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Comparison of Superoxide Dismutase and Peroxidase Activities in Rice Varieties

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ABSTRACT : Fifty-four Korean native and 28 foreign varieties harvested in 1998 and 1999 were examined for superoxide dismutase (SOD) and peroxidase (POD) activities. The SOD and POD activities of leaves extracts in Korean native and foreign rice varieties showed variation at the heading stage. The activities of SOD and POD changed with growth stage. In comparison of storage duration, the SOD and POD activities of the extract from three months stored seeds in Korean native (CV=53.3%) and foreign rice (CV=57.9%) varieties were higher than that of stored rices for a year in seed extracts although the activities among varieties did not show significant variation. Also, the averaged activity of foreign rice varieties (SOD= 12.9%) was relatively higher than that of korean native rice varieties (SOD=10.7%). The test of activity at the enzymatic level related to antioxidative activity suggests that the rice varieties with higher antioxidative potentials can be developed and also may provide information with rice breeder to breed rice variety with a high antioxidative activity.

Keywords : rice, variety, superoxide dismutase, peroxidase, activity.

A lot of attention has been recently directed to the development of natural antioxidants as biologically active substances that can exert considerable protection against aging and cancer caused by free-radicals in humans (Cutler, 1984). These studies have concentrated mainly on the application of these substances to foods and pharmaceutics.

A free radical is defined as any atom or molecule that possesses an unpaired electron (Punchard and Kelly, 1996). In biology and in related fields, the major free radical species of interest are those of oxygen, referred to as oxygen free radicals (OFRs). OFRs are part of a greater group of molecules often called reactive oxygen species (ROS). They all oxidize more strongly than molecular oxygen itself and include superoxide radical (O_2^-), hydrogen peroxide (H_2O_2), and hydroxy radical (HO^-) (Sies, 1993). The ROS generated by environmental stresses such as low and high temperatures and pollutants are associated with a number of physiological disorders in plants. The plants have evolved a wide range of enzymatic and non-enzymatic antioxidants to escape the oxidative stress by ROS, since antioxidants have common properties to be produced for a self-defense mechanism.

Superoxide dismutase (SOD, EC 1.15.1.1), discovered by McCord and Fridovich (1969), reacts with superoxide radicals at almost diffusion-limited rates to produce hydrogen peroxide ($2O_2^- + 2H^+ = H_2O_2 + O_2$). This enzyme is unique in that its activity determines the concentrations of O_2^- and H_2O_2. SOD is present in practically all organisms, from bacteria to humanbeings, and there are three types, classified by their metal cofactor, which depends on the metal found at the active site. The types are copper/zinc (Cu/Zn-SOD), manganese (Mn-SOD) and iron (Fe-SOD). In higher plants, the most prominent SODs are Cu/Zn isozymes found in the cytosol and plastids (Bannister et al., 1987; Sakamoto et al., 1992). The activity of plant SOD is known to increase in response to a variety of environmental and chemical stimuli (Fridovich, 1986; Peri-Treves, 1991). In addition, hydrogen peroxide produced by SOD is disposed by catalases (EC 1.11.1.6) and peroxidases (POD, EC 1.11.1.7). Catalase is found predominantly in the peroxisomes of plants where it functions chiefly to remove the H_2O_2 formed during photorespiration (Tolbert, 1981; Lazarow and Fujiki, 1985). These enzymes are indispensable for protecting organisms from damage caused by active oxygen molecules (O_2^-, OH^-).

Rice (*Oryza sativa* L.) is one of the most important food crops in Korea. The hope for improved nourishment of the world's population depends on the development of better rice varieties and improved methods for rice production and utilization. In order to breed rice varieties containing high antioxidative substances, screening methods need to be evaluated by enzymatic methods involved in antioxidative activity. Although purification and characterization of SOD in rice has been known (Kanematsu and Asada, 1989, 1990; Pan and Yau, 1991), there was no information on the SOD and POD activities in rice varieties. The objective of this study was to investigate SOD and POD activities using Korean native and foreign rice varieties.

