Effects of compound growth regulators on the anatomy of Jujube Leaf and Fruit

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Abstract

Effects of three compound growth regulators formulated with hypersensitivity protein, spermidine, salicylic acid and DA-6 (diethyl aminoethanol hexanoate) were tested on Xinjiang Jun Jujube. The doses of compound growth regulators were named as A (Hypersensitivity protein + spermidine + salicylic acid at the rate of 30 mg/L, 0.1 mmol/L and 0.25 mmol/L, respectively), B (Hypersensitive protein + spermidine + DA-6 at the rate of 30 mg/L, 0.1 mmol/L and 30 mg/L, respectively) and C (Spermidine + salicylic acid + DA-6 at the rate of 0.1 mmol/L, 0.25 mmol/L and 30 mg/L, respectively) versus a control group CK (contained only water). Fruit anatomical structures were compared after spraying. The results indicated that after spraying, the thickness of the upper and lower epidermal cells and the stratum corneum were increased. However, the upper epidermal stratum corneum became significantly thicker than the lower epidermis. Spraying with A improved the thickness of upper and lower epidermal cells, stratum corneum, the central vein and mesophyll. The cumulative effects of all these changes in leaf and fruit anatomical structures provided the resistance of the experimental fruit plant to stress. While the B and C regulators had inhibitory effects. So, the results obtained after spraying A category were beneficial to improve the stress resistance of the fruits. The length and cell area of pericarp and sarcocarp cells in the treatment groups were not changed significantly. But the length, number of sarcocarp cells and number of gaps were lower than those in the CK. This study can provide new measures for improving plant resistance in jujube production.

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1. Introduction

Ziziphus jujuba Mill. belonging to the botanical Family Rhamnaceae is a woody plant. Its common name is Chinese Date or Common Jujuba. This fruit tree resource has been considered unique in China. One of the cultivars of this fruit tree is called Jun Jujube (Z. jujuba CV. Junzao) which is native to Liuliang city of Shanxi province. The tree has not only strong resistance to drought, cold, barren, saline and alkaline conditions. But also strong body, high yield and long economic life. All these characters has made it suitable for growth in Xinjiang region. The plant not only produces high-quality fruits, but also increases farmers’ production and income and has earned a good reputation.

Because of dry and typical temperate continental climate, there have larger temperature difference between day /night and the long day light cycle in Xinjiang, these make the fruits rich in sugar and have unique taste. However, in the late summer and early autumn, unusually low temperature, drought, or excessive rain may occur in Xinjiang. This condition affects the growth and development of the fruit tree. Therefore, in order to harvest good quality jujube fruit, the plant resistance could be improved by spraying anti-stress inducers and hormones. For many years, there have been researches on the growth morphology of apple (Wang et al., 2014), grape metabolic pathway (Deluc et al., 2009), and pear gene expression (Li et al., 2015) of growth regulators, but they are mostly limited to the routine hormones such as IAA, GA3, BA, etc.
Hypersensitivity protein, spermidine, DA-6 (diethyl aminoethanol hexanoate) and salicylic acid are inducers to improve plant resistance. Hypersensitivity protein is a peptide class compound that do not directly affect pathogens, but can induce plant allergic necrosis (HR) responses, which can regulate plant growth, yield and disease resistance (Wang et al., 2014; Rodrigo-Garcia et al., 2019; Pasquale Crupi et al., 2019). DA-6 has the functions of auxin and cytokinin, which can effectively promote plant seed germination and seedling growth (Zhou et al., 2019) while slowing down the uptake of heavy metals which is harmful to plants (He et al., 2014). Spermidine (Spd) is a kind of plant growth regulator that can improve the resistance to salt stress (Li et al., 2014; Li et al., 2018). Salicylic acid (SA) is a phenolic compound commonly found in plants. It is a cell signaling molecule and an endogenous hormone, which is involved in regulating many physiological processes in plants. Salicylic acid can not only induce some plants to synthesize flavonoids with antioxidant properties, but also induce plant stress resistance by mediating signals (Somayeh et al., 2019; Zhang et al., 2019).

