Analysis of lipid peroxidation metabolism during florescence and senescence of Lily

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Abstract. In order to explore the correlation between the level of membrane lipid peroxidation and the physiological mechanism of senescence at different developmental stages of lily, the soluble protein content, soluble sugar content, malondialdehyde (MDA) content, superoxide radical (O^{2-}) production rate, hydrogen peroxide (H_2O_2) content, superoxide dismutase (SOD) activity, catalase (CAT) activity, and peroxidase (POD) activity in petal of *Lilium regale* and *Lilium leucanthum* were measured at different developmental stages. The results showed that during flowering and senescence, soluble protein content in petal of *L. regale* changed with a single peak curve, while the content of soluble protein in petal of *L. leucanthum* decreased steadily. Soluble sugar content, SOD activity, and CAT activity of the two lilies increased first and then decreased. MDA content, H_2O_2 content,and O^{2-} production rate continued to rise. POD activity of *L. regale* changed steadily, while POD activity of *L. leucanthum* increased significantly in the later stage. Therefore, the membrane lipid peroxidation caused by the accumulation of reactive oxygen species is one of the main physiological reasons for the senescence of lily.

1. Introduction

Lily (*Lilium* spp.) is a perennial bulb plant of *Lilium* in Liliaceae[1], it is one of the most important fresh cut flowers. Its fragrance is fragrant, its shape is elegant, and it has high economic and ornamental value, but the cut flower has a short life, usually around a week. The flowering and senescence of flowers is a very complicated physiological and biochemical process[2]. Free radical theory holds that plant senescence is a process of large accumulation of reactive oxygen species (ROS) and metabolic disorders[3]. The study on *bougainvillea glabra* have shown that the flowering and senescence of *b. glabra* were the processes of ROS accumulation and lipid peroxidation metabolism disorders[4]. The same study on *oncidium* also shown that MDA content in petal increased sharply after the senescence, soluble sugar content increased slowly in the early stage, and decreased from the early stage to the senescence stage, soluble protein in petal showed a downward trend, SOD activity increased first and then decreased, while POD activity continued to rise[5-6]. The exogenous free radical inducers promoted the senescence of cut flowers, increased the content of malondialdehyde and decreased the content of protein[7]. However, studies on the changes of ROS and reactive-oxygen-scavenging enzymes during natural blooming and senescence of lily are scarce. This experiment studied the change of soluble protein content, soluble sugar content, MDA content, superoxide radical (O^{2-}) production rate, hydrogen peroxide (H_2O_2) content, superoxide dismutase (SOD) activity, catalase (CAT) activity, and peroxidase (POD) activity in petal of lily at different developmental stages, aiming to understand the physiological causes of lily senescence from the level of lipid peroxidation, in order to provide a basis for delaying lily flowering.

2. Materials and Methods

2.1 Materials

The experimental materials are the wild species of *Lilium regale* (collected from Li County, Sichuan Province, 1430-2200 m above sea level) and *Lilium leucanthum* (collected from Fuling District, Chongqing City, 200-800 m above sea level). They are all grown in the No. 3 plastics greenhouse of Teaching Farm of Sichuan Agricultural University.

2.2 Sampling period and method

Collected materials separately during the following four periods: the color developing stage - 2/3 of tepal display color, and petal have not been unfolded; the initial flowering stage - tepal slightly unfolded, or 1-2 petal unfolded; the full flowering stage - tepal fully unfolded, and anthers fully unfolded; the initial downfalling stage - tepal appears wilting brown, and the color of anther becomes dark brown.

Picked fresh inner and outer petals. Immediately after sampling, wrap it with damp gauze, pack it in a sealed plastic bag, and put it into a storage box with an ice bag to bring it back to the laboratory. Rinsed them with tap water first, then rinsed them with distilled water three times. The samples were mixed evenly according to the upper, middle and basal parts, they were stored in -70 ℃ ultra-low temperature refrigerator by quick freezing of liquid nitrogen, which were used for the determination of physiological indexes.

2.3 Indexes analysis

1.0g of mixed materials were placed in a pre-cooled mortar, added pre-cooled 0.05 mol L^{-1} phosphate buffer (pH7.8) and rapid grinded to homogenization, fixed to 8 ml, then centrifuged 20 min at 5000 r min^{-1} at 4 ℃, that the supernatant as the crude enzyme solution.

