

# Study on Varieties Combination Suitable for Mutual Pollination of Yan Mountain Chestnut\*

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## ABSTRACT

In order to improve the yield and quality of new varieties *Castanea mollissima* cv. “Zipo”, *Castanea mollissima* cv. “Zunyu” and traditional Yan Mountain cultivars *Castanea mollissima* cv. “Duanci”, *Castanea mollissima* cv. “Yanhong”, *Castanea mollissima* cv. “Donglingmingzhu”, an experiment was carried out to study varieties combination that was suitable for their pollination. Fruiting rate and seed-setting rate were used to decide which the most suitable configuration was. The result indicated that: *Castanea mollissima* cv. “Zunyu” was the best pollination tree for *Castanea mollissima* cv. “Yanhong”, *Castanea mollissima* cv. “Zipo” and *Castanea mollissima* cv. “Duanci”; *Castanea mollissima* cv. “Duanci” was regarded as the best pollination tree for *Castanea mollissima* cv. “Zunyu”; the best variety to comply with *Castanea mollissima* cv. “Donglingmingzhu” was *Castanea mollissima* “Zunyu” and *Castanea mollissima* cv. “Duanci”; *Castanea mollissima* cv. “Zunyu” and *Castanea mollissima* cv. “Duanci” complied with each other best. Appropriate configuration of pollination varieties turned out to be one of the key measures to improve the yield and quality of chestnut, while irrational combination may even bring the reduction of output.

**Keywords:** *Castanea Mollissima*; Configuration; Fruiting Rate; Seed-Setting Rate

## 1. Introduction

Chestnut (*Castanea mollissima*) was considered as one of Chinese endemic species and the nutrient-rich nuts have unique flavor, with great economic value [1]. Yan Mountain chestnuts mainly grow in Qianxi, Zunhua, Xinglong, Kuancheng, Qinglong counties, Hebei province and Huairou, Miyun, Pinggu and Changping in Beijing. All of the chestnuts here have exquisite shape, delicate meat with high sugar content. The nuts are famous for fried with the trade name “Tianjin Sweet Chestnut” or “Jing-Dong Chestnuts.” The varieties mainly cultivated in Yan Mountain region are Yanghong, YanChang, YanMing, Timazhenzhu, Zipo, Zunyu, Huai Huang, Huaijiu, Zunhuaduanci and so on. *Castanea mollissima* is a typical kind of cross-pollination plants and its self-pollination seed-setting rate is very low which reached only 10% to 40% [2].

A number of related studies had been done by many scholars. Such as Mokay, he has already proven that the phenomenon self-infertility on some chestnut seedling trees was due to incompatibility between male gametes

and female gametes [3]. In addition, the stigma of pistil has strong selectivity on pollen, as a result, affinity among pollination combinations of different species are different. The test cross experiments on combinations of pollination varieties conducted by Wenbang Fan and other experts showed that fruiting rate was low when self-pollination and became high when cross-pollination [4].

Seedlings occupied the majority in all original chestnut parks. Therefore, each tree differs from others genotypically and they can be interactive with each other when pollination. However, with improvement of commercial value of chestnut, the fine varieties are vigorously promoted and all breeding varieties became almost the same genotype. Due to large-scale cultivation involving the exclusive use of the same species, high empty shell rate and low seed-setting rate increasingly appeared. Cross-pollination should be fully considered and pollination varieties should be well arranged in chestnut cultivation, otherwise production will be greatly reduced [5]. Therefore, to make clear of pollination and fruiting characteristics and appropriate combination of pollination varieties is one of the key measures to improve the yield and quality of chestnut. Although some research about cross-pollination effect on fruiting rate has been done, they have not been applied to production. And almost no study

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on combination of pollination trees about chestnuts has been reported till now.

In this experiment, new varieties *Castanea mollissima* cv. “Zipo”, *Castanea mollissima* cv. “Zunyu” which were bred during “The Eleventh Five-year Plan” in China and the traditional Yan Mountain cultivars *Castanea mollissima* cv. “Duanci”, *Castanea mollissima* cv. “Yanhong”, *Castanea mollissima* cv. “Donglingmingzhu” were chosen as materials for the study of varieties combination which is suitable for their pollination. In addition, fruiting rate and seed-setting rate of female parents were used to decide which the most suitable configuration was. The last purpose of this paper is to provide a theoretical basis for scientific combination of the pollination varieties for new chestnut orchard.

