



RESEARCH ARTICLE

OVERWINTERING PERFORMANCE OF SIX *BOUGAINVILLEA* VARIETIES IN JIANGHAN PLAIN

MA Xiaojie, XU Yaowen, LI Jiaqun and HU Die*

College of Horticulture and Gardening, Yangtze University, Jingzhou HuBei

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ABSTRACT

In order to introduce the planting and cultivation of *Bougainvillea spectabilis* in Jianghan area, domesticate the varieties that can adapt to the climate of Jianghan plain, improve the market competitiveness of *B. spectabilis* and make it more widely popularized. A comparative experiment on overwintering cold resistance was carried out with six *Bougainvillea* varieties introduced from Yunnan, China. By comparing the freezing injury degree of *B. spectabilis* under indoor and outdoor environmental conditions and measuring the physiological indexes such as electrical conductivity, soluble sugar content, soluble protein content, catalase activity and peroxidase activity of its leaves. The experimental results revealed that: under the same management conditions, *Yunnan Purple* bougainvillea suffered the least degree of freezing damage and could successfully overwinter, while other varieties suffered more damage. The comprehensive evaluation shows that *Yunnan Purple* and *Green Leaf Orange* have strong cold resistance. Except for *Yunnan Purple* bougainvillea, which can survive the winter smoothly outdoors in Jianghan Plain, other varieties are difficult to survive.

INTRODUCTION

Bougainvillea spectabilis is a kind of plant in *Bougainvillea*, Nyctaginaceae (Lu, 2016), which is native to South America. The optimum temperature for growth is 15 °C, the optimum temperature for flowering is 15-30 °C, the high temperature of 40 °C can be tolerated in summer, and the overwintering temperature should not be lower than 3-7 °C (Xu *et al.*, 2009). *Bougainvillea spectabilis* not only has high ornamental value; It can also absorb heavy metals such as lead, copper and cadmium in soil, and has the function of ecological restoration (Wang *et al.*, 2015). In addition, its leaf extract also has anti-inflammatory and antioxidant effects (Singh *et al.*, 2018). The stem bark extract can also reduce blood sugar and make drugs (Bhat *et al.*, 2011). At present, more studies on the cultivation, reproduction and variety classification of *Bougainvillea spectabilis* (Zeng *et al.*, 2018; Ye, 2011), but less on the cold tolerance. *Bougainvillea* likes a warm and moist, full of sunlight environment and is not resistant to cold. The biggest obstacle encountered in the process of introduction is the problem of overwintering in winter. In addition to overwintering in the open field in the south, outdoor open field cultivation in the north of South China and the vast areas of central and North China has serious freezing damage and is difficult to overwinter (Chen, 2009).

Nowdays China only depends on the trial planting experience to judge whether it is suitable for open field cultivation, and there is a lack of accurate understanding of its cold tolerance and cold resistance (Zhang, 2013). Therefore, researching the overwintering performance of different bougainvillea glabra varieties in Jianghan plain can provide scientific basis and guidance for the introduction, breeding and cultivation of new cold tolerant bougainvillea glabra varieties in Hubei and Northern China.

MATERIALS AND METHODS

Experimental materials: The varieties of bougainvillea glabra introduced from Yunnan in June 2018 with good growth and no diseases and pests were selected as the research object. The introduced varieties are: *Yunnan Purple* (*Bougainvillea spectabilis* Willd cv. 'Rabra-plena'), *Green Leaf Orange* (*Bougainvillea spectabilis* cv. 'Auratus'), *Baolao Orange* (*Bougainvillea buttiana* cv. 'Bao Lao Cheng'), *Pink Orange Princess* (*Bougainvillea glabra* cv. 'Elizabeth Doxey'), *Green Leaf Lemon Yellow* (*Bougainvillea buttiana* cv. 'Mrs Mc Lean'), *Sprinkle Golden Leaf Powder* (*Bougainvillea spectabilis* cv. 'Lateritia Gold'). There are 40 plants of each variety. Each variety is divided into two groups on average. They are placed in the indoor sunny place and outdoor balcony for natural overwintering respectively. The overwintering situation is observed after the temperature of the next year is stable at 0 °C.

*Corresponding Author: HU Die,

College of Horticulture and Gardening, Yangtze University, Jingzhou Hu Bei.

