2	26.8
HUADO	D	LM	HC-	A+	AC+	W	1.68	51.1	4.7	13.3	23
HWANGJO	D	LM	HC-	A+	AC+	W	1.64	10.9	9.8	19.6	13.4
HWANGJU	D	EM	HC-	A-	AC-	W	1.79	35.6	7.8	19.9	21.1
HWANGTODO	D	LM	HC-	A+	AC-	W	1.73	36.7	13.4	30.5	26.97
ILPUM	D	MM	HC-	A-	AC-	W	1.56	38.3	15.5	81.7	45.2
IR 1044-56	F	LM	HC-	A+	AC-	W	2.49	49	46.3	3.5	32.9
IR 329-19-5-2-2	F	MM	HC-	A-	AC-	W	3.01	59	45.2	51.4	51.9
IR 644-1-63-1-1	F	LM	HC-	A-	AC-	W	2.56	30.6	58.8	49.4	46.3
IRI 233	D	LM	HC+	A+	AC+	W	1.66	54.6	12	-10.1	18.8
IRI 268(NONGKWANG)	D	LM	HC-	A+	AC+	W	1.70	60.4	15.6	19.5	31.8
IRI 301(MANGYUNG)	D	LM	HC-	A+	AC-	W	1.84	65.9	16.5	20.5	34.3
JANGJO	D	EM	HC-	A+	AC+	W	1.52	35.3	28.5	90.6	51.5
JANGSAMDO	D	LM	HC-	A-	AC-	W	1.83	50.3	5.2	21.3	25.6

Table 1. Continued-2.

Variety	Varieties characteristics							SOD	DPPH	TBA	Total†
	Ori	M	HC	A	AC	P	G				
									----- Inhibition (%) -----		
JANGWANG	D	LM	HC-	A+	AC-	W	1.63	36.3	5.8	17	19.7
JEONA	D	EM	HC-	A-	AC-	W	1.71	48.7	21.4	22.6	30.9
JEONGDALDO	D	LM	HC+	A+	AC+	W	1.68	58.8	22.1	29.6	36.8
JEONGJO	D	LM	HC-	A-	AC-	W	1.77	49.7	12.7	25.9	29.4
JINHWA	D	LM	HC-	A+	AC+	G	1.60	52	2.5	50.3	34.9
KASARWALA MUNDARA	F	MM	HC-	A+	AC-	W	2.72	34.6	39	63.8	45.8
KINGMEN TOUMEN DHIU MU	F	EM	HC-	A+	AC-	W	2.28	36.1	57.6	65.6	53.1
MAMORIAK	F	EM	HC-	A+	AC-	W	2.20	20.5	42.7	64.5	42.6
MON-Z-WUAN	F	EM	HC-	A-	AC-	W	2.30	43	57	51.5	50.5
MUTANT 12-42	F	EM	HC-	A+	AC-	W	2.90	42.7	57	59.3	53
NAMKANGBAEKJO	D	EM	HC-	A+	AC-	W	1.72	37.7	41	47.6	42.1
