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THE BEST FRUIT LOADS FOR THE COLD RESISTANCE OF WINE GRAPE (*VITIS VINIFERA* L.) IN THE EASTERN FOOTHILLS OF HELAN MOUNTAIN, CHINA

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Abstract

Wine grape cannot grow well in cold areas. The cold resistance abilities of grape have become a very important problem to be solved. Effects of different fruit loads on the physiological and biochemical indexes of wine grape cultivar were studied through experiments. Results showed that different fruit loads had significant effects on the conductivity, soluble sugar content, Malondialdehyde (MDA), superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) of grape branches. Factor analysis and principal component analysis were used to analyze the physiological and biochemical indexes of cold resistance of wine grapes with different loads. Results showed that combined with the physical microstructure, physiological and biochemical data of branches, the best fruit load of wine grape was 5 kg in eastern foot of Helan Mountain, China.

Introduction

China is one of the origins of Grape vines *(Vitis vinifera* L.) belonging to the vitaceae. This plant is the second largest planted fruit in China. After China's Reform and Opening Up, the grape industry has developed rapidly, forming wine grape producing areas mainly in the north (Li 2009). Like other grape growing areas (Cragin *et al*. 2017, Antivilo *et al*. 2018, Kaya 2020), environmental stress is one of the limiting factors for the development of grape industry in China (Guo and Luo 2010). In particular, overwintering freezing damage has caused long-term low and unstable wine grape production in China (Sun *et al*. 2015, Wang *et al*. 2015). Therefore, it is necessary to solve the problem of wine grapes overwintering freezing damage.

Though there are many researches on the winter damage of wine grapes, they mainly focus on the causes of freezing damage, defense technology and cold resistance (Zulini 2010, Li *et al*. 2016, Todaro and Dami 2017). The wide range and frequent occurrence of overwintering freezing damage in northern China are still unexpected, which is closely related to the lack of understanding of the formation mechanism of wine grape wintering freezing damage and the lack of corresponding research support. At present, the formation process of overwintering freezing injury of wine grape is clearly understood, and the research on the formation mechanism of freezing injury is also more (Karimi and Ershadi 2015, Antivilo *et al*. 2019). However, the research on the effect of fruit loads on cold resistance of wine grapes has not been carried out in depth. Therefore, the present study was aimed to determine the best fruit loads capacity, defence technology of overwintering freezing injury, and this study has important significance for targeted research on wine grape overwintering.

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Materials and Methods

The site was selected at the east foot of Helan Mountain (37°43′~39°23′ north latitude and 105°45′~106°47′ east longitude) in Ningxia Hui Autonomous Region. The field experiments and sampling locations of this study are mainly located in the vineyard of Mehe Manor in the eastern foot of Helan Mountain in Ningxia and the Yuxiaying Xixia Wangyuquan International Winery in Yuquanying, Yongning County, and Yinchuan City.

Cabernet sauvgnon grape was used as the wine grape sample. In May 2019, three rows of vines with similar growth and uniform age were selected. Four consecutive pole distances were selected as the test area. There were 10 vines in each pole distance. The vines are designed with different load treatments, specifically 3 kg (A_1 treatment), 4 kg (A_2 treatment), 5 kg (A_3 treatment) and non-thinning control (CK treatment, 6 kg). There were 3 replicates test areas for each treatment. Annual branches were collected in the repeated plots before removing the grapevines from the shelves. Ten branches were collected in each treatment and were repeated for three times. The thickness is required to be between 0.5 and 1cm, and the 8 to 15 sections were selected as test materials.

In the laboratory, the test branches were treated with high and low temperature alternating test box (model: BC1300), the treatment temperature of the branches were -10, -15, -20, -25 and -30^{0}C. The cooling range during freezing and the heating range during thawing were both 5°C/hr. After 2 hrs restoration at room temperature, some parts were taken out and immediately tested for relative conductivity. All indexes were measured 3 times.

The logistic equation $y = k/(1 + ae^{-bx})$ was used to fit the relationship between the low temperature stress and the relative conductivity of the branches. In the equation, y is the low temperature semi-lethal temperature (LT_{50}) and x is the relative conductivity.

