Effects of different types of polyamine on growth, physiological and biochemical nature of lettuce under drought stress

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Abstract. This study explored the effects of drought stress on scattered leaf lettuce seedlings and the effects of different types of polyamines (PAs) on the morphological indexes and antioxidant enzyme activities of scattered leaf lettuce. The drought-sensitive variety “Victoria crispy” was used as the plant material, and 10% Polyethylene Glycol (PEG) 6000 was used to simulate drought stress. Foliar applications of 0.1 millimoles per litre of three polyamines [putrescine (Put), spermidine (Spd) and spermine (Spm)] were performed at the seedling stage (the control group was sprayed with water). The morphological indexes and antioxidant enzyme activity were recorded on the eighth day after treatment. The results showed that drought stress significantly decreased the morphological indexes of scattered leaf lettuce, and spraying different types of PAs had a mitigating effect. Drought stress significantly reduced the ascorbic acid peroxidase (APX), catalase (CAT), superoxide dismutase (SOD), and peroxidase (POD) activity, increased the content of malondialdehyde (MDA), and exogenous polyamine spraying significantly affected the antioxidant enzyme activity. Among the polyamines, Put showed the most noticeable effect of mitigation. Drought stress had a significant effect on the morphological and antioxidant enzyme activities of scattered leaves lettuce, and Put was the most effective PA to increase drought tolerance.

1. Introduction

Lettuce (Lactuca sativa L.) belonging to the family Compositae, is a one or two-year-old herb; its common name is lettuce [1]. Lettuce is a low-sugar, low-fat vegetable that is not only rich in vitamin [2], vitamin E [2-3], calcium, phosphorus, iron and other minerals and vitamins but also contains malic acid, tartaric acid, lactic acid, mannitol and the active thyroid hormone [4-5]; thus, it has a high nutritional value [6]. The stem and leaf extract of lettuce can enhance gastric juices, stimulate digestion, increase appetite, and has a hypnotic analgesic effect [2, 7]. At the same time, the extract of leaf lettuce can help treat neurasthenia and effectively lower cholesterol, in addition to other effects, which can reduce the risk of chronic diseases such as cancer, cardiovascular disease and age-related decline [8-10]. Thus, lettuce has gradually become a popular vegetable, and its productivity increases every year [11]. Lettuce has shallow roots, large leaf area characteristics, and does not tolerate drought.
Drought stress poses an immense challenge to global agricultural production. Due to its action, the balance between the generation and elimination of reactive oxygen species (ROS) disappears [13], causing plants to reduce the true potential for stress resistance. China is a country with a shortage of freshwater resources, and its per capita share is only one-fourth of the world average; there are drought and semi-arid phenomena in many areas. Water is the most important environmental factor in plant growth and development, and water deficits may be produced due to soil conditions and other factors, even if there is sufficient water. Photosynthesis, material transport, protein synthesis and other physiological processes are severely affected [14]. Drought stress affects plant growth and development in many ways, including reactive oxygen species, osmolytes, photosynthesis, and water use efficiency [13, 15-16]. To ensure their continuous growth and development under drought stress, plants act via a series of responses to stress, such as antioxidant regulation, stomatal regulation and osmotic adjustment [17-19]. Drought stress and subsequent plant responses have become a hot topic in plant physiology, and it is vital to cultivate drought-resistant varieties by studying plant drought resistance.

Polyamines (PAs) are ubiquitous low molecular aliphatic amines generated during the metabolic processes of an organism. They are the second messengers of signaling pathways, and have recently been added to plant growth regulators [20-22]. Putrescine (Put), Spermine (Spm), and Spermidine (Spd) play pivotal roles in plant growth and development [23]. Polyamines affect DNA, RNA and protein biosynthesis, promote plant growth and development, delay ageing, and improve disease resistance in plants [24]. Since PA can clean free radicals in plants, they also protect the membrane to some extent from oxidative damage [25]. And the most effective PA for alleviating drought stress in rice is Spm [26]. Exogenously applied PA also increased plant tolerance under several abiotic stresses [27-28]. Nayyar et al. found that drought tolerance in soybean can be increased with exogenous application of Put and Spd [29]. To our knowledge, previous studies on polyamines mostly involve studies on the drought resistance of crops; however, to date, no research has been found on the potential role, basis and influence of exogenous PA on the dryness resistance of vegetables such as leaf lettuce. In this study, we investigated the effects of different types of PAs on drought stress, determining its physiological indicators and screening out the most effective polyamines, which alleviate drought stress. This study aimed to provide a theoretical basis for screening more efficient polyamine types for leafy vegetables.