Plant leaf collection method: A total of 2 samples were taken in this experiment. The first sampling time was on December 10, 2018. The outdoor temperature was 5°C and the indoor temperature was 10°C; The second sampling time is December 31, 2018. The outdoor temperature is 3°C and the indoor temperature is 8°C. The sampling method is: select the plants with normal growth and consistent growth, collect the leaves with uniform size, consistent color, no diseases and insect pests and no damage from the 6th-10th spring branches around the crown from the top, and collect about 10 leaf samples for each variety indoors and outdoors to form a control. The collected leaves were classified and stored at - 80 °C in an ultra-low temperature refrigerator.

Determination method: In this experiment, HPCE-UV method of Wang *et al.* (2016) was used to determine catalase activity, peroxidase activity was measured by Li *et al.* (2008) guaiacol method, the soluble protein content was measured by Coomassie brilliant blue G-250 method, refer to Wang (2006) experimental method to determine the content of plant soluble sugar, the measurement of plant electrical conductivity refers to Zong (2013) experimental method, repeat the test for three times for each index to be measured, and then take the average value.

RESULTS AND ANALYSIS

Morphological expression: After observation, the same *Bougainvillea* variety has bright leaves, plump leaves and large and extended leaves indoors; The outdoor leaves are dim, wrinkled and slightly wilted, and the leaves are small and curly. According to the classification standard of winter freezing injury investigation in Guilin Botanical Garden in 1991 (Liet *et al.*, 1993). According to the overwintering performance of the outdoor *Bougainvillea* varieties, the freezing injury grades are shown in Table 1: the freezing injury grade of Yunnan Purple is grade 0, and there is no freezing injury to the branches and leaves during the whole overwintering period. Green Leaf Orange Green Leaves Lemon Yellow Pink Orange Princess have 25% of the leaves were frozen, and the frost damage level reached grade I; Among them, Green Leaf Orange began to show symptoms such as leaf shrinkage and leaf edge anti rolling in late December 2018; The leaves of Green Leaf Orange began to wilt from late December 2018; From mid December 2018, the leaves of Pink Orange Princess began to shrink, and then water immersion frostbite occurred. The freezing injury level of Sprinkle Golden Leaf Powder is grade II. 50% of the leaves are frozen, and the leaves curl, shrink and wither. From the middle and late December of 2018 to the early January of the next year, the shrinkage and anti curl are intensified, and the leaves are water soaked frostbite. The freezing injury grade of Baolao Orange is grade III. 75% of the leaves are frozen, the leaves shrink, dry and fall off. The leaves begin to curl in late December 2018, and gradually wither and fall off in early January 2019, and some summer shoot tips suffer from freezing injury.

Determination of electrical conductivity: It can be seen from Fig. 1. The conductivity of the six *Bougainvillea* varieties is higher than that in the room under low temperature conditions. Among the indoor plants, the conductivity of Yunnan Purple is the lowest and that of baolao orange bougainvillea is the highest; Among the outdoor plants, the conductivity of Pink Orange Princess is the lowest, and that of Green Leaf Lemon

Yellow is the highest; In indoor plants, there was no significant difference ($P > 0.05$). in conductivity between Baolao Orange and Yunnan Purple ($P < 0.05$). There was significant difference in electrical conductivity between Baolao Orange and Green Leaf Lemon Yellow in outdoor plants and Yunnan Purple, Green Leaf Orange, Pink Orange Princess and golden leaf pink ($P < 0.05$).