## 2. Materials and Methods

### 2.1. Varieties Selection and Combinations

According to research objectives, new varieties *Castanea mollissima* cv. “Zipo”, *Castanea mollissima* cv. “Zunyu”, which were bred during “The Eleventh Five-Year Plan” in China and the traditional Yan Mountain cultivars *Castanea mollissima* cv. “Duanci”, *Castanea mollissima* cv. “Yanhong”, *Castanea mollissima* cv. “Donglingmingzhu” were selected for mutual pollination on the basis of germplasm resources survey. Pollens of all 5 varieties were respectively collected for reciprocal pollination. Each variety was pollinated with the other four varieties and there were totally 20 combinations. Varieties selection and pollination combinations design are as followed in **Table 1**.

### 2.2. Test Site

The pollination experiment was carried out in the chestnut breeding base in Zunhua City, Hebei Province which is located at 40°11'50"N latitude and 117°58'30"E longitude. It is in the north of Tangshan City and belongs to warm temperate semi-humid monsoon climate and has four distinct seasons. The annual average sunshine hours is 2608.2 hours, annual average temperature is 10.9°C, and lowest temperature is -25.7°C. Frost-free period reaches 182 days and its precipitation is 724.7 millimeters. This

chestnut breeding base was selected as the test site was because Zunhua is one of the main chestnut production bases in north China.

Zunhua Chestnut mainly distributed in northern and western mountains areas along the Great Wall which is rich in gneiss. Weathered gneiss soils which contains large number of iron, managanese, sulfur, boron and other inorganic nutrients, coupled with low temperatures, large temperature difference and abundant rainfall here, make it suitable for growth of chestnut.

### 2.3. Test Method and Process

The research materials were healthy chestnut trees which were 20-year-old, non-pest, well-managed and in similar growth situations. 15 trees of *Castanea mollissima* cv. “Yanhong”, “Zunyu”, “Zipo”, “Donglingmingzhu” and “Duanci” were respectively and randomly selected as female parents. 7 - 8 similar female flowers in the east, west, north and south of each tree (totally 30) were picked out for pollination.

Chestnut flowers follow the leaves, appearing in middle of May and reached its full flowering stage onto late May. As a consequence, the pollination experiment was carried out during May and the follow-up testing was respectively conducted in June when all the male flowers withered and September when chestnut matured.

May 8, 2010-May 13, 2010: Ziplock bags were applied to bag the female flowers before their stigmas's appearing.

May 15, 2010-May 24, 2010: It was the most appropriate season for pollination when the angle between stigma and stigma became 30° - 45° and most anther of male inflorescence turned yellow from green at this time. Ziplock bags were removed before pollens of inflorescence were directly applied to female stigmas. Then they were put on to the female flowers again immediately after the pollination. The job was repeated 3 days later.

June 15, 2010: All male flowers have withered and bags were taken off from the female flowers. Tags were hanged to branches to indicate male parents and fruiting rates were calculated.

September 17, 2010-September 27, 2010: Gather chestnut and investigate their empty shell rate, seed-setting rate after the chestnut was harvested.

**Table 1. Pollination combinations design.**

male	female				
	A (“Yanhong”)	B (“Zunyu”)	C (“Zipo”)	D (“Dongling mingzhu”)	E (“Duanci”)
A (“Yanhong”)	-	A × B	A × C	A × D	A × E
B (“Zunyu”)	B × A	-	B × C	B × D	B × E
C (“Zipo”)	C × A	C × B	-	C × D	C × E
D (“Dongling mingzhu”)	D × A	D × B	D × C	-	D × E
E (“Duanci”)	E × A	E × B	E × C	E × D	-

## 2.4. Statistics

The numbers of female inflorescence was recorded when pollinating. The fruiting condition was written down while ziplock bags were removed after all male flowers had withered and the numbers of empty shell were recorded when chestnuts were collected.

The experimental data, in which self-pollination combinations were considered as the CK, were analyzed by average method and One-way ANOVA, applying mean square error test method by spss17.0 statistical software.

