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RESEARCH PAPERS

Ecophysiological Adaptation of *Calligonum roborovskii* to Decreasing Soil Water Content along an Altitudinal Gradient in the Kunlun Mountains, Central Asia[1]

Jun-Tao Zhu[a, b, c], Xiang-Yi Li[a, c], Xi-Ming Zhang[a, c], Fan-Jiang Zeng[a, c], Li-Sha Lin[a, c], Shang-Gong Yang[a, b, c], Dong-Wei Gui[a, b, c], and Hui Wang[a, b, c]

[a] *Xinjiang Institute of Ecology and Geography, Chinese Academy of Sciences, Urumqi 830011, China; e-mail: lixy@ms.xjb.ac.cn*

[b] *Graduate University of Chinese Academy of Sciences, Beijing, China*

[c] *Cele National Station of Observation and Research for Desert-Grass Land Ecosystem in Xinjiang, Cele, China*

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Abstract—To understand the ecophysiological adaptation mechanisms of *Calligonum roborovskii* to altitude variation, this study analyzed chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), Chl (*a* + *b*), carotenoid (Car), malondialdehyde (MDA), ascorbate (AsA), proline (Pro), membrane permeability (MP), reactive oxygen species (ROS), specific leaf area (SLA), leaf mass per area (LMA), leaf nitrogen content based on mass (N_{mass}), and the activities of peroxidase (POD), catalase (CAT), superoxide dismutase (SOD), and ascorbate peroxidase (APX) in leaves of plants inhabiting different altitudes (A1: 2100 m, A2: 2350 m, A3: 2600 m) on the northern slope of the Kunlun Mountains. The results showed that Chl *a*, Chl *b*, Chl (*a* + *b*), SLA, N_{mass}, and the activity of CAT increased with increasing altitude. LMA, MP, MDA, Car, Pro, AsA, O_2^-, H_2O_2 and the activities of SOD, POD, and APX decreased with increasing altitude. The test results also showed that, changes in venvironmental factors along an altitudinal gradient are not obvious. Soil water content is the main ecological factor. With increasing altitude, soil water content increased significantly. More non-enzymatic and enzymatic antioxidants played an important role in eliminating intracellular ROS. They kept the cell membrane in a stable state and ensured the normal growth of *C. roborovskii*.

Keywords: Calligonum roborovskii, altitude, leaf traits, antioxidant system, physicoecological indices, Kunlun Mountains.

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INTRODUCTION

High altitude (HA) environment is characterized by high solar radiation, low mean temperature, rapid temperature changes, and low partial pressure of the air [1]. The change in any of these parameters affects plant performance. Plant stature, structure, and physiology have been observed to change with the changing environmental variables with elevation. Plants at HA are exposed to greater irradiance, large diurnal fluctuations of temperature, high wind velocity, reduced partial pressure of gases, limited water and nutrient supply, narrow time window for growth and development and so on, as compared to low altitude (LA), and this may alter the morphological and physiological responses of plants [1–3].

The northern slope of the Kunlun Mountains is next to the Taklamakan Desert, which is the driest place in the Asian continent. The desert grassland at an altitude between 2100 and 2600 m consists mainly of *Calligonum roborovskii*, *Ceratoides latens*, *Sympegma regelii*, and *Seriphidium korovinii* Poljak. It is the main source of livestock food in winter at the southern part of the Taklamakan desert, and it conserves water, prevents wind erosion, and maintains ecological stability [4]. But currently, desertification becomes a serious problem. Sandstorms cause erosion as well as sand deposition. Therefore, research on protection and recovery of the vegetation on the northern slope of the Kunlun Mountains is necessary. *C. roborovskii*, an important vegatation component, does not only effectively prevent wind erosion but is also a good food for camels and sheep. In the present study, leaf traits and physiological properties at differ-

[1] This text was submitted by the authors in English.

Abbreviations: APX—ascorbate peroxidase; AsA—ascorbate; Car—carotenoid; CAT—catalase; Chl *a*—chlorophyll *a*; Chl *b*—chlorophyll *b*; Chl (*a* + *b*)—chlorophyll (*a* + *b*); HA—high altitude; LA—low altitude; LMA—leaf mass per area; MP—membrane permeability; N_{mass}—leaf nitrogen content based on mass; POD—peroxidase; Pro—proline; SLA—specific leaf area; SOD—superoxide dismutase.

ent altitudes were compared in order to reveal this species response to varying habitat conditions. A better knowledge of the adaptation mechanisms of this characteristic species to the unique environmental conditions can be contributed to the scientific basis for protection and recovery of the vegetation on the northern slope of the Kunlun Mountains.