Soluble protein content was measured by Coomassie Brilliant Blue G-250 method, soluble sugar was measured by Anthrone Colorimetry method, MDA was measured by Thiobarbituric acid Colouration method, SOD activity was measured by NBT Reductive method, CAT activity was measured by Potassium Permanganate Titration method, and POD activity was measured by Guaiacol method[8]. The content of H_2O_2 was determined by the method of Wen-liang He et al.[9]. O^{2-} production rate was determined by the method of Ai-guo Wang and Guang-hua Luo[10]. All tests were repeated three times.

3.Results and analysis

3.1 Changes of soluble protein content

The change trend of soluble protein content in petal of *L. regale* and *L. leucanthum* was obviously different during the development process (Fig 1). The content of soluble protein in petal of *L. regale* showed a single peak curve, increaed steadily from the color developing stage to the maximum of 1.786 mg g^{-1} at the full flowering stage, and then decreased. The content of soluble protein in petal of *L. leucanthum* decreased steadily, and soluble protein content in petal in the initial downfalling stage decreased by 42.85% compared with the color developing stage.

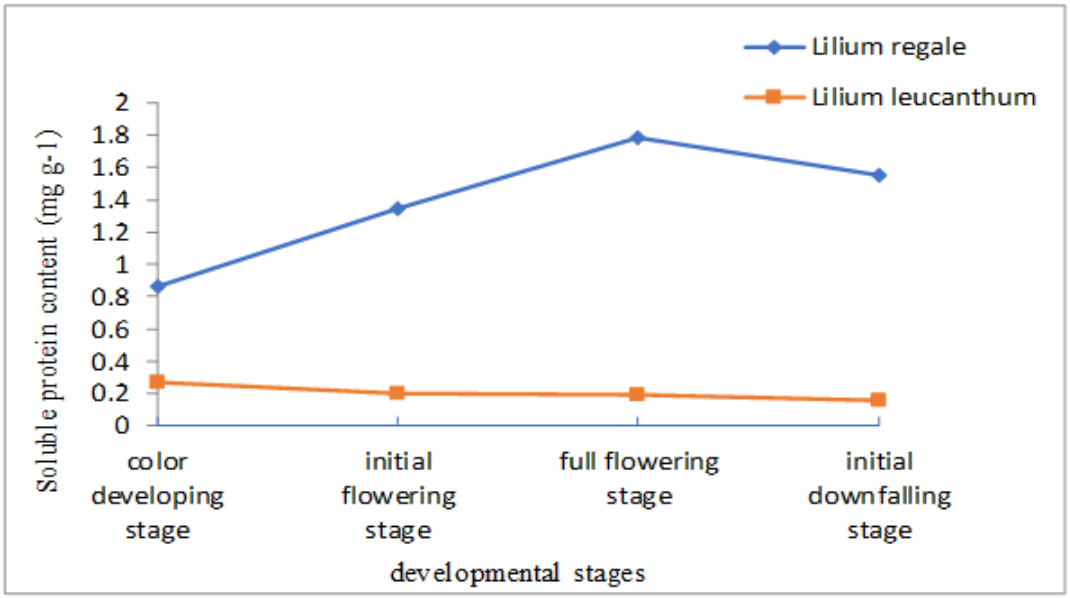

Figure 1. Changes of soluble protein content in different developmental stages of lilies

3.2 Changes of soluble sugar content

Soluble sugar content in petal of *L. regale* and *L. leucanthum* was basically the same during the development process, both increased first and then decreased (Fig 2). The content of soluble sugar in petal of *L. regale* and *L. leucanthum* increased steadily from the color developing stage to the full flowering stage, and reached the maximum value of 0.202% and 0.127% in the full flowering stage, respectively. Then decreased sharply from the full flowering stage to the initial downfalling stage.