The soluble sugar content of the test branches was determined using a kit of plant soluble sugar content produced by Shanghai Jianglai Biotechnology Co., Ltd. The four indicators contents (Malondialdehyde (MDA), superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) of the test branches were determined using the enzyme-linked immunoassay (ELISA) kit produced by Shanghai Jianglai Biotechnology Co., Ltd.

Data analysis and charts were performed with Excel 2019 and SPSS 20.0, the LT_{50} was calculated with DPS 9.05, relevant data of paraffin sections were observed and counted with Case Viewer 2.0, and significance test was performed with Duncan-style new double range test method.

Results and Discussion

In the early stage of the experimental design, each plot was treated with an estimated output of 0.5 kg as a spike of grapes, and the production was picked on September 26, 2019. The difference between the actual grape yield and the estimated yield in each area is within the range of ±10%, which meets the test demand.

It can be seen from Fig. 1 that during the cooling process of 4~-30°C, the relative conductivity of each treated branch showed an overall upward trend, and was approximately distributed in an "S" curve. Throughout the cooling process, the relative conductivity of each treatment increased differently.

As shown in Table 1, the correlation coefficient r of each logistic equation is greater than 0.900, all reaching a significant level ($p < 0.05$). According to the LT_{50} results, it can be preliminarily concluded that under different loads, the cold resistance of the total fruit loading of 3 kg is significantly weaker than other treatments.

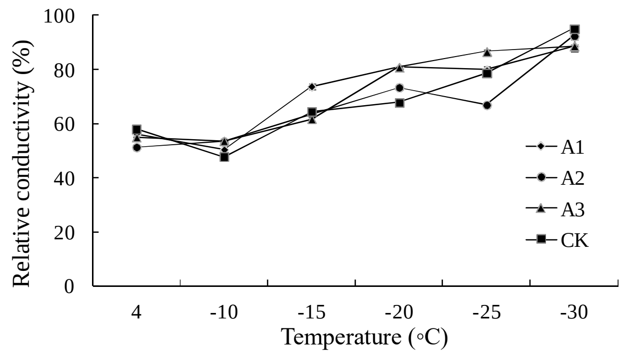

Fig. 1. Changes in relative conductivity of wine grape branches with different loads. CK, control group (6 kg load treatment); A1, 3 kg load treatment; A2, 4 kg load treatment; A3, 5 kg load treatment, the same as follows.

Table 1. Logistic equation of relative conductivity and LT_{50}.

deal with	Regression equation	decisive factor（r^2）	LT_{50} (℃)
A_1	$Y=61.25/(1+95.62e^{-6.42x})$	0.9035	-14.90
A_2	$Y=123.25/(1+3.94e^{-0.15x})$	0.9531	-26.62
A_3	$Y=105.95/(1+5.72e^{-0.22x})$	0.9768	-26.00
CK	$Y=203.88/(1+6.64e^{-0.25x})$	0.9023	-26.23

The soluble sugar content of grape branches under different treatments is shown in Fig. 2. During the temperature reduction of 4~-30℃, the soluble sugar content of the grape branches in each treatment showed a trend of slightly decreasing and then increasing and then decreasing, but the temperature at which the peak appeared was different. There was a significant difference in the soluble sugar content of the various treatments throughout the cooling process. Only at -25℃ there was no significant difference between the A1 treatment and the A2 treatment. It can be seen that there is a more obvious relationship between the load and the increase in soluble sugar. With the increase in load, the increase in soluble sugar gradually increases.