MATERIALS AND METHODS

Study site and plant species. This study was carried out on the northern slope of the Kunlun Mountains (36°23′41′′~36°27′45′′N, 80°43′11′′~80°43′27′′E, 2100~2600 m above the sea level), on the verge of the Taklamakan desert, the most arid center of the Asian continent belonging to the Xinjiang-Uighur Autonomous Region, NW China. Plant growth largely depends on the water from rainfall, although the annual precipitation is less than 60 mm (maximum in July and August). *Calligonum roborovskii* belongs to the Polygonaceae, it is distributed in various parts of southern Xinjiang and forms shrubs, the height of which is usually 0.3~1 m. It flowers from May to June. The fruiting period is June to July.

A comprehensive survey of *C. roborovskii* distribution was carried out on the northern slope of the Kunlun Mountains in July 2008. It is mainly distributed at an altitude between 2100 and 2600m. Three altitudes were chosen for the investigations: A1 (2100 m), A2 (2350 m), and A3 (2600 m). At each altitude, three plots (10 × 10 m) were selected. The leaf samples were collected from five different plants at each altitude. They were stored in liquid nitrogen until measurements in the laboratory.

Environmental factors. Measurements were performed every one hour in the period from 8:00 to 20:00 in a fine day of July with a Li-6400 photosynthesis system (LI-COR, United States), one apparatus was placed at each altitude; measurements were repeated 7 times a day. Soil water content was determined by drying at 115°C for 24 h; measurements were repeated 3 times per altitude. Soil surface samples (0–60 cm) at different altitudes were collected for the analysis of soil physical and chemical properties: pH value, organic matter, total salt, total N, total P, and total K [5].

Analysis of SLA, N_{mass}, MP, and the content of Chl, MDA, AsA, and Pro. Leaf discs with a definite area were taken from each leaf sample and oven-dried at 60°C for 48 h. SLA was calculated as the ratio of leaf area to mass. Leaf N content was determined by using a Kjeldahl apparatus (BUCHI Auto Kjeldahl Unit K-370, BUCHI Labortechnik AG, Switzerland). The measurements were performed in Biogeochemistry Laboratory of Xinjiang Institute of Ecology and Geography, Chinese Academy of Sciences. The contents of Chl, MDA, AsA, Pro and the membrane permeability (MP) were measured according to the method described by Li [6].

Analysis of O_2^- and H_2O_2 contents. The O_2^- content in the leaves was measured according to the method described by Wang [7], and the H_2O_2 content was measured according to the Ferguson method [8].

Analysis of antioxidant enzyme activities. Leaf samples were homogenized on ice with mortar and pestle in a 0.1 M potassium-phosphate buffer (pH 7.0).The homogenate was centrifuged at 12000 *g* for 15 min at 4°C. The supernatant was used immediately for enzyme assays. Total protein concentration was determined according to the Bradford method [9] using the Bio-Rad (United States) protein assay reagent. The activity of SOD was measured according to a method using xanthine, xanthine oxidase, and cytochrome *c* [10]. One unit of SOD was defined as the amount of enzyme that inhibits the rate of ferricytochrome *c* reduction by 50%. The activity of APX was assayed according to the method described by Nakano and Asada [11], using ascorbic acid as a substrate. The oxidation of ascorbate was initiated by H_2O_2, and a decrease of optical density at 290 nm was monitored for 1.5 min. One unit of APX was defined as the amount of enzyme required to oxidize 1 μmol of ascorbate. The activity of POD was assayed according to the method described by Kwak et al. [12], using pyrogallol as a substrate. One unit of POD activity was defined as the amount of enzyme necessary to obtain 1 mg of purpurogallin from pyrogallol in 20 s at 420 nm. CAT activity was assayed according to the method described by Aebi [13], by measuring a decrease of optical density at 240 nm for 1 min due to H_2O_2 consumption.

