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<th>Journal:</th>
<th>Canadian Journal of Forest Research</th>
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<tr>
<td>Manuscript ID:</td>
<td>cjfr-2018-0460.R2</td>
</tr>
<tr>
<td>Manuscript Type:</td>
<td>Article</td>
</tr>
<tr>
<td>Date Submitted by the Author:</td>
<td>05-Jun-2019</td>
</tr>
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</table>
| Complete List of Authors: | He, Liming; Northeast Forestry University, State Key Laboratory of Tree Genetics and Breeding; Northeast Forestry University, College of Life Science  
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| Keyword: | Interspecific hybridization of Fraxinus, F1 progenies, High voltage electrostatic field, Heterosis, Selection |
| Is the invited manuscript for consideration in a Special Issue?: | Not applicable (regular submission) |
Title:

Interspecific hybridization of *Fraxinus* Linn. (*F. mandshurica* × *F. americana* and

*F. mandshurica* × *F. velutina*) and heterosis analysis and selection of F1 progenies

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Abstract: The interspecific hybridizations of *Fraxinus mandshurica × Fraxinus americana* (MA) and *Fraxinus mandshurica × Fraxinus velutina* (MV) were conducted to solve the problems of poor cold-adaption associated with the introduction of *Fraxinus* in Heilongjiang province and high voltage electrostatic field (HVEF) treatment to pollen was performed to overcome the pre-fertilization barriers. The hybrids adapted more strongly and grew better than the pure species (heterosis over higher parent (HHP) of the 9-yr volume index was 5.5 % for MA and 23.1 % for MV) in Heilongjiang province. HVEF treatment greatly improved the number of seeds (0.25 folds ~ 5.52 folds) and seedlings (1.63 folds ~ 8.71 folds) of the hybrids. Additionally, 3 excellent female parents (No. 15, No. 16 and No. 17) and 7 hybrid combinations of MA (D94, D70 and D100) and MV (D103, D116, D105 and D104) with excellent growth traits were selected. The HHPs of volume index were 39.1 % ~ 112.5 % for selected hybrids. Additionally, growth trends of the hybrids predicted showed that the hybrids will maintain a 7.7% to 9.3% height advantage over *Fraxinus mandshurica* through ages 10 to 15 years in Mao Ershan. These findings will accelerate the genetic breeding process of *Fraxinus* species.

Keywords: Interspecific hybridization of *Fraxinus*; F1 progenies; High voltage electrostatic field; Heterosis; Selection
1. Introduction

Heterosis or hybrid vigour refers to the phenomenon in which the progeny of diverse varieties of a species or crosses between species confer growth, reproduction and adaptation advantages compared to that of their parents (Birchler et al. 2010; Riedelsheimer et al. 2012). The mechanism of heterosis has been debated for over a century, but is still far from clear. Several hypotheses including dominance, over-dominance, and pseudo-over-dominance are available to explain the phenomenon of heterosis (Chen 2010). However, besides these hypotheses, much evidence has shown that the differences in gene expression (Wang et al. 2007; Stupar et al. 2008), allele-specific expression (Wittkopp et al. 2004; Huang et al. 2015), epigenetics regulation of DNA methylation (Hofmann 2012; Shen et al. 2012), histone modification (Yang et al. 2016), regulation of small RNAs (Groszmann et al. 2011; Ng et al. 2012; Zhang et al. 2014) and regulation of product level and function of proteins (Goff 2011) are all involved in the formation of heterosis.

Regardless of the molecular mechanism of heterosis, tree breeders would like to take advantage of heterosis and obtain commercial varieties with ideal combinations of traits. For cross-pollinated trees, interspecific hybridization is an effective means to create new germplasm and create and utilize heterosis and is also a breeding strategy for stress tolerance breeding of trees (Li et al. 1998; Rebek et al. 2008; Shepherd et al. 2008; Cheng et al. 2009; Wang et al. 2009; Cheng et al. 2010; Wang et al. 2013). However, incompatibility or barriers in interspecific hybridization is a common phenomenon in nature and can prevent the production of first generation of hybrids.
Barriers have been divided into pre-fertilization and post-fertilization barriers (Van Tuyl et al. 1991), and specific techniques such as ploidy manipulation, bud pollination, ovary culture, ovule culture and embryo rescue can sometimes be successfully used to overcome them (Cheng et al. 2009; Wang et al. 2009). There is also evidence that some physical treatments to the pollen, such as appropriate laser, irradiation and high voltage electrostatic field (HVEF) can help to overcome pre-fertilization barriers (Li et al. 1995; Sarmah and Sarla 1995).