2. Materials and Methods

2.1. Experiment material

The lettuce varieties were screened based on their polyamine content. The variety Victoria crispy, (origin: Italy; manufacturer: Tianjin Priority Seed Co., Ltd.) contains polyamine species Spm, Spd and Put.

2.2. Experimental method

The seeds were germinated on a qualitative filter paper tile, wetted with distilled water, in a 9-cm-diameter dish. The seeds were selected such that they were the same size, whole, and showed no disease. The germinated seedlings were planted on a nursery sponge in water culture; half of the total seedlings were watered with the nutrient solution (the formula is shown in Table 1). At the four-leaf stage, the growing seedlings were placed in a hydroponic set-up to add the nutrient solution.

After slow seedling growth for 2-3 days, 10% polyethylene glycol (PEG 6000) was added for drought simulation. Different polyamines were sprayed after 6 pm every day (the control group was sprayed with water) until the leaves began to drip solution, and on the 8th day, the morphological index was measured. The test was carried out in a growth chamber; the temperature period was 20°C.
(14 h, 7:00 ~ 21:00) / 15°C (10 h, 22:00 ~ 6:00) and the photoperiod was 14 h (7:00 ~ 21:00) / night 10 h (22:00 ~ 6:00), with a light intensity 1200 Lux and 60% relative humidity.

Five test groups were set up for the experiment. Treatment I (CK-W) was set up as a control and treatment II (PEG-W) for drought stress. Two controls were maintained, one of them was maintained under drought conditions and the other was maintained under full watering conditions; both received no PA treatment as a foliar application or seed treatment. Treatment III (PEG-Spd) included drought treatment + 0.1 mmol / L of the spermine aqueous solution; treatment IV included (PEG-Spm) for drought + 0.1 mmol / L aqueous spermidine; treatment V (PEG-Put) included drought + putrescine (drought treatment 2-3 days after spraying 0.1 mmol / L aqueous solution of Put). The three groups were treated as described above to observe the reversal and alleviation effects of the three PAs on drought stress.

### Table 1. Nutrient solution.

| Name                                                                 | Consumption (1 L) |
|----------------------------------------------------------------------|------------------|
| Calcium nitrate tetrahydrate (Ca(NO$_3$)$_2$·4H$_2$O)                | 0.589 g          |
| Potassium nitrate (KNO$_3$)                                          | 0.887 g          |
| Ammonium nitrate (NH$_4$NO$_3$)                                       | 0.0571 g         |
| Magnesium sulfate heptahydrate (MgSO$_4$·7H$_2$O)                    | 0.183 g          |
| Potassium sulphate (K$_2$SO$_4$)                                      | 0.054 g          |
| Phosphoric acid (H$_3$PO$_4$)                                         | 0.223 mL         |
| Ethylenediaminetetraacetic Acid Ferric Sodium Salt (EDTA-FeNa)       | 0.020 g          |
| Perboric acid (H$_2$BO$_4$)                                           | 0.00286 g        |
| Manganese sulphate (MnSO$_4$)                                         | 0.002 g          |
| Zinc sulphate (ZnSO$_4$)                                              | 0.00022 g        |
| Copper sulfate (CuSO$_4$)                                             | 0.00008 g        |
| Ammonium heptamolybdate tetrahydrate ((NH$_4$)$_6$Mo$_7$O$_{24}$·4H$_2$O) | 0.00002 g        |

2.3. Determination of indicators and methods

Fresh and dry weights were recorded using a balance. Water content and relative moisture content of the samples were calculated. To determine the relative water content (RWC), fresh leaves (0.5 g) (Wf) were weighed to obtain a fresh weight. The leaves were then floated in water for 4 hours and the saturated weight (Wt) was measured. The same leaves were dried at 85 °C for 24 hours and the dry weight (Wd) was measured.

