Plant and Soil **155/156**: 497-500, 1993

Proline in relation to free radical production in seedlings of *Brassica juncea* raised under sodium chloride stress

ALIA[1], P. PARDHA SARADHI[1] and PRASANNA MOHANTY[2]
[1]*Centre for Biosciences, Jamia Millia Islamia, New Delhi-110025;* [2]*School of Life Sciences, Jawaharlal Nehru University, New Delhi-110067, INDIA*

Key words: Brassica juncea, free radicals, malondialdehyde, proline, sodium chloride stress.

Abstract

The production of malondialdehyde (MDA) was higher in cotyledons from NaCl-raised *Brassica juncea* seedlings than in control seedlings. Light accelerated the MDA-producing capacity of thylakoids isolated from both control and treated seedlings. When exposed to strong white light (920 μmol photons m^{-2} s^{-1}) the thylakoids from NaCl seedlings produced nearly 5 times more MDA than control thylakoids. In the cotyledons of NaCl seedlings, the proline level was 24-fold higher than in controls. The presence of proline during exposure of thylakoids to white light decreased MDA levels. The reduction in MDA production was higher in the thylakoids of NaCl seedlings than of controls. It is proposed that proline accumulation has an adaptive significance as it lowers the generation of free radicals and thus reduces the lipid peroxidation linked membrane deterioration under stress.

Introduction

Plants exposed to stress increase lipid peroxidation because of greater generation of free radicals (Price et al., 1989; De Vos and Schat, 1991; Price and Hendry, 1991; Somashekaraiah et al., 1992). Another frequently noted change in plants exposed to osmotic stress is the accumulation of proline (see Alia and Pardha Saradhi, 1991). Besides other roles assigned to proline, it is also reported to protect enzymes from denaturation induced by hydroxyl radicals by scavenging the free radicals (Smirnoff and Cumbes, 1989). In plants, chloroplasts are thought to be the major site for both the synthesis of proline (Rayapati et al., 1989) and generation of free radicals (Elstner, 1982; Asada, 1992; Scandalios, 1992).

In the present communication we propose that one of the adaptive roles of proline in plants exposed to salt stress is to reduce lipid-peroxidation-linked membrane deterioration, by suppressing salt-stimulated free-radical generation.

Material and methods

Seeds of *Brassica juncea* cv. DIRA 367 obtained from the Indian Agricultural Research Institute (New Delhi, India), were grown on modified B_5 medium (Gamborg et al., 1968) supplemented with 0, 50, 100, 150 or 200 m*M* NaCl, as described earlier (Alia et al., 1992). The cotyledons excised from 10 day old seedlings of *Brassica juncea* were used for all investigations.

Malondialdehyde (MDA) levels in cotyledons and thylakoids were determined by the procedure of Heath and Packer (1968) and proline levels in cotyledons by the procedure of Bates et al. (1973).

The thylakoids were isolated as described earlier (Alia et al., 1992) and then suspended in 50 m*M* tris-HCl buffer (pH 8.0) containing 175

m*M* NaCl. Thylakoids equivalent to 100 µg Chl/mL in a medium containing 175 m*M* NaCl and 50 m*M* Tris-HCl buffer (pH 8.0) were kept in the dark or exposed to strong white light (about 920 µmol photons m^{-2} s^{-1}) at 25±1°C in the absence or the presence of 1 *M* proline and their MDA content determined at regular intervals.

Results and discussion

The concentration of MDA, a product of lipid peroxidation and a major thiobarbituric acid reactive compound (Kunert and Ederer, 1985), was considerably higher in cotyledons of seedlings raised in the presence of NaCl than in control seedlings (Table 1). The MDA content increased progressively with increasing concentration of NaCl. The MDA level in cotyledons from seedlings raised in the presence of 200 m*M* NaCl was about 3 times higher than that of control seedlings (Table 1). Such an enhanced level of MDA suggests that NaCl stimulates free-radical generating capacity of seedlings. Exposure of plants to drought and heavy-metal stress have also been reported to be associated with excessive production of various activated forms of oxygen and free radicals (see Price et al., 1989; Buckland et al., 1991; Price and Hendry, 1991; Somashekaraiah et al., 1992). Enhanced free radical generation causes membrane deterioration through peroxidative degradation of poly-unsaturated fatty acids of membrane lipids (De Vos and Schat, 1991).