Statistical analysis. The experimental assays used were repeated at least three times, under the same conditions, and yielded essentially the same results. All measurements were subjected to analysis of variance (ANOVA) to discriminate the significant differences (defined as $P \leq 0.05$). Data are shown as the means ± standard deviations (SD).

RESULTS

Variations of Environmental Factors

As indicated in Table 1, there were no distinct differences between the altitudes in effective radiation, temperature, relative humidity, and the concentration of CO_2. As shown in Table 2, the soil water content increased, whereas the salt content showed a weak tendency to decrease with increasing altitude. However, some indices, including pH value, total content of soil organic matter, N, P, and K did not vary much at various altitudes.

Chlorophyll Content, N_{mass}, SLA, and LMA

Figure 1 shows changes in the chlorophyll content, N_{mass}, SLA, and LMA with altitude. As the altitude increased, the contents of Chl *a*, Chl *b*, and total chlorophyll tended to increase, whereas the content of Car

Table 1. The changes of environment factors with the altitude

Environmental factor	A1	A2	A3
Irradiance, μmol PAR/(m^2 s)	1604.45 ± 21.35	1615.45 ± 15.22	1672.03 ± 12.74
T, °C	32.81 ± 2.46^a	31.72 ± 2.58^a	28.24 ± 3.52^{ab}
RH, %	8.57 ± 1.65	8.73 ± 1.21	9.08 ± 1.05
CO_2, μmol/mol	392.15 ± 8.28	388.26 ± 5.34	382.45 ± 7.42

Notes: $n = 6$. Different letters designate significant differences according to a one-way ANOVA and Duncan test.

Table 2. Soil physicochemical properties at different altitudes

Altitude	Soil layer, cm	pH	Total salt, %	Organic matter, %	Total N, mg/kg	Total P, mg/kg	Total K, mg/kg	Soil water content, %
A1 (2100 m)	0–20	6.21	0.82	0.28	15.37	3.83	109.56	2.42
	20–40	6.59	0.84	0.22	23.91	4.34	131.84	2.37
	40–60	6.62	0.88	0.18	14.52	2.15	125.35	5.15
A2 (2350 m)	0–20	6.75	0.82	0.30	14.16	3.58	105.24	2.58
	20–40	6.81	0.83	0.26	23.54	4.30	129.52	4.25
	40–60	6.83	0.86	0.20	13.95	2.44	121.77	6.13
A3 (2600 m)	0–20	6.85	0.76	0.32	13.96	3.67	102.19	5.35
	20–40	6.86	0.79	0.27	24.35	4.13	133.46	8.68
	40–60	6.91	0.82	0.21	14.28	2.35	128.45	10.62

Note: $n = 3$.

decreased. The variations in the contents of different pigments are significant ($P < 0.05$). N_{mass} increased with increasing altitude, but only the difference between A3 and A1 reached a significant level. Similarly SLA increased with altitude. Again, the difference between A3 and A1 is significant. In contrast, LMA decreased with increasing altitude. Also in this case, the difference between A3 and A1 is significant.

Content of O_2^- and H_2O_2

As indicated in Fig. 2, the contents of O_2^- and H_2O_2 decreased with increasing altitude. In the case of O_2^-, the differences between all sites are significant. In the case of H_2O_2, only the difference between A3 and A1 is significant.

MDA Content and Membrane Permeability

Figure 3 shows that the content of MDA decreased greatly with increasing altitude and that the differences between all sites are significant. MP shows a similar trend with a significant difference between all sites.

Content of Pro and AsA

As indicated in Fig. 4, the contents of Pro and AsA decreased with increasing altitude. The content of Pro differs significantly between the altitudes; in the case of AsA, the differences between all sites are significant.

Activities of Antioxidant Enzymes

The activities of antioxidant enzymes are indicated in Fig. 5. The activities of SOD, POD, and APX decreased with increasing altitude, but the activity of CAT increased significantly. In the case of POD and CAT, the differences between all altitudes are significant; in the case of SOD and APX, only the difference between A3 and A1 is significant.