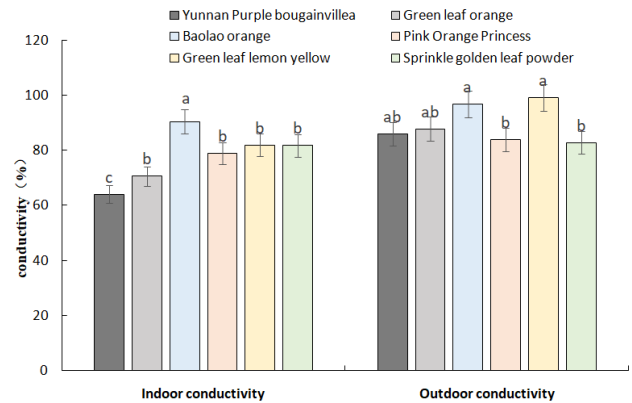


Fig.1 Electrical conductivity of different varieties of *Bougainvillea ssp*

Determination of soluble sugar: According to Fig. 2. The soluble sugar content of six *Bougainvillea spectabilis* varieties under outdoor natural wintering condition was lower than that under indoor protection. In both indoor and outdoor conditions, Yunnan Purple had the highest soluble sugar content and Baolao Orange had the lowest. There was no significant difference in the soluble sugar content between Pink Orange Princess and Sprinkle Golden Leaf Powder, Baolao Orange and Green Leaf Lemon Yellow in indoor plants ($P > 0.05$), but there was significant difference in the soluble sugar content between Baolao Orange and other *bougainvillea spectabilis* varieties ($P < 0.05$). In outdoor plants, there was no significant difference in soluble sugar content between Green Leaf Orange and Pink Orange Princess ($P > 0.05$); while the soluble sugar content between Yunnan Purple and Sprinkle Golden Leaf Powder, Baolao Orange, Green Leaf Lemon Yellow bougainvillea was significantly different ($P < 0.05$).

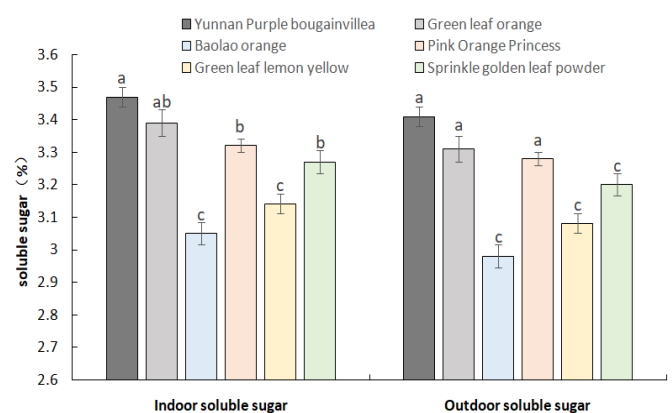


Fig.2 Soluble sugar content of different varieties of *Bougainvillea ssp*

Determination of soluble protein content: As can be seen from Fig. 3. The soluble protein content of indoor and outdoor plants of the same bougainvillea variety is similar, but the protein content of Green Leaf Orange, Green Leaf Lemon

Tab. 1 Leaf performance of different varieties of *Bougainvillea ssp.* in outdoor

Bougainvillea variety	Leaf performance	Frost damage grade	Time of occurrence
Yunnan Purple	Basically no freezing damage, and the leaves are in good condition	0	2019.1.10.
Green Leaf Orange	25% of the leaves are frozen, the leaves curl up and the color is dim	I	2018.12.20.
Baolao Orange	75% of the leaves are frozen, the leaves shrink, dry and fall off	III	2018.12.26.
Pink Orange Princess	25% of the leaves were frozen and wilted	I	2018.12.16.
Green Leaf Lemon Yellow	25% of the leaves were frozen, the leaves were wrinkled and moderately wilted	I	2018.12.23.
Sprinkle Golden Leaf Powder	50% of the leaves were frozen, curled, shrunk and withered	II	2018.12.21.

Yellow, Sprinkle Golden Leaf Powder is higher in outdoor low temperature than in indoor. However, under the same environmental conditions, there are obvious differences in the soluble protein content of each variety. Under indoor conditions, there is no significant difference in soluble protein content among Yunnan Purple, Green Leaf Orange and Pink Orange Princess ($P > 0.05$), but there is apparent difference in soluble protein content between Yunnan Purple and Baolao Orange, Green Leaf Lemon Yellow, Sprinkle Golden Leaf Powder ($P < 0.05$).

Among outdoor plants, there was no obvious difference in the content of soluble protein among Yunnan Purple, Green Leaf Orange, Pink Orange Princess and Sprinkle Golden Leaf Powder ($P > 0.05$), however, there was significant difference in the content of protein among Baolao Orange, Green Leaf Lemon Yellow and Sprinkle Golden Leaf Powder ($P < 0.05$).