Species of *Fraxinus* Linn. are important afforestation, timber and landscaping tree species. Among them, *Fraxinus mandshurica* Rupr. (M) is distributed in the Northwest and Northeast of China, the Russian Far East, the Northern part of the Korean Peninsula and Northern Japan. It is a valuable hardwood species in forest areas, and is a dioecious, wind-pollinated, cold-adapted, and broad-leaved tree species, but it is susceptible to drought and saline stress and has been listed as endangered species (Hu et al. 2008; Li et al. 2018). *Fraxinus americana* Linn. (A) has a long life, strong drought resistance and is easy to breed. It is an excellent tree species for soil and water conservation. However, *F. americana* cannot live through winter in high-latitude cold regions (Abrams and Mostoller 1995; Palla and Pijut 2015). *Fraxinus velutina* Torr. (V) is a species native to Southwestern North America, and it is widely distributed in coastal areas because of its rapid growth rate and tolerance to alkaline soils (Liu et al. 2003; Li et al. 2018). Similar to *F. americana*, *F. velutina* also does not adapt well to cold weather in Northeast China, such as in the Heilongjiang province.

Since the concept of crossbreeding was proposed in the early 20th century,
interspecific crossbreeding has been successful in a variety of tree species, such as *Betula* (birch, Atkinson et al. 1997), *Populus* (poplar, Wullschleger et al. 2005), *Pinus* (pine, Callister et al. 2007; Bradley and Will 2017; Lopez et al. 2017), *Eucalyptus* (Callister et al. 2007; Wu et al. 2012) and *Fraxinus* (ash). Among the hybrids of ash, only the hybrids of black ash (North American) × *F. mandshurica* (Rebek et al. 2008), the hybrids of *Fraxinus excelsior* × *Fraxinus angustifolia* (Thomasset et al. 2011) and, in recent years in our research, interspecific hybrid combinations with drought resistance and salt tolerance traits have been reported (Zeng et al. 2014; Zeng et al. 2015; He et al. 2015).

In this study, the technologies of interspecific hybridizations of *F. mandshurica* × *F. americana* (MA hybrid) and *F. mandshurica* × *F. velutina* (MV hybrid), the heterosis of hybrid F1 progenies in survival and growth traits of seedlings, the selection of excellent F1s and female parents and the selection effects of excellent F1s were systematically presented. Additionally, HVEF treatment to the pollen was used to overcome the pre-fertilization barriers in the interspecific hybridization of *Fraxinus*, and its effects on the hybridization, seed vitality, survival and growth traits of seedlings of the hybrid F1 progenies were investigated. Finally, the growth heterosis in tree height indexes in young stage (10 ~ 15A) of the F1 progenies were predicted by regression analysis. This work provides a strategy for solving the cold problems associated with the introduction of *Fraxinus* in Heilongjiang province.

2. Materials and Methods

2.1 Materials
2.1.1 Male parents

Totally, the pollen of 31 male parents was collected for this interspecific hybridization experiment. Among them, 15 male parents of *Fraxinus americana* were from Xinjiang province (10 male parents, 1 ~ 10) and Beijing city (5 male parents, 1 ~ 5) in China, and the other 16 male parents of *Fraxinus velutina* were from Panjin of Liaoning province (10 male parents, 1 ~ 10) and Tianjin city (6 male parents, 1 ~ 6) in China.

2.1.2 Female parents

15 female parents selected for interspecific hybridization were the 20 ~ 25A plus trees of *Fraxinus mandshurica* from the Dailing seed orchard in Yichun city (46°50’ ~ 47°20’ N, 128°37’ ~ 129°7’ E, located in the mid-temperate zone, with a continental humid monsoon climate) of Heilongjiang province in China.