RWC (%) were calculated as: RWC = (Wf − Wd)/(Wt − Wd)*100%

Plant height and root length were measured using a ruler, and the root / shoot ratio was calculated.

To analyze the activity of antioxidant enzymes, 0.2 g of fresh leaves were arbitrarily taken from each reservoir at each sampling time, immediately frozen in liquid nitrogen and stored at -80°C until use. For the extraction, frozen samples were crushed on ice with 4 ml of 50 mM cold phosphate buffer (pH 7.8) containing 1% (w/v) Polyvinylpyrrolidone. The homogenate was centrifuged at 12,000 g for 30 minutes at 4°C. The supernatant was used to determine the activity of the antioxidant enzyme and the MDA content, which was measured as the degree of lipid peroxidation. The activity of SOD enzyme is mainly determined by measuring the rate of decrease in absorbance of nitroblue tetrazolium...
chloride at 560 nm [30]. The activity information of CAT, POD and APX is determined by analyzing the changes in the absorbance values of CAT, POD and APX at 240, 470 and 290 nm [31-32].

2.4. Data processing and analysis
Data analysis was performed using SPSS 19.0 for determining variance and significance analysis.

3. Results and analysis

3.1. Morphological indicators

3.1.1. Effects of different polyamines treatments on fresh and dry weight of lettuce under drought stress. When the lettuce leaves are under drought conditions, their growth is affected by severe stress, but the exogenous application of polyamines reduces the damage caused by drought. Figure 1 shows the effect of different treatments on fresh weight and dry weight on the 8th day. In the control plants, the fresh weight of the shoots was significantly higher. Under drought stress treatment, exogenous application of Spm and Spd can restore the fresh weight to a certain extent, basically reaching the level of control treatment, but the difference is not obvious. However, spraying Put resulted in significantly higher fresh weight compared with that of other treatments, even more than that in the control treatment. The fresh weight of the roots was significantly higher in the control compared with the drought treatment. Under drought stress, the spraying of these three PAs could significantly increase the fresh weight of the roots, and there were significant differences among spraying Put Spm, and Spd, although the effect of Put was the most obvious.

Figure 1. Effects of different polyamine treatments on lettuce fresh weight and dry weight.
There was a small difference in the dry weight of the shoots treated with different treatments, and there were no significant differences among spraying Spm, Spd and water in drought treatment. The dry weight of plants treated with Put was slightly higher than the other treatments. Root dry weight differed slightly among different treatments; the dry weights were significantly different with Spd, Put and spraying water. However, Spm spraying did not result in significantly different dry weights compared with the control. It can be seen from the test conclusion that drought stress significantly reduces the dry weight and fresh weight of leaf lettuce.

3.1.2. Effects of different polyamines treatments on plant height, root length and root / shoot ratio of lettuce in drought stress. Figure 2 shows the significance of plant height, root length and root/shoot ratio at eight days after different treatments. Drought stress significantly reduced plant height. During drought treatment, spraying PA had a certain effect on plant height, but it was not apparent. Drought stress reduced the root length; spraying polyamine can increase root length, but Spm and Spd treatments did not show significant differences. The application of Put drought treatment showed increased root length, which was similar to the control group. Drought stress had no significant effect on the root/shoot ratio of seedlings. In the drought treatment, the effect of spraying Put on root/shoot ratio was the highest, but there was no significant difference between spraying and PA.

![Figure 2](image_url)

CK-W: well-watered, no polyamine treatment; PEG-W: drought stress, no polyamine treatment; Put: putrescine; Spm: spermine; Spd: spermidine
Note: Different letters showed significant differences (P<0.05)

Figure 2. Effects of different polyamines treatments on plant height, root length and ratio of root to leaf lettuce.

3.1.3. Effects of different polyamine treatments on water content and relative water content of lettuce in drought stress. Figure 3 shows the water content and relative water content on the 8th day after the different treatments; drought caused a decline in water content and relative moisture content in lettuce. Spraying polyamines, particularly putrescine, can significantly increase the water content of seedlings. Moreover, spraying PAs has little effect on plant relative water content.
CK-W: well-watered, no polyamine treatment; PEG-W: drought stress, no polyamine treatment;
Put: putrescine; Spm: spermine; Spd: spermidine
Note: Different letters showed significant differences (P<0.05)

Figure 3. Effects of different polyamine treatments on lettuce water content, relative water content.