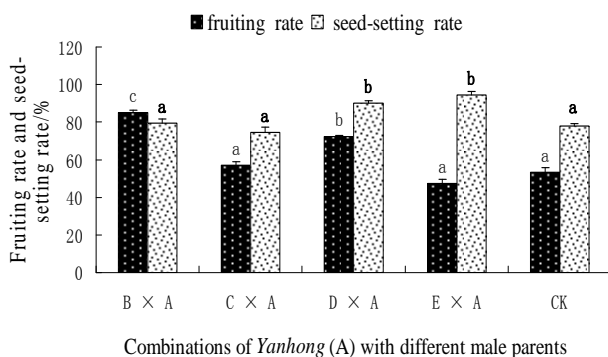
## 3. Result

### 3.1. Affect from Different Combinations on Fruiting Rate and Seed-Setting Rate

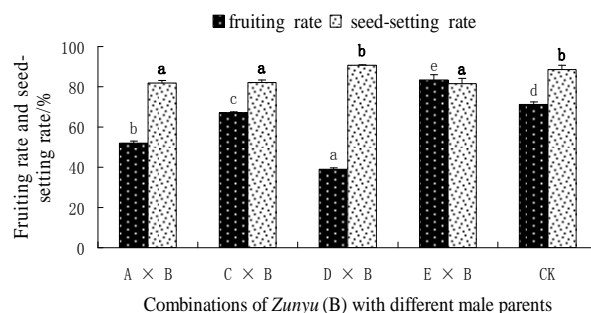
Empty shell of chestnut, commonly known as no fruit, means nuts in shells did not develop or just leave the seed coat [6-8]. It is considered as no empty shell even when there is only one seed in it. To some extent, the level of seed-setting rate can be used as indicator for strength of pollinator affinity. That is, the higher the seed-setting rate is, the stronger the affinity will be and it could be considered as a good combination [9].

The fruiting rate and seed-setting rate of 5 varieties *Castanea mollissima* cv. “Yanhong” (A), *Castanea mollissima* cv. “Zunyu” (B), *Castanea mollissima* cv. “Zipo” (C), *Castanea mollissima* cv. “Donglingmingzhu” (D), *Castanea mollissima* cv. “Duanci” (E) are followed as **Figures 1-5**.

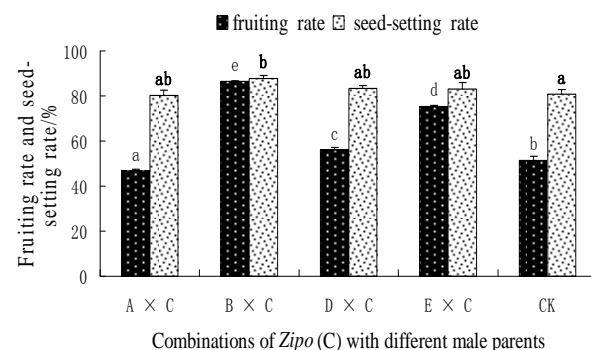
It can be seen from **Figure 1** that: Seed-setting rate of E × A, which reached 94.59%, was the highest in 4 combinations, but its fruiting rate was low, only 47.52%, indicating that fruiting rate and seed-setting rate is not so consistent with each other. In contrast, both fruiting rate (84.95%) and seed-setting rate (79.43%) of B × A were very high. Seed-setting rate of D × A was 90.00%, while fruiting rate which was 72.37% was lower than that of B



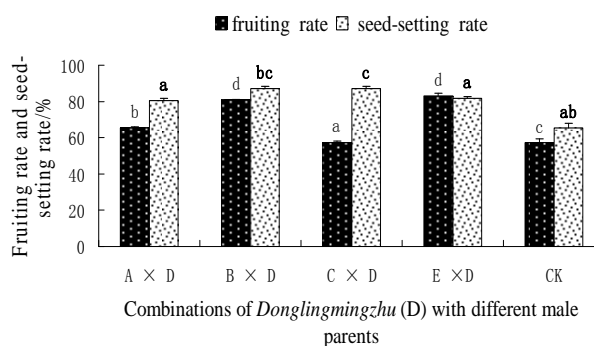
**Figure 1. Fruiting conditions of Yanhong (A) under different pollinations.** (a: From Figures 1-5 small letters on the post indicate differences in 5% level. Letters in bold described differences among seed-setting rate and those letters not in bold showed differences among fruiting rate.)



