BIOLOGIA PLANTARUM 42 (2): 313-316, 1999

BRIEF COMMUNICATION

Effects of abscisic acid and water stress on the senescence of detached rice leaves

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Abstract

The effects of abscisic acid (ABA) and water stress on senescence and enzyme activities of oxygen scavenging enzymes of detached rice leaves were compared. Exogenously applied ABA exhibited water stress-like effects by promoting senescence, by decreasing the activities of catalase, peroxidase, ascorbate peroxidase and superoxide dismutase. It seems that the effects of water stress on senescence and enzyme activities are possibly mediated through increased content of endogenous ABA.

Additional key words: *Oryza sativa*, oxygen scavenging enzymes.

The senescence of leaves is accompanied by a decrease in chlorophyll and protein contents. Among the various ideas regarding senescence initiation in leaves, the free radical hypothesis has attracted considerable attention. Several reports have shown that water stress promotes leaf senescence (*e.g.* Dwivedi *et al.* 1979, Kao 1981, Chen and Kao 1990) and is accompanied by the formation of free radicals. Exogenous application of ABA also promotes leaf senescence (Smart 1994). The levels of endogenous ABA rise markedly in response to water stress. Recently, we demonstrated that water stress increased lipid peroxidation and decreased activities of peroxidase, catalase, superoxide dismutase (SOD) and ascorbate peroxidase (APOD)

Received 10 November 1998, *accepted* 17 January 1999.

Abbreviations: ABA - abscisic acid; APOD - ascorbate peroxidase; GR - glutathione reductase; MDA - malondialdehyde; RWC - relative water content; SOD - superoxide dismutase.

Acknowledgements: This work was supported by the National Science Council, the Republic of China, grant NSC 88-2313-B-002-066.

This paper is part 46 of the series "Senescence of Rice Leaves".

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in detached rice leaves (unpublished). The present investigation was undertaken to examine whether exogenously applied ABA can mimic the effect of water stress on the senescence and the oxygen scavenging enzymes activities in excised rice leaves.

Rice (*Oryza sativa* cv. Taichung Native 1) seedlings were cultured as previously described (Kao 1980). Briefly, seedlings were planted on a stainless net floating on modified half-strength Johnson's nutrient solution in a 500-cm^3 beaker. The nutrient solution (pH 4.5) was replaced every 3 d. Rice seedlings were grown in a greenhouse with natural day light at day/night temperature 30/25 °C and humidity 95 %. The apical 3 cm segments were excised from the third leaves of 12-d-old seedlings. Groups of twenty leaf segments were exposed to vapour above a solution of 0.5 M NaCl to decrease air humidity. Similar segments of leaves were floated on 20 cm^3 of distilled water or a solution of natural (+)-abscisic acid (ABA, 45 μM) in a Petri dish to serve as turgid controls and ABA-treated samples, respectively. All samples were kept at temperature of 27 °C and irradiance of 40 μmol m^{-2} s^{-1} provided by fluorescent lamps for 8 h.

RWC was determined according to Mukherjee and Choudhuri (1983). Protein, proline and ABA were extracted and quantified as described previously (Kao 1981, Chen and Kao 1993, Chang and Kao 1998). Malondialdehyde (MDA) was extracted with 5 % (m/v) trichloroacetic acid and determined according to Heath and Packer (1960). MDA level is routinely used as an index of lipid peroxidation.

The H_2O_2 content was measured as described by Jana and Choudhuri (1981). H_2O_2 was extracted by homogenizing 50 mg leaf tissue with 3 cm^3 of phosphate buffer (50 mM, pH 6.5). The homogenate was centrifuged at 6 000 *g* for 25 min. Three cm^3 of extracted solution was mixed with 1 cm^3 of 0.1 % titanium sulphate in 20 % (v/v) H_2SO_4 and the mixture was then centrifuged at 6 000 *g* for 15 min. The supernatant was measured at 410 nm by spectrophotometer (*U-2 000, Hitachi,* Tokyo, Japan).

For extraction of enzymes, leaf tissues were homogenized with 0.1 M sodium phosphate buffer (pH 6.8) in a chilled pestle and mortar. The homogenate was centrifuged at 12 000 *g* for 20 min and the resulting supernatant was used for determination of enzyme activity. The whole extraction procedure was carried out at 4 °C. Catalase activity was assayed by measuring the decrease in H_2O_2 content according to Kato and Shimizu (1987). Peroxidase activity was measured using a modification of the procedure of MacAdam *et al.* (1992). SOD was determined according to Paoletti *et al.* (1986). APOD was determined according to Nakano and Asada (1981).

All experiments were repeated three times; within each experiment, treatments were replicated 4 times. Similar results and identical trends were obtained in all experiments. The data reported here are from a single experiment.

RWC of leaf segments exposed to the decreased air humidity under irradiance decreased significantly (Table 1). Proline and ABA contents increased markedly in detached rice leaves exposed to the decreased air humidity. These results indicate that exposure of detached rice leaves to the decreased air humidity caused water stress.

Externally applied ABA also resulted in an accumulation of proline and ABA in detached rice leaves. However, ABA treatment had no effect on RWC, suggesting

that the effect of ABA treatment on detached rice leaves is unlikely due to water stress but mainly due to the increase of endogenous ABA concentration. It is interesting to note that water stress resulted in 7-fold increase in ABA concentration whereas exogenously applied ABA caused only 4-fold increase in ABA concentration in detached rice leaves (Table 1).