NAMSEON 1	D	LM	HC-	A+	AC-	W	1.70	17.8	42.7	48.1	36.2
NOINDARI	D	MM	HC+	A+	AC-	W	1.65	44.2	48.3	57.8	50.1
NOINDO	D	LM	HC-	A-	AC-	W	1.69	38.5	47.1	46.7	44.1
OEGUKBYEO	D	LM	HC-	A+	AC+	W	1.77	53.6	11.5	26.7	30.6
OLBYEO	D	EM	HC-	A+	AC-	W	1.71	50.2	10.8	27.3	29.4
P 1279	F	LM	HC-	A-	AC-	G	2.77	53.1	52.6	64.3	56.7
PATBYEO	D	EM	HC-	A-	AC-	W	1.56	38.6	5	31.8	25.1
PHILIPPNE 2	F	EM	HC-	A-	AC-	W	2.05	46.4	87.3	59.6	64.4
PI 389011 OR SD	F	EM	HC-	A-	AC-	W	2.47	43.9	42.8	28.4	38.4
PYEONGBUK 4	D	MM	HC-	A+	AC+	W	1.98	51.9	11	38.5	33.8
PYEONGYANG	D	LM	HC-	A+	AC-	W	1.59	50.3	7.2	42	33.2
RED KHOSHA CERMA	F	EM	HC-	A-	AC-	W	2.99	36.9	52.8	60.4	50
REXMONT	F	MM	HC-	A-	AC-	W	2.34	47.1	54.6	57.7	53.1
RIKUU 132	F	MM	HC-	A+	AC+	W	2.06	45	58	59.9	54.3
SAN CHIAO TSWEN	F	EM	HC-	A-	AC-	Br	2.06	43.1	82.1	62.3	62.5
SANCHEONGDO	D	MM	HC-	A+	AC+	W	1.72	48.5	15.4	41.1	35
SANGPUNG	D	MM	HC+	A+	AC-	W	1.71	39.3	64.9	50.8	51.7
SANJO	D	MM	HC-	A+	AC-	W	1.56	51.9	13.5	32.8	32.7
SEUNGSILJO	D	LM	HC-	A-	AC-	W	1.80	2.6	13.5	38.1	18.1
SHALI I MAHIN	F	MM	HC-	A-	AC-	W	1.98	46.2	57.5	62	55.2
SHUANG CHIANG-30-21	F	MM	HC-	A-	AC-	W	1.96	45.6	55.3	31.8	44.2
SINBAEGSEOG	D	LM	HC-	A+	AC+	W	1.80	12.8	3.4	33.8	16.7
TAICHUNG NATIVE 1	F	MM	HC-	A-	AC-	W	2.05	45.4	52.3	59.5	52.4
TSAI YUAN CHON	F	MM	HC-	A-	AC-	W	1.96	50	24	30.3	34.8
WOO-CO-CHIN-YU	F	MM	HC-	A-	AC-	Br	2.08	44.8	51.3	62.7	52.9
MEANS							1.9	41.5	35.8	40.6	39.3
$LSD_{0.05}$							0.2	1.8	7.0	6.1	3.7