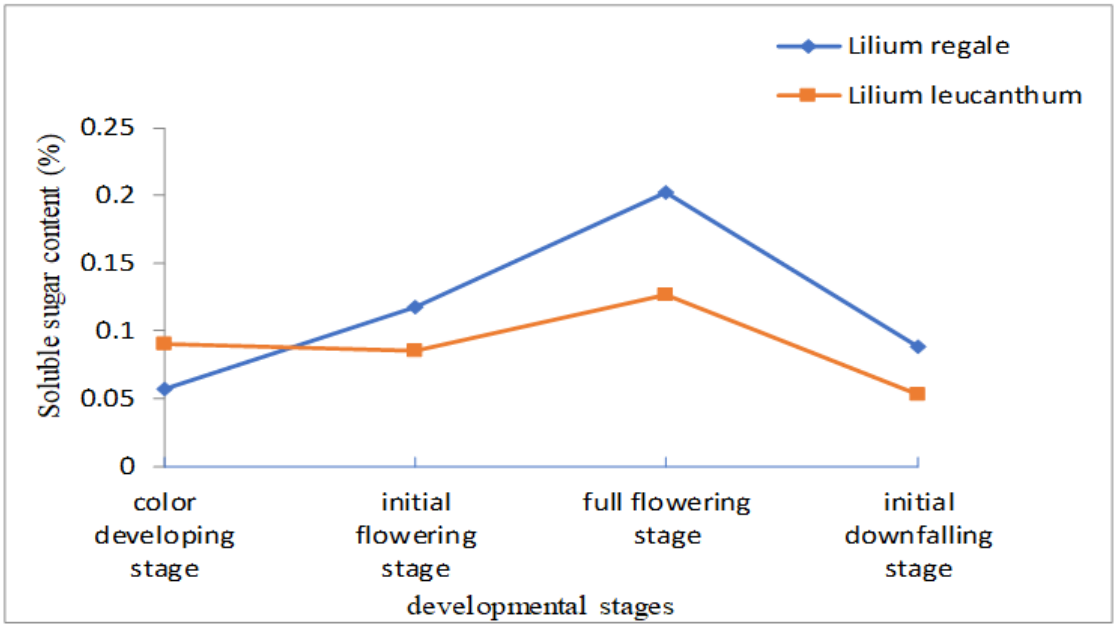

Figure 2. Changes of soluble sugar content in different developmental the stages of lilies

3.3 Changes of MDA content

The content of MDA in petal of *L. regale* and *L. leucanthum* continued to increase during the development process (Fig 3). From the color developing stage to the initial downfallling stage, *L. regale* and *L. leucanthum* increased by 101.16% and 86.32%, respectively.