2.1.3 Seedlings planted on Mao Ershan

The 2-yr seedlings of the hybrid combinations (≥ 30 plants) and their parental controls were planted in the Mao Ershan experimental forest farm at Northeast Forestry University under natural conditions (45°20’ ~ 45°25’ N, 127°30’ ~ 127°34’ E, located in the temperate zone, with a continental monsoon climate) in the spring of 2010. A complete randomized block design was used and there were 3 to 5 replicates for each combination. There were 10 trees per plot with a double row arrangement. The row spacing was 1.5 m × 2.0 m. These materials contained 32 hybrid combinations of MA, 42 hybrid combinations of MV, 24 open-pollinated male parental controls (consisted of 6 *F. americana* from Xinjiang, 6 *F. americana* from Beijing, 6 *F. velutina*
from Panjin and 6 *F. velutina* from Tianjin) and 14 open-pollinated female parental controls (there were no hybrid seedlings planted from No.10 female parent).

### 2.2 Methods

#### 2.2.1 Protocols for interspecific hybridization experiments

**Design for hybridization experiments.** In this experiment, the discontinuous test cross design was exploited, which comprised of 175 hybrid combinations for MA (85) and MV (90) totally (Table 1).

**Pollen collection, storage and processing.** The male flowering branches of *F. americana* and *F. velutina* were collected before flowering and cultivated in water indoors, and their pollen were collected in test tubes and stored at 4 °C and transported to Harbin for use. Before hybridization, HVEF treatments to the pollen of *F. mandshurica* with different strengths and processing distances were conducted and we proposed that 20 Kv at intervals of 10 cm for 30 min was the best treatment (Wan 2007). This optimal HVEF treatment was chosen for pollen treating. The pollen of the male parents mixed from the same provenance without HVEF treatment was the control group (CK in Table 1).

**Pollen germination.** After the HVEF treatment to pollen, the pollen germination rates of *F. americana*, *F. velutina* and *F. mandshurica* were investigated by germination. Details of the medium, cultural condition and statistical method of germination rates are displayed in Supplemental materials 1.1 (Wan 2007).

**Bagging isolation, pollination and bag-removal of female parents.** The interspecific hybridization experiment was carried out in the spring of 2007, which
consisted of artificial bagging, pollination and bag-removing as a conventional hybridization process. The artificial bagging took place when the lengths of the female flower inflorescences were 2.0 ~ 3.0 cm (approximately early to mid-May). Parchment bags (35 cm × 70 cm) with two open ends were used. The two ends of the hybrid bags were sealed with cloth-strips and paper clips to ensure that the pollen of *F. mandshurica* could not enter after artificial bagging. Approximately 4 ~ 10 female flower inflorescences were in each bag. Then, the artificial pollinations were carried out in mid-May when the female flowers were mature enough to receive the pollen (the lengths of the female flower inflorescences were 3.5 ~ 5.0 cm, and the stigmas were separated and the flowers shiny at the top). The pollen was spread evenly on the stigma of each flower in the inflorescence with a brush or duster. The bags were reclosed with paper clips and marked on the cloth-strips, with 20 ~ 50 bags for each hybrid combination. After the male flower of *F. mandshurica* powder was completely finished (the stigmas wilted, late May), the bags were removed and the cloth-strips were kept on the branches.

*Seed collection and sowing.* Mature seeds were harvested in mid-September of 2007 and kept separate by full-sib hybrid combination, and later sowed in the seedbed after the sand accumulation temperature treatment in the spring of 2008.

**2.2.2 Investigation of hybridization and the adaptability and growth traits of F1 progenies**

Next, the setting rate (SR), number of seeds per inflorescences (*N*<sub>P</sub>), number of seeds per 100 inflorescence (*N*<sub>A</sub>), thousand-grain weight (TGW), seed length (SL), seed
width (SW), seedlings emergence rate (SER) and number of seedlings per 100 inflorescences (N_C) of F1 progenies were investigated and calculated. The survival rates and growth traits of F1 progenies were also investigated in 2010, 2014 and 2016. The 2-, 3-, 6-, 7-, 8- and 9-yr tree height (TH) indexes, 3-, 7- and 9-yr ground diameter (GD) indexes, 9-yr diameter at breast height (DBH), volume and straightness (ST) indexes were then obtained. The details of formulas and evaluation methods of these indexes are displayed in Supplemental materials 1.2 ~ 1.3.

