Effect of Two Kinds of Antistaling Agent on The Pigments of Cutting Branch for *Cornus alba* L.[1]

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ABSTRACT

Aims: The research was aimed to study the effects of antistaling agent on the fresh-keeping of cutting branches in order to provide the foundation on sticks-cutting preservation in production.

Study Design: In order to analyze the function and action mechanism of antistaling agent A(3% sucrose +50mg·L^{-1} aluminium sulfate + 200mg·L^{-1} citric acid + 100mg·L^{-1} GA) and B (3% sucrose + 0.5% benzene propionic acid sodium), we used the *Cornus alba* L., as materials, observed fresh-keeping life and morphological changes, measured the fresh weight, the contents of chlorophyll, soluble sugar, red pigment and anthocyanin in cutting branchs of *Cornus alba* L.

Place and Duration of Study: College of Biological Science and Technology, Shenyang Agricultural University, between February 2017 and March 2018.

Methodology: The contents of soluble sugar, chlorophyll, anthocyanin and red pigment in stem bark were determined at 3d, 6d, 9d, 12d and 15d after treatment, respectively. The contents of chlorophyll, red pigment and anthocyanin in cutting branchs of *Cornus alba* L. were measured by method of spectrophotometer. The content of soluble sugar was determined by anthrone colorimetry.

Results: The effects of antistaling agent A (3% sucrose +50mg·L^{-1} aluminium sulfate + 200mg·L^{-1} citric acid + 100mg·L^{-1} GA) and antistaling agent B (3% sucrose + 0.5% benzene propionic acid sodium) were tested in this paper in order to investigate their effects on contents of chlorophyll, soluble sugar, red pigment and anthocyanin in cutting branchs of *Cornus alba* L., which provided foundation on sticks-cutting preservation in production. Early spring sticks of *Cornus alba* L. were used as materials by antistaling agent A and B treatment, respectively, and sterilized water as control. Results showed that contents of chlorophyll and soluable sugar in the antistaling agent A group were higher than those in B group. And the contents of anthocyanin and red pigments in the antistaling agent B group were higher than those in A group. Above all, the antistaling agent B had better preservation effect than the antistaling agent A.

Conclusion: The contents of chlorophyll and soluable sugar in the antistaling agent A group were higher than those in the antistaling agent B group. And the contents of anthocyanin and red pigments in the antistaling agent B group were higher than those in A group. Above all, the antistaling agent B had better preservation effect than the antistaling agent A.

Keywords: Antistaling agent; Cornus alba L.; Cutting branch

1. Introduction

Cornus alba L. is a deciduous shrub of Cornaceae. The old stem is dark red, the branches are blood-red with opposite leaves, which each leaf is elliptic. Cyme is terminal with milky white flowers and milky or blue-white fruits. Autumn leaves of *Cornus alba* L. are bright red, and fruits is small and white. After fallen leaves, the stem is bright red as coral [1-2], which is rarely enjoyed by bright-coloured sticks, but also a good cutting materials for flower arrangement. At present, the

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fresh-keeping of pruning branches is a hot topic, but there is no conclusion on the best formulation of antistaling agent for cutting branches of *Cornus alba* L.[3].

Gibberellin is a kind of plant hormone, which can be fresh-keeping for cutting branches and cutting flowers[4]. In addition, sodium benzoate and soluble sugar are also used in the formulation of fresh-keeping for cutting branches and cutting flowers[5]. Gibberellin can inhibit maturation, lateral bud dormancy and senescence, so it can be used as antistaling agent to maintain physiological activity of isolated organs[6]. Sodium benzoate ($C_6H_5CO_2Na$), belongs to acid preservative, and has good anticorrosion effect in acidic environment. It is commonly used in food preservative and has the effects of preventing deterioration and prolonging the shelf life. Sodium benzoate is easy to penetrate the cell membrane, interfere with the permeability of cell membrane, inhibit the absorption of amino acid, inhibit the activity of cell respiratory enzyme system, and prevent acetyl coenzyme A condensation, so as to play the role of food preservative[7-8]. It is also used in the preservation of isolated organs.