MATERIALS AND METHODS

Sample preparation

Fifty-four Korean native varieties including colored rice,

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'Jangsamdo', common rice, 'Arongbyeo', and 28 foreign rices including colored rice, 'HweiJu', and common rice, 'GPNO 12856' were cultivated at the Konkuk University farm by the conventional methods of rice cultivation in middle area of Korea, and were harvested in October 1998. The harvested seeds were stored for a year at a cold chamber (-35°C). In 1999, the leaves of same rice varieties were sampled at the intervals of a week, from 4 July to 8 August, for analyzing SOD and POD activity. Also, the harvested seeds were stored for three months at a cold chamber (-35°C) until use.

Tests of SOD and POD activity

Enzyme extraction

To measure the SOD and POD activity on each variety, fresh leaf samples (1.0 g) were broken up with 0.4 g polyvinylpolypyrrolidome (PVP) and 5 ml extraction buffer with pH 7.0, 100 mM potassium phosphate, 10 mM sodium ascorbic acid and 5 mM EDTA (Chung *et al*., 1995). The homogenate was centrifuged at 15,000 rpm for 20 minutes and the solution used for fractionation of a PD-10 column (bed volume : 9.1 ml and bed height : 5 cm) of Sephadex G-25. The extract solution was used to measure SOD and POD activity. Also, stored rice varieties were hulled and was ground in a milling machine through a 40-mesh screen. Brown rice powder samples (0.2 g) of the respective rice varieties were broken up with 0.4 g PVP and 2 ml extraction buffer. The next following procedures were the same as above mentioned.

SOD activity test

Test tubes containing reaction solution (3 ml assay buffer, 60 μl enzyme, 30 μl riboflavin) were illuminated with 20-W Sylvania Groiux fluorescent lamps for 7 minutes at 25°C. The SOD activity was then determined by the NBT (Nitro Blue Tetrazolium) reduction method (Beyer et al., 1987), with the absorbance measured by a spectrophotometer at 560 nm (Hitachi Ltd., Tokyo, Japan). A control solution was prepared in a similar manner, but without adding enzymes. SOD activity was measured as the inhibition percentage of NBT (Asada *et al*., 1974), and calculated as follows:

$$\text{SOD activity (\%)} = (1 - A / B)\ 100$$

where A is the absorbance of samples and B is the absorbance of the control.

POD activity test

POD activity was examined using the method of Raa (1971). The test tube containing the reaction solution (0.5 ml of 0.3% hydrogen peroxide, 0.5 ml of 1% *o*-phenylenediamine, and 7.9 ml of sodium phosphate buffer; pH 6.8) was left at room temperature for 30 minutes, then the absorbance of the solution was measured at 430 nm. The POD activity was calculated as the inhibition percentage.

$$\text{POD activity (\%)} = (1 - A / B)\ 100$$

where A is the absorbance of samples and B is the absorbance of the control.

Statistical analysis

Analysis of variance for all data was accomplished using the general linear model procedure of the statistical analysis system program (SAS Institute, 1992). The experiments were repeated in a completely randomized design with 6 replications and the pooled mean values were separated on the basis of least significant difference (LSD) at the 0.05 probability level.

RESULTS AND DISCUSSION

Change of SOD and POD activities in leaves extracts

SOD activities of Korean native and foreign rice leaves were examined weekly from 4 July to 8 August (Table 1). Rice varieties were classified into three groups based on the heading stage: A (~10 Aug.), B (11 Aug.~20 Aug.), C (21 Aug.~31 Aug.) and D (1 Sept.~20 Sept.).

For the Korean native rice varieties, groups A and B had the highest SOD activity, with means of 13.4% and 13.1%, respectively on 11 July, and those were decreased to 10.5% and 12.5%, respectively, on 18 July. The means of groups B, C and D of the foreign rice varieties were decreased to 4.9%, 5.7%, and 7.4%, respectively, on 11 July and increased to 9.2%, 12.3% and 16.2%, respectively, on 18 July. The means of group C of the Korean native rice varieties and group A of the foreign rice varieties were increased to 9.8% and 12.3%, respectively, on 18 July and decreased to 6.5% and 5.2%, respectively, on 25 July. Other than group C of the foreign varieties, SOD activity tended to decrease on 8 August.

