Article

Variation of Traits on Seeds and Germination Derived from the Hybridization between the Sections *Tacamahaca* and *Aigeiros* of the Genus *Populus*

Jialei Zhu ¹ , Ju Tian ², Jun Wang ^{3,*} and Shuijing Nie ⁴¹ College of Forestry, Beijing Forestry University, Beijing 100083, China; zhujialei@bjfu.edu.cn² Inner Mongolia Hesheng Institute of Ecological Science & Technology, Hohhot 011500, China; tianju@hsyl.com³ National Engineering Laboratory for Tree Breeding, Beijing Forestry University, Beijing 100083, China⁴ College of Science, Jiangxi University of Engineering, Xinyu 338209, China; nieshuijing@163.com

* Correspondence: wangjun@bjfu.edu.cn; Tel.: +86-010-6233-8105

Received: 8 July 2018; Accepted: 24 August 2018; Published: 27 August 2018

Abstract: Poplar is an important research organism, and species in sections *Tacamahaca* and *Aigeiros*, have advantages in terms of stress resistance, ease of propagation, and fast growth. Poplar species are widely distributed and well-adapted in the world, presenting a large potential for genetic improvement. Hybridization between different species allows us to generate offspring with a unique combinations of traits. This approach has a huge potential for breeding new poplar varieties that could aid in controlling desertification in the arid and semi-arid zones of the “Three-North” in China. In this study, we carried out a cross test scheme with nine female and thirteen male poplar trees. A total of 105,401 seeds were collected from 117 crosses. Flowering phenology and seed maturation differences of the hybrid progeny were monitored in greenhouses. For male trees, *Populus deltoides* had the longest flowering time. For female trees, *Populus pseudo-simonii* showed the longest seed maturity time. The number of carpals and ovules were not the same in different females. Meanwhile, three carpals were found in *P. pseudo-simonii*. A highly significant positive correlation was found between the seed size and the Thousand Kernel Weight, as well as the seedling cotyledon length. During seed germination, non-radicle and non-hypocotyl seedlings were observed. We also observed a number of cotyledon variants, including single and fused cotyledons, two cotyledons with one cotyledon cracking into two parts, three cotyledons, as well as four cotyledons. These results lay a favorable foundation for combining the research between the sections *Tacamahaca* and *Aigeiros* in future work.

Keywords: poplar; hybridization; cross compatibility; seed traits; cotyledon variation

1. Introduction

Desertification is one of the most serious ecological and environmental problems in the world [1–5]. It often causes water and wind erosion, vegetation degradation, and soil salinization [2]. Vast areas suffer from it in China, especially the “Three-North” (northeast, northwest, and north) regions (Figure 1). Inner Mongolia, for example, has seen a profound expansion of desertification and by 2008, it had reached nearly 185,000 km² [6]. Desertification contributes to poor soil fertility and economic losses in these afflicted areas, particularly in arid, semi-arid, and dry sub-humid zones [7–9]. Shelterbelt construction is one of the controlling measures for desertification. However, the vegetation of shelterbelt is not easily established [10] because of lack of tree varieties. Therefore, it is necessary to enhance variety breeding of afforestation trees in these regions and create new forest cultivars with a superior quality and high stress resistance.

Figure 1. Distribution map of “Three-North” region and location of flower branches collection.

Poplar trees are widely distributed over the world and play a significant role in ecological and environmental construction [11]. Taxonomically, poplar species are grouped into five sections, *Populus*, *Aigeiros*, *Tacamahaca*, *Turanga*, and *Leucoides* [12,13]. More than 100 poplar species have been discovered in the world, and 53 species exist in China [14]. The genus *Populus* L. has the characters of rapid growth, wide adaption, high fiber contents, and easy propagation [14–16]. For their rapid growth property, these trees are often used as a source of fiber, fuel, lumber, and to prevent soil erosion, they are also planted into windbreaks and protective stands [17]. Nevertheless, in the construction of shelterbelt in China, most of the poplar plantations have been showing low productivity and even more they are small, old trees, such aged ones lacking variety [18]. In order to solve this problem, it is better to create new poplar cultivars.

Hybridization is an efficient way to create a new genotype and plays an important role in the genetic improvement of numerous crops and animals [19–21]. Compared with other breeding methods, hybridization could recombine the parents’ genes, thus, combining the superior genes with different characters controlled by the parents into an organic whole or accumulate different minor genes with the same characters controlled by the parents, and contribute to a new genotype with all the characters. That is so-called heterosis, a complex genetical phenomenon [22]. It should be considered as the interaction of favorable combinations of genes at different loci for the parental lines [23]. Hybrid vigor may play an essential role in maintaining the genetic variation in populations [24]. Parental selection is very crucial during hybridization. Several principles should be considered, including the breeding objective, complementary characters, high combinability, heritability, far genetic relationship, and cross ability [19].

The history of poplar breeding spans more than a 100 year period [16]. In the hybridization experiment, interspecies hybridization within a section is easier than that between sections without manipulative techniques [16,25], whereas the crossability between sections is not the same. Crosses between sections *Aigeiros* and *Populus*, and *Tacamahaca* and *Populus* are quite difficult, while those between *Aigeiros* and *Tacamahaca* are easy [12,26,27]. The section *Tacamahaca* is the largest group of *Populus*, possessing an abundance of gene resources. *Populus simonii* and *P. pseudo-simonii*, are two widely distributed native tree species in China, and commonly appear in desertification regions, possessing features of easy rooting, drought tolerance, cold resistance, and wide adaptation [12,14,25]. In poplar genetic improvement, the section *Tacamahaca* frequently plays a role of the complementary parent. However, in contrast to other poplar groups, the level of genetic improvement is lower. Therefore, it is essential to enhance the genetic improvement level of the section *Tacamahaca* tree species. The section *Aigeiros* is distributed in North America, Europe, and Western Asia [17]. Among which, *P. deltoides*, *Populus nigra*, and their hybrids are important in the hybridization program of poplar

in China because they have the characteristics of rapid growth, a straight stem, and a resistance to diseases and pests, contrasting to *P. simonii*, *P. pseudo-simonii*, and especially *P. deltoides*, which are generally difficult to be propagated by hardwood cuttings [17]. Utilizing hybridization between section *Tacamahaca* and *Aigeiros* would allow for making the traits complementary and develop poplar varieties that are adaptable to use within shelterbelt programs in the “Three-North” regions.

In this work, we estimated the crosses compatibility among section *Tacamahaca*, section *Aigeiros*, and their hybrids. For this, we detected the seed and seedling traits of 117 crosses coming from a test cross scheme between 9 female parents and 13 male parents. The seed traits included seed length, seed width, and Thousand Kernel Weight (TKW). The seedling traits included the performance of the embryo root, the hypocotyl and cotyledon morphology, and length. In addition, seed formation rate and seed germination rate were also studied. Meanwhile, in order to describe the seed formation rate, the ovary observation and in vivo pollen germination test were performed.