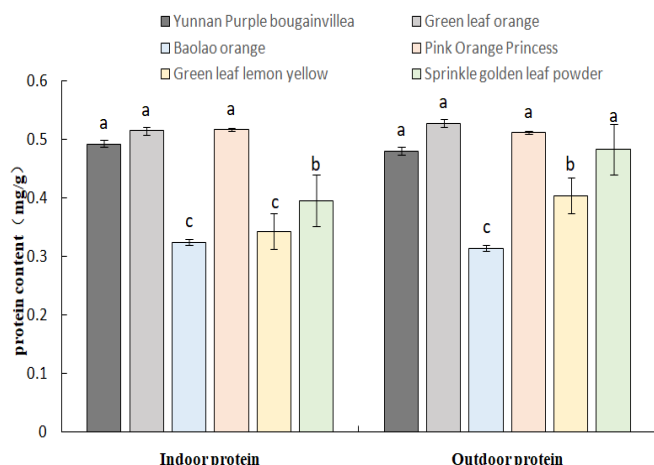


Fig.3. Soluble protein content of different varieties of *Bougainvillea ssp*

Peroxidase (POD) activity: From Fig. 4. The POD activity of outdoor plants is slightly lower than that of indoor plants. Both indoors and outdoors, the highest POD activity is Yunnan Purple bougainvillea, while Baolao Orange bougainvillea is the lowest. In indoor plants, there was no significant difference in POD activity among Yunnan Purple, Green Leaf Orange and Pink Orange Princess, and Baolao Orange, Green Leaf Orange, Sprinkle Golden Leaf Powder is the same; ($P > 0.05$)

However, there were significant differences between Yunnan Purple and Baolao Orange Green Leaf Orange, Sprinkle Golden Leaf Powder ($P < 0.05$). Among outdoor plants, there were no significant differences between Yunnan Purple, Green Leaf Orange and Pink Orange Princess, also between Baolao Orange, Green Leaf Lemon Yellow and Sprinkle Golden Leaf Powder ($P > 0.05$).

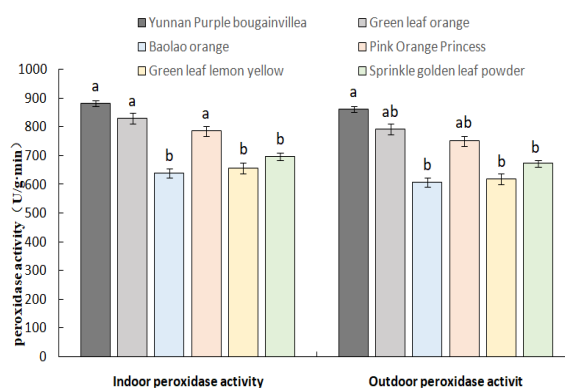


Fig. 4. Activities of POD in different varieties of *Bougainvillea ssp*

Catalase (CAT) activity: It can be seen from Fig. 5 that under low temperature, the CAT activity of six varieties of *Bougainvillea glabra* is slightly lower in outdoor than in indoor. The highest CAT activity of both indoor and outdoor plants is among Yunnan purple bougainvillea. The CAT activity of Yunnan purple is significantly different from that of other bougainvillea varieties ($P < 0.05$). However, there was no significant difference in CAT activity between Green Leaf Orange and Pink Orange Princess, and between Baolao Orange, Green Leaf Lemon Yellow and Sprinkle Golden Leaf Powder ($P > 0.05$).

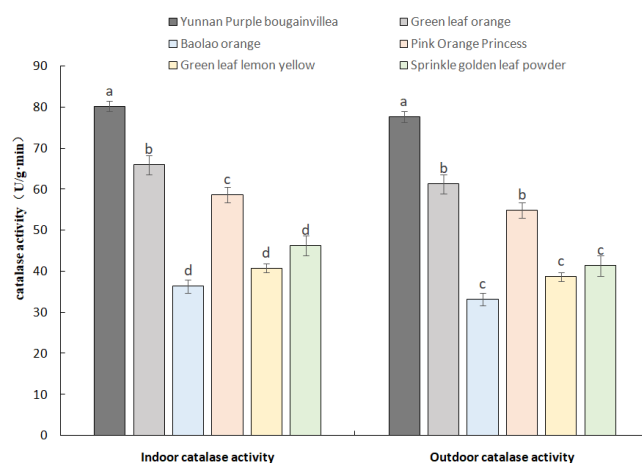


Fig. 5. Activities of CAT in different varieties of *Bougainvillea ssp*

DISCUSSION AND CONCLUSION

Under the outdoor low temperature condition, the six varieties of *Bougainvillea* were subjected to different degrees of freezing injury. Except that Yunnan Purple bougainvillea suffered less frost damage and only a few leaves were frostbitten, the leaves of other *Bougainvillea* varieties were curled, shrunk, withered and wilted in varying degrees and appear water stain like patches.