3.2. Effects of polyamines application on the antioxidants under drought stress in lettuce

Figure 4 shows a significant analysis of the antioxidant enzyme activity on day 8 after different treatments. APX content decreased due to drought stress, but the application of PA under stress conditions improved its content. Exogenous spraying of PAs under drought stress could significantly increase the activity of APX. The effect of Put was the most obvious, and the activity of APX was higher than that of the control. Compared with other PAs, there was a significant difference with Put treatment. Drought stress caused a significant increase in the MDA content in lettuce. Exogenous PAs application inhibited this increase such that there were no significant differences between the polyamine treatments and the control, and the MDA content was slightly reduced, which was significantly ameliorated by Spm and Put treatments, with Put effect being the most obvious. In the determination of CAT content, it can be found that the maximum amount can be obtained from fully watered lettuce, but it is significantly reduced under drought stress, and the application of exogenous PA shows a small increase in enzyme activity and is not obvious.
CK-W: well-watered, no polyamine treatment; PEG-W: drought stress, no polyamine treatment; Put: putrescine; Spm: spermine; Spd: spermidine
Note: Different letters showed significant differences (P<0.05)

**Figure 4.** Effects of different polyamine treatments on lettuce antioxidant enzyme activity.

Although the content of SOD was reduced due to drought stress, this was significantly improved by the application of PA. The Spd treatment and the control group showed almost no difference, the rest were significantly different, and the most obvious effect was shown by Put. Similarly, the activity of POD was significantly increased by exogenous polyamines, and the effect of putrescine was the most obvious. The results showed that APX, CAT, SOD and POD activities were significantly reduced due to drought stress treatment, increased MDA content, and produced different types of PA. In general, the activity of antioxidant enzymes is most obvious under exogenous application under drought stress.

**4. Discussion**

Polyamines are now considered as plant growth regulators and secondary messengers in signalling pathways [20, 22-33]. Studies have shown that PAs play an important role in the growth and development of plants under drought stress, the scavenging of reactive oxygen species and maintaining a stable membrane structure [34-35]. Therefore, it is important to study the use of PAs to alleviate the harm caused by drought stress and help reduce economic losses. The exogenous spraying of Spd could alleviate the increase in chili physiological indicators, and the initial injection of Spd in drought stress can make the mitigation effect more obvious [36]. And exogenous spermidine could significantly increase dry weight, relative water content, root number and the chlorophyll content of maize seedlings under drought stress; the cell membrane permeability of maize seedling leaves was significantly decreased, and the effect on the varieties with weak drought resistance was more apparent [37]. In this study, the morphological indexes on the 8th day after different treatments were assessed, and the results showed that drought stress caused a significant decrease in fresh weight, plant height, root length, water content and relative water content of lettuce seedlings but had no significant effect on dry weight and root/shoot ratio. In general, the exogenous spraying of polyamines has a
A also involves several other aspects of the elimination of reactive oxygen species in the antioxidant system. Under these circumstances, the antioxidant enzyme activity of seedlings under drought stress, and the effect was more evident in drought-stressed tomato seedlings, thus enhancing the ability of ROS removal and improving the resistance of plants under stress conditions. In addition, in plants, in addition to biotic and abiotic reactions, PA also involves several other physiological processes including embryogenesis, organogenesis, flower germination and development, fruit development, maturation and leaf senescence [38-39].