2. Materials and Methods

2.1. Plant Materials

Twenty-two cultivars were used in this study. Among which, nine were female and thirteen were pollen donors (Table 1). Their flower branches were collected from Tongliao city in Inner Mongolia, Wuwei city in the Gansu province, Yiling Plain Forest Farm in Sinkiang, and from other places in China during December 2012 (Figure 1). The flower branches were packaged tightly with a plastic film and put in low temperature specified storage.

Table 1. The original source of the materials used in the crosses, including the 9 female and 13 male cultivars belonging to section *Tacamahaca* and *Aigeiros*.

Cultivar	Latin Name	Sexuality	Collection Place
XY-1	<i>P. simonii</i>	Female	Manas Plain Forest Farm, Sinkiang
XY-2	<i>P. simonii</i>	Female	Tongliao, Inner Mongolia
XY-3	<i>P. simonii</i>	Female	Tongliao, Inner Mongolia
XY-4	<i>P. simonii</i>	Female	Tongliao, Inner Mongolia
XY-5	<i>P. simonii</i>	Male	Tongliao, Inner Mongolia
XY-6	<i>P. simonii</i>	Male	Tongliao, Inner Mongolia
XQ-1	<i>P. pseudo-simonii</i>	Female	Tongliao, Inner Mongolia
XQ-2	<i>P. pseudo-simonii</i>	Female	Tongliao, Inner Mongolia
XQ-3	<i>P. pseudo-simonii</i>	Female	Tongliao, Inner Mongolia
XQ-4	<i>P. pseudo-simonii</i>	Male	Tongliao, Inner Mongolia
OH-1	<i>P. nigra</i>	Male	Tongliao, Inner Mongolia
OH-2	<i>P. nigra</i>	Male	Manas Plain Forest Farm, Sinkiang
ZTY	<i>Populus nigra</i> var. <i>italica</i>	Male	Beijing Botanical Garden
137	<i>P. deltoides</i>	Male	Tongliao, Inner Mongolia
144	<i>P. deltoides</i>	Male	Tongliao, Inner Mongolia
154	<i>P. deltoides</i>	Male	Tongliao, Inner Mongolia
BJLY3	<i>Populus deltoides</i> × <i>Populus nigra</i>	Male	Yiling Plain Forest Farm, Sinkiang
BJY	<i>Populus deltoides</i> × <i>Populus nigra</i>	Male	Yiling Plain Forest Farm, Sinkiang
DZY	<i>Populus deltoides</i> × <i>Populus nigra</i>	Male	Yiling Plain Forest Farm, Sinkiang
XMH	<i>Populus simonii</i> × (<i>Populus pyramidalis</i> + <i>Salix matsudana</i>)	Male	Tongliao, Inner Mongolia
ZY3	<i>Populus pseudo-simonii</i> × <i>Populus nigra</i> var. <i>lica</i>	Female	Tongliao, Inner Mongolia
EBY	<i>Populus nigra</i> var. <i>thevestina</i> × <i>Populus simonii</i>	Female	Wuwei, Ganshu

2.2. Pollination Procedure

2.2.1. Water Culture

Male branches were cultured in water with all the flower buds and one leaf bud for each branch in the greenhouse of the Beijing Forestry University with an average temperature of 10–20 °C and 50%–70% humidity during January 2013. About two weeks later, the female branches were cultured,

keeping four robust and full flower buds and two leaf buds for each branch. Three leaves remained when the buds unfolded. The water was changed two or three times a week.

2.2.2. Pollen Collection and Pollination

A total of 117 cross combinations were made using an entire 9×13 test cross mating design scheme; that is, every female parent crossed with every male parent. The pollen was collected during anthesis of male flowers and immediately stored at -20°C until its utilization. A controlled pollination hybridization was carried out in February 2013. When the female's stigma became shiny, pollination was conducted by softly transferring pollen onto it with a brush and stopped when it was covered with pollen. In all cases, we performed three replicate pollinations for each stigma. We also strictly carried out work in isolation for all the materials before powdering the stamens and well bagging the pistils with paper bags after pollination until the stigma lost receptivity.

We noted the number of pollinated catkins per cross, and, meanwhile, five traits were recorded during the water cultured procedure, including the days before flowering (starting from water cultivation to first staminate flower anthesis), duration of pollen shedding, days before pollination (starting from water cultivation), days to seed maturation (starting from pollination), and number of capsules per catkin for each cross combination.

2.3. In Vitro Pollen Germination

The flowers were harvested 24 h after pollination and immediately fixed in Carnoy solution (3:1 ethanol/acetic acid) at 4°C . Over a 24 h fixation, they were stored in 70% ethanol. The stigmas were removed from the fixed flowers, washed three times using distilled water, and softened in 8 M sodium hydroxide at room temperature for 4 h, rinsed briefly, then mounted in a drop of 0.1% aniline blue prepared with 0.2 M dipotassium phosphate staining for 5–10 min on a microslide. Then the coverslips were lowered onto the tissue and extruded with the appropriate strength. The slides were observed under a fluorescent microscope (Olympus BX51, Tokyo, Japan) and the photos were taken with an attached charge-coupled device video camera (Olympus DP70, Tokyo, Japan).

2.4. Ovary Observation

Ten fixed florets were randomly chosen for each female parent. The numbers of carpel and ovule per capsule were counted under a stereo anatomical lens (Olympus SZX12, Tokyo, Japan) and photos were taken with an attached image capture camera (Olympus U-CMAD3, Tokyo, Japan).

2.5. Seed Traits Measurements

Seed traits including the TKW, seed length and width, and seed germination rate were collected for the per cross combination in this research. For the TKW of every cross, three 100-seed samples were randomly counted and weighed, then converted into TKW. A sample of 30-seed was randomly chosen for measuring the seed length and width of each cross. Five-hundred seeds were randomly extracted for germination for each treatment. We placed the seeds in glass culture dishes with wet filter paper in a climate chamber (24 h at 25°C under light and 50% relative humidity). After two days of germination, the number of germinated seeds was counted. The seed formation rate was defined as the percentage of seed formation from ovules in each capsule, which was calculated as follows:

$$\text{Seed formation rate} = (\text{Seed number per female catkin} \div \text{capsule number per catkin}) \div \text{ovule number per capsule} \times 100\%.$$

2.6. Seedling Traits Measurement

After two days of germination in the chamber, we found abnormal development of the radicle and cotyledon, and counted the number of normal rooting seedlings, non-radicle, and non-hypocotyl

seedlings. The cotyledon length of the 30 seedlings was measured for each cross after one month of growth in the soil under the greenhouse condition.

