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PROLINE ACCUMULATES IN PLANTS EXPOSED TO UV RADIATION AND PROTECTS THEM AGAINST UV INDUCED PEROXIDATION

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Proline accumulated in the shoots of seedlings of rice (*Oryza sativa*), mustard (*Brassica juncea*) and mung bean (*Vigna radiata*) exposed to UV radiations. The level of proline in the seedlings increased significantly with increase in UV exposure time. The production of malondialdehyde (an indice of lipid peroxidation) was also higher in the shoots of seedlings exposed to UV radiation as compared to controls, suggesting that UV radiations promote lipid peroxidation. The extent of UV radiation promoted enhancement in the levels of proline as well as that of malondialdehyde was higher in the seedlings of rice than those of mung bean or mustard. This lead us to believe that UV radiation induced proline accumulation protects plants against UV radiation promoted peroxidative processes. UV radiations also promoted peroxidation in linolenic acid micelles. The presence of proline along with linolenic acid micelles during UV exposure caused a considerable reduction in the production of malondialdehyde. This study, for the first time shows that plants exposed to UV radiations accumulate proline and proline can protect plant cells against UV radiation induced peroxidative processes.

The UV-component of solar radiations reaching the earth's surface is increasing due to ongoing depletion of stratospheric ozone layer (1). This increase in the ground level UV radiation results in a wide range of adverse effects on vegetation. UV radiations have been reported to retard plant growth and development by altering a number of metabolic events such as photosynthesis, respiration, lipid and protein metabolism (2,3,4,5). The mechanism of action and damage caused by different ionizing radiations in biological system is receiving increased attention. There are some circumstantial evidences suggesting that UV light induced damage is related to acceleration in free radical generation (1,6).

Proline is one of the organic molecules that accumulate in plants exposed to environmental stresses such as salt, drought, temperature and heavy metal stress (7,8). Accumulation of this molecule under extremely different stresses prompted us to check if plants can accumulate proline upon exposure to UV radiations.

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In this paper we report for the first time that UV radiation induces proline accumulation in plants and this accumulation may be a means for protecting the plants against free radical generation.

MATERIALS AND METHODS

Seeds of rice (*Oryza sativa*, Poaceae) cv. Ratna, mustard (*Brassica juncea*, Brassicaceae) cv. Dira 367 and mung bean (*Vigna radiata*, Fabaceae) cv. Pusa 105, were procured from Indian Agricultural Research Institute (New Delhi, India). Seedlings were raised in petriplates on cotton watered with mineral growth medium (9), under continuous illumination (~16 W/m^2). Five day old plants were exposed to UV radiation (~120 uW/cm^2; consisting largely of UV-C radiation), provided by four 15 W NIS G15T8 Germicidal tubes (Japan), for different time intervals. Proline content in shoots was measured according to Bates et al. (10). Malondialdehyde content in shoots was determined by using thiobarbituric acid reaction as described earlier (11).

The linolenic acid micelle solution was prepared following the method of Tien et al. (12). A suitable aliquot of freshly prepared fatty acid micelles were irradiated by UV radiation in the absence and the presence of proline (1M). Peroxidation of fatty acid micelles was measured in terms of production of malondialdehyde by following the procedure of Heath and Packer (13).

RESULTS AND DISCUSSION

A sharp increase in the level of proline was observed in seedlings of rice, mustard as well as mung bean upon exposure to UV-radiation (Fig. 1). The accumulation of proline increased with increase in the time of exposure of seedlings to UV-radiation, in all the three plant species used (belonging to three distant families) (Fig. 1). No significant change in the level of proline was observed in seedlings of any of the crop plants kept in white fluorescent light for the same time interval. Seedlings of rice had higher potential to accumulate proline in response to UV exposure as compared to those of mustard or mung bean. The rise in proline level, after 24 h of UV exposure was 7.7, 2.8 and 3.0 fold higher in rice, mustard and mung bean, respectively, as compared to the control plants maintained in white fluorescent light for the same duration.

Proline accumulation is one of the most frequent metabolic responses exhibited by plants exposed to environmental stresses (7). Earlier, we have suggested that proline accumulation in plants, during salt stress and under zinc toxicity, helps in protecting plants against stress induced free radical generation (11, 14).