2.2.3 Variance analysis and estimation of genetic parameters of growth traits

**Variance analysis.** The variance analysis of observed values of the combinations is repeated at least three times and the linear model is as follows (Dieters et al. 1997):

\[ Y_{ijkl} = \mu + F_i + M_j + B_k + FM_{ij} + FB_{ik} + MB_{jk} + MFB_{ijk} + E_{ijkl} \]

where \( Y_{ijkl} \) is the \( l \)th tree of the \( ij \)th family, in the \( k \)th block; \( \mu \) is the overall mean; \( F_i \) is the random effect of the \( i \)th female parent, \( E(F_i) = 0 \), \( \text{Var}(F_i) = \sigma^2_f \); \( M_j \) is the random effect of the \( j \)th male parent, \( E(M_j) = 0 \), \( \text{Var}(M_j) = \sigma^2_m \); \( B_k \) is the random effect of the \( k \)th block, \( E(B_k) = 0 \), \( \text{Var}(B_k) = \sigma^2_b \); \( FM_{ij} \) is the random effect of the interaction between the \( i \)th female parent and the \( j \)th male parent, \( E(FM_{ij}) = 0 \), \( \text{Var}(FM_{ij}) = \sigma^2_{fm} \); \( FB_{ik} \) is the random effect of the interaction between the \( i \)th female parent and the \( k \)th block, \( E(FB_{ik}) = 0 \), \( \text{Var}(FB_{ik}) = \sigma^2_{fb} \); \( MB_{jk} \) is the random effect of the interaction between the \( j \)th male parent and the \( k \)th block, \( E(MB_{jk}) = 0 \), \( \text{Var}(MB_{jk}) = \sigma^2_{mb} \); \( MFB_{ijk} \) is the random effect of the interaction between the \( jk \)th family and the \( i \)th block, \( E(MFB_{ijk}) = 0 \), \( \text{Var}(MFB_{ijk}) = \sigma^2_{fmb} \); \( E_{ijkl} \) is the random effect of tree-to-tree within a block, \( E(E_{ijkl}) = 0 \), \( \text{Var}(E_{ijkl}) = \sigma^2_e \).
σ₂ᵣ and σ²ₘ are estimates of one quarter of the additive genetic variance, σ²_A; σ²(fm) is an estimate of one quarter of the dominance variance, σ²_D; σ²(fe) and σ²(me) are estimates of one quarter of the additive–environment interaction, σ²_AE; and σ²(fme) is an estimate of one quarter of the dominance–environment interaction, σ²_DE (Dieters et al. 1997).

Estimation of genetic parameters. The variance component estimates were used to estimate heritability (h²), the proportion of dominance (d²), the ratio of dominance to additive variance (σ²_D/σ²_A) and the phenotypic coefficient of variation (CV). The formulae used to estimate these genetic parameters are listed below (Dieters et al. 1997; Hai et al. 2008):

\[
\text{female } h^2 = \frac{4\sigma^2_r}{(\sigma^2_r + \sigma^2_m + \sigma^2_{fm} + \sigma^2_{rb} + \sigma^2_{mb} + \sigma^2_e)}
\]

\[
\text{male } h^2 = \frac{4\sigma^2_m}{(\sigma^2_r + \sigma^2_m + \sigma^2_{fm} + \sigma^2_{rb} + \sigma^2_{mb} + \sigma^2_e)}
\]

\[
d^2 = \frac{4\sigma^2_{fm}}{(\sigma^2_r + \sigma^2_m + \sigma^2_{fm} + \sigma^2_{rb} + \sigma^2_{mb} + \sigma^2_e)}
\]

\[
\sigma^2_D/\sigma^2_A = 2\sigma^2_{fm}/(\sigma^2_r + \sigma^2_m)
\]

\[
CV (\%) = (\sigma_f / X) \times 100 \%
\]

Where σᵣ is the phenotypic standard deviation, X is the mean of the hybrid.