2.7. Statistical Analysis

The seed formation rate and seed germination rate were subjected to arcsine root square transformation for statistical analysis. The analyses of variance (ANOVA), Pearson's correlation coefficients, and Duncan's multiple range test were performed for the days before flowering, the duration of pollen shedding, the days before pollination, the days to seed maturation, the days for seed development, the ovule number of per capsule, the seed formation rate, the seed length, the seed width, the seed germination rate, the seedling no radicle rate, and the seedling cotyledon length, using SPSS 16.0 (SPSS Inc., Chicago, IL, USA).

3. Results

The male parents showed variability in the days before flowering and for the duration of pollen shedding during the flowering time in this study within a greenhouse. The means of the days before flowering ranging from 22 ± 2 to 27 ± 3 days for "XY-5", "XY-6", "XQ-4", "OH-1", "OH-2", "ZTY", "BJLY3", "BJY", "DZY", and "XMH" (Figure 2). However, for *P. deltooides* ("137", "144", and "154"), it ranged from 35 ± 2 to 44 ± 1 days, particularly, the cultivar "154" required the longest time. Additionally, the duration of pollen shedding for cultivar "154" was 22 ± 2 days, which was the longest time among the male parents and was accompanied by the phenomenon of some staminate catkins failing to release pollen grains and falling off.

Figure 2. The flowering phenology on days before flowering and for duration of pollen shedding for the 13 male parents under the condition of water culturing in a greenhouse (mean + s.e.). The bars followed by the same letter are not statistically different ($p < 0.05$, $n = 3$). Duncan's multiple range test was taken.

The days before pollination was significant ($p = 0.0057$), it was about 26 days for female parents (Figure 3), while the time for the crosses from pollination to seed maturation was not exactly the same. ANOVA revealed that there was a highly significant effect ($p = 0.0001$) on the days to seed maturation according to the choice of females. The crosses that took *P. pseudo-simonii* as the female parent required the longest time for seed maturation, which was above 40 days (Figure 3). However, non-significant differences were observed in seed maturation for different male parents ($p = 0.7324$).

Figure 3. The phenology of the days before pollination and seed maturation for the 9 female parents under the condition of water culturing in a greenhouse (mean + s.e.). The bars followed by the same letter are not statistically different ($p < 0.05$, $n = 13$). Duncan's multiple range test was taken.

3.1. Variation in Ovary Traits of Female Parents

The anatomical observation revealed that there was a highly significant difference in the number of carpels and ovules per capsule for the female parents ($p = 0.0001$ both for the carpel and ovule). Most of the capsules had two carpels per capsule (Figure 4a), while three carpels were found in *P. pseudo-simonii* (Figure 4b). Cultivar “XQ-2” was at an 80% proportion of tree-carpel. The number of ovules per capsule for *P. simonii* was minimum, especially for the cultivar “XY-2”, which merely had 5.2 ± 0.3 ovules per capsule. Higher numbers were observed in other females.

Figure 4. The ovary anatomy of female parents. (a) Ovary anatomy for *P. simonii* “XY-4” showing two carpels. (b) Ovary anatomy for *P. pseudo-simonii* “XQ-2” showing three carpels. Bar = 1 mm.

3.2. Seed Formation Rate and Seed Morphological Variation

A total of 117 cross combinations were made by us and a total of 105,401 seeds were harvested. Most of the combinations hybridized between section *Tacamahaca* and *P. deltoidea* only obtained shriveled seeds (Figure 5a,b). However, the germination of pollen on the stigma was quite well done (Figure 6a,b). The combinations which took *P. pseudo-simonii* as the female parent and “ZY3”, “EBY”, or hybrid “BJLY3” as the male parent could obtain full seeds (Figure 5c,d). The results of the hybridization between section *Tacamahaca* as well as their hybrids and *P. nigra* were generally fine with plump seeds (Figure 5e–h). A few combinations did not obtain seed, especially the combinations which took hybrids “ZY3” or “EBY” as the female parent and *P. deltoidea* as the male parent with a

serious problem of falling catkins and fruits. Additionally, this situation also occurred in the crosses taking *P. simonii* as the female parent and cultivars “137”, “144”, or hybrid “BJY” as the male parent. ANOVA indicated that the parents had a highly significant impact on the seed formation rate (Table 2). The crosses which took “EBY” or “BJY” as parents expressed a low seed formation rate. The highest seed formation rate was the inter-specific cross “XY-3 $\times$ XQ-4”, reaching 79.26% while the intersectional cross, “XY-3 $\times$ OH-1”, only had a seed formation rate of 55.93%. According to the analysis of different cross combinations, the ones taking *P. simonii* cultivars “XY-2”, “XY-3” or “XY-4” as the female parent showed higher seed formation rate.

Figure 5. The morphology of the different hybrid seeds derived from hybridization between section *Tacamahaca* and *Aigeiros*. (a) Seeds of “XY-1 $\times$ 154”; (b) Seeds of “XY-2 $\times$ 137”; (c) Seeds of “XQ-5 $\times$ BJLY3”; (d) Seeds of “EBY $\times$ BJLY3”; (e) Seeds of “XY-1 $\times$ OH-1”; (f) Seeds of “XQ-2 $\times$ OH-2”; (g) Seeds of “X ZY3 $\times$ ZTY”; (h) Seeds of “EBY $\times$ OH-2”. The bars are equal to 3 mm.

Figure 6. The behavior of in vitro pollen germination, taken at 24 h after pollination. (a) *P. deltooides* “144” pollen on *P. simonii* “XY-2” stigma; (b) *P. deltooides* “154” pollen on *P. pseudo-simonii* “XQ-3” stigma. The bars are equal to 200 μ m.

Table 2. The analysis of variance showing the mean squares of the agronomic related parameters for hybrids crossing between section *Tacamahaca* and *Aigeiros*.

Evaluated Traits	Sources Variation							
	Female		Error (Female)		Male		Error (Male)	
	df	Mean Squares	df	Mean Squares	df	Mean Squares	df	Mean Squares
Days for seed development	8	377.23 **	93	12.82	-	-	-	-
Ovule number of per capsule	8	171.97 **	81	2.16	-	-	-	-
Seed formation rate (%)	8	0.11 **	93	0.03	12	0.20 **	89	0.02
Seed length (mm)	8	0 **	90	0	12	0 ns	86	0
Seed width (mm)	8	0.11 **	90	0.01	12	0.06 **	86	0.02
Seed germination rate (%)	8	0.26 ns	93	0.27	12	1.63 **	89	0.09
Seedling non-radicle rate (%)	8	504.21 ns	77	543.41	12	1692.17 **	73	350.27
Seedling cotyledon length (mm)	8	2.15 **	59	0.44	9	2.28 **	59	0.39

** Significant at 0.01% levels; ns, non-significant result; “-” no data.

3.3. Variation in Seed Traits

There was a very significant difference in the seed length and width for different cross combinations according to the variance analysis ($p = 0.0001$ both for seed length and width). Female parents had a highly significant effect on seed length, while there was no significant difference for male parents (Table 2). However, for seed width, both parents had a highly significant effect. The seed width of the combinations which took “EBY” or “ZY3” as the female was the largest ($p = 0.05$). No significant difference was detected for it, contrasting to the combinations taking “XY-4” as the female parents. Nonetheless, it was significantly bigger than the combinations taking the other tree species as the female parent. For the effect of the male parents, crosses that took “DZY” or “BJLY3” had the largest seed width ($p = 0.05$). The combination “ZY3 $\times$ BJLY3” represented the longest seed length and width, while the shortest seed length occurred in the cross “XY-1 $\times$ OH-2”, and the shortest seed width in cross “XY-1 $\times$ 137”. There was a highly positive significant correlation between seed length and seed width with different combinations according to the correlation analysis ($r = 0.4892$, $n = 99$, $p < 0.0001$).