DISCUSSION

In the present study, with increasing altitude, SLA, N_{mass}, and Chl content increased, whereas LMA decreased, together with trend to an increase in soil water content. Thus, the reponses of *C. roborovskii* are in accordance with the general adaptation strategies. In arid habitats, plants often invest more carbohydrates and nitrogen into the leaf cell wall to enhance its strength. At the same time, the leaves become smaller and thicker, resulting in an increase of dry matter per unit area (i.e., SLA decreases and LMA increases). Moreover, relatively less nitrogen is allocated to the

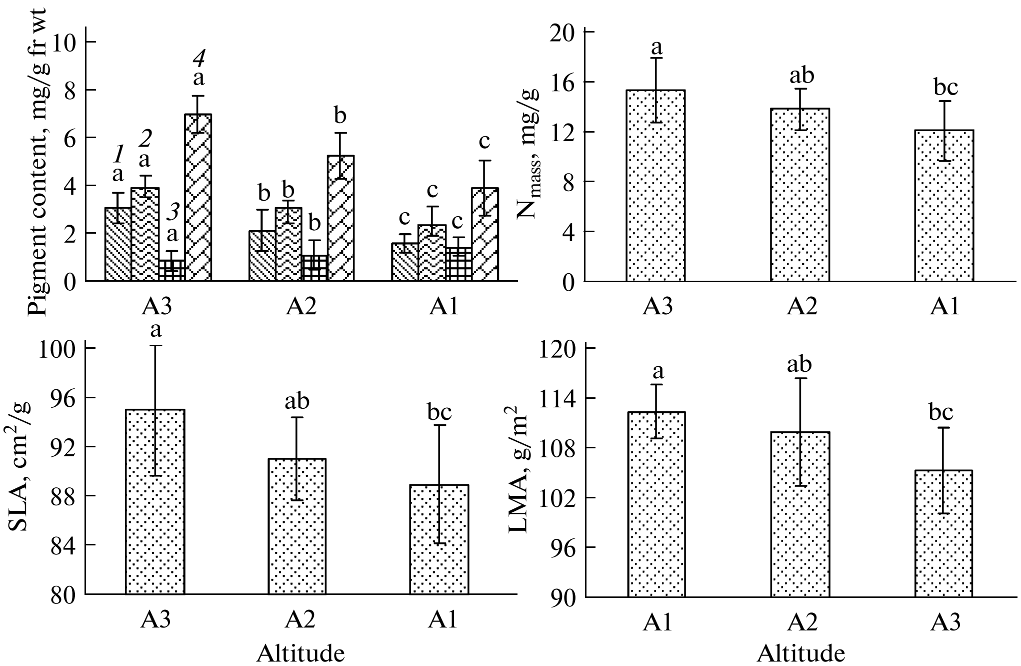

Fig. 1. The changes in the chlorophyll and carotenoid contents, leaf nitrogen content based on mass, specific leaf area, and leaf mass per area with the altitude.

(*1*) Chl *a*; (*2*) Chl *b*; (*3*) Car (*4*) Chl (*a* + *b*) $n = 6$. Different letters on the same index indicate a significant difference at the $P <$ 0.05 level.

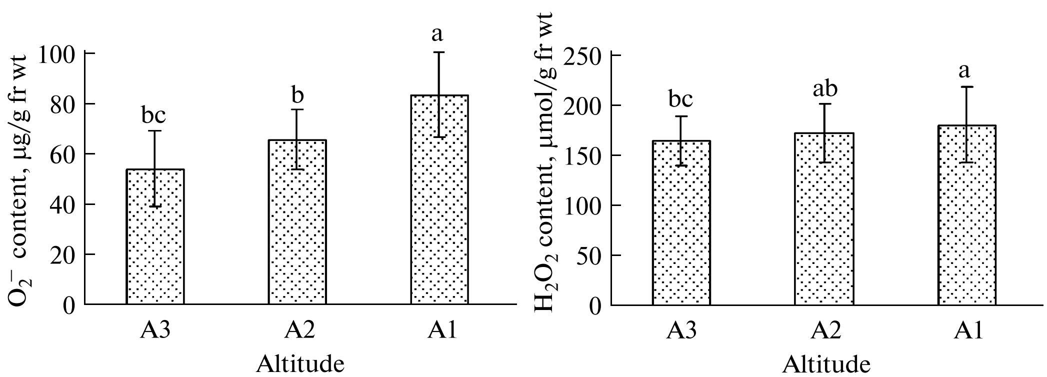

Fig. 2. The changes in the O_2^- and H_2O_2 contents with the altitude.