In this study, two kinds of antistaling agent A(3% sucrose +50mg·L^{-1} aluminium sulfate + 200mg·L^{-1} citric acid + 100mg·L^{-1} GA) and B (3% sucrose + 0.5% benzene propionic acid sodium) were used to study the effects on the contents of chlorophyll, soluble sugar, anthocyanin and red pigments in cutting branches of *Cornus alba* L. in order to provide the basis for fresh-keeping on the production of cutting branches .

2. Materials and Methods

2.1 Materials

Annual branches, which were healthy, disease-free, well-developed, were harvested, immediately cutting strip about 30cm length. The upper end was straightly sniped from the upper bud about 15-20mm, which was conducive that the upper bud did not dry. The lower end was beveled from the lower bud about 15-20mm. The cut should be smooth to prevent the splitting of the epidermis and xylem, so as to prevent the water from rotting. After cutting, the initial fresh weight of the cutting branches was weighed, then inserted into the bottles with different antistaling agents.

2.2 Methods

The antistaling agents A(3% sucrose +50mg·L^{-1} aluminium sulfate + 200mg·L^{-1} citric acid + 100mg·L^{-1} GA) and B (3% sucrose + 0.5% benzene propionic acid sodium) were prepared for 200mL with distilled water, respectively, and filled in 250 mL conical bottles. A group of control (CK- distilled water) was set up. Each treatment was repeated three times, and the three branches were placed in the same triangular bottle. The branches were inserted into the conical bottle and sealed with degreased cotton in order to reduce the consumption of transpiration. The branches were recorded once every 3d and the bottle solutions were changed once. The inserted branches were placed in scattering light, 20-28 , and the relative humidity was kept at 60% -70% in order to observe the fresh-keeping days and determinate the fresh weight, contents of pigment in stem bark.

2.3 Assay methods

The contents of soluble sugar, chlorophyll, anthocyanin and red pigment in stem bark were determined at 3d, 6d, 9d, 12d and 15d after treatment, respectively.

2.3.1 Determination of chlorophyll content

Fresh samples of stem bark about 0.2g were cut and extracted with acetone and anhydrous ethanol, used to determine the absorbance at 663 nm and 645 nm, then the chlorophyll content was calculated.

2.3.2 Determination of anthocyanin content

Material of 0.2g was taken in triangle bottle with 10mL hydrochloric acid ethanol solution, and heated in 60 water bath for 30 min, then poured the solution into 25mL volumetric bottle. Extraction was repeated 3 times with 5mL extracted solution for 15 min. The total extraction time was 1h, and the final volume was 25 mL.

The concentration of anthocyanin was calculated by spectrophotometer with 0.1 mol.L^{-1} ethanol hydrochloric acid solution as reference solution. The optical density of extract solution was determined by spectrophotometer at 530nm, 620nm, 650nm, respectively.

2.3.3 Determination of red pigment

The red pigment was extracted by ultrasonic method. The extraction condition was as follows: 60 , 250W, 70 min, solid-liquid ratio 1: 30. The extraction solution was 30% ethanol hydrochloric acid, and the absorbance was determined at 524nm wavelength. The content of red pigment was proportional to OD value.

2.3.4 Determination of soluble sugar content

Fresh sample of 1g was sheared and mixed, placed in test tube. Test tube was placed in 50mL triangle bottle with 25 mL boiling water, extracted in boiling water bath for 10 min, cooled and filtered into 50mL volumetric bottle. The residue was washed in hot water for 2-3 times, then filtered into the volumetric bottle, then cooled to room temperature, fixed volume to calibration. The content of soluble sugar was determined by anthrone colorimetry.

2.4 Statistical analysis

SPSS 17.0 software was used to perform the statistical analysis of data. The data represent the mean±SD of n= 3 independent experiments. P< 0.05 was considered as statistically significant.

3. Results and Discussion

3.1 Effects of two kinds of antistaling agent on the fresh weight of the cutting branches in *Cornus alba* L.

The fresh weight of the cutting branch of *Cornus alba* L. decreased with the increase of the treatment time, the reduction in the fresh weight of the two kinds of antistaling agent was lower than that of the control, indicating that two kinds of antistaling agent could maintain the water absorption of the branches. The antistaling agent B was a little better than A group (Table 1) .