Therefore, the aim of the present research was to observe the anti-stress increasing effects of three compound growth regulators on jun jujube plant. The result of which could help the plant to overcome the unusual seasonal stresses and can provide high quality fruits.

2. Materials and methods

2.1. Materials and test design

In this experiment the test materials were healthy jun jujube plants (Z. jujuba CV. Junzao). Three different compound regulators (A-C) were used to spray the jun jujube plants. The combination of which has been provided in Table 1. The sprays were conducted on July 15th and 25th, August 10th, 20th and 30th of 2017 and 2018. The control plants (CK) were sprayed only with water. The compound regulators were sprayed for a total of 5 times following a progressive manner based on the line. It means that the first to the fifth line were sprayed once, twice, thrice, four and up to five times, respectively. Ten days after spraying, mature and healthy fruits from each experimental group were collected rinsed and dried. Several small pieces of the outer skin of equatorial part of the fruits were cut, fixed with FAA and sealed for future use.

2.2. Methodology

2.2.1. Paraffin section cutting

The method of making paraffin section was based on Li et al. (2009). The section thickness ranged from 8 to 10 μm and saffron and fast green were used as staining material. The section material was dehydrated with a gradient of ethanol and xylene was used to make the sections transparent. Then it was embedded with paraffin, and the cut sections were processed further following dewaxing, staining, gradient dehydration with ethanol and mounting on clear glass slides. Finally, the images of the sections were viewed and collected through OLYMPUSBX53 microscope.

2.2.2. Jun jujube leaf and fruit data determination

The structural indexes of leaves and fruits had been measured. Three sections were selected for each group of treatments and the vision of three fields were observed in each section. A total of nine fields of vision corresponding to the tissue structure were observed. The anatomical structure of the leaves, including the thicknesses of central vein, leaf, upper and lower epidermal cells were recorded. In addition, the thicknesses of upper and lower epidermal stratum corneum were also observed. During the observation of the fruit structure, the thickness of the stratum corneum and epidermis, the number of layers of exocarp cells, the size of sarcocarp cells and sarcocarp cell cavities were observed and their number were recorded.

2.2.3. Statistical analysis

The observed data was statistically analyzed by SPSS 20 software and the significance between the measured values of the anatomical structure of leaves and fruits in each group was tested by Duncan method.

3. Results and analysis

3.1. Structural characteristics of leaves

Jun jujube leaves are composed of epidermis, mesophyll, and veins, which are typical equifacial leaves (Fig. 1). The upper and lower epidermis of the leaf are composed of a layer of cells, nearly equal to each other in diameter, arranged very regularly and compactly. The outer wall of the cells has stratum corneum. The upper epidermal cells are larger than the lower epidermal cells but both of them are almost rectangular. The tangential wall of the upper epidermal cells is thicker than the lower epidermis. It is observed that the stratum corneum thickens after spraying the three compound regulators. While the upper epidermal stratum corneum thickens much more strongly than that of the lower one.

The leaf and mesophyll consists of 6–7 layers of thin-walled cells, without obvious differentiation of palisade tissue and spongy tissue. This is in consistent with the characteristics of equifacial leaf. The mesophyll tissue near the upper epidermis is composed of 3 layers of long columnar, palisade-like cells, which vary in size and are arranged closely. The cells are significantly larger than the cells of the palisade tissue near the lower epidermis. While the mesophyll tissue near the lower epidermis consists of 3–4 short columnar cells and a small number of oval cells. The cells are loosely arranged having well-developed intercellular spaces. From the structural point of view, spraying the compound regulators showed no obvious effect on the cell type, layer number, size and arrangement of mesophyll tissue. After spraying C, the cells of the mesophyll palisade tissue are smaller and more closely arranged compared with CK, treatment A and treatment B.