$n = 6$. Different letters on the same index indicate a significant difference at the $P < 0.05$ level.

photosynthetic organelles [14]. This general tendency is confirmed in our study. Soil drought stress increases with decreasing altitude, and thus, *C. roborovskii* allocates more nitrogen to the cell wall in lower regions in order to enhance the resilience of the cell wall and to reduce water loss. A lower allocation of nitrogen into the photosynthetic organelles, such as the chloroplasts, leads to lower chlorophyll content. In order to adapt to the drought stress, *C. roborovskii* shows a high morphological and physiological plasticity.

Drought is a stress factor that can interfere with cell metabolism in such a way that ROS are generated and thus oxidative stress arises [15]. Under normal conditions, plant cells are able to keep a balance between ROS production and their scavenging, but under long-term environmental stress, this balance can be disturbed and ROS can accumulate and lead to damage.

ROS include O_2^- and OH^-. [16]. The level of ROS determines the type of the response: while their low concentrations induce defense gene expression, the high concentration leads to the cell death. ROS induce membrane lipid destruction (MDA accumulation) and disturb many biological functions via damaging

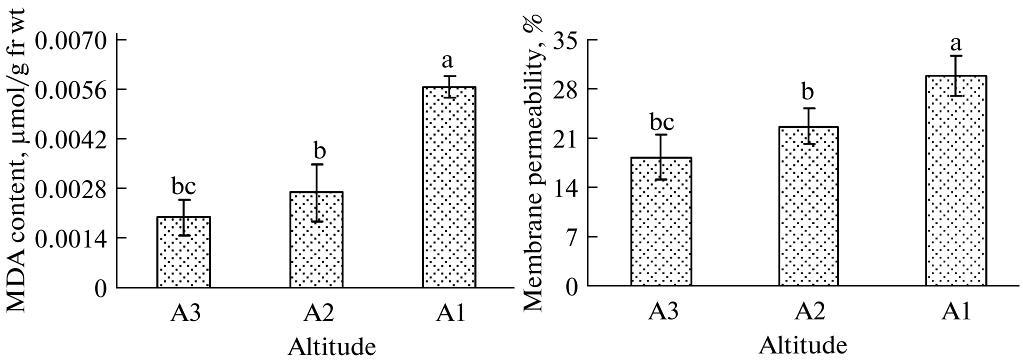

Fig. 3. The changes in the MDA content and membrane permeability with the altitude.
$n = 6$. Different letters on the same index indicate a significant difference at the $P < 0.05$ level.

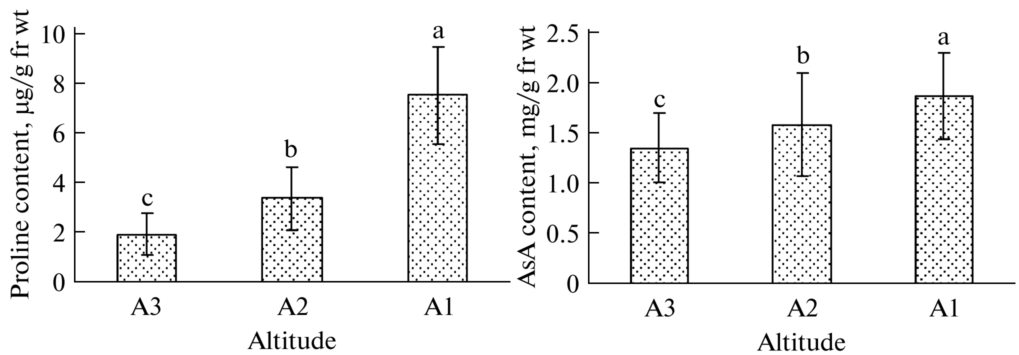

Fig. 4. The changes of proline and ascorbate contents with the altitude.
$n = 6$. Different letters on the same index indicate a significant difference at the $P < 0.05$ level.

