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Analysis of the Inclusion in Xylem Sap and the Effects on Grape Growth

Zhenqiang Xie, Ting Zheng, Xudong Zhu, Xiaopeng Li, Haifeng Jia, Jinggui Fang*

College of Horticulture, Nanjing Agricultural University, Nanjing, China

ABSTRACT

Growth of grapevine and fruit quality was compared with the treatment of bleeding sap, to explore the influence of xylem sap on the grape growth and development. By the analysis on growth dynamics, it was found that xylem sap postponed the phenophase, decreased the rate of germination and the growth of new shoot, and the shedding of flowers and fruitlets was higher than control group. For fruit quality, bleeding of xylem sap decreased the cluster weight, and heavy bleeding also decreased the weight of single fruit and content of soluble solids. Therefore, attention must be paid to the bleeding of xylem sap in order to reduce the undesirable influence to grapevine. The inclusion in the xylem sap of grape was analyzed by different methods with different instruments. On the basis of previous research, the different of the inclusion in xylem sap between different varieties or times was compared, which can provide the base for the insight of the roles of xylem sap during grape growth and development.

Key words: *Grapevine; Xylem sap; Growth and development; Inclusion; Composition*

Introduction

The growth and development process of grapes mainly includes the changes in the various phenological phases of grape growth and development, which mainly include the germination period, leaf development period, inflorescence budding period, inflorescence separation period, inflorescence display period, blooming period, physiological fruit dropping period, and fruit Expansion period, hard core period, color conversion period, full maturity period and dormant period. Grape is a berry fruit, and its yield and fruit quality are also the focus of scientific research and production. The yield of grape fruit is mainly affected by factors such as cultivation method, density, water and fertilizer management, variety, etc., while fruit quality mainly includes the influence of fruit color, single seed, sugar and acid content and hardness. The production of bleeding sap often occurs on grapes, so the experiment systematically studied the effects of bleeding on the growth and development of grapes and fruit quality, in order to derive the role of plant sap in growth and development.

Wound sap refers to the sap that flows from the xylem of plant wounds. The wound fluid is subjected to the root pressure of the plant, moves to the above-ground part in the duct and flows out. Wound fluid contains part of plant tissue fluid, cell fluid and organic matter between cells. Predecessors' research on the bleeding fluid of grapes is only limited to the determination of hormones and oxidative active substances in the bleeding fluid, and the data collected and measured are also relatively single. Therefore, through the determination of the contents of the wounded flow of the grape xylem, the changes of the wounded flow and the content of related contents at different times in the wounded flow period can be obtained.

The main research in this experiment is the bleeding fluid of the xylem. Long distance transportation in plants mainly includes xylem transport and phloem transport. Among them, phloem transport mainly transports organic synthetic substances in leaves, while xylem transport mainly involves transpiration and roots. Transport water and minerals under pressure. Since grapes have not yet sprouted during the bleeding stage of grapes, the activity of grape bleeding sap is mainly related to the root activity of grapes in spring, and the active substances in bleeding sap are mostly related to the root active substances transported through xylem. Studies have shown that in addition to common mineral elements, the bleeding fluid of plants also contains a certain number of soluble sugars, soluble proteins, amino acids, enzymes, and a small number of nucleic acids [1-3]. In this regard, this experiment analyzed the differences in the bleeding sap of different varieties through the determination of the contents in the bleeding sap of different varieties.

Materials and Methods

Sample Preparation and Collection of Wound Fluid

The tested tree comes from the Jiangsu Agricultural Expo Garden, the tree is 5-6 years old, the tree is in good vigor, and the vineyard is managed regularly. The grape varieties tested were 'Summer Black, Rosario Biancorio, Kyoho, Wink. The tested tree species were pruned in normal winter, 3 trees of each type were treated with bleeding and 3 trees were compared. The growth dynamics of buds, branches and fruits of each grape variety were measured.

The bleeding fluid required for the experiment was collected during the sprouting period of the vine. Cut the annual shoots short, and gird the phloem 3~5 cm away from the cut. Rinse the cut with sterilized double distilled water (ddH_2O), then wipe dry with sterilized absorbent cotton, then put on the sterilized glass bottle, seal the mouth of the bottle with absorbent cotton, and collect the wound fluid. Choose 3 trees for each species. Take 3 time periods of wound fluid every day (8:00-10:00, 12:00-14:00, 15:00-17:00), and collect the outflow wound fluid in a 50mL centrifuge tube. Place it in a refrigerator at -40°C for related experiments.

Observation of Phenological Period and Rate of Germination, Length of New Shoots, Rate of Falling Flowers and Fruits

For the trees treated with blemish flow, select the annual branches whose tops were cut due to blemish flow treatment, the annual branches in the middle of the main branches, and the annual branches near the main branches at the end of the main branches, and count the total number of buds on the branches. After the buds begin to sprout, count the number of buds every other day.

After the new shoots start to grow, on the annual shoots treated by the wound, each tree chooses a new shoot with the same length and starts to measure its length; at the same time, select the middle section of the tree and a position close to the main trunk, and choose the same length for the new shoot, start measuring its length change.

For the determination of fruit drop rate, the annual branches treated with wounding flow were selected, and the inflorescences of similar size were measured. When the grapes are in full bloom, wrap the inflorescences to be measured with a fine-grained mesh bag, and count the flower and fruit drops every other day. For each tree, three inflorescences are taken from the annual branches and the average value is taken. Since the number of flowers and fruits of different varieties is quite different, the percentage is used instead of the specific number to calculate the drop rate. Carry out simple rain-proof treatment on the measured inflorescence.

Determination of Fruit Quality

The determination of fruit quality mainly includes the ear weight of the fruit, the single seed of the fruit, the hardness of the fruit, the soluble solid content of the fruit and the titratable acid content. The ear weight and single-grain weight of the fruit are weighed with an electronic balance; for the determination of fruit hardness, 10 fruit kernels are randomly selected, using the GY-1 fruit hardness meter, two symmetrical positions of the fruit are selected for measurement, and the average value is selected, soluble solid form The content of the substance is measured with the LB50T handheld sugar tester; the titratable acid of the fruit is determined by the acid-base neutralization method, the specific method is: Weigh 5 g of grape pulp, grind it with 5 ml of distilled water and centrifuge at 12000 g for 10 min, take 5 ml of supernatant to a new test tube, add 5 ml of distilled water and second phenolphthalein solution, and use 0.01 mol/L NaOH standard solution Titration. Take 10 fruits for each treatment and repeat the measurement 3 times.

Soluble Protein and Soluble Sugar Determination

Refer to whose method; use the anthrone colorimetric method to determine the soluble sugar content. Aspirate 0.5 mL of wound fluid into a 20 mL test tube, repeat 3 times, add 1.5 mL of distilled water, and then add 0.5 mL of ethyl anthrone and 5 mL of concentrated sulfuric acid, and carry out related reactions according to the method of drawing a standard curve.

Refer to whose method, use Coomassie brilliant blue method to determine the content of soluble protein. Take 3 clean centrifuge tubes, draw 1 mL of the wound fluid, and add 5 mL of Coomassie Brilliant Blue G-250. After mixing thoroughly, let it stand for 2 min. Use distilled water as a control and use a colorimetry of 1 cm light path. The absorbance of the dish was measured at a wavelength of 595 nm, and the protein content X (μg) in the sample was obtained from the standard curve.