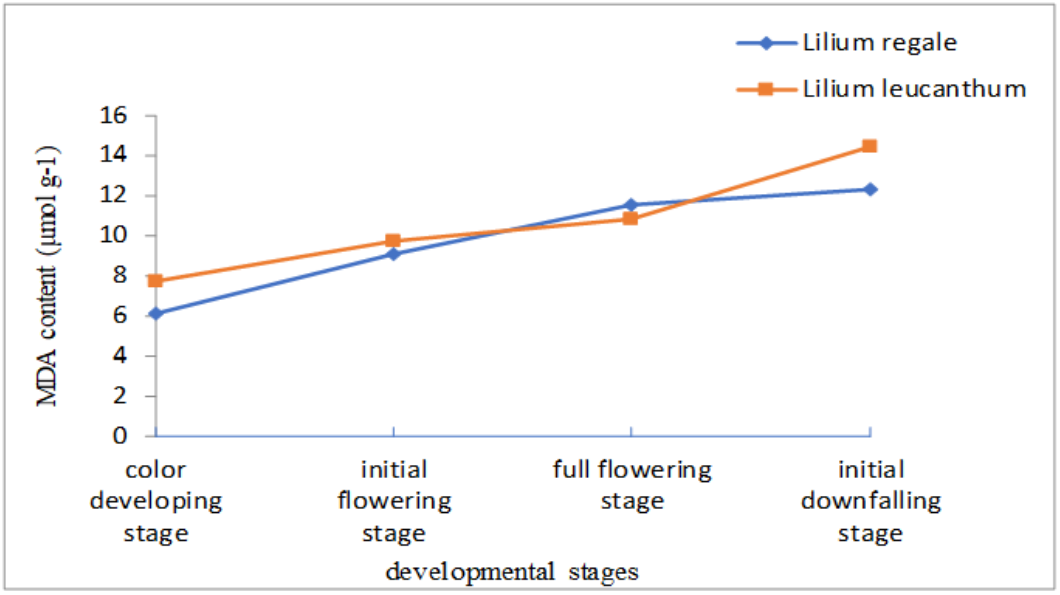

Figure 3. Changes of MDA content in different developmental the stages of lilies

3.4 Changes of ROS

O^{2-} production rate and H_2O_2 content in petal of *L. regale* and *L. leucanthum* showed an upward trend, and the increment speed of *L. leucanthum* was faster than that of *L. regale* (Fig 4 and Fig 5). The O^{2-} production rate and H_2O_2 content of *L. regale* increased rapidly from the full flowering stage and reached the maximum 0.127 nmol mg^{-1} min^{-1} and 29.545 μmol g^{-1} to the initial downfalling stage, respectively. The O^{2-} production rate and H_2O_2 content of *L. leucanthum* continuously increased from the color developing stage to the initial downfalling stage and reached the maximum 0.192 nmol mg^{-1} min^{-1} and 104.924 μmol g^{-1}, respectively.

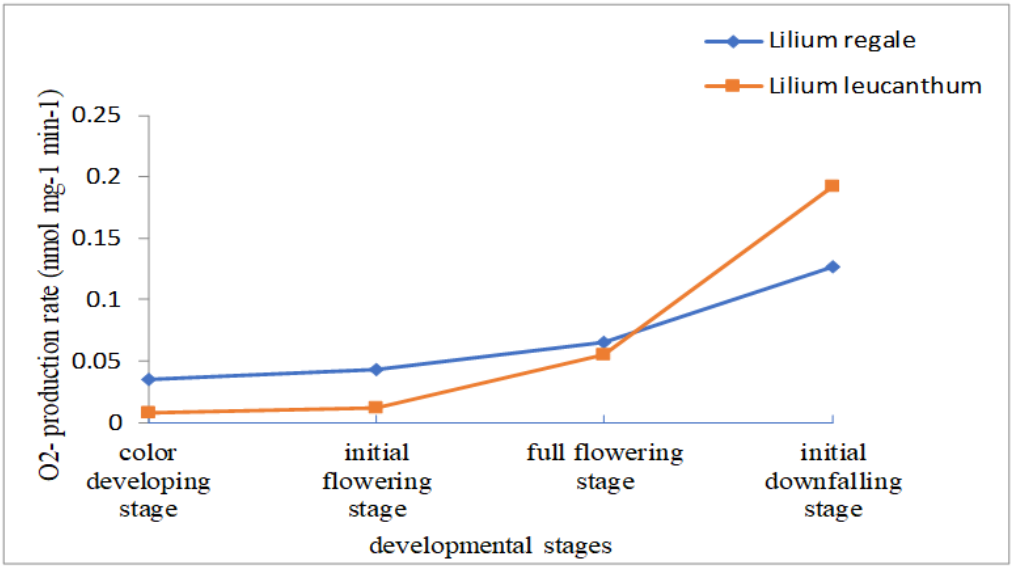

Figure 4. Changes of O^{2-} production rate in different developmental stages of lilies