†(SOD + DPPH + TBA) / 3
Ori (regional origin): F, foreign origin; D, domestic origin;
M (maturity): EM, early maturity; MM, middle maturity; LM, late maturity;
HC (hull color): HC+, colored hull; HC-, colorless hull;
A (awn): A+, presence of awn; A-, absence of awn;
AC (awn color): AC+, colored awn; AC-, colorless awn;
P (pericarp color): Br, brown; G, green; w, white;
G (grain length/width) (mm)

RESULTS AND DISCUSSION

Evaluation of antioxidative capacity of 96 rice varieties

Ninety six rice varieties were investigated for antioxidative capacity of brown rice grain using SOD, DPPH and TBA assays. As shown in Table 1, the activity varied widely depending on the variety and measurement method. This is expected because different assays measure the effects of different free radical species generated in dissimilar amounts

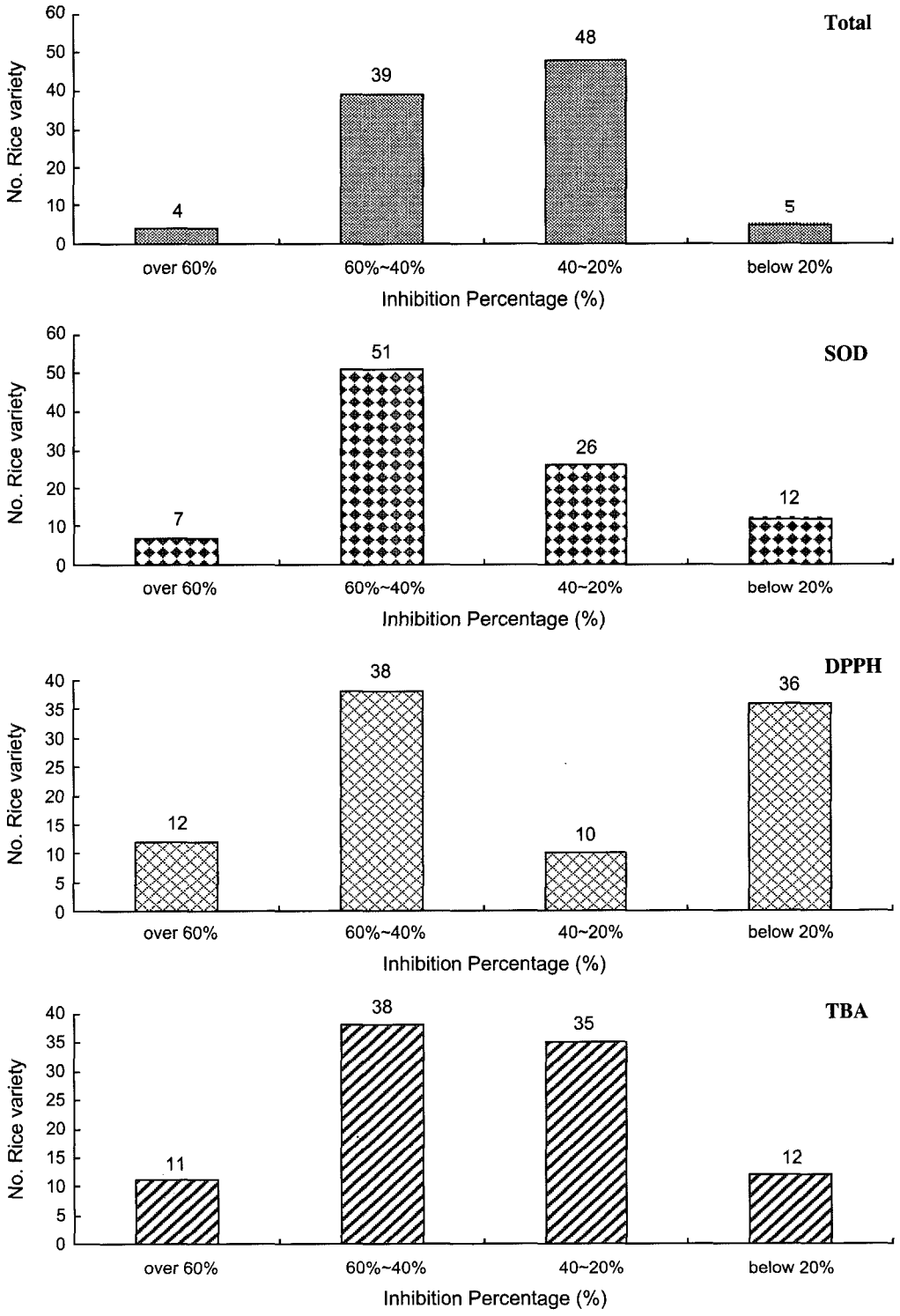

Fig. 1. Four groups of rice varieties based on the extent of antioxidative activity.

by different varieties. With respect to the individual measurement, an average activity of 96 varieties was of the following order: SOD (41.5%) > TBA (40.6%) > DPPH (35.8%). This gave 39.3% of total activity on the average, which ranges from 64.4% (Philippine 2) to 13.4% (Hwangjo). Overall, the total activities were of a very wide range; four varieties were over 60%, 39 varieties were between 40% and 60%, and 48 varieties were between 20% and 40% (Fig. 1).

