Current Status of Research on Pollen Vitality and Stigma Receptivity of Wuren Walnut (*Juglans sigillata*) in Guizhou Province

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Abstract Wuren walnut is a unique germplasm resource of *Juglans sigillata* in Guizhou Province, named after its purple and black seed coat. It is a typical medicinal and edible plant with high nutritional value and good taste compared with ordinary walnut. The color of its inner seed coat is different from that of ordinary walnut, which is mostly light yellow. Wuren walnut has great market potential for development. To lay a theoretical foundation for the future breeding of improved varieties, pollination tree configuration, and hybrid breeding of Wuren walnut in Guizhou Province, this paper summarized the current research status of pollen vitality and stigma acceptability of Guizhou Wuren walnut in recent years, and looked forward to future research and application.

Key words *Juglans sigillata*; Pollen vitality; Stigma; Cross breeding

Wuren walnuts (*Juglans sigillata*), also known as "Wumi walnut", "Zirang walnut", or "Xiaowuhe" in Guizhou, are characterized by its purple inner seed coat in a fresh fruit state. After the nuts are dried, the inner seed coat is mostly black or partially purplish brown, which is different from the light yellow inner seed coat of ordinary walnut fruit. The nutritional value of Wuren walnuts is higher than that of ordinary walnuts, and the taste is more fragrant and pure than ordinary walnuts, so it can be developed and utilized with emphasis[2]. Wuren walnuts with a strong aroma can fill the gap in the walnut variety market and meet the market demand for high-quality varieties[3]. The male flowers of walnut are in catkin, and the flowering periods of male and female flowers are mostly inconsistent, so its self-pollination condition is inhibited, and the fruit setting rate of self-pollination is low. The amount of pollen in different types of fruit trees varies, and the difference is significant even for different varieties of the same tree species. The amount of pollen has a significant impact on pollination results[4]. The number of walnut pollen is relatively large, but studies have shown differences in pollen vitality among different varieties[5-6], and the pollen vitality is relatively low[7-8]. The outer wall of pollen is relatively sturdy and has strong genetic conservation, which has been widely applied in plant classification systems, origins, and evolution at present. The unique morphology, ornamentation, number and location of germination pores of each variety are commonly used for plant classification and identification, and have been successfully applied. The morphology of pollen is also closely related to its vitality. At present, research on walnut pollen vitality and stigma receptivity has focused on ordinary walnut[9], and there have been no reports on related aspects of Wuren walnut. Guizhou Province has a wide planting area of walnut and abundant germplasm resources. Utilizing Guizhou's abundant germplasm resources to select walnut varieties with wider adaptability and better quality is an important content of current walnut cultivation work in Guizhou. Understanding the pollen vitality and stigma receptivity of different performance types (female first and male first) of Wuren walnut germplasm resources in Guizhou Province is of great significance for the future breeding of Wuren walnut varieties in Guizhou Province, pollination tree configuration, and hybrid breeding. It also provides a scientific basis for the later development and utilization of Guizhou's unique walnut germplasm resources.

Current Status of Research on Wuren walnut

Wuren walnut is mainly distributed in southwestern China, and the research on *J. sigillata* is mainly conducted in Yunnan and Guizhou. Wen *et al.*[10] conducted an experimental study on the factors affecting the survival rate in grafting propagation of Wuren walnut using Wuren walnut as scions and Tie walnut (*J. sigillata*) as rootstocks. It was found that environmental factors such as different grafting periods, degree of scion lignification, light and humidity have a significant impact on the survival rate in grafting propagation of Wuren walnut. Tong *et al.*[1] investigated the germplasm resources of Wuren walnut in northwestern Guizhou Province and conducted seed selection work. Six excellent strains were selected. Based on quality analysis, it was also found that Wuren walnuts have characteristics such as high protein, low sugar, low fiber, high phosphorus, and high carotene compared with ordinary walnuts, and thus has a great nutritional value. Wen *et al.*[11] measured the fruit traits and main nutritional