Table 1. Levels of proline and malondialdehyde (MDA) in cotyledons of *Brassica juncea* seedlings raised in B_5 medium in the absence or presence of varying concentrations of NaCl.

NaCl (m*M*)	MDA[a]	Proline[b]
0	12.3 ± 0.32	0.36 ± 0.07
50	18.4 ± 0.41	1.20 ± 0.09
100	21.7 ± 1.09	2.91 ± 0.12
150	29.1 ± 0.91	4.22 ± 0.30
200	36.8 ± 2.20	8.52 ± 0.72

The data are means ± SD (n=4)
[a] nmol/g fresh weight
[b] mg/g fresh weight

Thylakoids isolated from seedlings raised in the presence of NaCl contained about 30% more MDA than those from seedlings without NaCl (Figs. 1 A and B). Exposure of thylakoids isolated from control seedlings to strong white light for 50 minutes increased their MDA content 2.3-fold and that from NaCl seedlings 7-fold. However, little change in the MDA content occurred during dark incubation (Figs. 1 A and B). The light-induced production of MDA in thylakoid preparations has also been reported earlier (Heath and Packer, 1968; Alia et al., 1991). Upon illumination, chloroplasts generate superoxide, hydrogen peroxide and hydroxyl radicals (Halliwell and Gutteridge, 1986) which, in turn, cause lipid peroxidation (Heath and Packer, 1968).

The presence of NaCl in the growth medium also induced accumulation of free proline in the cotyledons of *Brassica juncea* (Table 1). The cotyledons of seedlings raised in the presence of 200 m*M* NaCl showed about 24-fold enhancement in the level of proline compared to controls (Table 1). A wide range of organisms from bacteria to higher plants accumulate proline when exposed to various environmental stresses (see Aspinall and Paleg, 1981; Alia and Pardha Saradhi, 1991). It has been postulated that proline acts as a compatible cytoplasmic osmoticum and protects enzyme and membrane functions (Jolivet et al., 1982; Nikolopoulos and Manetas, 1991).

Earlier Rayapati et al. (1989) showed that Δ-pyrroline-5-carboxylate reductase enzyme, which catalyses the last step of proline synthesis, is localised in chloroplasts so that proline like many other amino acids is synthesised in chloroplasts. Smirnoff and Cumbes (1989) have shown that proline can protect the activity of malate dehydrogenase by scavenging free radicals. As proline might be synthesised in chloroplasts, experiments were carried out to see if proline can influence the free radical generating capacity of the thylakoids.

The MDA-producing potential of the thylakoids from control and NaCl-raised plants in light was reduced when 1 *M* proline was added to the incubation mixture (Figs. 1 A and B). In thylakoids from NaCl seedlings, proline