2.2.4 Selection of female parents

The general combining abilities (GCAs) of different female parents were calculated as follows:

\[\text{GCA}_i = X_i - X\]

Xᵢ is the mean of progenies of i-th female parents; X is the mean of all progenies

2.2.5 Selection of F1 hybrid combinations with excellent growth traits

Then, the membership function method of fuzzy mathematics was used for
transforming the data (Zeng et al. 2015) and the growth traits of F1 progenies were then
evaluated using the CSI (Comprehensive selection index) method, and the formulas are
as follows:

\[
U(\text{X}_{ij}) = \frac{(\text{X}_{ij} - \text{X}_{i-min})}{(\text{X}_{i-max} - \text{X}_{i-min})};
\text{CSI} = \sum \text{W}_i \times U(\text{X}_{ij})
\]

U(\text{X}_{ij}) is the membership function value; \text{X}_{ij} is the value of the i-th index of the
j-th hybrid combination; \text{X}_{i-min} is the minimum value of the i-th index; \text{X}_{i-max} is the
maximum value of the i-th index; \text{W}_i is the weight of i-th index. In this selection, the
weights of each index were 90 % (9-yr V index) and 10 % (9-yr ST indexes).

**2.2.6 Heterosis evaluation of F1 hybrids**

The heterosis of a growth index is evaluated by the HHP superiority (Springer
and Stupar 2007) and the formula is as follow:

\[
\text{HHP} \% = \frac{\text{X}_{F1} - \text{X}_{HP}}{\text{X}_{HP}} \times 100 \%
\]

\text{X}_{F1} is the mean of an index of the F1; \text{X}_{HP} is the higher of two parental controls.

**2.2.7 Statistical analysis**

The data were analysed using SPSS 19.0 (SPSS IL, USA). One-way ANOVA,
two-way ANOVA and Duncan's test were used to assess the differences. The figures
were drawn by Prism 7.0.

**3. Results**

**3.1 No significant differences occurred in setting, and most seed trait indexes
between the hybrids and the female parents and the seed vitalities of the hybrids
were significantly higher than the female parents**
The hybridization results showed that over 20,000 seeds for the hybrids and over 19,000 seeds for female open-pollinated parents were obtained in this hybridization experiment, and in the SR, N_A, TGW and SL indexes, there were no differences in MA-1 (male parents from Xinjiang provenance of A), MA-2 (male parents from Beijing provenance of A), MV-1 (male parents from Panjin provenance of V), MV-2 (male parents from Tianjin provenance of V) and the female parents. In the SW index, MA-1 and MV-2 were significantly higher than the female parents. Additionally, the seed vitalities (SER, seedlings emergence rate) of the hybrids (31.6 % ~ 43.8 %) were significantly higher than the female parents (15.7 %) (Table 2).

3.2 The F1 progenies that were obtained by interspecific hybridization adapted more strongly and grew better than their double parental progenies in Heilongjiang province

To determine the adaptability in Heilongjiang province of F1s and their double parental progenies, the survival rates in different years were investigated. The 3-yr survival rates for MA and MV hybrids were 85.0 % or more and the survival rates decreased because of biotic and abiotic stresses. There was no significant difference between the two hybrids in the same year. The F1 progenies showed stronger adaptability, whose 3-, 7- and 9-yr survival rates were significantly (P < 0.05) higher than their parental controls. The 3-yr, 7- and 9-yr survival rates for the MA hybrid were 86.2 %, 63.6 % and 47.9 %, in comparison with survival rates of 77.9 %, 55.3 %, 40.2 % for M and 79.7 %, 30.6 %, 20.3 % for A. The 3-yr, 7- and 9-yr survival rates for the MV hybrid were 85.7 %, 65.5 % and 50.5 %, in comparison with survival rates of 76.1
%, 52.8 %, 37.6 % for M and 67.0 %, 11.7 %, 6.3 % for V (Figure 1a). The HHPs of
the 3-yr, 7-yr and 9-yr survival rate indexes were 8.1 %, 15.0 %, 24.1 % for the MA
hybrid and 12.6 %, 19.1 %, 34.2 % for the MV hybrid in three different years,
respectively.