TKW ranged from 0.1550 to 0.8900 g among the crosses. There was no significant difference for the crosses taking “XY” or “XQ” as the female parent ($p = 0.1045$ for “XY” and $p = 0.3438$ for “XQ”, respectively). The TKW ranged from 0.1550 to 0.7155 g when the female was *P. simonii*, especially the combination “XY-4 $\times$ XY-5” having the largest TKW. Similarly, when the female was *P. pseudo-simonii*, it ranged from 0.1515 to 0.6433 g, particularly the combination “XQ-3 $\times$ DZY” which had the largest TKW. Furthermore, there was a highly positive significant correlation between TKW and the seed length ($r = 0.4325$, $n = 88$, $p < 0.0001$) as well as the seed width ($r = 0.7893$, $n = 88$, $p < 0.0001$).

3.4. Seed Germination Characteristics

The germination rate of hybrid seeds which were obtained from the section *Tacamahaca* crossing with *P. deltooides* was very low, even as low as zero. A non-significant difference was present in the seed germination rate for female parents while the male parents had a very significant effect on it (Table 2). The seed germination was generally good for the combinations between section *Tacamahaca* along with its hybrids and *P. nigra* but presented differences. The seed germination rate was above 45% when the hybrids of section *Tacamahaca* and *P. pseudo-simonii* crossed with “BJLY3” and “DZY”; while, the seed germination rate was below 25% when the female was instead *P. simonii*. The cross combinations among section *Tacamahaca* had a high seed germination rate, and the crosses which took *P. pseudo-simonii* as the female parent had a better seed germination situation compared with *P. simonii* as the female parent.

3.5. Variations in Seedling Cotyledon Length and Morphology

The parents’ combination had a highly significant effect on the seed cotyledon length (Table 2). It was not significant on the seed cotyledon length for the combinations which took *P. simonii* or

P. pseudo-simonii as the female parent ($p = 0.0770$ for the former and $p = 0.3836$ for the latter). The seed cotyledon length of the crosses which took “ZY3” as the female parent was longer than the others and there was a tendency that the crosses taking hybrids as parents had a longer cotyledon length.

Seedlings without a visible root organ and hypocotyl were also observed. When the radicle of the normal seedlings was elongated (Figure 7a), in the others it had not yet been seen (14.24%) (Figure 7b). Moreover, a few seedlings only presented with two cotyledons without a hypocotyl (Figure 7c). There was no regular segregation ratio of non-rooting to normal rooting seedlings. The seedlings without a hypocotyl accounted for a percentage of 1.13%. When it came to the individual cross, the results were rule less. ANOVA indicated that there was no significance on the non-radicle rate of seedlings for female parents (Table 2), whereas it was highly significant for the male parents (Table 2). The non-hypocotyl rate of crossbred seedlings was generally low. It was relatively higher for the combinations which took the hybrids as one of the parents; such as the crosses “EBY $\times$ OH-2” and “XQ-3 $\times$ BJLY3” (11.43% for the former and 9.34% for the latter). Furthermore, during the germination stages, the color of the hypocotyl was not the same for different crosses. Parts of them were peak green (Figure 7b) and the others were red (Figure 7a,d–g). However, the peak green would turn red due to illumination during later growth.

Figure 7. The different morphological variations during the seed germination of the hybrids crossing between sections *Tacamahaca* and *Aigeiros*. (a) Seedling of hybrid between *P. simonii* “XY-4” and *P. nigra* “OH-1”; note: two opposite and approximate cotyledons; (b) seedling of hybrid between *P. simonii* “XY-1” and *P. nigra* var. *italica* “ZTY”; note: no radical and peak green hypocotyls; (c) seedling of hybrid between *P. nigra* var. *thevestina* $\times$ *P. simonii* “EBY” and *P. nigra* “OH-1”; note: no hypocotyls; (d) seedling of hybrid between *P. pseudo-simonii* “XQ-3” and “XQ-4”; note: fused cotyledons; (e) seedling of hybrid between *P. pseudo-simonii* $\times$ *P. nigra* var. *lica* “ZY3” and *P. nigra* “OH-1”; note: two cotyledons with one cracking into two parts; (f) seedling of hybrid between *P. simonii* “XY-4” and *P. nigra* “OH-1”; note: three cotyledons; and (g) seedling of hybrid between *P. pseudo-simonii* “XQ-2” and “XQ-4”; note: four cotyledons.

Seedlings with normal cotyledons generally had two opposite and approximate ones in dicot species (Figure 7a). However, there were various other types in our study, such as the single and fused cotyledons (Figure 7d), two cotyledons with one cotyledon cracking into two parts (Figure 7e), three cotyledons (Figure 7f), and four cotyledons (Figure 7g). Among them, the three cotyledons type appeared frequently. The four cotyledons type only appeared in the combinations taking *P. pseudo-simonii* as the female parent and occupied quite a low ratio (0.01%). The parents were not significant on the seedling cotyledons variation ($p = 0.1212$ for female and $p = 0.4244$ for male). The seedling cotyledons variation ratio was higher for the combinations which took *P. pseudo-simonii* as the female parent rather than the *P. simonii* ones. Between them were the combinations which took the hybrids as the female parents. The combinations taking “BJLY3” as the male parent had a high cotyledon variation rate. The crosses “XQ-2 $\times$ XMH”, “XY-4 $\times$ OH-2”, “XY-4 $\times$ XY-5”, and so forth had a higher cotyledon variation than others (2.55%, 2.54%, and 2.31%, respectively).