Table 1. Relative water content, contents of endogenous ABA, proline, protein, MDA and H_2O_2 and activities of oxygen scavenging enzymes in rice leaves treated for 8 h by water (control), exogenous ABA and the decreased humidity under light. The data are expressed on the basis of initial f.m. and represent means ± SE (n = 4). U for catalase, peroxidase, SOD, APOD and GR were defined as the amount of enzyme which breaks down 1 μmol of H_2O_2 per min, causes the formation of 1 μmol tetraquiacol per min, inhibites 50 % the rate of NADH oxidation observed in control, breaks down 1 μmol of ascorbate per min, and decreases absorbance (A_{340}) per min, respectively.

	Control	ABA	Decreased humidity
RWC [%]	99.20 ± 0.20	98.40 ± 0.60	86.50 ± 1.30
ABA [nmol g^{-1}(f.m.)]	0.19 ± 0.01	1.13 ± 0.05	2.00 ± 0.13
Proline [μmol g^{-1}(f.m.)]	0.57 ± 0.06	1.14 ± 0.04	4.26 ± 0.10
Protein [nmol g^{-1}(f.m.)]	40.20 ± 0.60	36.90 ± 0.50	34.40 ± 1.00
MDA [nmol g^{-1}(f.m.)]	35.50 ± 0.70	37.60 ± 0.50	38.20 ± 0.40
H_2O_2 [μmol g^{-1}(f.m.)]	28.30 ± 0.50	32.90 ± 0.20	32.00 ± 0.20
Catalase [U g^{-1}(f.m.)]	15.80 ± 0.60	14.00 ± 0.60	14.30 ± 0.50
Peroxidase [U g^{-1}(f.m.)]	19.30 ± 0.50	14.70 ± 0.50	17.60 ± 0.40
SOD [U g^{-1}(f.m.)]	47.60 ± 0.80	42.20 ± 1.00	41.60 ± 1.00
APOD [U g^{-1}(f.m.)]	51.40 ± 1.30	48.70 ± 0.70	48.00 ± 1.00
GR [U g^{-1}(f.m.)]	6.35 ± 0.18	6.25 ± 0.11	6.11 ± 0.03

Both ABA-treated and water-stressed detached rice leaves had lower protein level than the controls (Table 1). Lipid peroxidation, as judged by the level of MDA, and H_2O_2 content increased in detached rice leaves exposed to water stress or ABA. This increase may be a reflection of the decline in activities of protective enzymes such as catalase, peroxidase, SOD, APOD and GR.

Both water stress and exogenously applied ABA slightly reduced activity of catalase, peroxidase, APOD and SOD in detached rice leaves when compared with controls (Table 1). However, GR reductase does not seem to be affected by ABA and water stress treatments.

The evidence presented in this paper shows that the effects of exogenously applied ABA on the oxygen scavenging enzymes in detached rice leaves are similar to those of water stress. Simultaneously, endogenous ABA content increased in detached rice leaves exposed to water stress (Table 1). Therefore, it seems possible that the water stress-induced senescence and changes in the enzyme activities in detached rice leaves are mediated through accumulation of ABA.

References

Chang, C.J., Kao, C.H.: H_2O_2 metabolism during senescence of rice leaves: Changes in enzyme activities in light and darkness. - Plant Growth Regul. **25**: 11-15, 1998.

Chen, C.T., Kao, C.H.: Osmotic stress and water stress have opposite effects on putrescine and proline production in excised rice leaves. - Plant Growth Regul. **13**: 197-202, 1993.

Chen, C.T., Kao, C.H.: Comparative study of the metabolism of 1-aminocyclopropane-1-carboxylic acid and senescence of water-stressed and ABA-treated excised leaves. - Plant Cell Physiol. **31**: 463-468, 1990.

Dwivedi, S., Kar, M., Misra, D.: Biochemical changes in excised leaves of *Oryza sativa* subjected to water stress. - Physiol. Plant. **45**: 35-40, 1979.

Foster, J.G., Hess, J.L.: Response of superoxide dismutase and glutathione reductase activities in cotton leaf tissue exposed to an atmosphere enriched in oxygen. - Plant Physiol. **66**: 482-487, 1980.

Heath, R.L., Packer, L.: Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. - Arch. Biochem. Biophys. **125**: 189-198, 1960.

Jana, S., Choudhuri, M.A.: Glycolate metabolism of three submerged acquatic angiosperm during aging. - Aquat. Bot. **12**: 345-354, 1981.

Kao, C.H.: Senescence of rice leaves IV. Influence of benzyladenine on chlorophyll degradation. - Plant Cell Physiol. **21**: 1255-1262, 1980.

Kao, C.H.: Senescence of rice leaves VI. Comparative study of the metabolic changes of senescing turgid and water-stressed excised leaves. - Plant Cell Physiol. **22**: 683-688, 1981.

Kato, M., Shimizu, S.: Chlorophyll metabolism in higher plants VII. Chlorophyll degradation in senescing tobacco leaves: phenolic-dependent peroxidative degradation. - Can. J. Bot. **65**: 729-735, 1987.

MacAdam, J.W., Nelson, C.J., Sharp, R.E.: Peroxidase activity in the leaf elongation zone of tall fescue. - Plant Physiol. **99**: 872-878, 1992.

Mukherjee, S.P., Choudhuri, M.A.: Implications of water stress-induced changes in the leaves of endogenous ascorbic acid and hydrogen peroxidase in *Vigna* seedlings. - Physiol. Plant. **58**: 166-170, 1983.

Nakano, Y., Asada, K.: Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. - Plant Cell Physiol. **22**: 867-880, 1981.

Paoletti F.D., Aldinacci, D., Mocali, A., Caparrini, A.: A sensitive spectrophotometric method for the determination of superoxide dismutase activity in tissue extracts. - Anal. Biochem. **154**: 536-543, 1986.

Smart, C.M.: Gene expression during leaf senescence. - New Phytol. **126**: 419-448, 1994.