The changes in POD activities of Korean native and foreign rice leaves is given in Table 2. Korean native rice varieties had maximum POD activity on 1 August. For foreign rice varieties, groups A and C had maximum POD activity (95.8% and 97.9%, respectively) on 8 August and groups B and D (94.5% and 95.9%, respectively) on 25 July. POD activity of all groups changed irregularly during the six weeks. The value of SOD and POD value showed some

Table 1. Change SOD activity of Korean native and foreign rice varieties on leaves for six weeks in 1999.

Type	Heading stage	No. of var.		4/7†	11/7	18/7	25/7	1/8	8/8
				Activity (%)					
Korean native rice varieties	A‡	18	Maximum	13.4	27.0	20.9	32.3	37.8	15.5
			Minimum	0.1	4.6	3.7	1.9	4.9	3.0
			Mean	5.7	13.4	10.5	7.0	11.6	7.5
	B	25	Maximum	10.0	22.0	24.7	8.5	34.9	12.3
			Minimum	1.5	3.5	4.3	1.6	1.2	1.8
			Mean	5.2	13.1	12.5	4.9	9.1	5.4
	C	11	Maximum	19.5	16.5	19.0	16.2	10.8	15.5
			Minimum	2.7	3.7	4.1	1.2	3.5	2.6
			Mean	7.6	9.4	9.8	6.5	7.5	7.2
CV (%)				58.8	48.8	56.1	46.4	40.4	60.4
LSD (0.05)				5.18	8.71	8.78	4.22	5.43	6.03
Foreign rice varieties	A	12	Maximum	10.6	18.8	24.2	8.1	14.4	9.9
			Minimum	1.9	2.4	4.2	0.6	1.3	2.4
			Mean	5.7	6.8	12.3	5.2	9.0	5.6
	B	9	Maximum	10.3	6.3	12.5	12.4	17.8	13.1
			Minimum	1.0	2.8	4.2	2.7	4.9	1.7
			Mean	6.3	4.9	9.2	7.3	10.9	7.5
	C	4	Maximum	11.9	9.0	16.8	7.0	8.5	11.9
			Minimum	7.4	1.5	8.5	2.7	3.1	4.4
			Mean	8.9	5.7	12.3	4.7	5.6	7.4
	D	3	Maximum	8.9	7.8	24.5	7.0	11.3	8.0
			Minimum	7.2	7.1	8.0	4.0	5.8	6.6
			Mean	8.1	7.4	16.2	5.4	7.9	7.2
CV (%)				51.5	68.8	34.9	52.3	47.6	58.5
LSD (0.05)				5.82	6.74	6.88	4.87	7.02	6.33
CV (%)				52.5	53.4	47.4	45.9	43.8	60.4
LSD(0.05)				5.24	8.64	8.56	4.18	6.07	6.15

†Sampling day/month., ‡A; ~10 Aug., B; 11 Aug. ~20 Aug., C; 21 Aug. ~31, D; 1 Sept. ~20 Sept.

variation.

The marked variability in the SOD and POD activity of rice leaf extracts with different physiological growth stages were easily detected. Differences in activity obtained from this study now reported correspond to their suggestion (Pan and Yau, 1991) since the highest activity recorded during the sampling period was for August samples, the month in which the heading stage in most cultivars. The cause of the fluctuation in SOD and POD activity observed in our study could not be decided because environment on the stages of development were not recorded. Further studies are needed to measure the relationships between the environmental parameters and the activity of SOD and POD. In order to do this, plots would need to be established in areas with different rainfall and temperature regimes. Measurements of other variables such as soil temperature, moisture, and pH would also be needed.

SOD and POD activities tests on seed extracts

The values of SOD and POD enzymatic activities on rice seed extracts are given in Table 3. The SOD activity between Korean native and foreign rice varieties differed significantly (CV=53.3%). In 1999, the SOD activity of the foreign rice varieties (12.9%) was higher than for Korean native rice varieties (10.7%). POD activity was also statistically different between the Korean native and foreign rice varieties (CV=22.6%). However, the POD activity of Korean native rice varieties (51.1%) was higher than for foreign rice varieties (45.9%).