POD, CAT and Malondialdehyde Content Determination

The activity of peroxidase (POD) was determined with reference the guaiacol method of Kochba [4]. The change in absorbance at 470 nm per minute was 0.01 as one unit of POD activity, and the result was expressed in U/mg protein. Catalase (CAT) activity was determined with reference to the method of Cakmak [5]. The change in absorbance at 240 nm per minute of the reaction solution at 0.001 was one unit of enzyme activity, and the result was expressed in U/mg protein. Referring to the method of Malondialdehyde (MDA) contents were quantified by using thiobarbituric acid method. Three technical repeats were generated for all the quantifications, and results were analyzed using [6,7].

IAA, ABA and GA3 Content Determination

Accurately weigh 2 g of the sample, grind it with liquid nitrogen, add 10 ml of 80% cold methanol, and extract overnight at 4°C (for extracting the wound stream, take 50 ml of the wound stream, vacuum dry, and add 80% methanol for extraction). The next day, vortex, centrifuge (4000 g, 4°C, 10 min), and take the supernatant. Add 10 ml of 80% cold methanol to the residue, vortex and shake, extract and centrifuge, take the supernatant, and repeat. Combine the supernatants, filter with suction, and lyophilize; add 3 ml of pH=3.0 buffer, add 2 volumes of petroleum ether, vortex and centrifuge (9000g, 4°C, 5 min), discard the ether phase, save the water phase, and repeat twice. Add 6 ml of ethyl acetate, extract, (9000g, 4°C, 5 min), remove the water phase and leave the ester phase. Add 3 ml of ethyl acetate, extract, (9000 g, 4°C, 5 min), remove the water phase and leave the ester phase. Then add 1 ml of ethyl acetate, extract, (9000 g, 4°C, 5 min), remove the water phase, leave the ester phase, combine the ester phase, and freeze-dry; dissolve in 10 ml of pure methanol, pass through a C18 pre-column, 0.22 μm Filter by suction and store at 4°C for testing.

Mobile phase: methanol-water (containing 0.075% glacial acetic acid)=45:55 (V/V), pH=3.0. Flow rate: 0.3ml/min; Sensitivity: 0.3 AUFS; Detection wavelength: n=254 nm. Time program control, concentration gradient elution. The standard 11.4 mg/L GA3, 10.4 mg/L IAA, and 11.2 mg/L ABA (SIGMA products) were used as standard products. The peak times were 8.5 min, 16.35 min, and 20.3 min, respectively. Injection volume: 5 ul, repeat three times.

Results and Analysis

Determination of Wound Flow during Grape Bleeding Period

Analyze the volume of bleeding fluid collected from grapes. According to the date of collecting wound fluid, the amount of wound fluid collected every day is counted. Different varieties of grapes have obvious differences in the outflow of bleeding fluid on different dates. Among them, compared with the three varieties of 'Summer Black', 'Wink' and 'Kyoho', 'Rosario Bianco' has 8 days ahead of the start date of the injury. The injury began on March 11. The daily injury flow has been at a relatively high level for a long time. From March 21 to 31, the daily injury flow reached more than 20 ml. The three varieties of 'Summer Black', 'Summer Black' and 'Kyoho' all started to suffer injuries from March 19, and the number of injuries was much smaller than that of 'Rosario Bianco'. Among them, 'Summer Black' and 'Wink' reached the maximum daily wound flow rate on the second day (March 23) after the wounding fluid was produced, and then the wound flow rate dropped rapidly, starting on March 29th, at Low level (<2 mL); and after the occurrence of the 'Kyoho' injury flow, its daily injury flow slowly increased. On March 25, its daily injury flow reached its maximum value, and then gradually decreased slowly Figure 1. At the same time, all 4 varieties started to stop bleeding around April 8, indicating that the germination of spring buds of grapes requires the tree to provide sufficient water through the sap and nutrients to participate in the physiological metabolic processes such as cell formation, and inhibit the occurrence of bleeding. At the same time, the metabolic activity of the wound becomes more active with the buds sprouting, completing wound healing. In the process of collecting wound fluid, it is found that the amount of wound fluid produced at different times of the day is not exactly the same, as shown in Figure 2.

It can be seen that the temperature changes from low to high during the day from 8:00 to 12:00, and the root activity becomes more and more active as the temperature rises, and the flow of sap strengthens. In the afternoon, the root activity is the opposite. In addition, the soil temperature in spring is relatively low, and the water absorbed by root activity in the afternoon cannot make up for the water deficit caused by the injury in the morning. Therefore, the overall performance of the grape injury in the day is more in the morning than in the afternoon.

The Effect of Bleeding on Grape Germination Rate

The germination rate is determined by buds with no obvious damage on the annual branches. The germination rate is

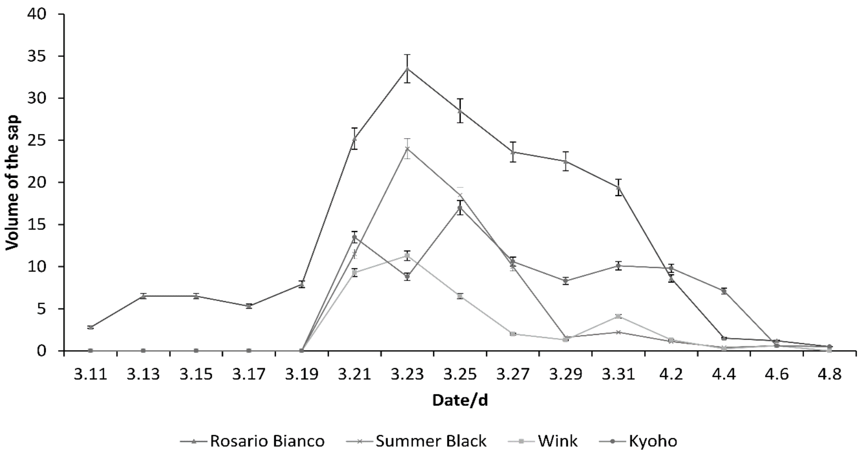

Figure 1: Volume changes of xylem sap between different grape varieties.

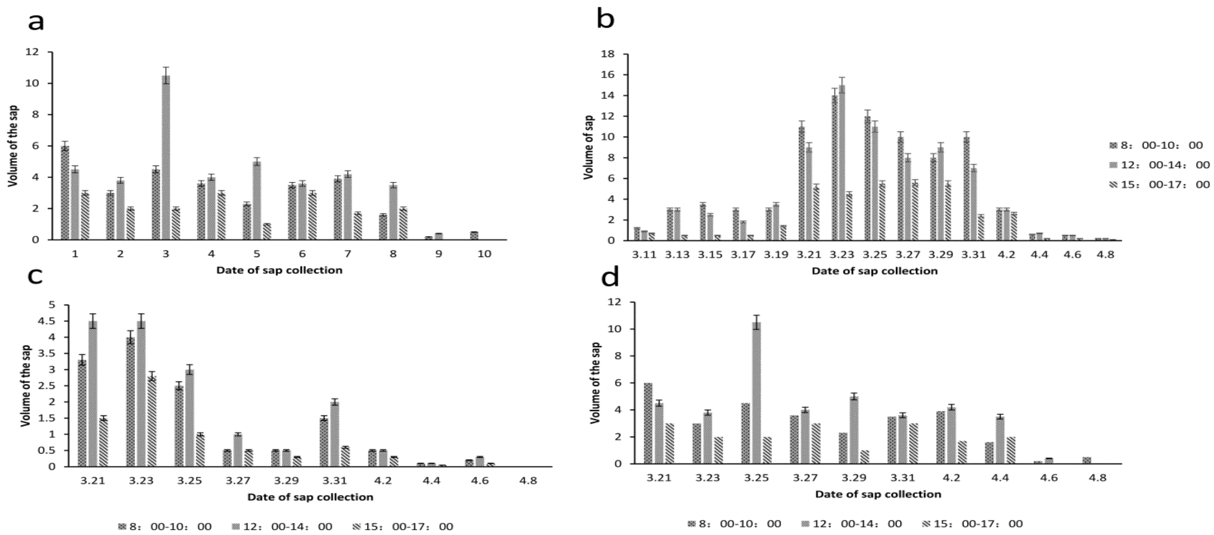

Figure 2: Volume of the sap collected during different time.