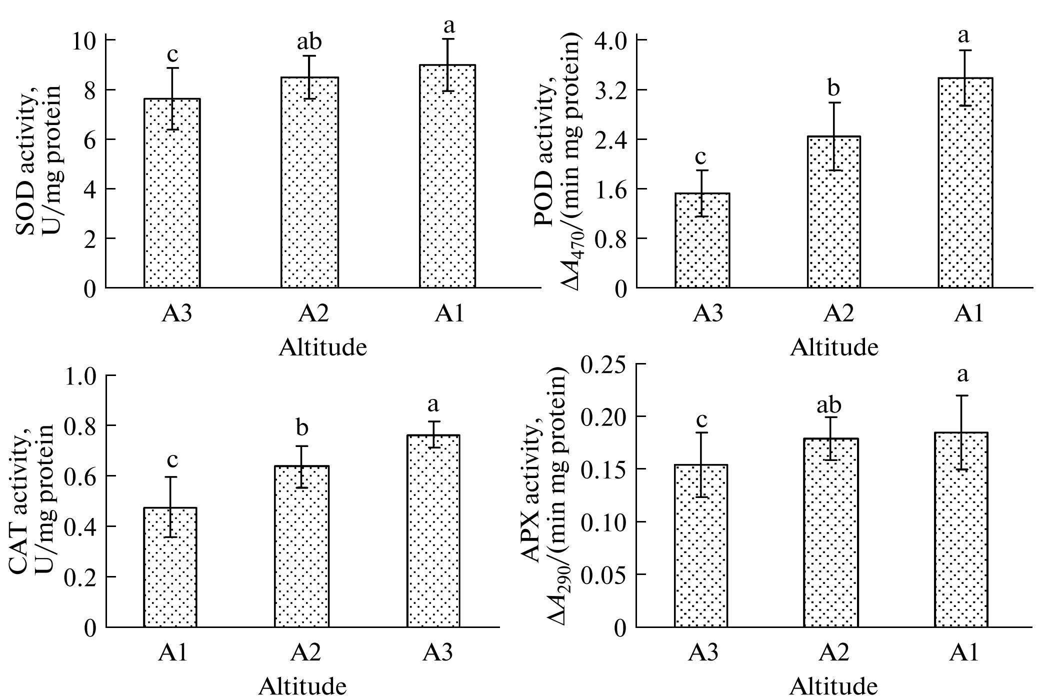

Fig. 5. The changes of the activities of superoxide dismutase, peroxidase, catalase, and ascorbate peroxidase with the altitude.
$n = 6$. Different letters on the same index indicate a significant difference at the $P < 0.05$ level.

the structure of proteins and enzymes. The concentration of the peroxide shows the degree of membrane injury, usually as a physiological indicator of stress [17]. The results of our study show (Figs. 2, 3) that, with the decrease in altitude, the body of *C. roborovskii* generates more O_2^- and H_2O_2. ROS accumulation leads to membrane lipid peroxidation and membrane injury. But even in A1 region, ROS remain within a low concentration range, and the low ROS concentration induces defense gene expression, which timely remove ROS. Therefore, *C. roborovskii* can keep normal growth and metabolism on the northern slope of the Kunlun Mountains and evolve into a predominant community.

The accumulation of proline and ascorbate play an important role to counteract environmental stress [18, 19]. Nicholas and Quinton also found that Pro can effectively scavenge OH^- [20]. As shown in Fig. 4, Pro content in leaves decreased with increasing altitude. This result contradicts the results of Rada [21] obtained for the elevation of 4200~4500 m above the sea level.

This may be because ecological conditions become better with increasing altitude at low altitude regions. AsA in A1 and A2 regions was significantly higher than in A3. This shows that soil drought stress intensifies with decreasing altitude.

SOD is an inducible enzyme capable of converting superoxide anion into molecular oxygen and H_2O_2. Other enzymes, such as POD and CAT, convert H_2O_2 into water and molecular oxygen [22, 23]. APX and glutathione reductase are the components of another scavenging system. They directly destroy H_2O_2 in the chloroplasts [24]. In our study, as shown in Fig. 5, the activities of SOD, POD, and APX in A1 were significantly higher than in A3. Thus, *C. roborovskii* activates SOD firstly under drought stress. SOD converts superoxide anion into molecular oxygen and H_2O_2, and then POD and APX remove H_2O_2. But the activity of CAT is decreased in A1 and A2. Its inactivation could be induced by H_2O_2 accumulation, or it occurred in the light [23].

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