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components of 11 types of Wuren walnuts in Guizhou, and analyzed their main fatty acid composition. Hu[2] conducted superior tree selection and comprehensive trait evaluation on Wuren walnut in Ziyang County, Ankang City. Ma *et al.* [3] selected a new walnut strain with local characteristics from agricultural varieties, Longhe 3, a new walnut strain of Wuren walnut. Song *et al.* [12] selected a new walnut variety called "Zhenhe 2". Wen *et al.* [13] studied the growth characteristics of excellent individual seedlings of Wuren walnut during the seedling stage, revealing the differences in the growth rhythm of annual seedlings of different excellent individual plants of Wuren walnut. There are few research reports on Wuren walnut, and overall, basic research has been conducted.

Current Status of Research on Walnut Pollen Vitality and Stigma Receptivity

Current status of walnut pollen vitality

Pollen viability refers to the ability of pollen to survive, grow, germinate, or develop[14]. Studies have shown that artificial pollination during the peak flowering period of walnut female flowers can increase the fruit setting rate by 17.3% – 19.1%, and two rounds of artificial pollination could increase the fruit setting rate by 26%[15]. Before pollination, it is necessary to evaluate the quality of pollen vitality. Currently, there are four methods for measuring pollen vitality: *in-vivo* germination, *in-vitro* culture, inorganic acid methods, and staining methods[16-17]. Among them, common staining methods usually include triphenyl tetrazolium chloride staining (TTC method), fluorescence staining, proline-isatin fast staining, benzidine fluorescence staining, Saerdakeke staining, Kew staining, FCR staining, I-KI staining, and catalase staining.

Research has shown that the germination rate of walnut pollen is relatively low[18-19]. Previous studies have measured the vitality of walnut pollen using different methods, and the results indicate that there are certain differences in the vitality of walnut pollen among species and among varieties. Sucrose plays an important role in pollen germination and pollen tube elongation in *in-vitro* culture. It is the main nutrient for pollen germination and pollen tube wall synthesis, and participates in pollen metabolism and transmembrane transport, and maintaining osmotic pressure[20]. Qi *et al.* [21] found that four walnut varieties had the highest pollen germination rates in the medium with a low sugar content and a high boric acid content (*i. e.* 5% sucrose + 0.5% boric acid). Wu *et al.* [6] reported that the most suitable culture medium for walnut pollen was 100 g/L sucrose + 10 mg/L H_3BO_3 + 40 mg/L $CaCl_2$, and the pollen germination rate could reach 45.24%. Zhang *et al.* [22] measured that the pollen viability of 'Lvzao' and 'Liao 3' were both above 93.00% by peroxidase staining, and believed that the optimal temperature for walnut pollen germination was 24 – 25 ℃, and it took 33 h to germinate under 20 ℃ conditions.

Relationship between the length of pollen viability and environmental factors

The length of pollen's vitality is determined by both genetic factors and environmental factors[23]. Wang *et al.* [24] reported that walnut pollen had a relatively short storage time at room temperature, and generally, after 2 – 6 d of pollination, its viability was almost zero. And the pollen viability at the lower end of the male inflorescence was higher than that at the upper end, and the pollen viability at the inner cavity was slightly higher than that at the periphery. Zhang *et al.* [25] used the FCR method to study the vitality of *in-vitro* pollen from hickory. They found that during the peak period of pollen dispersal, the pollen had the highest vitality, and within 30 d of storage at room temperature, the vitality value was only 8%, while under dry conditions at room temperature, the pollen still had vitality within 90 d; under 4 ℃ storage conditions, 9% of pollen remained active after 180 d; and under storage conditions of – 40 ℃, both dry and non-dry pollen would maintain high activity (48% and 81%). Ren *et al.* [26] measured the pollen vitality of 15 early-fruiting walnut varieties using staining method and solid culture medium method, respectively, and pointed out that they all had strong vitality and could still maintain it for 4 – 7 d at room temperature. Liu *et al.* [27] studied the vitality of walnut pollen by the MTT staining method and found that the pollen could be preserved for 96 h in its natural state, but after 4 h, its vitality decreases to 33%. When the male flowers first bloomed, the pollen vitality reached a maximum of 95%.