Table 1 Changes of the fresh weight on the cutting branches in *Cornus alba* L. by two kinds of antistaling agent treatment

Days		0d	3d	6d	9d	12d	16d	Fresh weight reduction
Fresh weight （g）	CK	7.21±0.59	7.14±0.60	6.67±0.38	5.88±0.61	5.53±0.29	5.35±0.21	1.86
	A	7.23±0.23	7.19±0.39	6.87±0.28	6.56±0.47	6.34±0.38	5.98±0.34	1.25
	B	6.46±0.31	5.85±0.44	5.64±0.55	5.31±0.33	5.22±0.25	5.29±0.26	1.17

Note: CK-sterilized water; A-3% sucrose +50mg·L^{-1} aluminium sulfate + 200mg·L^{-1} citric acid + 100mg·L^{-1} GA; B-3% sucrose + 0.5% benzene propionic acid sodium

3.2 Effects of two kinds of antistaling agent on the fresh-keeping life and morphological changes in *Cornus alba* L.

Both the antistaling agents A and B could effectively prolong the fresh-keeping life of *Cornus alba* L. The fresh-keeping life was longer by antistaling agent B treatment, which the branches were full, and the phenomenon of water loss wilting was late. But the fresh-keeping life of antistaling agent A was a little worse than that of B group(Table 2).

Table 2 Effects of two kinds of antistaling agent on the fresh-keeping life and morphological changes of the cutting branches in *Cornus alba* L.

Treatment	Fresh-keeping days(d)	Morphological changes of cutting branches
CK	9	The buds of the branches were withered, the colors of the branches were dim, and the leave buds were withered.
A	15	The buds on the branches germinated 0.5d later than the CK, the leave buds were full and strong, and the branches were full.
B	17	The buds on the branches germinated later and stronger, the branches were bright in color, and the branches were full.

Note: CK-sterilized water; A-3% sucrose +50mg·L^{-1} aluminium sulfate + 200mg·L^{-1} citric acid + 100mg·L^{-1} GA; B-3% sucrose + 0.5% benzene propionic acid sodium

3.3 Effects of two kinds of antistaling agent on chlorophyll content in the cutting branches of *Cornus alba* L.

After the antistaling agents A and B treatment, the contents of chlorophyll in the cutting branches were shown in Fig. 1. The chlorophyll content in stem bark of group A was higher than that in control group, but group B was lower than that in control group.

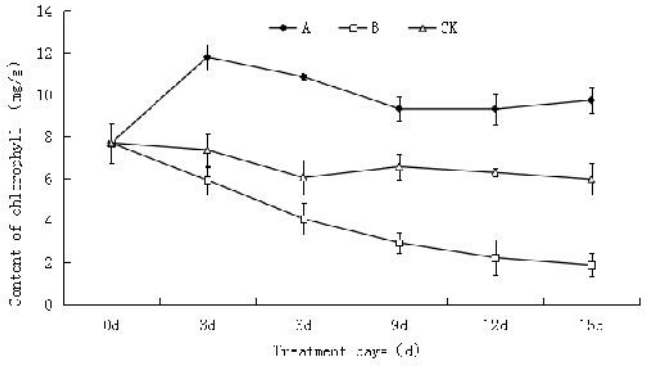

Fig.1 Effects of two kinds of antistaling agent on chlorophyll content in cutting branches of *Cornus alba* L.

Note: CK-sterilized water; A-3% sucrose +50mg·L^{-1} aluminium sulfate + 200mg·L^{-1} citric acid + 100mg·L^{-1} GA; B-3% sucrose + 0.5% benzene propionic acid sodium

3.4 Effects of two kinds of antistaling agent on anthocyanin content in the cutting branches of *Cornus alba* L.

After treated with the antistaling agents A and B, the anthocyanin contents in the cutting branches were shown in Fig. 2. The content of anthocyanin in group B was higher than that in group A and control group. In the first 6d, the anthocyanin content of antistaling agent B treatment was

significantly higher than those in antistaling agent A treatment and the control group, while the anthocyanin contents of two treatment groups were slightly higher than that in control group in the 9-12d.

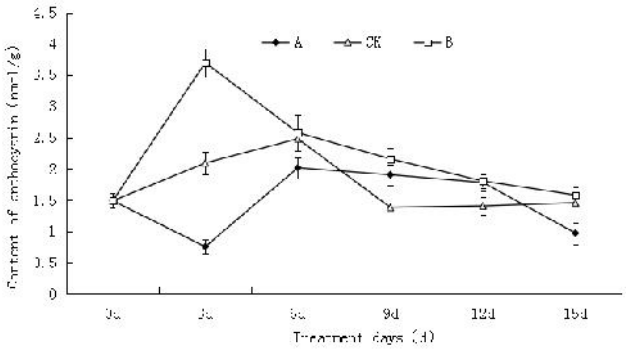

Fig.2 Effects of two kinds of antistaling agent on anthocyanin content in cutting branches of *Cornus alba* L.