4. Discussion and Conclusions

Flowering asynchronization can lead to a barrier to hybridization, while flowering synchronization is a stronger selector in dioecious plants [28–30]. Hence, mastering the flowering phenology plays a guiding role in the breeding program. Flowering phenology also strongly affects reproductive success [31]. Changes in the flowering time and flowering duration may modify the overall ontogeny and the architecture of plant individuals [32]. In our study, there was a significant difference in the flowering time for different kinds of poplars in the greenhouse. The results revealed that it took 44 days from water planting to powdering and 22 days for the powdering period for male *P. deltoides*, while Zhao et al. [33] showed that *P. deltoides* took five days from water planting to powdering, and three days for the powdering period in a greenhouse. Broeck et al. [29] also indicated that the powdering period was three and 14 days, respectively, in 1999 and 2000. The flowering phase was connected with the environment, genes, and other endogenous factors [29,31,34–36]. Due to diversified impact factors, it should be a reasonable arrangement of the hydroponic time to make sure the female and male flowering phase encounter each other when carrying out cross breeding work between section *Tacamahaca* and *Aigeiros*.

Seed size is an essential functional character in flowering plants [37–39], influenced by environmental factors and genetic traits [40]. In our work, we found that the influence of female parents for seed length was significant, while for seed width, both females and males had a significant influence on it. This suggests that the seed length is mostly controlled by females in poplars. The TKW ranged from 0.3333 to 0.7155 g for section *Tacamahaca* in our research. However, species in section *Populus* ranged from 0.1250 to 0.1990 g [41,42] and in North America, *Aigeiros* ranged from 2.2222 to 3.3333 g [41]. These data showed that there is a wide variation in the TKW for *Populus* L. Zhang et al. [43] pointed out that several kernel traits, including kernel length and kernel width, controlled the TKW in wheat. Similarly, there was a highly positive significant correlation between TKW with the seed length and seed width in our study. According to the correlation analyses results, the seed width was more relevant to the TKW than the seed length.

Root apical meristems are at the bottom of the hypocotyl and can lead to the formation of a radical or embryonic root [44]. The normal development of the hypocotyl and radicle is essential to the seedling's growth. During embryogenesis, the embryonic root and its shoot apical meristems are established in angiosperms [28,45–47] and they initiate post-embryonic development by producing a visible root organ. Zhang et al. [48] discovered that the separation ratio of the non-rooting to normal rooting was 1:3 in F1 offspring for *P. tomentosa*. However, we did not find the same segregation ratio in our study. Zhang et al. [48] indicated that it was a qualitative trait for the radical development character and a non-rooting trait due to the allelic mutation in *P. tomentosa*. However, some *Arabidopsis* embryo defective mutants lack a primary root but are capable of forming roots (hobbit and bodenlos mutants) and hypocotyl (monopteros and fackel mutants) in the post-embryonic stage, which changes the function of some housekeeping genes, the metabolic pathway, secretory

pathway, or homeodomain transcription factor [49–52]. These mutant genes have an effect on the formation of the apical-basal polarity pattern for zygotic embryo [53]. Apical-basal pattern controls important elements of the seedling body, such as the shoot meristem, root meristem, embryonic root, hypocotyl, and cotyledons [44]. It is clear to see that the regulation of genes has a huge effect on the growth of the plant. The traits of the seedling radicle and hypocotyl constitute a reference for the reselection of the parents.

Cotyledons provide nutrition and are able to carry out photosynthesis for some young plants in angiosperm [54]. Generally, there are two bilaterally symmetric and almost equally sized cotyledons for most dicot species. However, research has revealed that there are numerous cotyledon mutants in many plant species. The partial or complete fusion of the cotyledons was presented in species of *Calophyllum*, *Swietenia*, and *Guarea* [55]. Karschon [56] reported that the seedlings of the *Chenopodiaceae* occurred schizocotily in order to give hemitrocotily, where one of two cotyledons might be a cleft. Moreover, some species of *Acer*, *Coffea*, *Raphanus*, and *Sesamum* have three cotyledons [57–60]. Additionally, there are four cotyledons in some *Ranales* and *Persoonia* [55,61]. Similar morphological variations of cotyledons were shown in our study and the seedlings with three cotyledons occurred at a high frequency among the variable seedlings. It was the same for the results of triploid *P. tomentosa* [62], whereas, when it comes to *Arabidopsis*, there were more morphological variations of the cotyledons, such as cup-shaped cotyledons, malformed cotyledons, and cotyledon absence [63–65]. Kang et al. [62] pointed out that the equilibrium of cell chromosomes might be related to these variations in triploid *P. tomentosa*, as genes were the main factor of controlling cotyledons variations in *Arabidopsis* [54,65]. At the same time, auxin, which is essential for organ formation, may also be the ultimate reason. The variation of cotyledons may have an influence on the nutrient uptake and preliminary photosynthesis in epigeal seedlings in which the cotyledons appear above ground. As a consequence, it finally affects the growth of seedlings.

Intersectional hybridization plays an important role in the genetic improvement of the species of the genus *Populus* and wide hybridization easily contributes to hybrid incompatibility and hybrid sterility, but it is a way to produce transgressive hybrids. Cross-incompatibility usually occurs in wide hybridization and it generally takes place as the dropping of flowers and fruits, low seed set, shriveled seeds, and low seed formation percentage [66–68]. In our study, the combinations which had *P. deltoides* as the male parent had a low seed formation percentage and seed set, shriveled seeds, and low seed germination rate, some having no collected seed. Similarly, the crosses involving male *P. deltoides* and female *P. balsamifera* produced few seeds while these seeds revealed a low viability [67]. Meanwhile, Mahama et al. [68] pointed out that the crosses involving male *P. deltoides* and female *P. maximowiczii* were less successful and rarely acquired seedlings. However, when *P. deltoides* was taken as the female and some species of section *Tacamahaca* were taken as male, the crosses showed a high success rate, obtaining many seedlings [33,66,67]. The results in our research suggested that cross-incompatibility was occurring. Many stages in the hybridization process, including the germination of the pollen grains and the failure of the pollen tube penetrating the stigma and ovary as well as the failure of seed development and hybrid sterility, may contribute to cross-incompatibility [68,69]. These stages could be classified into prezygotic and postzygotic stages [70,71]. The prezygotic stage is closely influenced by pollen tube behavior [68,69,72] and, if the cross-barrier occurred in the postzygotic stage, the embryo rescue and tissue culture technique could overcome it [73–75]. In our study, numerous germinated pollen grains were clearly observed and penetrated into the stigma according to aniline blue stain observation in crosses taking *P. deltoides* as the pollen donor, and a few shriveled seeds were collected. Therefore, the reason for the incompatibility most probably occurred in the postzygotic stage. Further work is still needed to elucidate what factors led to the incompatibility.

Hybridization breeding plays an important role in poplar breeding engineering and lays the foundation for polyploidy breeding. The parental selection is essential for hybridization. At the same time, it is beneficial to improve the effectiveness of polyploidy breeding in the genus *Populus*, according to the experience of parent selection in hybridization breeding [76]. Polyploidization is essential in

speciation, evolution, and creating elite varieties for poplar plantations [77–79]. Moreover, triploid *Populus* is more widely used for the production of lumber, fiber, and fuel, in contrast to diploids [80,81]. At the same time, it is also an essential part of the control of desertification. Since the application of polyploid *Populus* is important, it is necessary to conduct a study of polyploid induction. Therefore, we will carry out research of general and special combining ability in the next study.