The highest SOD activity (67.0%) was found in Baekkyeongjo, Basmati and Dadajo, whereas the lowest activity (0.9%) was in Danganeuibangju. More than 50% of rice varieties displayed SOD activity between 40% and 60%. As for the DPPH inhibition, the activity also widely ranges from 90.1% (AC 1423) to 2.5% (Jinhwa); 38 varieties were between 40% and 60%, 10 varieties were between 40% and 20%, and 36 varieties were below 20%. A similar observation was made in TBA activity with the highest inhibition in Jangjo (90.6%). Significant differences in the three activities were noted depending on the variety and evaluation method. For example, Danganeuibangju had about 38% activity of DPPH and TBA with 0.9% SOD activity, whereas Jinhwa retained about 50% activity of SOD and TBA with 2.5% activity of DPPH. On the other hand, Gangreungdo and Geumjeomdo showed very similar percentages in all three types of activities.

Generally, measured antioxidative activities are assumed to differ with varieties, growth stages and conditions, extraction methods, and types of assays. For instance, the antioxidative activity could be changed by sample havesting time, cultivars, storage periods, and experiments (Kim *et al.*, 2004; Lee *et al.*, 2002). In addition to, Kong *et al.*(2004) reported that antioxidative activites depend on various genetic and environmental factor such of their components, where and when they were collected, and their path of action. Chung *et al.*(2000c) measured SOD and POD activities of rice leaf tissue taken every week from 4 July to 8 August and found significant variations between 4.9% and 13.1% with a higher activity at a heading stage. In addition, all 16 Korean rice varieties differed in SOD, DPPH and TBA activity from each other (Chung *et al.*, 2000a). Ethanolic extract gave a higher activity than extracts of other solvents, and colored rice bran contained much greater activities than noncolored bran (Kang *et al.*, 2003; Nam *et al.*, 2003, 2005). This finding suggests that unknown soluble substances as well as pigments in colored rice may contribute to antioxidative capacity.

In the present study, total inhibition activity was significantly correlated with SOD ($r=0.29^{***}$, $P<0.0001$) DPPH ($r=0.82^{***}$, $P<0.0001$) and TBA ($r=0.76^{***}$, $P<0.0001$). Between the three activities, SOD was not positively correlated with DPPH ($r=-0.15^{*}$, $P=0.0450$), while TBA was significantly correlated with DPPH ($r=0.51^{***}$, $P<0.0001$) Thus, the higher DPPH and TBA value, the higher the total antioxidative activity. This is consistent with a previous report of a positive correlation between TBA and DPPH activity in rice grains (Chung *et al.*, 2000b).

Assessment of antioxidative capacity in relation to seed traits

In an attempt to find a simple and fast approach to screening rice varieties for high antioxidative power, we examined the activities in relation with genetic and phenotypic traits of seed. Genetic traits were of the regional origin, the time of maturity, and the ratio of grain length to width, whereas phenological traits were of the presence and absence of an awn and a color of awn and hull. Firstly, rice varieties were grouped in two major clusters of domestic and foreign origin, and antioxidative activities of the two groups were compared (Table 2, Fig. 2a). DPPH (55.20%) and TBA (50.36%) were significantly higher in foreign rice, while SOD activity (44.29%) was significantly higher in domestic rice. However, an average total activity was significantly higher in foreign rice (47.31%) than in domestic rice (35.92%). Secondly, rice varieties were divided into three

Table 2. Comparison of antioxidative activities in relation to seed traits.

Traits†	Inhibition Activity (%)		
	SOD	DPPH	TBA
D (67 varieties)	44.29	26.95	36.51
F (29 varieties)	36.37	55.20	50.36
$LSD_{0.05}$	4.42	5.88	4.61
EM (32 varieties)	38.01	34.67	40.79
MM (33 varieties)	46.74	42.72	45.41
LM (31 varieties)	41.17	29.91	36.19
$LSD_{0.05}$	5.04	7.73	5.58
HC+ (19 varieties)	44.64	41.08	37.71
HC- (97 varieties)	41.16	34.56	41.57
$LSD_{0.05}$	5.39	8.42	5.94
A+ (57 varieties)	44.20	32.69	40.18
A- (39 varieties)	38.17	40.50	41.86
$LSD_{0.05}$	4.23	6.67	4.75
AC+ (28 varieties)	44.81	25.24	37.35
AC- (68 varieties)	43.60	40.14	42.81
$LSD_{0.05}$	5.12	7.81	5.71
HG (6 varieties)	29.13	55.17	38.40
MG (20 varieties)	37.58	57.40	52.55
LG (70 varieties)	43.66	27.94	37.37
$LSD_{0.05}$	1.93	1.89	2.78