Note: CK-sterilized water; A-3% sucrose +50mg·L^{-1} aluminium sulfate + 200mg·L^{-1} citric acid + 100mg·L^{-1} GA;

B-3% sucrose + 0.5% benzene propionic acid sodium

3.5 Effects of two kinds of antistaling agent on red pigment in cutting branches of *Cornus alba* L.

After treated with antistaling agents A and B, the contents of red pigment in the cutting branches were shown in Fig.3. During the whole treatment time, the content of red pigment of treatment group B was higher. But in 3d treatment, the content of red pigment of group B was higher than that of the control group, and both of them were higher than that of the group A. At 9d after treatment, the treatment groups were higher than the control group.

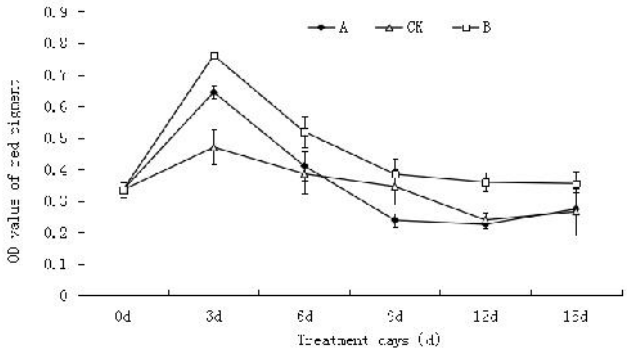

Fig.3 Effects of two kinds of antistaling agent on red pigment in cutting branches of *Cornus alba* L.

Note: CK-sterilized water; A-3% sucrose +50mg·L^{-1} aluminium sulfate + 200mg·L^{-1} citric acid + 100mg·L^{-1} GA;

B-3% sucrose + 0.5% benzene propionic acid sodium

3.6 Effects of two kinds of antistaling agent on the content of soluble sugar in cutting branches of *Cornus alba* L.

Treated with antistaling agents A and B, the contents of soluble sugar in the cutting branches were shown in Fig. 4. Treatment for 3d, the content of soluble sugar in group B was the highest, but in

the group A was the highest at the 6d treatment. There was no significant difference among the three groups in other time.

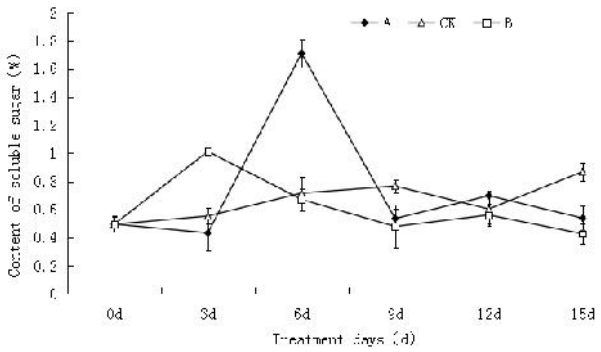

Fig.4 Effects of two kinds of antistaling agent on soluble sugar content in cutting branch of *Cornus alba* L.

Note: CK-sterilized water; A-3% sucrose +50mg·L^{-1} aluminium sulfate + 200mg·L^{-1} citric acid + 100mg·L^{-1} GA; B-3% sucrose + 0.5% benzene propionic acid sodium

3.7 Comparison of related substances in cutting branches of *Cornus alba* L. after treatment by two kinds of antistaling agent

After treated with two antistaling agents A and B, the comparison of the contents of chlorophyll, anthocyanins, red pigment and soluble sugar obtained from the cutting branches of *Cornus alba* L. was shown in Table 3.

The contents of chlorophyll, anthocyanin and red pigment in group A and B were all decreasing, and the content of chlorophyll in group A was higher than that in group B, while the contents of anthocyanin and red pigment were slightly higher in group B. Soluble sugar content in group B was lower than that in group A.