Author Contributions: J.W. conceived and designed the experiments; J.Z. performed the experiments; J.T. and S.N. collected samples and analyzed the data; J.Z. wrote the paper.

Funding: This study was supported by the Fundamental Research Funds for the Central Universities (2018ZY30), the National Natural Science Foundation of China (31470662) and the program from the Beijing Municipal Education Commission (CEFF-PXM2018_014207_000024).

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Liu, Y.H.; Dong, G.R.; Li, S.; Dong, Y.X. Status, causes and combating suggestions of sandy desertification in Qinghai-Tibet plateau. *Chin. Geogr. Sci.* **2005**, *15*, 289–296. [[CrossRef](#)]
2. An, P.; Inanaga, S.; Zhu, N.W.; Li, X.J.; Fadul, H.M.; Mars, M. Plant species as indicators of the extent of desertification in four sandy rangelands. *Afr. J. Ecol.* **2006**, *45*, 94–102. [[CrossRef](#)]
3. Ikazaki, K.; Shinjo, H.; Tanaka, U.; Tobita, S.; Funakawa, S.; Kosaki, T. “Fallow Band System,” a land management practice for controlling desertification and improving crop production in the Sahel, West Africa. 1. Effectiveness in desertification control and soil fertility improvement. *Soil Sci. Plant Nutr.* **2011**, *57*, 573–586. [[CrossRef](#)]
4. Peters, D.P.C.; Yao, J.; Sala, O.E.; Anderson, J.P. Directional climate change and potential reversal of desertification in arid and semiarid ecosystems. *Glob. Chang. Biol.* **2012**, *18*, 151–163. [[CrossRef](#)]
5. Wang, X.M. Sandy desertification: Borne on the wind. *Chin. Sci. Bull.* **2013**, *58*, 2395–2403. [[CrossRef](#)]
6. Zhang, J.T.; Liu, Z.; Zhao, X.Y.; Wen, C.Z.; Meng, Q.H.; Wu, F.J. Prophylaxis and treatment of land desertification in Inner Mongolia. *Inner Mong. Pratacult.* **2008**, *20*, 37–39. (In Chinese)
7. Zhou, R.L.; Li, Y.Q.; Zhao, H.L.; Drake, S. Desertification effects on C and N content of sandy soils under grassland in Horqin, Northern China. *Geoderma* **2008**, *145*, 370–375. [[CrossRef](#)]
8. Albalawi, E.K.; Kumar, L. Using remote sensing technology to detect, model and map desertification. *J. Food Agric. Environ.* **2013**, *11*, 791–797.
9. Duan, H.C.; Wang, T.; Xue, X.; Liu, S.L.; Guo, J. Dynamics of Aeolian desertification and its driving forces in the Horqin Sandy Land, Northern China. *Environ. Monit. Assess.* **2014**, *186*, 6083–6096. [[CrossRef](#)] [[PubMed](#)]
10. Yan, P.; Dong, G.R.; Su, Z.Z.; Zhang, D.S. Desertification problems in the Yangtze River source area, China. *Land Degrad. Dev.* **2004**, *15*, 177–182. [[CrossRef](#)]
11. Feng, J.J.; Jiang, D.C.; Shang, H.Y.; Dong, M.; Wang, G.N.; He, X.Y.; Zhao, C.N.; Mao, K.S. Barcoding Poplars (*Populus* L.) from Western China. *PLoS ONE* **2013**, *8*, e71710. [[CrossRef](#)] [[PubMed](#)]
12. Zsuffa, L. A summary review of interspecific breeding in the genus *Populus* L. In *Proceedings of the 14th Meeting of the Canadian Tree Improvement Association, Part 2*; Department Environment, Canadian Forestry Service: Ottawa, ON, Canada, 1975; pp. 107–123.
13. Thieret, J.W. Trees and shrubs hardy in the British Isles. *Econ. Bot.* **1982**, *36*, 273. [[CrossRef](#)]
14. Xu, W.Y. *Poplar*; Heilongjiang People’s Publishing House: Harbin, China, 1988; pp. 1–74.
15. Tuskan, G.A. Short-rotation forestry: What we know and what we need to know. *Biomass Bioenergy* **1988**, *14*, 307–315. [[CrossRef](#)]
16. The Organisation for Economic Co-operation and Development (OECD). *Consensus Document on the Biology of Populus L. (Poplars)*; Presented at the Environmental Health and Safety Publications Series on Harmonization of Regulatory Oversight in Biotechnology, Environment Directorate Organisation for Economic Co-operation and Development; OECD: Paris, France, 2000; pp. 1–53.
17. Rae, A.M.; Street, N.R.; Rodríguez-Acosta, M. *Populus* Trees. In *Forest Trees*; Springer: Berlin/Heidelberg, Germany, 2007; pp. 1–28.
18. Lyu, W.; Zhang, W.D. Function and status of *Populus* L. in the construction of three-north protection forest. *For. Sci. Technol.* **2000**, *5*, 7–9. (In Chinese)