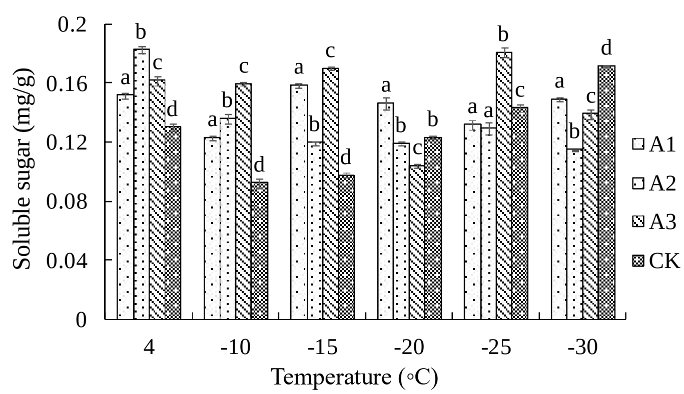

Fig. 2. Changes in soluble sugar content of wine grape branches with different loads.

During the temperature reduction of 4~-30℃, the MDA content of grape branches in each treatment showed a trend of first increasing and then decreasing (Fig. 3.). The changes in the early stage were stable, and the growth rate accelerated in the range of -15 to -20℃. After -20℃, there were different degrees of decline for the grape branches of different loads. In the whole cooling process, there is basically a significant difference in the MDA content of each treatment. It can be seen that the greater the amount of change in the MDA content during the whole freezing process, the greater the increase, and the greater the load of the grape branches, the greater the maximum MDA content reached.

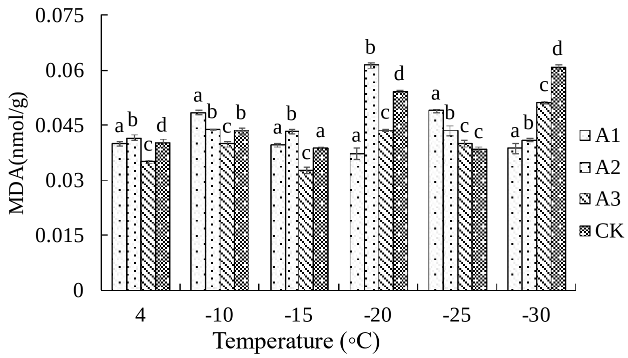

Fig. 3. Changes in MDA content of wine grape branches with different loads.

The SOD activity of grape branches under different treatments is shown in Fig. 4. During the temperature reduction of 4~ -30℃, the SOD activity of the grape branches in each treatment showed a trend of first increasing and then decreasing. The changes in the early stage were stable, and the growth rate accelerated in the range of -20℃ to -25℃, and then declined to varying degrees. The maximum increase in SOD activity was A3 treatment.

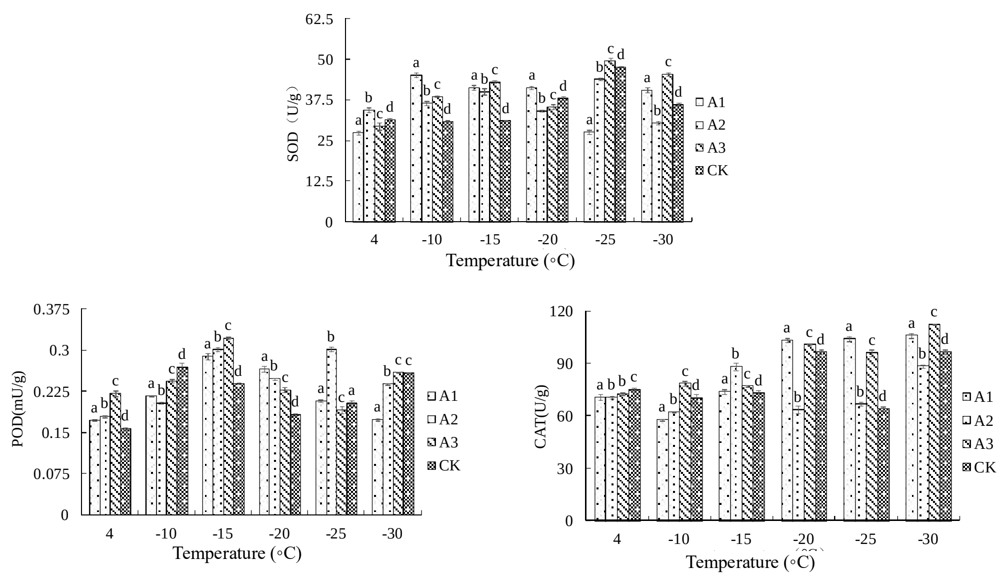

Fig. 4. Changes in SOD, POD, CAT activity of wine grape branches with different loads.