| Treatment | Reagent | Concentration |
|-----------|---------|---------------|
| A         | Hypersensitivity protein + spermidine + salicylic acid | 30 mg/L + 0.1 mmol/L + 0.25 mmol/L |
| B         | Hypersensitive protein + spermidine + DA-6 | 30 mg/L + 0.1 mmol/L + 30 mg/L |
| C         | Spermidine + salicylic acid + DA-6 | 0.1 mmol/L + 0.25 mmol/L + 30 mg/L |
| CK        | Water | |
The main vein of leaves was well developed but formed upward and downward protrusions on the leaf surface along with the larger lateral veins. The veins of lower epidermis have more prominent protrusions than the upper epidermis. The main vein is principally composed of vascular bundles and basic tissues. The main vascular tissue is predominantly composed of xylem, formation layer and phloem. The xylem is close to the adaxial, while the phloem is to the abaxial surface forming the external vascular bundle. The xylem was more developed. The xylem of the CK consisted of about 15 rows of ducts. The diameter of the ducts was small and closely arranged. After spraying A and B, the xylem of the main veins of the leaves of the test groups were arranged in a fan-shaped pattern with about 15 rows of ducts. The number of rows of ducts in the main vein after spraying C was about 20. The number is higher than that in the CK and A and B. The formation layer was not obvious and it was composed of flat, discontinuous 1–3 layers of cells. Among them, the group sprayed with C had more obvious formation layer with larger number of cells. The formation layer was not obvious for the test group sprayed with A. For B, phloem cells were small, large in amount and were arranged closely. There were 1–2 layers of collenchyma on the inner side of the upper and lower epidermis. On the other hand, there existed 4–5 layers of thin-walled cells of various sizes between the vascular bundle and collenchyma. After spraying the compound regulator, the main vein structure of leaves did not change significantly.

3.2. Characteristics of fruit structure

The results showed that the average stratum corneum thickness in CK was 5 μm. But the stratum corneum thickness of fruits of tested plants had increased. The stratum corneum became thickest after spraying C for 5 times, the thickness being 7.4 μm. The number of subepidermal cell layers changed slightly among different treatment groups. It can be seen from Fig. 2 that after spraying A and B, the test plants produced irregular fruit subepidermal cell shapes compared CK. The arrangement was tight, while there was no significant difference in the fruits of C, but the number of subepidermal layers increased significantly.

The parenchyma cells are relatively large in volume, loosely arranged, irregular in size and have obvious intercellular spaces or cell cavities. The cells become larger toward the inner cavity and they even can form a network. For the fruit of CK, close to the peel i.e., outside the sarcocarp and in the middle of the sarcocarp, the size of the cells did not change significantly. But the num-
ber of gaps between the cells increased significantly and the gap areas became relatively larger. Trend of this change remained the same after the spraying. The number and area of cell gaps in C were significantly increased.

3.3. Spraying times effect on the main vein, leaf and mesophyll thickness of the test plants

The effects of spraying 1–5 times on the test plants the anatomical structure of veins, mesophyll and leaves have been shown in Fig. 3. The number of spraying significantly changed the thickness of the leaves and mesophyll tissues. The changes in the mesophyll tissue was the smallest which is between 0.158 and 0.2325 mm. The leaf thickness changed slightly more than the mesophyll. There were significant differences between the main vein groups after spraying the three compound regulators (P < 0.05). But there was no significant difference of the mesophyll and leaf thickness (P < 0.05). After spraying, the main vein diameter of leaves changed inconsistently. However, in A and B, the main vein thickness increased significantly when sprayed 1–2 times. The maximum values is 1.4363 and 1.3832 mm for A and B respectively. But the thickness of the main vein decreased significantly after 3 times of spraying and reached the minimum at the fifth time spraying, being 2.014 and 0.7003 mm, respectively. The leaf vein thickness was not significantly different among the treatment groups after spraying the three compound regulators. The thickness of leaf veins showed a downward trend when C compound regulator was sprayed for 1–2 times, and its thickness increased significantly after three sprays. After spraying A 1–5 times the thickness of mesophyll changed slightly from 0.1546 to 0.1719 mm. However, after spraying B, the thickness of mesophyll basically changed insignificantly. Thickening trend was obvious after spraying C. Where, after
spraying 5 times, the thickness increased significantly reaching a maximum of 0.1926 mm. But when B was sprayed 4 times, the result was significantly different from the other groups.