Free radical generation is one of the initial cytochemical responses of plants exposed to UV radiation. Malondialdehyde is a major cytotoxic product of lipid peroxidation and acts as an indicator of free radical production (15). The production of malondialdehyde was higher in shoots of seedlings exposed to UV radiations compared to controls and the extent of

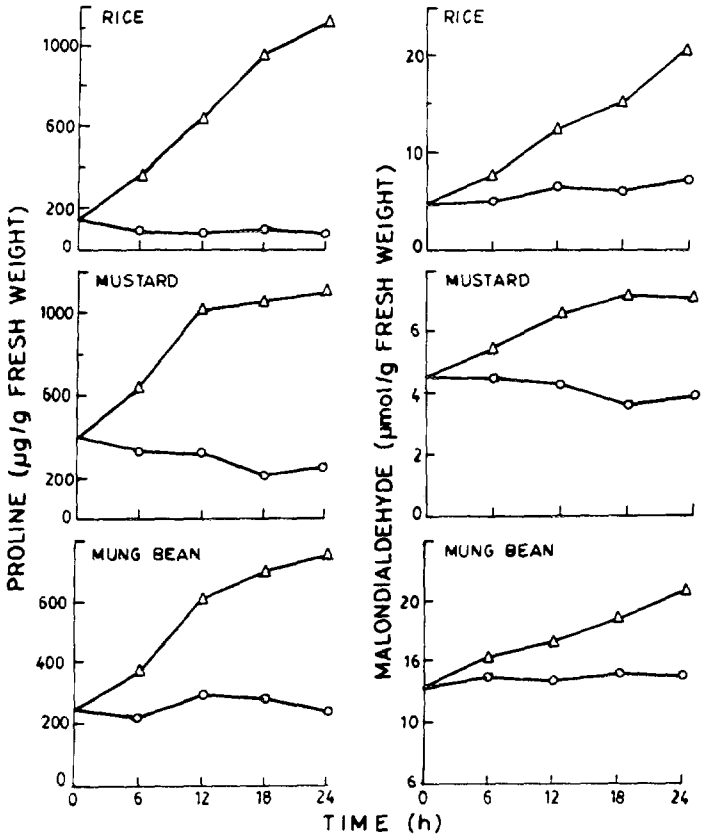

FIG. 1. Changes in the level of proline and malondiadehyde in shoots of rice, mustard and mung bean seedlings upon exposure to white light (-○-) and UV radiation (-△-) for different time intervals. Data are the mean of three independent experiments.

enhancement increased with increase in duration of UV exposure in all the three plant species (Fig. 1). Like proline, the extent of UV promoted free radical generation was higher in rice than in mung bean or mustard. This lead us to believe that UV radiation induced proline accumulation have an important role in protecting plants against UV radiation promoted peroxidative processes.

Linolenic acid micelles exposed to UV radiation showed a time dependent increase in the level of malondialdehyde (Table 1). UV-induced peroxidation of linolenic acid micelles is reported to be mediated through the generation of free radicals (16). Presence of proline along with linolenic acid micelles brought about considerable reduction in time dependent increase in the level of malondialdehyde during UV-exposure (Table 1). These results show that proline has the capacity to reduce the level of free radicals produced during UV exposure.

Organisms have evolved numerous biochemical mechanisms to protect their cells against free radicals, which includes the production of free radical scavenging enzymes and quenching

TABLE 1. Time dependent increase in Malondialdehyde content (% increase over the zero time value) in linolenic acid micelles upon exposure to UV-radiation in the absence and the presence of proline (1M). Data are the mean of three independent experiments.

	Time (min)						
Treatment	0	10	20	30	40	50	60
- Proline	100	736.6	990.8	1300.6	1543.3	1643.3	1717.4
+ Proline	100	217.0	364.8	466.2	564.5	659.0	750.8

molecules (14, 17). Proline accumulation in plants during UV-exposure might be one of the modes to conteract UV radiation promoted free radical generation. Proline might be having the capacity to scavenge and/or to reduce the production of free radicals.

In summary, these results, for the first time show that plants (irrespective of their family) exposed to UV radiations accumulate proline which protects plant cells against UV radiation induced peroxidative processes.

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