Xu[28] believed that the pollen viability of actual walnut trees was higher than that of grafted walnut trees, making them more suitable for pollination as walnut trees. Wang *et al.* [24] observed through a microscope that the shape of walnut pollen was elliptical or circular, and the average diameter of pollen grains was 41.00 μm. Ren *et al.* [26] determined by the same method that the pollen shape of early-fruiting walnuts was mostly nearly circular, and the average diameter of pollen grains was 42.96 μm. Li *et al.* [29] conducted electron microscopy scanning on the pollen of several walnut clones and compared the differences in polar axis, equatorial axis, germinal pores, germinal pore size, and pollen grain decoration among several varieties. They believed that there were differences among the varieties. Li *et al.* [30] reported that the mature pollen of walnuts is spherical, medium-sized, and had a diameter of about 50 μm, and there were 13 – 15 germination pores, all distributed on the hemisphere of the pollen.

Effects of genetic factors on walnut pollen vitality

The amount of pollen directly affects the pollination and fertilization process of flowers, which is related to the yield of the year. Generally, varieties used as a pollination variety in production have more pollen grains. In other fruit trees, there have been reports, such as 13 large cherry varieties with a single pollen yield ranging from 810 to 3 323 grains[31], Xinjiang wild apricot with an average pollen yield of 1 516 grains per anther[32], apple with an average pollen yield of 8 428 grains per anther, and plum with only 1 197 grains[33]. There is a significant difference in the pollen content of individual anthers among different apricot varieties[34]. Jia *et al.* [35] believed that the pollen contents of different types of fruit trees were different, with an average of 626 – 6 541. The

pollen contents of fruit trees in the kernel type were larger, while those of fruit trees in the stone type were smaller. Walnut has a large number of male flowers, with each inflorescence producing approximately 1.8 million pollen grains. During the development process, they consume a large amount of water and nutrients stored in the tree body, which to some extent reduces trees' vigor. If the male flowers are manually removed during the blooming of the male inflorescence, it is estimated that a mature walnut tree with 90% – 95% male flower buds removed can save 50 kg of water and 1.1 – 1.2 kg of dry matter.

Hu *et al.* [9] clarified the pollen vitality and stigma receptivity of two walnut varieties, Yangbi Dapao and Yunxin Gaoyuan, and concluded that the female flower stigmas of Yangbi Dabao and Yunxin Gaoyuan had a certain receptivity starting from the V-shaped stage, and strong receptivity during the inverted V-shaped stage, which was the optimal pollination period.

Chen *et al.* [36] studied the effects of pollen viability and stigma receptivity of *Juglans hopeiensis* on fruit setting, and found that the fruit setting of *J. hopeiensis* was not only related to parental traits such as pollen viability, stigma receptivity, and compatibility between pollen and stigma, but also to environmental factors such as temperature and humidity during the pollination period. Liu *et al.* [37] analyzed the flowering dynamics, pollen vitality and stigma receptivity of the introduced *J. sigillata*, and found that falling flowers were the main reason for the low seed setting rate and fruit yield of the two varieties. A large number of falling flowers were related to ovule atrophy and abortion.

Prospects

Many scholars have conducted different studies on pollen characteristics and stigma receptivity on various tree species, and have achieved certain results. Some studies have been carried out on Wuren walnut, and the quality and development value of Wuren walnut have been recognized. However, the pollen vitality and stigma receptivity of Wuren walnut germplasm resources in Guizhou Province have not been reported. Therefore, it is particularly important to conduct research on the pollen vitality and stigma receptivity of Wuren walnut germplasm resources in Guizhou Province, so as to provide a theoretical basis for the future breeding of Guizhou Wuren walnut varieties, pollination tree configuration, and hybrid breeding. The research on the seed coat composition of Wuren walnut in Guizhou Province and the molecular mechanism of the formation of the seed coat color difference will help the directional improvement and molecular breeding of walnut varieties, and have important guiding significance for the breeding of walnut varieties with specific seed coat and high nutritional value.

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