Notes: CK is the control group, sprayed with water; A1-A5 is sprayed with A (hypersensitive protein + spermidine + salicylic acid) 1–5 times, B1-B53 is B (hypersensitive protein + spermidine + DA-6) sprayed 1–5 times, C1-C5 is C (spermidine + salicylic acid + DA-6) sprayed 1–5 times.

3.4. Effects on the thickness of the upper and lower epidermis and stratum corneum of leaf

The spraying frequency caused relatively small changes in the thickness of the epidermal cells and the thickness of the stratum corneum of the upper and lower epidermis of leaves. The trends were similar as shown in Fig. 4. When A was sprayed 1–4 times, the upper epidermal cells on the leaves were slightly thickened but the minimum thickness 0.0369 mm occurred when sprayed five times. The thickness of upper epidermal cells became smaller i.e., ranging from 0.0351 to 0.0411 mm after B was sprayed 1–5 times. After spraying C, the thickness of upper epidermal cells of leaves in each treatment group clined to an overall thickening trend. After the 5th spraying, it was significantly thickened (0.043 mm) compared with upper epidermal cell thickness of CK (0.0195 mm). After spraying A and B, the thickness of the lower epidermal cells of leaves increased. But after the 5th spray, the thickness decreased to the minimum, which was 0.0218 and 0.0222 mm respectively for A and B. However, after spraying 1–3 times, the thickness of upper epidermal leaf cells increased slightly, and the thickness decreased when sprayed 4–5 times, the thinnest being recorded was 0.0185 mm.

Fig. 5 shows the changes in the thickness of the stratum corneum on the upper and lower epidermis after been sprayed 1–5 times. After spraying A and B 1–4 times, the stratum corneum on the upper and lower epidermis was thickened each time. And the stratum corneum was thinner than the CK leaves after the 5th spraying, which were 0.0033 and 0.0031 mm, respectively for upper and lower epidermis. After spraying C the thickness of the stratum corneum of the upper and lower epidermis was larger than that of the CK, but the pattern of thickening was not obvious.

3.5. Effects of different treatments on the thickness of the exocarp and stratum corneum of fruit

After spraying 1–5 times to all the test plants, the thickness of exocarp and stratum corneum has been presented in Fig. 6. The number of sprays increased the thickness of the stratum corneum. After spraying B, the increase of thickness of the stratum corneum was the smallest which ranged from 0.005 to 0.00 65 mm. But spraying with C, the thickness increase of the stratum corneum was the largest (0.005–0.0074 mm). The thickness of the exocarp showed an upward trend as a result of spraying. The exocarp cells were significantly thickened when sprayed 1–2 times. They fluctuated after three sprays but still showed a thickening trend, yet the thickening was slow. The largest change happened when C was sprayed 1–5 times, during which the change ranged from 0.1337 to 0.976 mm. However, smallest change (0.1287–0.976 mm) happened when sprayed 1–5 times with A.

3.6. Effects on the length and area of fruit sarcocarp cells

The effects of spraying times on the fruit sarcocarp cell length and sarcocarp cell area were relatively small in all the test plants (Fig. 7). After spraying, the length of sarcocarp cells showed a downward trend. The cell length was still smaller than that of the CK when sprayed 4–5 times. But when formula B was sprayed
for the second time, the length of sarcocarp cells became the shortest (0.0698 mm). The length of the sarcocarp also reached the shortest, when A was sprayed for the 4th time (0.0738 mm) (see Fig. 8).