Plants produce reactive oxygen free radicals (ROS) in normal and adverse environments; the ROS react with proteins, lipids and DNA, thus impairing normal cellular functions [40]. Under non-stress conditions, plants maintain a balance between producing and scavenging ROS through a well-coordinated and rapidly responsive antioxidant system. Under these circumstances, the antioxidant enzymes CAT, SOD, POD and others coordinate to promote low levels of free radicals to prevent cell damage [41-43]. Water stress obliterates the equilibrium state in the production and removal of reactive oxygen species in plants, thus causing the accumulation of free radicals of active oxygen, which accelerates the oxidation of many vital substances on the cell membrane, resulting in the membrane lipid peroxidation product malondialdehyde (MDA) [26]. The accumulation of MDA can be used as a measure of the degree of cell damage and as an indicator of drought. Previous studies showed that the accumulation of MDA increased with the increase in the time of stress; the degree of membrane lipid peroxidation increased, but there were differences among the cultivars [44]. Under drought stress, the degree of membrane lipid peroxidation was lower compared with that in non-drought-resistant varieties. Under drought stress, the activities of malondialdehyde (MDA), superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) in cucumber leaves increased significantly [45]. Moreover, MDA content in tomato seedlings increased first and then decreased, and the content of MDA also decreased after exogenous Spd treatment. The content of MDA was lower, and the alleviation effect was more obvious in drought-treated plants. This indicated that drought stress in tomato seedlings could accumulate reactive oxygen species (ROS) in plant cells and attack the cell membrane, which would increase the MDA content in membrane lipid peroxidation products. However, exogenous spermidine can reduce the MDA content, enhance membrane stability, and relieve drought damage in plants [46].

SOD is the first line of defense that constitutes the elimination of reactive oxygen species in plants. The high expression of these enzymes can reduce the damage of reactive oxygen species to plants under stress conditions. Under drought stress, the activity of SOD in drought-resistant cultivars was higher than that in susceptible cultivars, which indicated that drought-tolerant varieties had a higher ability to scavenge superoxide free radicals, and similar results were obtained in other crop studies [47]. CAT and POD can decompose H$_2$O$_2$ [48]. The activity of SOD and POD in drought stress increased during a specified period, probably because of the increase in active oxygen free radicals, such as O$_2^-$ and other active oxygen in drought stress, and the accumulation of reactive oxygen species in the plant. Adaptability regulation reduces the increase in reactive oxygen free radicals caused by cell damage; however, prolonged accumulation of reactive oxygen free radicals and the resulting accumulation of MDA in the cells leads to decreased enzyme activity, which was significantly lower in drought-resistant varieties compared with drought-sensitive varieties. Under drought stress, CAT, SOD and POD activities increased first and then decreased. Exogenous Spd treatment significantly increased the protective enzyme activity of seedlings under drought stress, and the effect was more evident in drought-sensitive cultivars. Under drought stress, the APX activity of tomato seedlings continued to increase, and exogenous Spd treatment significantly improved its APX activity [46]. Moreover, under drought stress, exogenous Spd helps improve the antioxidant enzyme activity of tomato seedlings, thus enhancing the ability of ROS removal and improving the resistance of seedlings.

In this study, drought stress could decrease the activity of APX, CAT, SOD and POD, and increase the content of MDA in the leaves of lettuce seedlings. The exogenous spraying of different polyamines could alleviate the activities of antioxidant enzymes in leaves under drought stress.
Exogenous spraying of Put showed the best effect in alleviating drought effects. The results are similar to those of previous studies, but there are some differences. According to the study of Muhammad Farooq Abdul Wahid-Dong-Jin Lee, 10 μM Spm, Spd and Put can significantly alleviate the net photosynthetic rate and water use efficiency of the leaves after drought stress [26]. Moreover, among the three types of PAs, the effect of Spm was the most apparent. The main reasons for these differences are as follows: First, there are differences in the tested varieties. The drought resistance of rice and lettuce is different, and the physiological structure and the number of polyamines required are different. The concentration of the three polyamines was different. In this experiment, the concentration of polyamines used was 0.1 mM, which is 10 times the concentration described by Muhammad Farooq et al., so there was a difference. Finally, foliar spraying did not result in seed irrigation, but it may be a reason for the differences in test results. There are many studies on the relationship between protective enzyme systems and plant drought tolerance, but the results are different [49]. This may be related to the activity of protective enzyme systems in plants, different drought resistance in different plants and the content of nutrients, such as calcium ion distribution and antioxidant content, and other factors related to the root. Moreover, the drought stress response was also different in different ground parts, and this view can be obtained from the experimental results.

In summary, the effect of drought stress in lettuce lowers the morphological indicators and antioxidant enzyme activities, and exogenous polyamine sprays can effectively alleviate the situation; Putrescine showed the most significant effect in alleviating drought stress in lettuce seedlings.

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