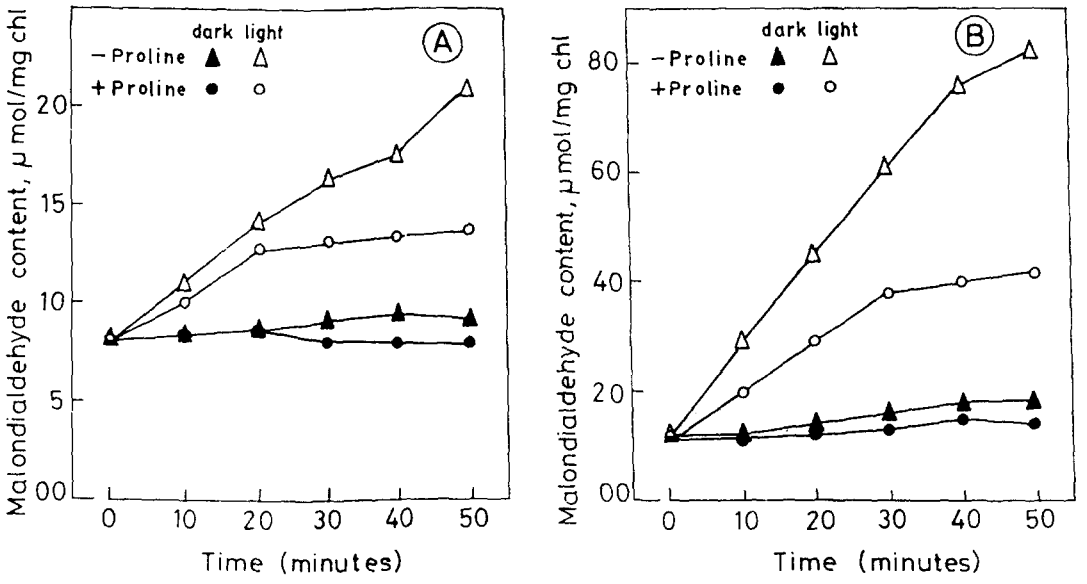

Fig. 1. Change with time in the malondialdehyde content of thylakoids isolated from cotyledons of *Brassica juncea* seedlings raised in the absence (A) or the presence (B) of NaCl during incubation in the presence or absence of 1 *M* proline in the dark or in strong white light of 920 μmol m^{-2} s^{-1} intensity. The data are the means of three independent experiments.

depressed MDA production by 50% compared with 30% in control thylakoids. The reduction in the level of MDA with proline reflects the reduction in free radical production possibly due to its ability to act as a free radical scavenger (Smirnoff and Cumbes, 1989) and/or to its capacity to reduce the production of free radicals.

In summary, our results suggest that the accumulation of proline during NaCl stress has an adaptive significance as it suppresses the stress-induced enhancement in free radical production by the thylakoid membranes and thereby reduces the lipid peroxidation linked membrane deterioration.

Acknowledgments

This work was supported, in part, by the Department of Science and Technology grant no. SR/OY/B-50/91 and University Grants Commission grant no. F.3-268/90 (SR-II). Alia thanks the Plant Nutrition Colloquium Fund (Royal Society) for financial support in attending the Colloquium.

References

Alia and Pardha Saradhi P 1991 J. Plant Physiol. 138, 554-558.

Alia, Mohanty P and Pardha Saradhi P 1992 Biochem. Physiol. Pflanzen. 188, 1-12.

Alia, Pardha Saradhi P and Mohanty P 1991 Biochem. Biophys. Res. Commun. 181, 1238-1244.

Asada K 1992 *In* Molecular Biology of Free Radical Scavenging System. Ed. J G Scandalios. pp 173-192 Cold Spring Harbor Laboratory Press.

Aspinall, D., and Paleg, L.D. 1981 *In* The Physiology and Biochemistry of Drought Resistance in Plants. Eds. L G Paleg and D Aspinall. pp. 215-228. Academic Press, Sydney.

Bates L S, Waldren, R P and Teare, I D 1973 Plant Soil 39, 205-207.

Buckland S M, Price A H and Hendry G A F 1991 New Phytologist 119, 155-160.

De Vos C H R and Schat H 1991 *In* Ecological Response to Environmental Stresses. Eds. J Rozema and J A C Verkleij. pp 22-30. Kluwer Academic Publishers, The Netherlands.

Elstner E F 1982 Ann. Rev. Plant Physiol. 33, 73-96.

Gamborg D L, Miller R A and Ojima K 1968 Exp. Cell Res. 50, 151-158.

Halliwell B and Gutteridge J M C 1986 Trends Biochem. Sci. 11, 372-375.

Heath R L and Packer L 1968 Arch. Biochem. Biophys. 125, 189-198.

Jolivet Y, Larher F and Hamelin J 1982 Plant Sci Lett. 25, 193-201.

Kunert K J and Ederer M 1985 Physiol. Plant. 65, 85-88.

Nikolopoulos D and Manetas Y 1991 Phytochemistry 30, 411-413.

Price A H and Hendry G A F 1991 Plant Cell Environ. 14,477-484.

Price A H, Atherton N M and Hendry G A F 1989 Free Radical Res. Comm. 8, 61-66. Rayapati P J, Steward C R and Hack E 1989 Plant Physiol. 91, 581- 586.

Scandalios J G 1992 *In* Molecular Biology of Free Radical Scavenging System. Ed. J G Scandalios. pp 117-152 Cold Spring Harbor Laboratory Press.

Smirnoff N and Cumbes Q J 1989 Phytochemistry 28, 1057-1060.

Somashekaraiah B V, Padmaja K and Prasad A R K 1992 Physiol. Plant. 85, 85-89.