3.7. Effects of number of spraying on sarcocarp cells and intercellular

Fig. 3 shows the effects of spraying times on number of sarcocarp cell and intercellular among the fruits of the test plants. The effect of spraying formular A on the number of sarcocarp cells showed a downward trend (21.6–13.6). While the effect on the number of sarcocarp cell gaps was fluctuating. The number of gaps between sarcocarp cells reached 13.5–14.4, while under other conditions, the number of gaps was smaller (12.3) than CK. The number of sarcocarp cells and the number of gaps were unstable when B was sprayed. The number reached the maximum (32.6) when sprayed twice, the number of gaps recorded was 18.9. Cell number reached 24 when sprayed 4 times with gap number 19. The num-

Fig. 4. Changes in the thickness of the upper and lower epidermal cells of jujube leaves in different treatment group. Notes: CK is the control group, sprayed with water; A1-A5 is sprayed with A (hypersensitive protein + spermidine + salicylic acid) 1–5 times, B1-B5 is B (hypersensitive protein + spermidine + DA-6) sprayed 1–5 times, C1-C5 is C (spermidine + salicylic acid + DA-6) sprayed 1–5 times.

Fig. 5. Changes in the thickness of the upper and lower epidermal stratum corneum of jujube leaves in different treatment groups. Notes: CK is the control group, sprayed with water; A1-A5 is A (hypersensitive protein + spermidine + salicylic acid) sprayed; B1-B5 is B (hypersensitive protein + spermidine + DA-6) sprayed; C1-C5 is C (spermidine + salicylic acid + DA-6) sprayed.
4. Discussion

The leaf is the vegetative organ of the angiospermic plant and possessing largest transpiration area. In general, the xerophytes are adapted to the drought environment wherein the characteristics of leaf epidermis possess stratum corneum and well-developed palisade cells. Thick epidermal cells and stratum corneum have functions or resistance to some environmental factors. These are heat insulation, water retention and damage resistance. As a result, the plants become suitable to adapt to low temperature, strong light, and physiological drought (Cai and Song, 2001; Li et al., 2009). The well-developed mesophyll palisade cells of xerophyte can not only improve photosynthetic efficiency, but also avoid burns of mesophyll cells caused by intense light in arid regions. The smaller and denser the cells of the palisade, the higher the efficiency of the plant’s use of light energy (Li et al., 2002).

Fig. 6. Changes of stratum corneum and exocarp thickness of jujube fruits in different treatment groups. Notes: CK is the control group, sprayed with water; A1-A5 is A (hypersensitive protein + spermidine + salicylic acid) sprayed; B1-B5 is B (hypersensitive protein + spermidine + DA-6) sprayed; C1-C5 is C (spermidine + salicylic acid + DA-6) sprayed.