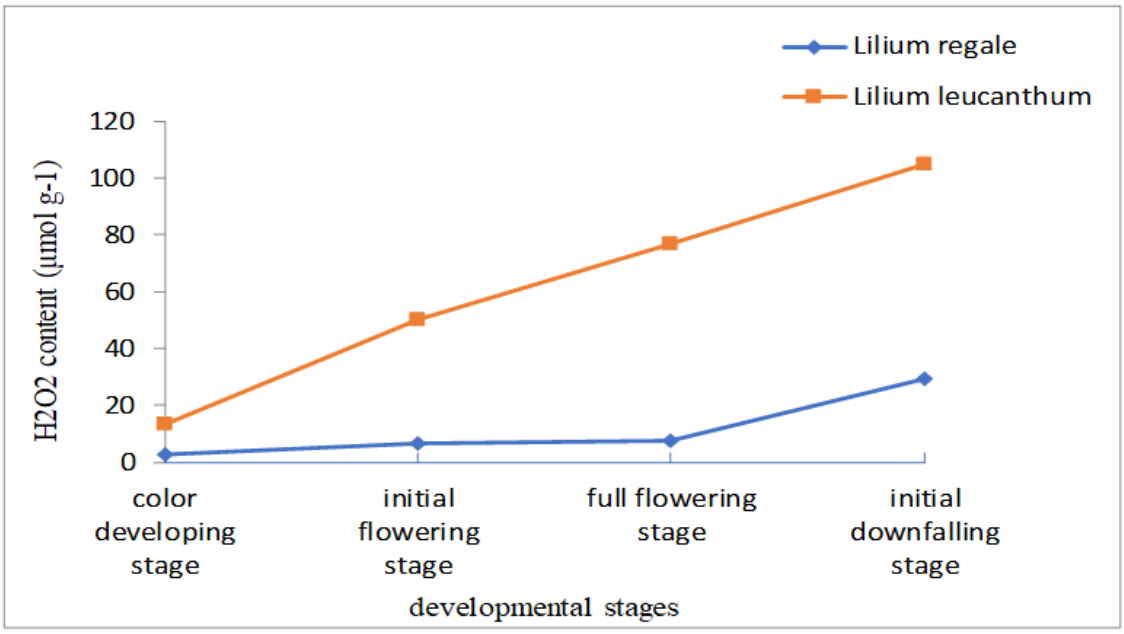

Figure 5. Changes of H_2O_2 content in different developmental stages of lilies

3.5 Changes of antioxidant enzyme activity

SOD activity in petal of *L. regale* began to decrease sharply after the peak of the initial flowering stage, POD activity in petal changed more smoothly, and the fluctuation during the whole development process was small, while CAT activity in petal showed a small peak in the full flowering period (Fig 6, Fig 7, and Fig 8). SOD and CAT activities in petal of *L. leucanthum* began to decreased after reached the maximum in the full flowering stage, and compared with the full flowering stage, SOD activity and CAT activity in the initial downfalling stage decreased by 70.98% and 11.67%, respectively. POD activity changed more smoothly from the color developing stage to the full flowering stage, and then increased rapidly, compared with the full flowering stage, POD activity at the initial downfalling stage increased by 618.66%.