A: Summer Black; b: Rosario Bianco; c: Wink; d: Kyoho

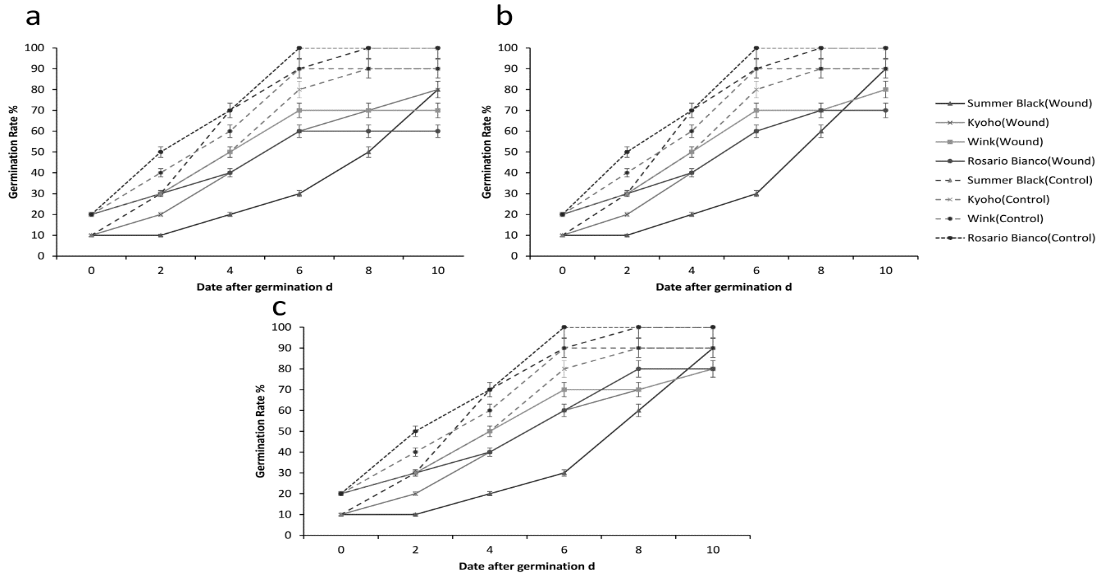

Figure 3: Germination rate of branches between different varieties.

A: front branches; b: medium branches; c: basal branches.

affected by the natural environment such as temperature and moisture, as well as the level of fertilization, pruning and cultivation methods and other human factors [7]. Since the cultivation and management methods of the trees used in the experiment are the same, the germination rate can reflect the impact of the wound treatment from a certain angle. The shoot germination rate of different varieties and different positions of the wound treatment is shown in Figure 3.The difference in the germination rate is mainly reflected in the shoots treated by the wounded stream. The shoots that have been short-cut and injured have a greatly reduced germination rate. Among them, the germination rate of ‘Rosario Bianco’ was the most significantly lower than that of the control group. The germination rate of the shoots in the control group was 100%, while the average germination rate of the shoots in the treatment group was only 60%, much lower than the control group. The germination rate of "Summer Black" in the treatment group was 70%, which was much lower than that of the control group. The germination rate of 'Summer Black' and 'Kyoho' were 20% and 10% lower than the control, respectively. The germination rate of annual shoots in the middle of the treatment group was also lower than that of the control group, but the degree of decrease was lower than that of the shoots treated with apical cuts. Among them, the ‘Rosario Bianco’ was only 60%. The germination rate of the end shoots is not much different, generally 10% lower than the control group, and only the ‘Rosario Bianco’ treatment group is 20% lower than the control group.

The size of the germination rate can reflect the viability of the branches. Statistics have found that after the branches of the grape main branches far away from the main branches are cut, they lose minerals, hormones, sugar, protein and other nutrients due to the wound. The main trunk nutrition first supplements the nutrient loss of the end branches of the main branches. This results in nutrient deficiency and reduced viability of the branches at the front end, which is manifested as a reduced germination rate.

The Impact of Injury Flow on Flower and Fruit Drop

During the growth of grapes, flower and fruit drop often occurs. Normal flower and fruit drop are a process of self-regulation and nutrient balance during the grape growth cycle. However, in the cultivation process, often due to improper cultivation and management measures, or natural environmental influences, the phenomenon of grape falling and fruit falling is very serious, which greatly reduces the yield and often causes serious economic losses. In production, it is possible to appropriately reduce the fall of flowers and fruits through methods such as rational use of fertilizers, balanced nutrition, and protected rain cultivation.

The flower and fruit drop rate of "Summer Black" is shown in Figure 4. Compared with the control group, the flower drop rate at each position of the tree in the treatment group of 'Summer Black' was about 5% higher; and the flower drop rate at the front position was also higher than that of the middle inflorescence. The difference in fruit drop rate is even greater. The fruit drop rate on the front branches of the tree treated with 'Summer Black' is 20% higher than the control group, while the fruit drop rates on the middle and end branches are about 3 ~ 4% higher than the control group.

The flower and fruit drop rate of ‘Rosario Bianco’ is shown in Figure 4. 'Rosario Bianco' was the variety that produced the most bleeding among the four treated varieties. Its front-end flower drop rate was higher than that of the control group by more than 15%, and the middle-end and end-end percentages increased by about 10% and 7%. Compared with the control group, the fruit drop rate has increased by about 20%. It can be seen that the bleeding has seriously affected the normal fruit setting of the grapes. Compared with the control group, the rate of flower and fruit drop in the treatment group of 'Summer Black' was lower among the 4 varieties, and the rate of flower drop and fruit drop in the control group itself was also 4 The lower in the variety. The law of the flower and fruit drop rate of 'Kyoho' grapes (Figure 4) is basically the same as that of the 'Summer Black' grape flower and fruit drop rate. Among the above 4 varieties, in the control group, the flower and fruit drop rate of 'Kyoho' was the highest, followed by 'Summer Black' and 'Rosario Bianco', and the flower and fruit drop rate of 'Summer Black' was the lowest; while the treatment group Compared with the control group, the increase in the rate of flower and fruit drop was still the highest in 'Rosario Bianco' compared to the control group. The increase in the rate of flower drop in 'Kyoho' compared to the control group was not very large, but its fruit drop rate was also much higher than that of the control group. The rate of flower and fruit drop in different positions is not exactly the same in response to the wound flow. The increase in the rate of flower drop and fruit drop in the branches treated with wound flow is higher than that of other parts.