This is consistent with the conclusion that Lu *et al.* (2008) studied different varieties of *Photinia*. When the plants were subjected to freeze injury, the leaves of the plants appeared brown patches of varying degrees, and the leaves were wilting and curling. When plants are affected by adversity, it will make electrolyte extravasation in cells and increase electrical conductivity (Li, 2020). In this study, the electrical conductivity of *Prunus mume* under outdoor low temperature was higher than that in indoor, which showed that low temperature damaged the tissue of *Prunus mume* plant and increased its electrical conductivity. The results were the same as those of Yang *et al.* (2021) on the cold resistance of different apricot varieties. The lower the temperature, the greater the electrical conductivity of the plant and the weaker its cold resistance. The increase of soluble sugar content can improve the cold resistance of plants (Li *et al.*, 2016). From the results of this experiment. In both indoor and outdoor environments, the soluble sugar content of Yunnan Purple *Bougainvillea* is the highest, and that of Baolao Orange *Bougainvillea* is the lowest. The soluble sugar content of outdoor plants was slightly lower than that of indoor plants. This shows that Yunnan Purple *Bougainvillea* has strong cold resistance, while Baolao Orange *Bougainvillea* has weak cold resistance, and the outdoor low temperature conditions reduce the soluble sugar content of triangular plum plants. The results were basically consistent with the conclusion of Huet *al.* (20202) on the study of *Camellia oleifera* seedlings.

The increase of soluble protein content can improve the tolerance of plants to low temperature stress (Yuan *et al.*, 2020). In this study, Yunnan Purple, Green Leaf Orange and Pink Orange Princess had higher soluble protein content under outdoor and indoor conditions, which showed that they had strong cold resistance; Under outdoor conditions, the soluble protein content of Green Leaf Orange, Green Leaf Lemon Yellow and Sprinkle Golden Leaf Powder increased, indicating that the soluble protein content in the plant will increase in the face of low temperature stress. This is roughly the same as the conclusion of Ma *et al.* (2021) on the cold resistance of four different varieties of *Bougainvillea glabra*. The increasing trend of soluble protein content makes *Bougainvillea* resistant to low temperature. Catalase (CAT) generally exists in plants and can improve the cold resistance of plants (Sun, 2012). From the results of this experiment, the CAT activity of Yunnan Purple *Bougainvillea* was the highest and that of baolao orange *Bougainvillea* was the lowest among the plants placed outdoors for natural winter.

And the cat activity of outdoor plants was slightly lower than that of indoor plants. The results showed that Yunnan Purple *Bougainvillea* had the strongest cold resistance and Baolao Orange *Bougainvillea* had the weakest cold resistance. Under the outdoor low temperature environment, the cat activity of *Bougainvillea spectabilis* decreased due to the influence of low temperature. The observations of the current study is falling in line with the study of *Ligustrum japonicum* conducted by Gao *et al.* (2019), that is, the higher the catalase activity of the plant, the stronger the cold resistance, and the more seriously the plant is frozen, the lower the catalase activity. The physiological indexes showed that among the six varieties, the varieties with strong cold tolerance were Yunnan Purple and Green Leaf Orange; Except Yunnan Purple *Bougainvillea*, the other five varieties of *Bougainvillea spectabilis* can not overwinter normally in Jiangnan Plain and need overwintering protection.

This paper only studies different *Bougainvillea* varieties from the aspects of overwintering performance and physiology. In the next research, we can start from the aspect of genes and select transgenic *Bougainvillea* varieties with strong cold resistance, so as to contribute to the introduction and popularization of *Bougainvillea spectabilis* in Jiangnan Plain and cold areas in the north.

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