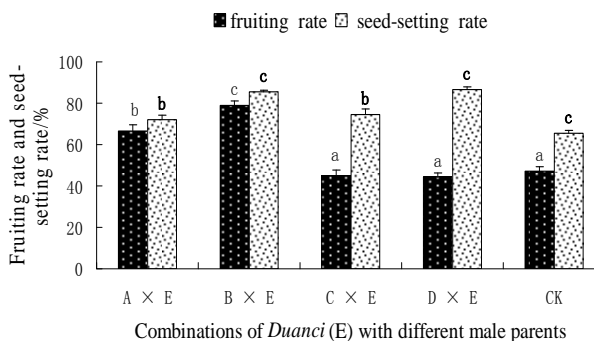
**Figure 2. Fruiting conditions of Zunyu (B) under different pollinations.**



**Figure 3. Fruiting conditions of Zipo (C) under different pollinations.**



**Figure 4. Fruiting conditions of Donglingmingzhu (D) under different pollinations.**



**Figure 5. Fruiting conditions of Duanci (E) under different pollinations.**

× A. Fruiting rate and seed-setting rate of *Castanea mollissima* cv. “Yanhong” (A) who had been under natural pollination were 53.45% and 77.78%. As a result, *Castanea mollissima* cv. “Zunyu” (B) was the best pollination tree for *Castanea mollissima* cv. “Yanhong” (A).

As we can see from **Figure 2**: Fruiting rate of E × B, which reached 83.33%, was significantly higher than that of the other 3 combinations. Seed-setting rate of E × B was 81.60%, although lower than combination D × B, whose seed-setting rate was 90.54%, significantly higher than its fruiting rate 38.95%. Fruiting rate of *Castanea mollissima* cv. “Zunyu” (B), who had been in natural pollination, was only 71.27%, far below fruiting rate of E × B. Thus, *Castanea mollissima* cv. “Duanci” (E) was regarded as the best pollination tree for *Castanea mollissima* cv. “Zunyu” (B).

It can be seen from **Figure 3** that fruiting rate and seed-setting rate of combination B × C were 86.61% and 87.33%, both of which were significantly higher than that of other 3 combinations. Fruiting rate and seed-setting rate of *Castanea mollissima* cv. “zipo” (C) were 51.34% and 80.65% in natural pollination, and they respectively increased significantly by 35.27% and 7.08% after pollinated by *Castanea mollissima* cv. “zunyu” (B). So the result presented here indicated that the best variety to comply with *Castanea mollissima* cv. “zipo” (C) is *Castanea mollissima* cv. “zunyu” (B).

It can be observed from **Figure 4** that seed-setting rate of C × D is significantly higher than that of other 3 combinations, who reached 87.21%, but fruiting rate was low, only 57.33%. Both fruiting rate and seed-setting rate of A × D became lower than that of B × D. In contrast, fruiting conditions of B × D and E × D showed significant advantages than that of *Castanea mollissima* cv. “Donglingmingzhu” (D) under natural pollination for fruiting rate and seed-setting rate of B × D achieved 80.86% and 86.96%, and E × D reached 83.15%, 81.65%. Thus, the best variety to comply with *Castanea mollissima* cv. “Donglingmingzhu” (D) were *Castanea mollissima* cv. “Zunyu” (B) and *Castanea mollissima* cv. “Duanci” (E).

**Figure 5** showed that fruiting condition of B × E was obviously better than that of A × E, C × E and D × E, whose fruiting rate got to 79.00% and seed-setting rate reached 89.45%. Though seed-setting rate of D × E became the highest of the 4 combinations, its fruiting rate was very low, only 44.50%, significantly lower than that of *Castanea mollissima* cv. “Donglingmingzhu” (D) under natural pollination. As a result, *Castanea mollissima* cv. “Zunyu” (B) turned out to be the best pollination tree for *Castanea mollissima* cv. “Duanci” (E).