Table 3 Comparison of pigment related substances in pruning branches of *Cornus alba* L. after treatment by two kinds of antistaling agent

Days of treatment		3d	6d	9d	12d	15d
Chlorophyll	A	11.81±0.93a	10.87±0.20a	9.34±0.82a	9.33±0.75a	9.75±0.59a
	B	5.93±0.38b	4.09±0.77b	2.94±0.30b	2.23±0.88b	1.88±0.56b
Anthocyanin	A	0.75±0.18b	2.02±0.16b	1.90±0.36a	1.78±0.34a	0.97±0.37b
	B	3.70±0.03a	2.58±0.06a	2.16±0.03a	1.80±0.18a	1.58±0.16a
Red pigment	A	0.65±0.01b	0.41±0.05b	0.24±0.02b	0.23±0.01b	0.28±0.05b
	B	0.76±0.01a	0.52±0.03a	0.39±0.11ba	0.36±0.02a	0.36±0.04a
Soluble sugar	A	0.43±0.13b	1.71±0.10a	0.54±0.07a	0.70±0.03a	0.54±0.45a
	B	1.01±0.04a	0.67±0.08b	0.48±0.01a	0.56±0.02a	0.43±0.04a

Note: A-3% sucrose +50mg·L^{-1} aluminium sulfate + 200mg·L^{-1} citric acid + 100mg·L^{-1} GA; B-3% sucrose + 0.5% benzene propionic acid sodium. Letters indicate significant differences at $P<0.05$ compared with the root by Student's *t*-test.

3.8 Discussion

Autumn leaves of *Cornus alba* L. are bright red with small white fruits. Branches are bright red behind fallen leaves, which are the good cutting materials. The ornamental value of cutting

branches is mainly reflected in the color of stem bark. How to make its bright color last is a hot research topic at present. The changes of color are mainly related to the contents of chlorophyll, anthocyanin and red pigment[9-10], and soluble sugar is the synthetic substrate of anthocyanin and red pigment[11-12]. In this study, the contents of chlorophyll, anthocyanin, red pigment and soluble sugar in the cutting branches of *Cornus alba* L. were determined after different antistaling agents A (3% sucrose +50mg·L^{-1} aluminium sulfate + 200mg·L^{-1} citric acid + 100mg·L^{-1} GA) and B (3% sucrose + 0.5% benzene propionic acid sodium) treatment.

The results showed that the contents of chlorophyll and soluble sugar in stem bark by the antistaling agent A treatment were higher than those in the antistaling agent B group. The contents of anthocyanin and red pigment were higher in the antistaling agent B group than those in the antistaling agent A group. Gibberellin can promote growth, inhibit maturation, dormancy and senescence of lateral buds, and reduce the degradation rate of chlorophyll. Therefore, the chlorophyll content of group A was higher[7]. The soluble sugar is the synthetic substrate of anthocyanin and red pigment[9]. The contents of anthocyanin and red pigment in the antistaling agent B group maintained a high level, which was consistent with the decrease of soluble sugar content. Above all, antistaling agent B had better preservation effect on the color of stem bark than that of antistaling agent A.

4. Conclusions

The effects of antistaling agent A (3% sucrose +50mg·L^{-1} aluminium sulfate + 200mg·L^{-1} citric acid + 100mg·L^{-1} GA) and antistaling agent B (3% sucrose + 0.5% benzene propionic acid sodium) were tested in this paper in order to investigate their effects on contents of chlorophyll, soluble sugar, red pigment and anthocyanin in cutting branchs of *Cornus alba* L., which provided foundation on sticks-cutting preservation in production. Early spring sticks of *Cornus alba* L. were used as materials by the antistaling agent A and B treatment, respectively, and sterilized water as control. Results showed that contents of chlorophyll and soluable sugar in the antistalin agent A group were higher than those in the antistalin agent B group. And the contents of anthocyanin and red pigments in the antistalin agent B group were higher than those in the antistalin agent A group. Above all, the antistalin agent B had better preservation effect than the antistalin agent A.

Acknowledgement

This study was supported by the Guiding Project of Liaoning Provincial Natural Science Foundation(20180550074); The Key Project of Basic Scientific Research of Liaoning Provincial Colleges(2017004).

AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration between all authors. Authors CN designed the study. performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author SY managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

Competing Interests

Authors have declared that no competing interests exist.

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