The Impact of Bleeding on Fruit Quality

The quality of the fruit mainly includes the ear weight, single seed, color, sugar and acid content of the fruit [8]. For fruit trees, the improvement of fruit quality has always been the focus and focus of research. Figure 5 shows the change of ear weight of different varieties. The average ear weight of the 'Summer Black' treatment group was 375g,

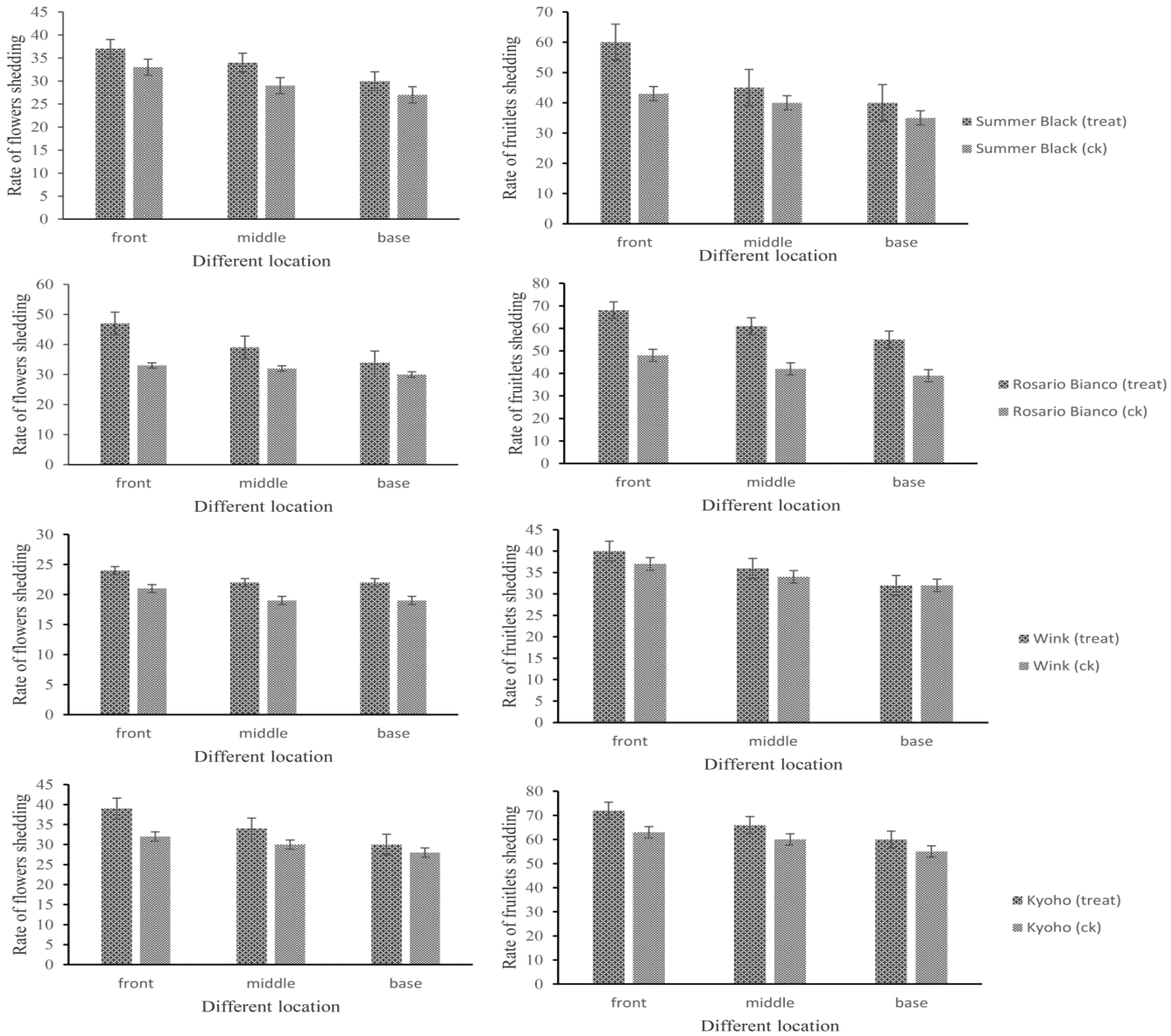

Figure 4: Rate of flowers and fruits shedding.

the average ear weight of the control group was 415g; the average ear weight of the 'Rosario Bianco' treatment group was 290 g, and the average ear weight of the control group was 448g; the average ear weight of the 'Summer Black' treatment group The ear weight was 390 g, the average ear weight of the control group was 445g; the average ear weight of the 'Kyoho' treatment group was 312g, and the average ear weight of the control group was 402g. The ear weight of the 'Rosario Bianco' treatment group was 35.5% lower than that of the control group, which was the highest among the 4 varieties. The ear weight of 'Kyoho' dropped by 22% followed by the ear weight of 'Summer Black' and 'Summer Black'. The weight drop is relatively small. As shown in Figure 5, the single grain weight of 'Summer Black' is the largest among the four varieties, about 10g, followed by 'Kyoho' and 'Rosario Bianco', about 8.5g, and 'Summer Black' 'The single grain weight is the smallest, about 3.8g. Among them, the single-grain weight of "Rosario Bianco" in the treatment group was 8.22g, and the control group was 8.45g; the "Summer Black" treatment group was 3.68g and the control group was 3.89g. There was no significant change in the single-grain weight of 'Summer Black' and 'Kyoho'.

The analysis shows that although bleeding has a small effect on the single-grain weight of fruits, it aggravates the fall of flowers and fruits of various varieties, which will still cause different degrees of reduction in single ear weight, which will inevitably manifest as a decrease in yield in production. For varieties with high fruit setting rates such as 'Summer Black' and 'Summer Black', a reasonable fruit drop rate can reduce the workload of thinning flowers and fruits, but for the 'Kyoho' variety, it will not only cause a decline in yield, but also the commerciality of spike type is reduced.

Fruit hardness is shown in Figure 5. According to the experiment, the average fruit hardness of the 'Summer Black' treatment group was 6.15 N, and that of the control group was 6.21 N; the average fruit firmness of the 'Rosario

Bianco' treatment group was 6.39 N, and the control group was 6.42 N The average hardness of the 'Summer Black' treatment group was 7.04 N, and the control group was 6.97 N; the average hardness of the 'Kyoho' treatment group was 5.41 N, and the control group was 5.52 N. Comparative analysis shows that there are differences in hardness between the varieties, but there is no significant difference between the treatment group and the control. The soluble solids and titratable acid content are shown in Figure 5. Among them, the soluble solid content of the 'Summer Black' and 'Rosario Bianco' treatment groups was significantly lower than that of the control group. Compared with the control, the soluble solids content of 'Summer Black' and 'Kyoho' were not significantly reduced, and the acid was titratable. From the perspective of the effects of bleeding on the hardness and soluble sugar and soluble protein content of grape berries, the bleeding did not have a significant and substantial impact on the internal quality of grapes.

Determination of Soluble Sugar in Grape Wound Fluid

Soluble sugar plays an important role in the growth and development of plants, and is also an important stress and signaling substance in plants [9,10]. The content of soluble sugars four types of wound fluid was determined by the enthrone method. The determination of the soluble sugar content in the sap of grapes can provide guidance for future research on the role of sap in the growth and development of grapes. The content of soluble sugar in grape bleeding sap is shown in Figure 5.