The POD activity of each treatment of grape branches generally showed a trend of increasing first and then decreasing (Fig. 4.). Among them, the A1, A2 and A3 treatments reached the peak at -15℃ treatment while the CK treatment reached the peak at -10℃. The maximum increase in the POD activity was at A3 treatment, followed by For A2 treatment.

The CAT activity of grape branches in each treatment changed relatively slowly within the range of 4~-15℃ (Fig. 4.). The activity increased rapidly between -15~-20℃, and then the growth rate slowed down. The four treatments all peaked at -30℃. The largest increase in CAT activity was at A1 treatment, the smallest at A2 processing.

The change rate α (equation (1)) between the maximum value reached by each index in the whole temperature-reducing process and its control value is taken as the original data of comprehensive evaluation.

Rate of change $\alpha = （X_{imax} - CK_i）/CK_i$ (1)

Among them, X_{imax} is the maximum value of the index i in the whole cooling process, and CK_i is the measured value of the CK of the index i without freezing. The result of factor contribution rate calculated by SPSS is shown in Table 2. The first two principal components are selected as effective principal components according to the principle of extracting the number of principal components. The first two principal components can contain 88.218% of the information in the data used, which is representative and can be analyzed further.

Table 2. Explanation of variance of principal component analysis.

main ingredient	Initial eigenvalue		
	Eigenvalues	Variance contribution rate	Cumulative variance contribution rate
1	4.794	68.482	68.462
2	1.381	19.736	88.218
3	0.825	11.782	100

The main component load table calculated by SPSS is shown in Table 3. From the table, we can see that the main component 1 mainly carries the information of relative conductivity, MDA content, SOD activity, POD activity and CAT activity, and the main component 2 mainly carries the information of LT_{50}.

Table 3. Principal factor load table.

Physiological and biochemical indicators	main ingredient 1	main ingredient 2
Relative conductivity	-0.924	-0.367
Soluble sugar content	0.672	-0.277
LT_{50}	0.336	0.931
MDA content	0.908	0.009
SOD activity	0.986	-0.052
POD activity	-0.834	0.547
CAT activity	0.940	0.035

According to the component matrix obtained by factor analysis, the eigenvector matrix is calculated, and the two principal components extracted can be expressed as a linear combination of each variable, and the score function of the two principal components (equation (2) and equation (3) can be obtained. In the formula, X_1~X_7 respectively represent relative conductivity, soluble sugar content, LT_{50}, MDA content, SOD activity, POD activity and CAT activity.

After normalizing all variables, the values of the two principal components corresponding to each harvest period, that is, the scores of *F1* and *F2*, can be calculated, and the respective variance contribution rates of the two principal components are used as weights for the *F1* and *F2*. The scores are weighted, and the comprehensive score equation *F* (Equation (4)) of each treatment is obtained, and the overall cold resistance of the wine grape branches with different loads is ranked according to the size of the *F* value. The higher the overall score, the higher the ranking, which means that the load resistance of the grape branches is better. The comprehensive score is greater than 0, indicating that the overall cold resistance of the grape branches under the load is above the average level, otherwise it is below the average level.

$$F_1=0.422X_1+0.307X_2+0.153X_3+0.415X_4+0.450X_5-0.381X_6+0.429X_7 \quad (2)$$

$$F_2=-0.312X_1-0.236X_2+0.792X_3+0.008X_4-0.044X_5+0.465X_6+0.030X_7 \quad (3)$$

$$F=(68.482F_1+19.736\ F_2)/100 \quad (4)$$

The comprehensive score ranking of the cold resistance of wine grape branches with different loads is shown in Table 4. A3 treated principal component 1 scored the highest at 1.775. The cold resistance of grape branches with different loadings was ranked as 5 kg total fruit> 3 kg total fruit> 6 kg total fruit (CK) > 4 kg total fruit.