†Abbreviations are the same as footnotes in Table 1 except for the ratios of grain length to width that are denoted by HG, 3.0-3.6; MG, 2.0-3.0; and LG, 1.5-2.0.

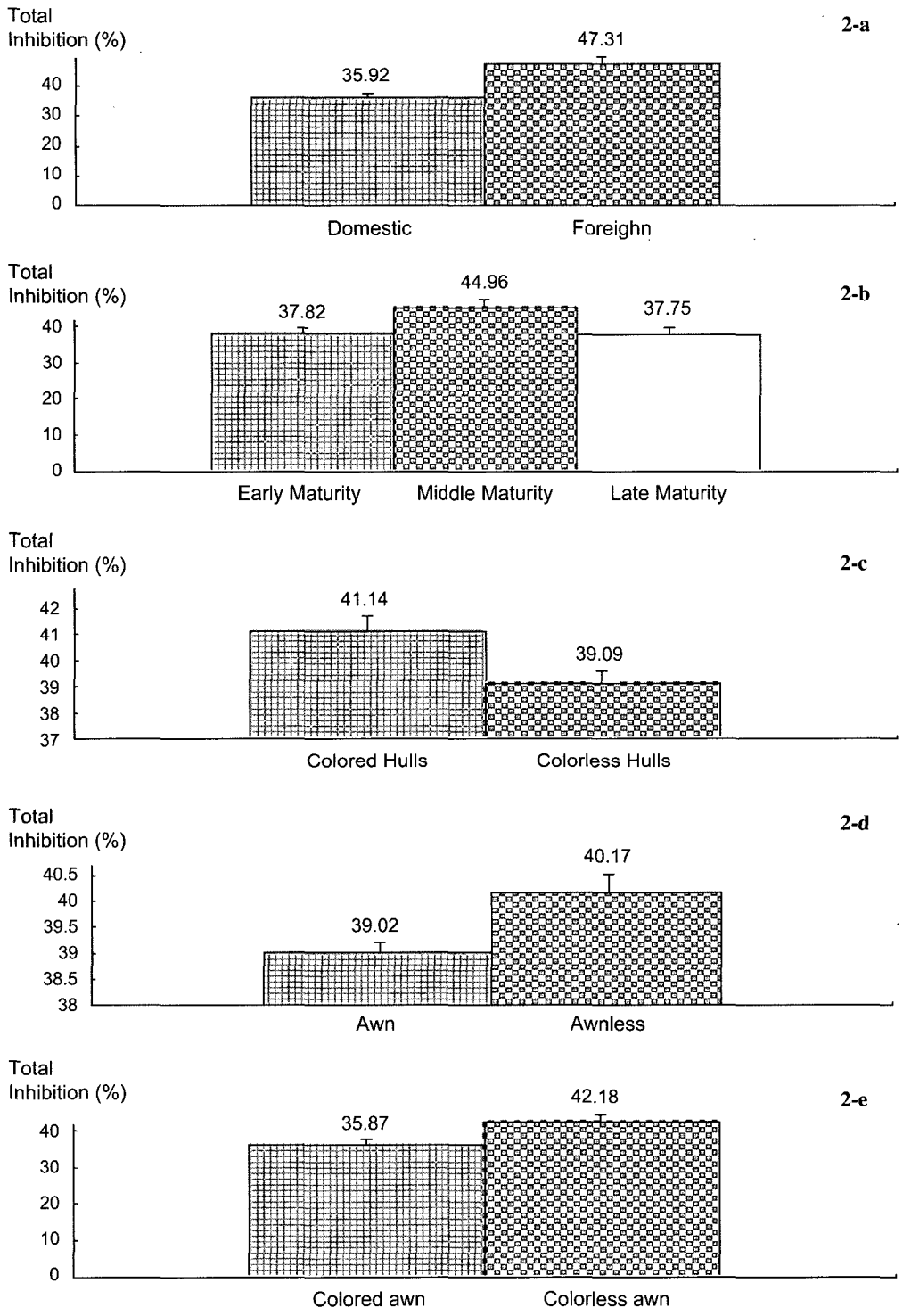

Fig. 2. Total antioxidative activities according to the genetic and phenological characteristics. Comparison with total antioxidative activity among domestic and foreign (2-a), maturing time (2-b), color of hull (2-c), existence of awn (2-d), and color of awn on rice varieties (2-e).

groups according to the maturing time. The activities of SOD, DPPH and TBA were all highest in rice of middle maturity, which as a result, had the highest total activity (44.96%) and significantly differed from the other two groups (Table 2, Fig. 2b). Thirdly, antioxidative activities were compared based on the hull color. None of the

activities were significantly different between colored and colorless hulls, leading to no significant difference in total activity (Table 2, Fig. 2c). This could be due to the fact that activity was derived from dehulled brown rice grain. Fourthly, antioxidative activities were compared according to the presence/absence of awn and awn color. The rice with awn had higher SOD activity but lower DPPH activity than the rice without awn (Table 2). The presence and absence of awn in rice did not make any significant differences in total activity (Fig. 2d). By contrast, the rice with colored awn had higher DPPH and TBA activities than the rice with colorless awn. Total activity was significantly higher in rice with a colorless awn (42.18%) than with a colored awn (35.87%), as shown in Fig. 2e. Finally, rice varieties were grouped in three categories according to the ratio of grain length to width (Table 2). Remarkably all the domestic rice except for one variety Daegudo belongs to the low ratio category, whereas virtually all the foreign rice belongs to either high or middle ratio category with the ratios of only four foreign varieties, GPNO 12856, Shalimahin, Shuang Chiang-30-21, and Tsai Yuan Chon, being close to a middle ratio. This finding indicates a strong positive correlation between the ratio and the regional origin.