According to the determination of soluble sugars, it is found that different varieties have certain differences in different stages of wounding. Observed from the overall content of soluble sugar, the content of soluble sugar in the wound fluid of 'Summer Black' was higher than that of the other three varieties, and during the period from March 31 to April 2,'Summer Black' 'The content of soluble sugar in the wound fluid reached the maximum, about 0.06 mg/mL, and the other three varieties were significantly less than this value. There is also a certain pattern in the change of soluble sugar with date. Among them, the content of soluble sugar in the bleeding fluid of the three varieties of 'Summer Black', 'Wink' and 'Kyoho' all had a significant gradual increase. 'Wink' and 'Kyoho' were on March 27, respectively. Sun and March 29 reached the maximum, "Summer Black" was slightly delayed, and reached the maximum on April 2. Compared with the other three varieties, the soluble sugar content of 'Rosario Bianco' has always been maintained at a lower level, and there is no obvious change; on the contrary, on March 31, when the secretion of wounds is about to stop, a certain amount of Increase.

Grape bleeding caused the tree's water to decrease, forming a stress state similar to drought. Soluble sugars can regulate the osmotic potential of cells, and the increase in its content is conducive to improving the drought resistance of plants. Soluble sugar is also an important regulator to improve the cold resistance of plants. However, after April 4, the temperature rose steadily, the root water absorption activity strengthened, the low temperature stress factor was gradually relieved, and the soluble sugar content in the wound fluid appeared slightly decreased.

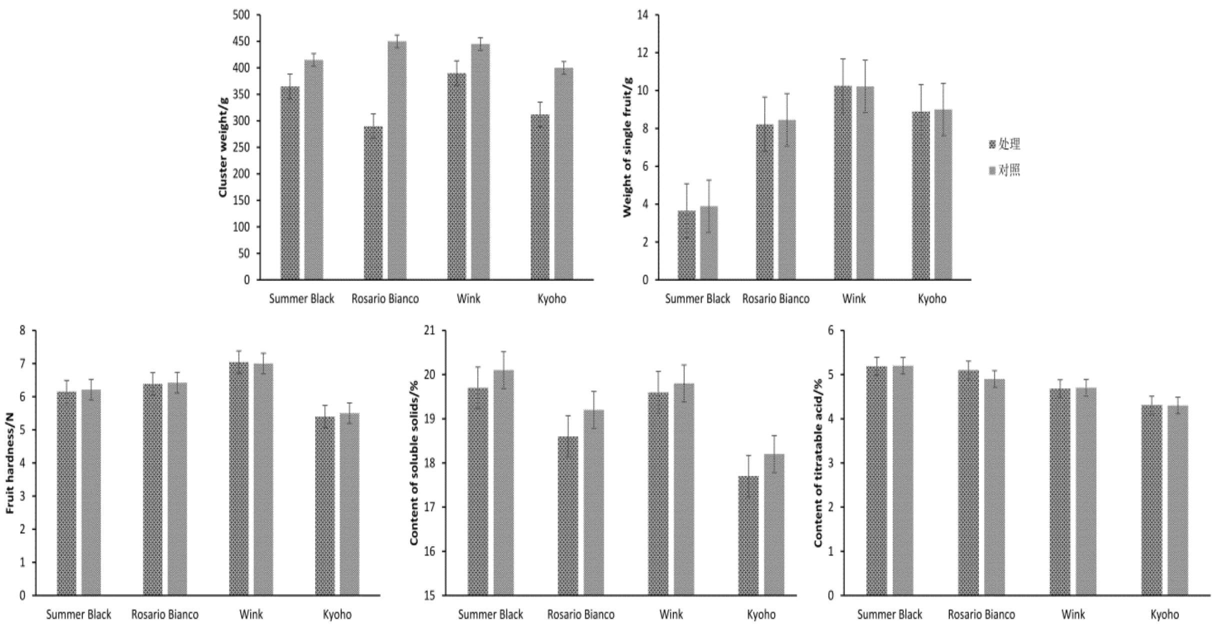

Figure 5: Cluster weight, single fruit weight, fruit hardness, soluble solids content and titratable acids content of different varieties.

Determination of Soluble Protein in Grape Wound Fluid

Protein is an important part of all living organisms, and it accounts for about 18% of the total mass in living organisms. The role of protein in life activities is also diverse. People's research on protein has experienced processes from morphology and organization, to adversity physiology, to functional expression, and signal transduction [11-13]. To this end, this study used the determination of the soluble protein content in wound fluid to reflect the content of soluble protein in wound fluid from a certain angle and the influence of wound fluid treatment on the dynamic changes of soluble protein expression. Lay an important foundation for proteomics analysis [14,15].

The determination of soluble protein in wound fluid is shown in Figure 6. The content of soluble protein in the bleeding fluid of the above four grape varieties showed a certain upward trend as a whole. Specifically, there are differences in the time when the soluble protein rises in different varieties. Among them, the content of soluble protein in the wound fluid of 'Summer Black' was higher than other varieties at the beginning (March 21) and the maximum date (March 31). Unlike other varieties, 'Summer Black' had no obvious change in the soluble protein content in the wounding fluid during the initial stage of sprouting (March 21 to March 25). After March 27th, the content of soluble protein showed a more obvious increase, and reached the maximum on March 31, and then there was a slow decline. The soluble protein in "Summer Black" wound fluid also appeared similar to "Summer Black" from March 21 to March 25, with a certain amount of decline, and then began to gradually increase. From March 21st to April 2nd, the soluble protein content of the ‘Kyoho’ grape wound fluid rose slowly, and reached the maximum on April 2nd, and then there was a slight decrease. The soluble protein in the wound fluid of 'Rosario Bianco' also rises overall, but there are certain fluctuations, which may be related to its larger wound volume.

Determination of POD and CAT in Wound Fluid

Peroxidase (POD) is a ubiquitous active enzyme in plants. POD can effectively remove H2O2 formed by superoxide free radicals in plants, and it also plays an important role in Reactive Oxygen Species (ROS) in plants. Its role as a key enzyme in plants has been valued Studying the change of POD content in wounding fluid can reflect the effect of grape bleeding and the possible response of pruning. The POD activity in different wound fluids [16,17]. According to the dynamics of POD, these four grape varieties are all rising dynamically. Among them, the change trend of POD activity in the bleeding fluid of 'Summer Black' and 'Kyoho' grapes was basically the same. It rose slowly from March 21 to April 4, and then decreased slightly. The activity of POD in the wound fluid of 'Summer Black' reached its maximum on March 23, and then its enzyme activity maintained a small fluctuation of 135-145 U/ml·min. The POD activity in the wound fluid of 'Rosario Bianco' increased to a certain extent from March 21 to March 25, followed by a slight decrease on March 27, and then continued to rise. On April 4, its activity reaches its maximum. Interruption damages the tree, and the wound accelerates the loss of nutrients in the tree, causing the accumulation of active oxygen and free radicals in the tree. The increase in POD activity may be related to the mechanical damage of the tree.