Fig. 7. Changes in sarcocarp cell length and area of Jun jujube in different treatment groups. Notes: CK is the control group, sprayed with water; A1-A5 is sprayed with A (hypersensitive protein + spermidine + salicylic acid), and B1-B5 is sprayed with B (hypersensitive protein + spermidine + DA-6), C1-C5 is C spermidine + salicylic acid + DA-6) sprayed.
sub-epidermis consisted of 4–6 layers of cells but arranged in a flat elongated rectangle manner. It gradually became smaller from outer to the inner cells direction and arranged compactly. The cell walls were relatively thick, and the lignification was obvious. Sub-epidermal cells were tightly connected to sarcocarp cells and the outermost epidermal cells. Wang et al. (1985) opined that jujube leaves are bilateral, and their palisade tissue is lesser than spongy parenchyma. However, the leaves of sour-, jun-, apple-, pear- and dog head jujube are considered to be isolateral (Wei and Bi, 1997; Cao et al. 2004). In the present study the mesophyll part of the leaf is composed of columnar cells of different lengths. Cells closer to the upper epidermis are long columnar. The parts near the lower epidermis are composed of short columnar and a few oval cells. There is no obvious differentiation of palisade tissue and spongy tissue, so jujube leaves were judged to be isolateral (Liang et al., 2018; Nie et al., 2017). This indicates that the leaf structure is conducive to improving photosynthetic efficiency and adapting to arid environments. At the same time, the thickness of the lower epidermal cells of the leaves is increased by spraying the compound regulators. But whether this can promote transpiration and photosynthesis needs further study. The present study revealed that after spraying 1–5 times, the thickness of the stratum corneum of the leaves showed an upward increasing trend. The upper and lower epidermis of it was measured 0.0057 and 0.0048 mm, respectively (Guo et al., 2017). The upper epidermis had longer exposure to sunlight than the lower epidermis, which could enhance the effects of jun jujube resistance. After spraying, the mesophyll and thickness of the leaves of each experimental group did not change significantly, but the main vein thickness (P < 0.05). The thicker the central vein of the leaf, the stronger its water control ability will be when suffered high temperature. This relationship accelerated transpiration in arid environment and high temperature to reduce leaf temperature and avoid damage to plants (Liang et al., 2018; Nie et al., 2017). So, the result of the present investigation shows that after spraying, the jun jujube leaves have enhanced transmission ability, which is conducive to improving their resistance. Spraying with formul A is better than that of B and C.

In the study of Lingwu long jujube, it is believed that multiple fruit epidermal cell layer, thicker epidermis and closer outer epidermal cell arrangement made it difficult for individual fruit to crack. On the contrary, fewer layers of epidermal cells, thinner epidermis, and looser outer epidermal cell arrangement made individual fruit susceptible to cracking (Yang, et al., 2010; Wang et al., 2017). In the present study, the stratum corneum and exocarp thickness of jun jujube fruits in the control group were 0.005 and 0.0976 mm, respectively. The thickness of stratum corneum and exocarp was significantly increased after spraying the regulators, indicates that it could increase the quantity and help to reduce the occurrence of cracked fruits. Li and Gao (1990) pointed out that the thickness of stratum corneum of huping-, black leaf - and langzao jujube were 6.81, 6.41 and 8.28 µm, respectively. Stratum corneum of those plants were thicker than that of jun jujube. The average thickness of stratum corneum of po jujube has been reported as 4.81 µm (Wang, 1974). This value was slightly thinner than the stratum corneum of jun jujube fruit. In the study of cracked fruit it has been seen that the fresh jujube cracked fruit had a certain insignificant negative correlation with the thickness of the stratum corneum (Shi and Wang, 2003).

5. Conclusion

The stratum corneum and epidermis are thick, the cells are tightly arranged, and they are tightly bound to sub-epidermal cells, so they have good resistance to storage. The peel and epidermis of jun jujube control group are slightly thinner than other varieties of jujube, but they are closely bound to subepidermis. The tightness of the sub-epidermal cell arrangement and the connection with the mesocarp, directly affect the water retention and anti-wrinkle properties of jujube fruits. This study reports that sub-epidermal cells of jun jujube possess similar kind of properties. However, the application of the compound regulators and the number of sprays also affected the exocarp cells. After the application of the A compound regulator, the subepidermal cells of the jun
jujube exocarp had irregular appearances and characteristics. There were fewer flat and regular cells, showing that the number of sprays of the regulators should be controlled. So, the relationship between the number of sprays and their effects need further study. In the present study, it appeared that the spraying of the three compound regulators and the number of sprays all made no significant difference in the number of mesocarp cells, the tightness of the arrangement of cells, and the number of gaps between cells of jujube fruits. The number of cells per unit area and the number of gaps of the fruits increased significantly after spraying compound regulators B and C. The number of cells per unit area and the number of gaps in the sarcocarp after application of A, basically lowered than those in the CK, which was beneficial for improving fruit yield and quality. Therefore, spraying A compound regulator is more helpful than the other two in improving fruit yield and quality.

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