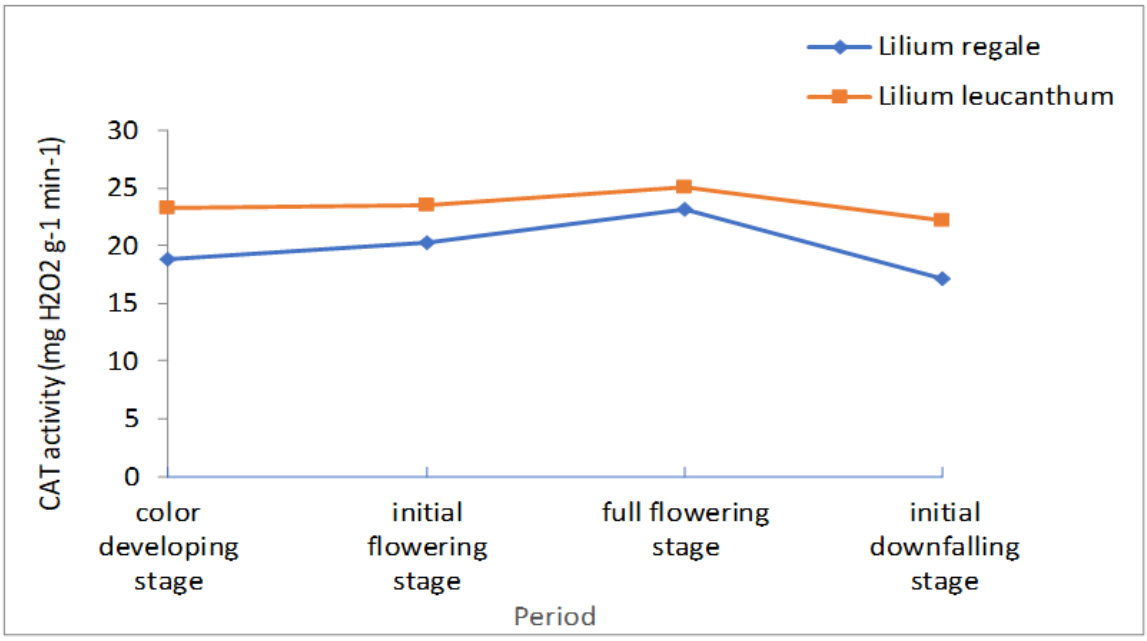

Figure 6. Changes of SOD activity in different developmental stages of lilies

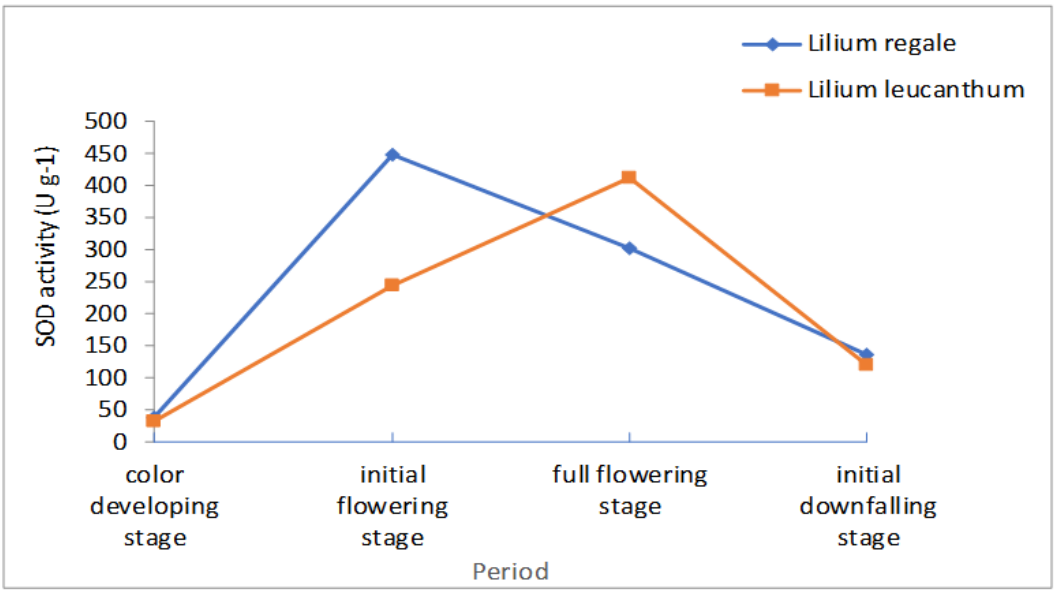

Figure 7. Changes of CAT activity in different developmental stages of lilies

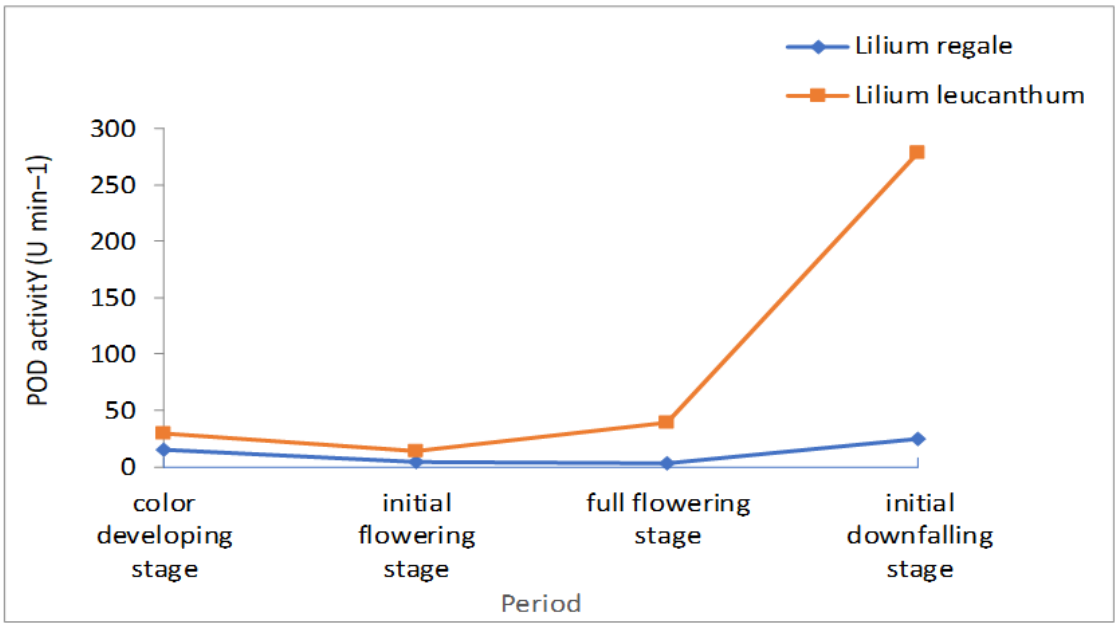

Figure 8. Changes of POD activity in different developmental stages of lilies

4.Discussion

Soluble protein is the main form of nitrogen in plants, including most of the enzymes that support life activity. Therefore, many scholars regard the content of soluble protein as one of the important indexes of senescence[11]. In this experiment, the content of soluble protein in petal of *L. regale* and *L. leucanthum* decreased after full flowering stage, this indicates that with senescence of lilies, protein in petal gradually degraded and the protein content decreased, which is roughly consistent with the results of previous studies[12, 5].

Soluble sugar content reflects the energy supply basis of plants[13]. In this experiment, soluble sugar content in petal of lilies decreased significantly after the peak at the full flowering stage, which indicated that with the beginning of the decay process of lilies, sugar in petal was consumed greatly. During the developing process of tree peony, the content of soluble sugar (glucose and fructose) increased rapidly until reaching the peak after blooming[14]. Soluble sugar content in *oncidium* also

increased in the early stage, and decreased from the decay period[5], which is basically consistent with the results of this experiment.

Many enzymatic reactions in aerobic plant metabolism and automatic oxidation of some proteins and low molecular compounds can produce ROS such as O^{2-}, O^{2-} has toxic effects on plants, and it also induces the formation of poisons such as H_2O_2[3]. The protective enzyme system such as SOD, CAT, and POD can scavenge the ROS in plant cells and prevent the accumulation of ROS from poisoning the plants. SOD specifically catalyzes the conversion of O^{2-} to less toxic hydrogen H_2O_2 and molecular oxygen, which protects plant cells from O^{2-} toxicity, maintain the structure and function of cell membrane[3]. CAT and POD can scavenge excessive H_2O_2, transform H_2O_2 into H_2O, and reduce the damage caused by excessive accumulation of ROS in plants[2]. Under normal conditions, the synergism of SOD, CAT, and POD makes the ROS in at a lower level[15]. During plant senescence, the balance between generation and removal of ROS was destroyed. The activity of SOD was decreased, and the concentration of O^{2-} was increased greatly, which increased the