19. Chen, X.Y.; Shen, X.H. *Forest Tree Breeding*; Higher Education Press: Beijing, China, 2005; pp. 71–78. (In Chinese)
20. Falconer, D.S. *Introduction to Quantitative Genetics*, 4th ed.; Longman Scientific and Technical: London, UK, 1989.
21. Stuber, C.W. Heterosis in plant breeding. *Plant Breed. Rev.* **1994**, *12*, 227–251.
22. Jinks, J.L.; Jones, R.M. Estimation of components of heterosis. *Genetics* **1958**, *43*, 223–234. [[PubMed](#)]
23. Hayman, B.I. Interaction, heterosis and diallel crosses. *Genetics* **1957**, *42*, 336–355. [[PubMed](#)]
24. Crow, J.F. Basic concepts in population, quantitative, and evolutionary genetics. *Trends Genet.* **1988**, *4*, 1–25. [[CrossRef](#)]
25. Villar, M.; Gaget, M.; Said, C.; Knox, R.B.; Dumas, C. Incompatibility in Populus: Structural and cytochemical characteristics of the receptive stigmas of *Populus alba* and *P. nigra*. *J. Cell Sci.* **1987**, *87*, 483–490. [[PubMed](#)]
26. Stettler, R.F.; Kostob, R.; Steenackers, V. Interspecific crossability studies in poplars. *Theor. Appl. Genet.* **1980**, *58*, 273–282. [[CrossRef](#)] [[PubMed](#)]
27. Li, W.D.; Xu, H.; Cheng, X.-F. The behavior of pollen tubes on the stigma in the intersectional crosses in Populus and its relation to seed-setting. *Chin. J. Bot.* **1981**, *3*, 102–109. (In Chinese)
28. Laux, T.; Jürgens, G. Embryogenesis: A new start in life. *Plant Cell* **1997**, *9*, 989–1000. [[CrossRef](#)] [[PubMed](#)]
29. Broeck, A.V.; Cox, K.; Quataert, P.; Van Bockstaele, E.; Van Slycken, J. Flowering phenology of *Populus nigra* L., *P. nigra* cv. *italica* and *P. x canadensis* Moench. and the potential for natural hybridisation in Belgium. *Silvae Genet.* **2003**, *52*, 280–283.
30. Munguia-Rosas, M.A.; Ollerton, J.; Parra-Tabla, V. Phenotypic selection on flowering phenology and size in two dioecious plant species with different pollen vectors. *Plant Spec. Biol.* **2011**, *26*, 205–212. [[CrossRef](#)]
31. Kobayashi, M.J.; Shimizu, K.K. Challenges in studies on flowering time: Interfaces between phenological research and the molecular network of flowering genes. *Ecol. Res.* **2013**, *28*, 161–172. [[CrossRef](#)]
32. Sola, A.J.; Ehrlén, J. Vegetative phenology constrains the onset of flowering in the perennial herb *Lathyrus vernus*. *J. Ecol.* **2007**, *95*, 208–216. [[CrossRef](#)]
33. Zhao, L.; Fan, J.F.; Gao, J.S.; Zhou, Y.X. Artificial hybridization and seedling traits test of *Populus deltoides* and *P. cathayana*. *Acta Agric. Boreali-Occident. Sin.* **2010**, *19*, 108–112. (In Chinese)
34. Farmer, R.E. Sexual reproduction of eastern cottonwood. In Proceedings of the Symposium on Eastern Cottonwood and Related Species, Greenville, MI, USA, 28 September–2 October 1976; Louisiana State University: Baton Rouge, LA, USA, 1976; pp. 89–98.
35. Pilson, D. Herbivory and natural selection on flowering phenology in wild sunflower, *Helianthus annuus*. *Oecologia* **2000**, *122*, 72–82. [[CrossRef](#)] [[PubMed](#)]
36. Weis, A.E.; Kossler, T.M. Genetic variation in flowering time induces phenological assortative mating: Quantitative genetic methods applied to *Brassica rapa*. *Am. J. Bot.* **2004**, *91*, 825–836. [[CrossRef](#)] [[PubMed](#)]
37. Harper, J.L. *Population Biology of Plants*; Academic Press: London, UK, 1977.
38. Leishman, M.R.; Wright, I.J.; Moles, A.T.; Westoby, M. The evolutionary ecology of seed size. In *Seeds: The Ecology of Regeneration in Plant Communities*; Fenner, M., Ed.; CABI Publishing: Oxford, UK, 2000; pp. 31–51.
39. Lönnberg, K.; Eriksson, O. Rules of the seed size game: Contests between large-seeded and small-seeded species. *Oikos* **2013**, *122*, 1080–1084. [[CrossRef](#)]
40. Liu, B.; Zhou, X.M.; Qu, D.N. Relationship among seed size from different seed positions at several-seeded pod in soybean. *J. Food Agric. Environ.* **2012**, *10*, 768–771.
41. Schreiner, E.J.; Populus, L. *Seeds of Woody Plants in the United States*; Agricultural Handbook No. 450; Schopmeyer, C.S., Ed.; USDA, Forest Service: Washington, DC, USA, 1974; pp. 645–655.
42. Allen, T. *The Occurrence, Morphology, and Reproductive Biology of Natural Hybrids of Populus alba in Southeastern MICHIGAN*; University of Michigan, School of Natural Resources: Ann Arbor, MI, USA, 1978; p. 125.
43. Zhang, X.Y.; Deng, Z.Y.; Wang, Y.R.; Li, J.F.; Tian, J.C. Unconditional and conditional QTL analysis of kernel weight related traits in wheat (*Triticum aestivum* L.) in multiple genetic backgrounds. *Genetica* **2014**, *142*, 371–379. [[CrossRef](#)] [[PubMed](#)]
44. Jürgens, G. Apical-basal pattern formation in Arabidopsis embryogenesis. *EMBO J.* **2001**, *14*, 3609–3616. [[CrossRef](#)] [[PubMed](#)]
45. Benfey, P.N.; Schiefelbein, J.W. Getting to the root of plant development: The genetics of Arabidopsis root formation. *Trends Genet.* **1994**, *10*, 84–88. [[CrossRef](#)]