Recent studies have reported that substances of potential health benefits are retained in rice with colored pericarp. A feeding experiment demonstrated that rabbits fed with red and black rice varieties showed an improved antioxidant status in their blood and a decreased atherosclerotic plaque formation (Ling *et al.*, 2001). Moreover, red and black pericarp rice was found to have significantly higher DPPH radical-scavenging activity than white rice (Oki *et al.*, 2002). Along this line, the higher antioxidative power of red pericarp rice than other white rice varieties was shown to positively correlate with phenolic content (Chi *et al.*, 2006). This is consistent with previous reports that phenols in rice contribute to antioxidative activity (Bunzel *et al.*, 2002; Hudson *et al.*, 2000). In fact, rice with white pericarp occupies over 85% of rice varieties worldwide, and the rest has most commonly green, black and red pericarp (Simmons and Williams, 1997). In the present study, rice varieties with brown, green and white pericarp were used, and most of them were of white pericarp. The average total antioxidative activity varied widely within the same rice category of pericarp color, brown pericarp is between 27.3 and 62.5%; green pericarp is between 34.9 and 56.7%; white pericarp is between 16.7 and 64.4% (Table 1). No significant correlations were found between the three pericarp colors and antioxidant activity. The phenolic contents of rice varieties within the same pericarp category remain to be determined to further verify the linear relationship between the phenolic content and antioxidant capacity.

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