peroxidation of membrane lipid. Free radicals, the intermediate product of membrane lipid peroxidation, and MDA, the final product of membrane lipid peroxidation, can both seriously damage biofilm, cause the polymerization and crosslinking of membrane proteins, deactivate the structure and catalytic function of membrane proteins, and make the membrane lose its function, cause plant cells to die and speeds up senescence[16].

In this experiment, the content of O^{2-} in petal of lilies was lower and the change was small, while the activity of SOD and CAT increased gradually in the flowering stage, which indicated that the production and elimination of reactive oxygen species in the cells were in a dynamic balance, and O^{2-} production rate and H_2O_2 content was controlled at a lower level, and the content of MDA in the petal was low. After the flowering stage of lily, the production rate of O^{2-} was increased, then induced a large amount of H_2O_2, which led to the increase of POD activity to remove H_2O_2. However, the activities of SOD and CAT were decreased, the protective system of lilies was weakened, then the production and elimination of reactive oxygen species were out of balance, which resulted in the accumulation of O^{2-}, the rapid increase of MDA content and the increase of membrane damage by membrane lipid peroxidation, thus accelerating the senescence of lilies. The same study on the *oncidium* showed that the content of MDA in petal increased sharply after the early stage of senescence, and the activity of SOD increased continuously in the early flowering stage, then decreased sharply, while the activity of POD increased continuously during the development process[5-6]. Therefore, it can be inferred that the level of membrane lipid peroxidation has a certain correlation with the physiological mechanism of flower senescence. So the results of this experiment can provide theoretical basis for the regulation of preservation of lily, thus prolonging the flowering period of lily, raising the utilization rate of flower resources.

5.Conclusions

During the whole development period of lilies, the results showed that the accumulation of O^{2-} and H_2O_2 was one of the important factors leading the increase of membrane lipid peroxidation, which resulted in the short flowering period of lilies. Therefore, enhancing the activity of antioxdant enzyme and maintaining the dynamic balance between the production and elimination of reactive oxygen species may be an important way to prolong the flowering period of lily cut flowers.

Acknowledgements

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