46. Vernoux, T.; Wilson, R.C.; Seeley, K.A.; Reichheld, J.P.; Muroy, S.; Brown, S.; Maughan, S.C.; Cobbett, C.S.; Van Montagu, M.; Inzé, D.; et al. The ROOT MERISTEMLESS1/CADMIUM SENSITIVE2 gene defines a glutathione-dependent pathway involved in initiation and maintenance of cell division during postembryonic root development. *Plant Cell* **2000**, *12*, 97–110. [CrossRef] [PubMed]
47. Zhang, D.; Zhang, Z.; Yang, K. Identification of AFLP markers associated with embryonic root development in *Populus tomentosa* Carr. *Silvae Genet.* **2005**, *56*, 27–32. [CrossRef]
48. Brown, J.D.; Beggs, J.D. Roles of PRP8 protein in the assembly of splicing complexes. *EMBO J.* **1992**, *11*, 3721–3729. [PubMed]
49. Shevell, D.E.; Leu, W.M.; Gillmor, C.S.; Xia, G.; Feldmann, K.A.; Chua, N.H. EMB30 is essential for normal cell division, cell expansion, and cell adhesion in Arabidopsis and encodes a protein that has similarity to Sec 7. *Cell* **1994**, *77*, 1051–1062. [CrossRef]
50. Long, J.A.; Moan, E.I.; Medford, J.I.; Barton, M.K. A member of the KNOTTED class of homeodomain proteins encoded by the STM gene of Arabidopsis. *Nature* **1996**, *379*, 66–69. [CrossRef] [PubMed]
51. Hamann, T.; Mayer, U.; Jürgens, G. The auxin-insensitive bodenlos mutation affects primary root formation and apical-basal patterning in the Arabidopsis embryo. *Development* **1999**, *126*, 1387–1395. [PubMed]
52. Mayer, U.; Büttner, G.; Jürgens, G. Apical-basal pattern formation in the Arabidopsis embryo: Studies on the role of the gnom gene. *Development* **1993**, *117*, 149–162.
53. Chandler, J.W. Cotyledon organogenesis. *J. Exp. Bot.* **2008**, *59*, 2917–2931. [CrossRef] [PubMed]
54. Datta, S.C. *Systemic Botany*, 4th ed.; Wiley Eastern Ltd.: New Delhi, India, 1988.
55. Karschon, R. Seedling Morphology and Schizocotily in *Hammada salicordia* (Moq.). Iljin. Forestry Division Agricultural Research Organisation. *Iianot* **1973**, *46*. Available online: <http://agris.fao.org/agris-search/search.do?recordID=US201303127995> (accessed on 8 July 2018).
56. Eames, A.J. *Morphology of the Angiosperms*; McGraw-Hill Company: New York, NY, USA, 1961.
57. Duke, J.A. On tropical tree seedlings. Seeds, seedlings, systems and systematics. *Ann. Mo. Bot. Gard.* **1969**, *56*, 125–161. [CrossRef]
58. Dube, V.P.; Awasthi, D.K.; Singhal, V.P. Comparative anatomical observations on the dicotylous and tricotylous seedlings of *Raphanus sativus* L. (Brassicaceae). *Acta Bot. Indica* **1981**, *9*, 134–147.
59. Pillai, A.; Goyal, S.C. Developmental anatomy of some oilyielding plants IV. Normal and tricotylous seedlings of *Sesamum indicum* L. *Feddes Rep.* **1983**, *94*, 87–90.
60. Fletcher, J.J. Illustrations of polycotyledony in the genus *Persoonia*, with some reference to *Nuytsia*. *Proc. Linn. Soc. NSW* **1909**, *33*, 867.
61. Kang, X.Y.; Mao, J.F. Gamete fertility and morphological variations in offsprings of triploid clones *Populus tomentosa*. *J. Beijing For. Univ.* **2001**, *23*, 20–23. (In Chinese)
62. Aida, M.; Ishida, T.; Fukaki, H.; Fujisawa, H.; Tasaka, M. Genes involved in organ separation in Arabidopsis: An analysis of the cup-shaped cotyledon mutant. *Plant Cell* **1997**, *14*, 841–857. [CrossRef] [PubMed]
63. Schrick, K.; Mayer, U.; Horrichs, A.; Kuhnt, C.; Bellini, C.; Dangl, J.; Schmidt, J.; Jürgens, G. FACKEL is a sterol C-14 reductase required for organized cell expansion in Arabidopsis embryogenesis. *Gene Dev.* **2000**, *14*, 1471–1484. [PubMed]
64. Trembl, B.S.; Winderl, S.; Radykewicz, R.; Herz, M.; Schweizer, G.; Hutzler, P.; Glawischnig, E.; Ruiz, R.A. The gene ENHANCER OF PINOID controls cotyledon development in the Arabidopsis Embryo. *Development* **2005**, *132*, 4063–4074. [CrossRef] [PubMed]
65. Fu, Y.Q.; Liu, Y.Y.; Li, J.A. Sexual hybridization and genetic analysis of *Populus deltoides* and section *Tacamahaca*. *Shaanxi For. Sci. Technol.* **1983**, *2*, 2–23. (In Chinese)
66. Hamzeh, M.; Sawchyn, C.; Perinet, P.; Dayanandan, S. Asymmetrical natural hybridization between *Populus deltoides* and *P. balsamifera* (Salicaceae). *Can. J. Bot.* **2007**, *85*, 1227–1232. [CrossRef]
67. Guries, R.P.; Stettler, R.F. Pre-fertilization barriers to hybridization in the poplars. *Silvae Genet.* **1976**, *25*, 37–43.
68. Mahama, A.; Hall, R.B.; Zalesny, R.S. Differential interspecific incompatibility among *Populus* hybrids in sections *Aigeiros* Duby and *Tacamahaca* Spach. *For. Chron.* **2011**, *87*, 790–796. [CrossRef]
69. Hirose, T.; Ujihara, A.; Kitabayashi, H.; Minami, M. Interspecific Cross-compatibility in *Fagopyrum* according to Pollen Tube Growth. *Breed. Sci.* **1994**, *44*, 307–314. [CrossRef]
70. Stebbins, G.L. *Variation and Evolution in Plants*; Columbia University Press: New York, NY, USA, 1950.
71. Rieseberg, L.H.; Willis, J.H. Plant speciation. *Science* **2007**, *317*, 910–914. [CrossRef] [PubMed]
72. Morris, M.R. Cytogenetic studies on buckwheat. *J. Hered.* **1951**, *42*, 85–89. [CrossRef] [PubMed]

73. Lee, B.S. Breeding of interspecific hybrids in genus *Fagopyrum*. (4) Production of interspecific hybrids ovule culture among *F. esculentum*, *F. tataricum* and *F. cymosum*. *Breed. Sci.* **1994**, *44* (Suppl. 1), 183. (In Japanese)
74. Stanton, B.J.; Villar, M. Controlled reproduction of *Populus*. In *Biology of Populus and Its Implications for Management and Conservation*; Stettler, R.F., Bradshaw, H.D., Heilman, P.E., Hinkley, T.M., Eds.; NRC Research Press: Ottawa, ON, Canada, 1996; pp. 113–138.
75. Zsuffa, L.; Lin, D.; Payne, P. One-way crossing barriers in some interspecific crosses of Aigeiros and Tacamahaca poplars. *For. Chron.* **1999**, *75*, 833–836. [[CrossRef](#)]
76. Kang, X.Y. Some understandings on polyploid breeding of poplars. *J. Beijing For. Univ.* **2010**, *32*, 149–153. (In Chinese)
77. Wang, J.; Kang, X.Y.; Zhu, Q. Variation in pollen formation and its cytological mechanism in an allotriploid white poplar. *Tree Genet. Genomes* **2010**, *6*, 281–290. [[CrossRef](#)]
78. He, Y.C.; Ge, J.; Wei, Q.; Jiang, A.M.; Gan, L.; Song, Z.J.; Cai, D.T. Using a polyploid meiosis stability (PMes) line as a parent improves embryo development and the seed set rate of a tetraploid rice hybrid. *Can. J. Plant Sci.* **2011**, *91*, 325–335. [[CrossRef](#)]
79. Kong, F.M.; Liu, J.J.; Chen, Y.N.; Wan, Z.B.; Yin, T.M. Marker-aided selection of polyploid poplars. *Bioenergy Res.* **2013**, *6*, 984–990. [[CrossRef](#)]
80. BanBuijtenen, J.P.; Joranson, P.N.; Einspahr, D.W. Diploid versus triploid aspen as pulpwood sources with reference to growth, chemical, physical and pulping differences. *Aspen Bibliogr.* **1958**, *41*, 170–175.
81. Zhu, Z.T.; Lin, H.B.; Kang, X.Y. Studies on allotriploid breeding of *Populus tomentosa* B301 clones. *Sci. Silvae Sin.* **1995**, *31*, 499–505. (In Chinese)

© 2018 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).