### 3.2. Relationship between Cross-Pollination and Fruiting Conditions

Fruiting conditions of 20 hybrid combinations and 5 fe-

male parents pollinated under natural conditions were analyzed through variance analysis, applying Duncan comparison test method, and the data come to the top of each post. The data presented above indicate that significant differences were brought into fruiting rate and seed-setting rate between hybrid combinations and female parent. Moreover, much variation appeared in fruiting rate and seed-setting rate between different hybrids. From **Figure 1** we can see that fruiting condition of chestnut has been greatly affected by cross-pollination. However, because affinity among different pollination combinations vary widely, fruiting conditions of cross-pollination trees may become worse than the trees pollinated under natural conditions. Such as fruiting rates of combinations A × B, D × B, C × D even became significantly lower than the female parent pollinated under natural condition. So, appropriate configuration of pollination varieties would become one of the key measures to improve the yield and quality of chestnut, while irrational configuration may even bring the reduction of output.

What's more, fruiting conditions of cross combination and inverse cross combination were significantly different with each other. Such as fruiting rate of D × B, C × E were 38.91% and 45.02%, while B × D, E × C became 80.87% and 75.33%; seed-setting rate of E × A was 94.56%, but A × E only turned out to be 72.24%. Different varieties could comply with each other well when both fruiting conditions of cross combination and inverse cross combination go very high. From the second group of **Figure 1** we can see *Castanea mollissima* cv. “Duanci” (E) was regarded as the best pollination tree for *Castanea mollissima* cv. “Zunyu” (B) with fruiting rate and seed-setting rate respectively coming to 84.33% and 81.76%; moreover, the last group of **Figure 1** showed the best variety to comply with *Castanea mollissima* cv. “Duanci” (E) was *Castanea mollissima* cv. “Zunyu” (B) with fruiting rate and seed-setting rate getting to 79.07% and 85.46%. Consequently, *Castanea mollissima* cv. “Duanci” (E) and *Castanea mollissima* cv. “Zunyu” (B) could comply with each other quite well for configuration.

## 4. Discussion

### 4.1. The Best Configurations for Pollination Trees

The artificial pollination experiment which has been done by Renxue Xia and other experts on chestnut tree indicated that: empty shell rate of self-pollination tree was higher than that of cross-pollination tree and the number of nut decreased by 0.74 per shell [10]. Female flower has strong selectivity on pollen of different varieties. Consequently, to make clear of pollination and fruiting characteristics and appropriate combination of pollination varieties is crucial to improve fruiting condition of chestnut.

The cross-pollination experiment on 5 chestnut varieties indicated that: *Castanea mollissima* cv. “Zunyu” (B) was the best pollination tree for *Castanea mollissima* cv. “Yanhong” (A), *Castanea mollissima* cv. “Zipo” (C) and *Castanea mollissima* cv. “Duanci” (E); *Castanea mollissima* cv. “Duanci” (E) was regarded as the best pollination tree for *Castanea mollissima* cv. “Zunyu” (B); the best variety to comply with *Castanea mollissima* cv. “Donglingmingzhu” (D) was *Castanea mollissima* cv. “Zunyu” (B) and *Castanea mollissima* cv. “Duanci” (E); *Castanea mollissima* cv. “Zunyu” (B) and *Castanea mollissima* cv. “Duanci” (E) complied with each other best.

#### 4.2. Assessment on Relationship between Cross-Pollination and Fruiting Conditions

The results presented here indicated that fruiting conditions were significantly different between hybrid combinations and parents. What is more, fruiting rate, seed-setting rate also differ obviously among different hybrids. Therefore cross-pollination influenced fruiting rate, seed-setting rate a lot. What's more, fruiting conditions may become worse than that of under natural conditions. So, appropriate configuration of pollination varieties would become one of the key measures to improve the yield and quality of chestnut, while irrational combination may even bring the reduction of output.

#### 4.3. Actual Significance of the Results

One of the main reasons of low yield and low economic benefit of chestnut has been poor fruiting rate and seed-setting rate. Hence, more attention should be paid to pollination tree assignment. However, disadvantages such as no pollination trees, mess of mixed species and low yield per unit area are standing problems in Zunhua chestnut. What is more, no study on chestnuts pollination trees has been done in this region. Therefore, the best configurations above could help local planters avoid low fruiting rate and seed-setting rate problems. Meanwhile, assessment on relationship between cross-pollination and fruiting conditions highlighted irrational cross-pollination should be avoided, which corrected the long-terms errors that all the cross-pollination are beneficial.

All in all, it resolved the pollination tree problem which would increasingly improve chestnut yield in

future in this area, as well as provided a theoretical basis for scientific pollination tree assignment for new chestnut orchard here.

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