Table 4. Comprehensive scores of cold resistance of wine grape branches with different loads.

deal with	*F1*	*F2*	*F*	Rank
A1	0.162	1.668	0.440	2
A2	-1.750	-0.023	-1.203	4
A3	1.775	-0.871	1.044	1
CK	-0.188	-0.774	-0.281	3

Comprehensive physical microstructure and physiology and biochemistry data of branches were observed. The comprehensive score of physiology and biochemistry is similar to the result of the proportion of xylem, which means that the growth of the branches is the best under the appropriate amount of fruit, so the resistance Cold ability is also the strongest. If the load is too much, the branches will not get enough nutrients, and the growth degree is not enough to resist the low temperature stress; if the load is too little, the branches will get too many nutrients, and the growth amount is too large, and more osmotic adjustment is required during low temperature stress substances and protective enzymes to resist low temperature, and the rate of production of synthetic substances has an upper limit, it is impossible to fully meet the needs of the branches, so it is not conducive to resistance to low temperatures. Combined with the above analysis, it can be concluded that the branch has the best cold resistance when the fruit is 5 kg, so it can be determined that the optimal amount of fruit is 5 kg.

In the wine grape cultivation technology, the regulation of the load is important. It can not only regulate the quality of the grape fruit, but also affect the resistance of the grape itself. The load can regulate the degree of vegetative growth of the tree, and can also regulate the degree of reproductive growth (Liu *et al*. 2015, Chen 2016), only the appropriate load can achieve the purpose of the best wine grape quality and the best wine quality (Shi *et al*. 2016, Zhang 2016).

At present, most of the researches on the fruit load has focused on the effects on fruit quality (Zhang 2013, Man *et al*. 2011) and fruit disease resistance (Yu *et al*. 2010, Wen 2016), and few articles related to the resistance of trees. In this study, the growth of the branch is the best in the right amount of fruit hanging, so the cold resistance is also the strongest. If the load is too much, the branches cannot get enough nutrition and the growth degree is not enough to resist the stress of low temperature. Under low temperature stress, more osmotic adjustment substances and protective enzymes are needed to resist low temperature, and the rate of production of synthetic substances has an upper limit, which cannot fully meet the needs of branches, so it is not conducive to resist low temperature. The results of this study showed that the electrical conductivity, enzyme content and other physical and chemical properties of branches could reach a more appropriate level under the condition of appropriate fruit setting. Combined with the above analysis, it can be concluded that the cold resistance of branches of Cabernet Sauvignon is the best when the fruit bearing capacity is 5 kg.

Shi *et al* (2015a) found that the branches with the most fruit grape varieties with a slight fruit ratio of 1:1 in western Liaoning had the strongest cold resistance capacity. Some scholars have also found that in the eastern foothills production area of Helan, the semi-lethal temperature of the branches was the lowest when the plant load of the Cabernet Sauvignon variety was 10 ears in the irrigated silt planting area (Shi *et al*. 2015b), and when the plant load of the Cabernet Sauvignon variety was 15 ears in the gravel sand soil planting areas. The semi-lethal temperature of the branches is the lowest. The above results are consistent with the conclusions of this study.

The research showed that in the cooling process, the relative conductivity of different load wine grape branches showed an upward trend. Overall, the more the fruit load, the higher the semi-lethal temperature of grape branches. The soluble sugar content of wine grape branches with different loading showed a trend of slightly decreased at first, then increased and then decreased. The MDA content of different load wine grape branches showed a trend of first increased and then decreased. In the cooling process, the three kinds of anti-adversity enzymes through the synergistic effect together against low temperature stress, different load wine grape branches of anti-adversity enzyme activity were significantly improved.

The results of factor analysis and principal component analysis showed that the cold resistance of grape branches with different loads was in the order of total fruit 5 kg > total fruit 3 kg > total fruit 6 kg (CK) > total fruit 4 kg. Based on the physiological and biochemical data, it can be concluded that the cold resistance of branches is the best when the fruit load is 5 kg, so the best fruit load of wine grapes in the eastern foot of Helan Mountain is 5 kg.

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