Catalase (Catalase, referred to as CAT) is a very important protective enzyme in plants. Its main role is specifically aimed at the effects of plant bodies under various stresses, decomposing and removing the large amount of H2O2 produced by plants under adversity conditions, reducing its adverse effects on plants protect various membranes

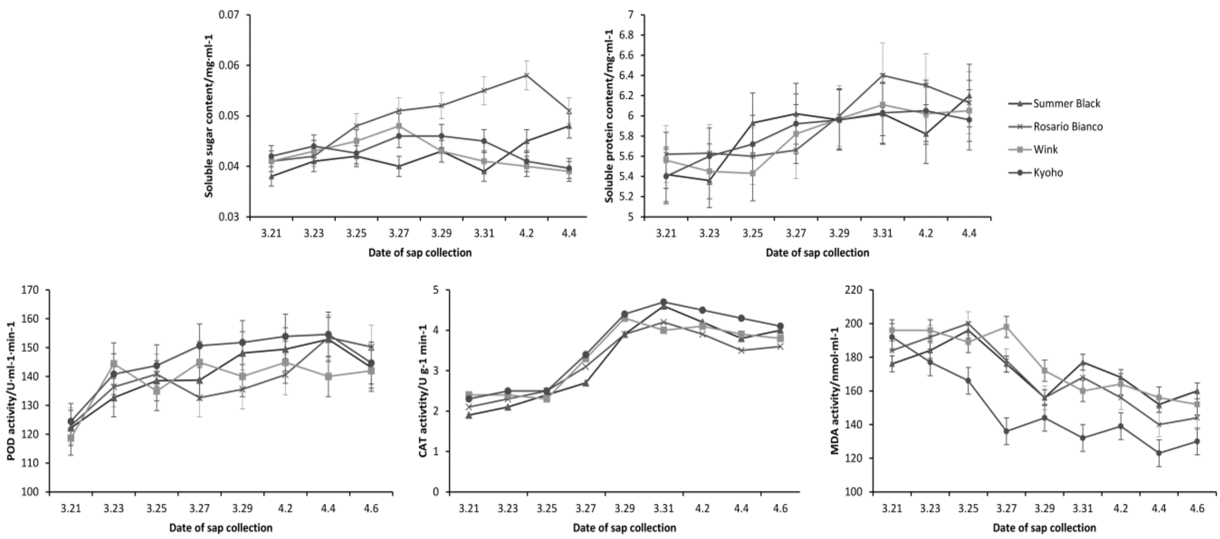

Figure 6: Change on content of soluble sugar, soluble protein and MDA in sap of grape. Changes in SOD and CAT activity in sap of grape.

and organs in plants from damage caused by H2O2. Like POD, the level of CAT activity can also reflect the stress resistance of plants from a certain level. The CAT activity in the wound fluid of each species used in the experiment is shown in Figure 6. The law of CAT activity in the wound fluid of these four species is very obvious. From March 21st to March 25th, its activity relatively little changed. From March 27th to March 29th, CAT activity there has been a significant improvement. CAT activity declined slightly from March 31 to April 6, but it was still at a high level.

Determination of Hormones in Wound Fluid

Phytohormones are a class of organic substances produced by the metabolism of plants. They move from the production site to the action site, and can produce obvious physiological effects at very low concentrations. They are also called endogenous hormones. Hormones exist in all stages of plant growth and development, from plant embryogenesis, seed germination, growth and development, fruit maturity to senescence and death, etc. all are regulated by hormones [18]. The general chemical structure of endogenous hormones is relatively simple, but their physiological effects in plants are very complicated, and their mechanism of action is also hot research topic [19]. The measurement results of IAA, GA3, and 6-BA in the above-mentioned four types of wound fluid are shown in Figure 7.

The overall trend of the IAA content in the bleeding fluid of these four varieties was to increase first and then decrease, and there were certain differences among different varieties. The specific analysis results are as follows: On March 14, the IAA content of 'Kyoho' was relatively high. Subsequently, the content of IAA in wounds of various varieties began to increase. Among them, the IAA in the wound fluid of 'Rosario Bianco' increased rapidly, but decreased rapidly after reaching the maximum on March 24, and began to be at a low level. After March 29, its IAA content was 4 varieties the lowest within. Although the IAA content of 'Kyoho' grapes was relatively low in the early stage, its increase was obvious. After March 24, it began to be at the highest level of the four varieties. The trends of the three varieties of 'Summer Black', 'Summer Black' and 'Kyoho' are basically similar.

The overall trend of GA3 content in the wound fluid of the 4 varieties is gradually increasing. Among them, from March 14 to March 24, the GA3 content in the wound fluid of 'Rosario Bianco' did not change significantly, but then it began to increase rapidly, and it was still at a relatively high level at the end of the wound and fluid period on April 11. High level. The content of GA3 in the wound fluid of the other three varieties all gradually increased, and then from March 29 to April 1, the GA3 content in the wound fluid began to fluctuate between 0.11 and 0.16 μg/ml.

The content of 6-BA in the wounding fluid of the four varieties all increased firstly and then decreased. Among them, the 6-BA content of 'Summer Black', 'Wink' and 'Kyoho' increased slowly from March 14th to March 24th, and began to decrease after reaching the maximum value on the 24th. From 24th to March 29th, the content of 6-BA in the wound

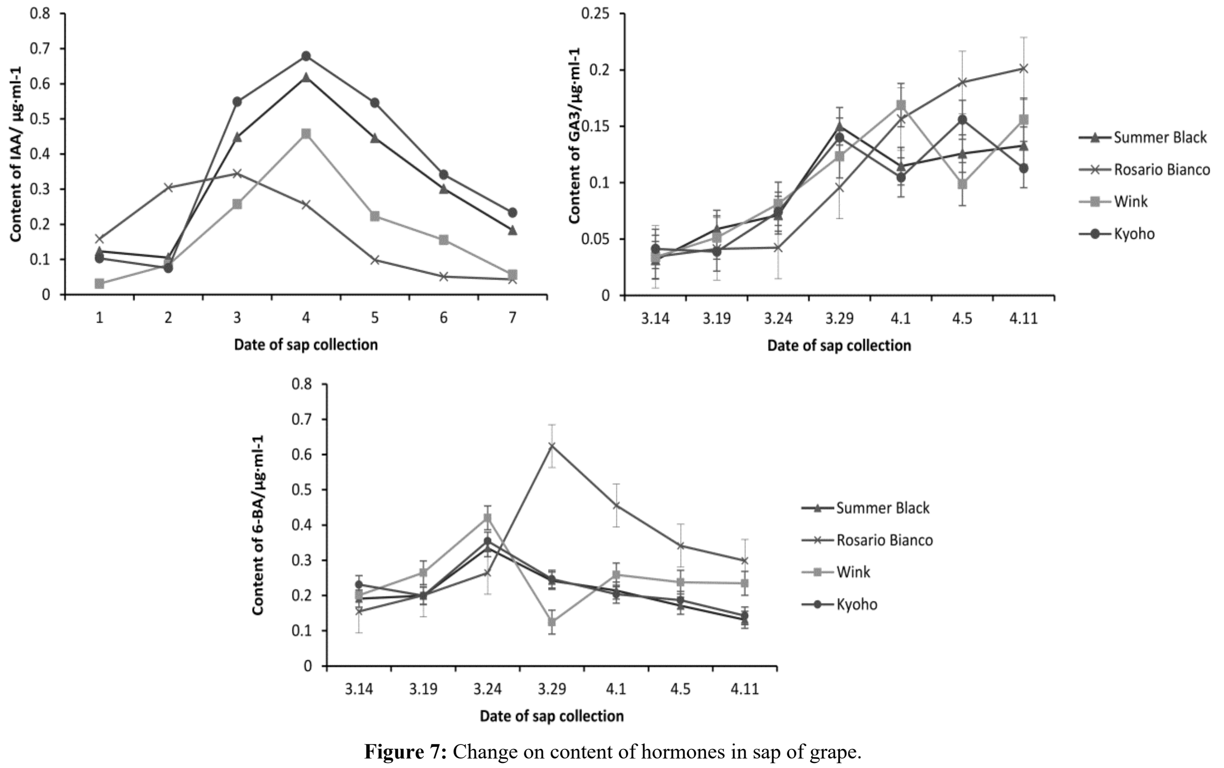

Figure 7: Change on content of hormones in sap of grape.

fluid showed a significant decrease, but then increased and remained at a relatively constant level. Although the 6-BA content in the injury stream of 'Rosario Bianco' was at a relatively low level in the early stage, it then increased significantly from March 24 to March 29, and reached on March 29 A maximum value then began to decline, but its value is still higher than the other three varieties.