The SOD and POD activity of seeds of all Korean native and foreign rice varieties stored for three months were higher than those stored for a year. These results are similar to those of Cho *et al.* (1995) and Navasero *et al.* (1975), who noted that seeds stored for a year after harvest did not con-

Table 2. Change POD activity of Korean native and foreign rice varieties on leaves for six weeks in 1999.

Type	Heading stage	No. of var.		4/7[†]	11/7	18/7	25/7	1/8	8/8
				Activity (%)					
Korean native rice varieties	A[‡]	18	Maximum	97.8	97.1	98.4	98.3	97.8	98.5
			Minimum	14.5	33.1	48.4	12.8	81.9	66.7
			Mean	87.2	90.7	82.6	87.3	95.9	92.4
	B	25	Maximum	97.6	96.5	98.0	98.2	97.4	98.2
			Minimum	18.6	13.6	74.3	52.1	77.4	68.2
			Mean	73.3	86.4	94.2	90.9	94.0	89.9
	C	11	Maximum	96.8	96.9	98.0	97.9	97.6	98.0
			Minimum	34.9	70.9	44.6	25.9	85.7	52.3
			Mean	73.4	90.1	89.0	79.9	94.9	87.3
CV (%)				7.2	3.0	4.8	6.9	1.4	2.2
LSD (0.05)				8.42	4.33	6.86	8.68	2.09	3.24
Foreign rice varieties	A	12	Maximum	95.2	94.7	96.8	98.5	96.7	98.0
			Minimum	14.5	2.3	50.3	79.8	55.1	82.9
			Mean	61.4	60.4	91.1	95.0	81.7	95.8
	B	9	Maximum	93.9	93.8	96.5	98.0	94.9	98.6
			Minimum	49.0	26.8	73.2	85.7	38.9	49.2
			Mean	82.7	67.8	91.1	94.5	83.1	84.0
	C	4	Maximum	90.4	92.8	96.6	97.7	92.0	98.2
			Minimum	22.2	79.2	90.9	12.9	56.8	97.5
			Mean	68.3	90.3	94.5	91.9	81.9	97.9
	D	3	Maximum	90.1	92.6	96.2	97.0	74.2	98.0
			Minimum	76.2	39.9	92.3	94.2	51.0	76.6
			Mean	83.1	67.4	94.8	95.9	63.6	90.7
CV (%)				8.1	6.1	2.3	2.5	8.4	2.7
LSD (0.05)				8.23	6.71	3.38	10.85	4.00	3.03
CV (%)				52.5	53.4	47.4	45.9	43.8	60.4
LSD(0.05)				5.24	8.64	8.58	4.18	6.09	6.15

[†]Sampling day/month., [‡]A; ~10 Aug., B; 11 Aug. ~20 Aug., C; 21 Aug. ~31, D; 1 Sept. ~20 Sept.

Table 3. Comparison with SOD and POD activities among Korean native and foreign rice varieties.

Stored period	Type	SOD	POD
		Activity (%)	
One year	Korean native rice varieties	6.6	23.6
	Foreign rice varieties	8.5	13.7
CV (%)		45.8	24.5
LSD (0.05)		ns	7.53
Three months	Korean native rice varieties	10.7	51.1
	Foreign rice varieties	12.9	45.9
CV (%)		31.5	22.6
LSD (0.05)		ns	ns
CV (%)		53.3	57.9
LSD (0.05)		5.54	22.23

tain enzymes such as peroxidase. SOD is one of the house-keeping enzymes that acts in defense against reactive oxygen species in rice. High constitutive levels of antioxidants in a plant tissue are supported to provide resistance to a environmental stress. Further studies of SOD isozyme pattern on seed extracts and crude leaf extracts sampled from different stages are needed, since Cu/Zn SOD, among the activity of total SOD, is most active in rice seeds.

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