Discussion

The process of grape growth and development can intuitively reflect various factors such as the light, temperature, moisture and soil fertility of the grape tree. The experiment statistically analyzed the growth and development changes of different varieties of grapes after treatment. The study found that the phenological period of grapes and fruit trees can be delayed to varying degrees after wound treatment, and there is a certain correlation between the delay of the phenological period and the amount of wound flow: the variety 'Rosario Bianco' that produces more wound flow has a phenological period. The delay is the most serious, the germination period is delayed by about 8d to 9d, and the varieties that produce less injury flow have relatively few days of delay in the phenological period. It can be seen that the strength of the injury flow in early spring can affect the phenological period of grapes, and the degree of influence may be related to the amount of injury flow produced by the grapes. Generally speaking, the main factors affecting the phenological period of plants are rainfall, temperature and other natural causes, and are also related to the growth of the plants themselves and the age of the trees. The occurrence of wounds may affect the nutrition of the tree and may also lose a certain number of hormones. Hormone measurement found that the content of GA3 and 6-BA in the wound fluid of 'Rosario Bianco' was higher during the wound period, and due to its large wound flow, a large amount of GA3 and 6 must be lost during the wound flow-BA. The main function of GA3 is to break dormancy, promote germination, and accelerate the elongation and growth of stems [20,21]. The function of 6-BA is mainly to promote plant cell differentiation and accelerate plant growth. Inducing the growth of dormant buds, etc [22,23]. During the sprouting period of the blemish stream, a certain amount of blemish stream flowed out of the experimentally treated tree, which caused a large number of hormones in the blemish stream to be depleted, which caused the phenological period of the treated tree to be delayed to varying degrees, the germination rate and the length of new shoot (Taking "Rosario Bianco" as an example, Figure 2) and others are also lower than the control group. During the physiological fruit drop period, the fruit drop rate of "Rosario Bianco", which produces the most injury flow, increased the most than the control group. The increase in fruit drop rate will directly affect the change of fruit tree yield (Figure 2).

Fruit quality has always been a hotspot in fruit tree science research. In the measurement statistics, it is not difficult to find that the ear weight of the severely wounded 'Rosario Bianco' was reduced by more than 30% compared with the control group. The other three varieties also showed varying degrees Decrease; but the single-kernel weight of the fruit is not significantly reduced; the soluble solid content, 'Rosario Bianco' and 'Summer Black' are 1% lower than the control group, and the other two varieties have smaller values. The changes in hardness and titratable acid content among various varieties are not obvious.

Integrating the entire grape growth dynamics and fruit quality, the occurrence of bleeding will delay the phenological period of grapes, reduce the germination rate, and delay the growth of new shoot lengths, resulting in a large number of flowers and fruits, and a significant reduction in single ear weight, which will reduce the yield of grapes. Regarding fruit quality, general bleeding does not have a significant impact on fruit quality. The soluble solid content of 'Rosario Bianco' with severe bleeding and the soluble solid content of 'Summer Black' and 'Kyoho' with moderate bleeding there is a certain decrease, but other physiological indicators related to fruit quality have little change.

Bleeding flow is an important physiological preparation stage before grape germination. Its occurrence is affected by temperature, moisture, nutrition and root pressure of the grape itself. Severe bleeding caused the loss of water and nutrients before germination. This study shows that bleeding caused a certain amount of hormone loss, causing early germination and new shoot growth of plants, increasing the rate of flower and fruit drop and reducing yield. Therefore, in the production of grapes, it is necessary to avoid the occurrence of a large amount of bleeding and reduce its impact on the yield. In production, it is generally through proper early winter pruning to deposit the fillings in the grape branch tissue to prevent or appropriately reduce the occurrence of bleeding in early spring.

Bleeding period is an important physiological stage of grapes. It generally refers to the time period when the root system of grapes starts to move in early spring and the sap in the grapes starts to move, until before and after germination or when the grapes start to spread the leaves. Grape bleeding is considered as a physiological preparation process before germination. Its occurrence is affected by temperature, moisture status and root pressure of the grape itself. The amount of bleeding and the duration of bleeding between different grape varieties vary greatly. The occurrence of normal blemishes indicates the beginning of grape root activity, but excessive blemishes often cause a large amount of water and nutrient loss, which affects the growth of budding and inflorescence. Therefore, proper early winter cutting can promote the accumulation of tissue fillers in the cutting mouth, thereby reducing the occurrence of wounds to a certain extent.

The content of substances contained in grape wound sap is very rich. Metal elements mainly include Ca, Fe, K, Mg, etc., and organic substances mainly include various sugars, amino acids, malic acid, citric acid, and some hormones. Based on previous research trends, this study combined with previous research results to further analyze and identify the contents of grape bleeding from the perspective of enzyme activity.

The amount of bleeding caused by the four varieties of grapes measured in the experiment is very different. Among them, the amount of 'Rosario Bianco' is much greater than the other three varieties, followed by 'Summer Black' and 'Kyoho', and 'Summer Black' The flow is relatively small. The measured content can be divided into the following categories: soluble protein and soluble sugar, which are widely present in various parts of grapes, and are generally stabilized in plants at a certain content; POD, SOD and CAT are three key enzymes in adversity Class; malondialdehyde and proline, these two substances are stress products accumulated in a large amount under the stress of plants; the last class is plant hormones with very low content.

The soluble sugars and soluble proteins in the bleeding fluid of the four varieties are all increasing. This may be related to the transportation of part of the accumulated material caused by the start of plant root activity, or the cutting of new shoots in early spring. The damage formed is related. The content of soluble sugars in the sap of grape wounds changed relatively less than that of its soluble proteins.

The activities of the three protective enzymes POD, SOD and CAT in the wound fluid have a certain increasing trend. However, the activities of POD and CAT are constantly increasing during the production of wounds, while the activity of SOD starts to decrease to a certain extent after reaching the maximum value within 5-8 days of wounding. As far as the functions of the three enzymes are concerned, SOD first removes O_2- produced by plants and converts it into H_2O_2. Although H_2O_2 is still persecuting plants, the high-efficiency CAT and POD in plants can effectively remove it. Converted to H_2O. Therefore, in the experiment, when SOD activity began to decrease, the CAT and POD activities were still at a higher level. Since there are few studies on grape bleeding, the conclusions in this regard need to be further experimentally verified, but the preliminary results are basically similar to those of other species.

As typical stress substances, MDA and PRO content can directly reflect the stress of plants. Since the bleeding sap of each grape variety in the treatment is produced from the truncated annual branches in early spring, it may have a certain stress effect on the grapes [24]. However, at this stage, the grapes have not yet sprouted, the root system activity has just begun, and the tree is still in a semi-dormant state. Studying the content of MDA and PRO can supplement the research of adversity. In terms of the changes in the content of MDA and PRO, the content of MDA was higher in the early stage, but then began to decrease. The general stress often causes a large accumulation of MDA, which is generally related to the influence plant membrane oxidation and respiratory chain [25-26]. It can be preliminarily concluded that the truncation of the annual branches of grapes in the semi-dormant state does not have too much influence on the respiration of grape roots. However, the content of PRO increased continuously with the progress of wounding, which may be related to the amount of PRO produced by root activity. MDA is produced by membrane oxidation, while SOD's main role is to clear O_2^- in plants.

Conclusion

The injury rate of the four grape varieties is very different. Among them, the injury rate of 'Rosario Bianco' is much larger than the other three varieties, followed by 'Summer Black' and 'Kyoho', and the injury rate of 'Wink' is relatively small. Statistics found that the occurrence of bleeding reduces the germination rate and increases the rate of flower and fruit drop. In terms of fruit quality, blemishes significantly reduced the ear weight. For varieties with severe blemishes, the single kernel weight and soluble solid content also decreased to a certain extent. Therefore, in production, we should pay attention to the bleeding phenomenon and reduce its adverse effects on the growth and development of grapes. The overall increase in soluble sugar and soluble protein in the bleeding sap of the four varieties may be related to the movement of plant roots in spring and the transportation of some accumulated substances, or it may be related to the damage to the grape cambium caused by the cutting of new shoots in early spring. The activities of the three protective enzymes, CAT and POD, in the wound fluid have a certain upward trend. In addition, wound fluid treatment also increased the content of plant hormones, including IAA, GA3 and 6-BA. Therefore, wound fluid treatment plays an important role in plant resistance.

REFERENCES

1. Masuda S, Kamada H, Satoh S. Chitinase in cucumber xylem sap. *Bioscience, Biotechnology, and Biochemistry*. **2001**, 65(8):1883-1885.
2. Iwai H, Usui M, Hoshino H, Kamada H, Matsunaga T, et al. Analysis of sugars in squash xylem sap. *Plant and cell physiology*. **2003**, 44(6):582-587.
3. Buhtz A, Kolasa A, Arlt K, Walz C, Kehr J, et al. Xylem sap protein composition is conserved among different plant species. *Planta*. **2004**, 219(4):610-618.
4. Kochba J, Lavee S, Spiegel-Roy P. Differences in peroxidase activity and isoenzymes in embryogenic ane non-embryogenic 'Shamouti'orange ovular callus lines. *Plant and Cell Physiology*. **1977**, 18(2):463-467.
5. CAKMAK I, STRBAC D, MARSCHNER H. Activities of hydrogen peroxide-scavenging enzymes in germinating wheat seeds. *Journal of Experimental Botany*. **1993**, 44(1):127-132.
6. Schmedes A, Hølmer G. A new thiobarbituric acid (TBA) method for determining free malondialdehyde (MDA) and hydroperoxides selectively as a measure of lipid peroxidation. *Journal of the American Oil Chemists' Society*. **1989**, 66(6):813-817.
7. Wakana A, Hiramatsu M, Park SM, Hanada N, Fukudome I, et al. Degree of abortion and germination rates in triploid seeds from crosses between diploid and tetraploid grapes (*Vitis vinifera L*. and V. complex). *Journal of the Faculty of Agriculture*. **2002**, 46(2):281-294.
8. Mirdehghan SH, Rahimi S. Pre-harvest application of polyamines enhances antioxidants and table grape (*Vitis vinifera L*.) quality during postharvest period. *Food Chemistry*. **2016,** 196:1040-1047.
9. Monti A, Barbanti L, Venturi G. Photosynthesis on individual leaves of sugar beet (Beta vulgaris) during the ontogeny at variable water regimes. *Annals of Applied Biology*. **2007**. 151(2):155-165.
10. Krousky J. Signing of an agreement and a long-term contract between the Czech Sugar Beet Producers Union and the Czech Eastern Sugar Association. **2002**.
11. Dahlback B. (1986). Inhibition of protein Ca cofactor function of human and bovine protein S by C4b-binding protein. *Journal of Biological Chemistry*. 261(26):12022-12027.
12. Black BL, Rhodes RB, McKenzie M, Lyles DS. The role of vesicular stomatitis virus matrix protein in inhibition of host-directed gene expression is genetically separable from its function in virus assembly. *Journal of virology*. **1993**, 67(8):4814-4821.
13. Kavanaugh MP, Wang H, Zhang Z, Zhang W, Wu YN, et al. Control of cationic amino acid transport and retroviral receptor functions in a membrane protein family. *Journal of Biological Chemistry*. **1994,** 269(22):15445-15450.
14. Purvanov V, Koval A, Katanaev VL. A direct and functional interaction between Go and Rab5 during G protein–coupled receptor signaling. *Science signalling*. **2010***,* 3(136):65.
15. Noel EA, Kang M, Adamec J, Van Etten JL, Oyler GA, et al. Chlorovirus Skp1-binding ankyrin repeat protein interplay and mimicry of cellular ubiquitin ligase machinery. *Journal of virology*. **2014,** 88(23):13798-13810.
16. Liu N, Lin Z, Guan L, Gaughan G, Lin G, et al. Antioxidant enzymes regulate reactive oxygen species during pod elongation in Pisum sativum and Brassica chinensis. *PloS one*. **2014**, 9(2):87588.
17. Uarrota VG, Moresco R, Schmidt EC, Bouzon ZL, da Costa Nunes E, et al. The role of ascorbate peroxidase, guaiacol peroxidase, and polysaccharides in cassava (Manihot esculenta Crantz) roots under postharvest physiological deterioration. *Food chemistry* **2016**, 197:737-746.
18. Wolters H, Jurgens G. Survival of the flexible: hormonal growth control and adaptation in plant development. *Nature Reviews Genetics*. **2009,** 10(5):305-317.
19. Santner A, Calderon-Villalobos LIA, Estelle M. Plant hormones are versatile chemical regulators of plant growth. *Nature chemical biology*. **2009,** 5(5):301-307.
20. Su P, Tong Y, Cheng Q, Hu Y, Zhang M, et al. Functional characterization of ent-copalyl diphosphate synthase, kaurene synthase and kaurene oxidase in the Salvia miltiorrhiza gibberellin biosynthetic pathway. *Scientific reports*. **2016**, 6:23057.

21. Kong L, von Aderkas P, Zaharia LI. Effects of exogenously applied gibberellins and thidiazuron on phytohormone profiles of long-shoot buds and cone gender determination in lodgepole pine. *Journal of plant growth regulation*. **2016**, 35(1):172-182.

22. Aslam A, Ali A, Naveed NH, Saleem A, Iqbal J et al. Effect of interaction of 6-benzyl aminopurine (BA) and sucrose for efficient microtuberization of two elite potato (Solanum tuberosum L.) cultivars, Desiree and Cardinal. *African Journal of Biotechnology*. **2011**, 10(59):12738-12744.

23. Ugare B, Banerjee K, Ramteke SD, Pradhan S, Oulkar DP, et al. Dissipation kinetics of forchlorfenuron, 6-benzyl aminopurine, gibberellic acid and ethephon residues in table grapes (*Vitis vinifera*). Food chemistry. **2013**,141(4):4208-4214.

24. Abdel Latef AA, Tran LSP. Impacts of priming with silicon on the growth and tolerance of maize plants to alkaline stress. *Frontiers in plant science*. **2016**, 7:243.

25. Gaoxing D, Keqin P, Langtao X, Guofu D. Effect of drought stress simulated by PEG on malonaldehyde, proline contents and superoxide dismutase activity in low potassium tolerant rice seedlings. *Zhongguo Shuidao Kexue*. **2006,** 20(5):557-559.

26. Shashamene E. Flowering and fruiting phenology and floral visitation of four native tree species in the remnant moist Afromontane forest of Wondo Genet, south central Ethiopia. *Tropical Ecology*. **2016**, 57(2):299-311.