To investigate the growth traits, the TH and GD indexes in different years and the
9-yr indexes of the V, the DBH and the ST were examined and calculated. The mean
values of TH in six years of MA were 19.54 cm (2A), 45.65 cm (3A), 136.99 cm (6A),
192.79 cm (7A), 277.76 cm (8A) and 342.43 cm (9A), and there were significant
differences between the MA hybrid and its male parents in six different years, but no
significant difference with its female parents. The above 6-yr TH indexes for the MA
hybrid were better than those of its parents, and its HHPs were 3.0 % ~ 5.6 %. Unlike
MA, the mean values of the TH indexes for the MV hybrid were 19.62 cm (2A), 47.73
cm (3A), 146.38 cm (6A), 205.46 cm (7A), 283.73 cm (8A) and 350.96 cm (9A),
respectively, and the above 6-yr TH indexes for the MV hybrid were significantly
higher than its double parents (HHPs were 5.3 % ~ 14.4 %) (Figure 1b). In addition,
the mean GD values for the MA hybrid were 11.16 mm (3A), 30.89 mm (7A) and 47.09
mm (9A), and for the MV hybrid, they were 11.27 mm (3A), 32.43 mm (7A) and 49.74
mm (9A). The HHPs for the MA hybrid were 3.5 % (3A), 4.9 % (7A) and 2.8 % (9A).
Similar to MA, the heterosis for the MV hybrid was also obvious, with HHPs of 11.7 %
(3A), 12.6 % (7A) and 9.1 % (9A) (Figure 1c). The mean values of the 9-yr DBH
indexes for MA and MV hybrids were 28.42 mm and 30.15 mm, respectively, and their
HHP values reached 3.1 % (MA) and 9.4 % (MV) (Figure 1d). Moreover, the mean
values of 9-yr volume indexes for MA and MV hybrids were 1,836.0 cm$^3$ and 2,182.3 cm$^3$, respectively, and the F1 progenies were higher than their parents, but the differences were not significant. The HHPs of the volume index for the hybrid progenies reached 5.5 % (MA) and 23.1 % (MV) (Fig. 1e). Finally, the mean values of 9-yr ST index were 3.07 (MA) and 2.99 (MV), with corresponding HHP values of 5.3 % (MA) and 1.7 % (MV). Only the difference between the MA hybrid and the A control was significant (Figure 1f).

In short, the F1 progenies adapted more strongly to Mao Ershan in Heilongjiang province and showed growth heterosis to their double parental free pollinated progenies.

### 3.3 HVEF treatment to pollen helps to improve the pollen and seed vitalities and the number of the seeds and seedlings without significantly changing the seed traits and the survival and growth traits of seedlings (≥ 3A) in the F1 progenies

Pollen germination rates of *F. americana*, *F. velutina* and *F. mandshurica* under HVEF treatment indicated that, compared to the control group, the pollen germination rates increased by 0.13, 0.13 and 0.26 folds, respectively. The change for *F. mandshurica* was significant, and the pollen germination rate was 94.7 % under HVEF treatment (Figure 2a). The hybridization results showed that the SRs for MA-1 and MA-2 were 21.0 % (0.35 fold) and 41.5 % (0.71 fold) (Figure 2b), the N$_{PS}$ for MA-1 and MV-1 were 8.8 (3.83 fold) and 8.3 (1.41 fold) (Figure 2c), and the N$_{AS}$ for MA-1, MA-2, MV-1 and MV-2 were 5.52, 0.89, 1.53 and 0.25 folds, respectively, higher than the control group under the HVEF treatment, and these differences were significant. In addition, there were no significant effects of the HVEF treatment on the seed traits of
the F1 progenies (Figure 2g ~ 2j). After the seeds were sowed in the soil, the SER
indexes of the four kinds of hybrids were significantly improved by 10% or more after
HVEF treatment (Figure 2d). Compared to the control group, the \( N_{BS} \) of MA-1, MA-2,
MV-1 and MV-2 increased by 8.71, 2.59, 2.76 and 1.63 folds, respectively (Figure 2f).

In the investigations of the survival and growth traits, although the effects of the
HVEF treatment on the hybrids from different female parents were not identical, the
differences in survival rates and growth indexes between the HVEF treatment and
control groups in two kinds of hybrids from different female parents in the same year
were not significant (Figure 3 and S1).

In summary, in the interspecific hybridization experiment of *Fraxinus*, the HVEF
treatment to the pollen of *F. americana* and *F. velutina* helped to overcome pre-
fertilization barriers and improved seed vitalities, number of seeds and yielded more
seedlings without significantly changing the seed trait and the survival rates, growth
traits of seedlings (≥ 3A) in the F1 progenies.

### 3.4 Analysis of the general combining ability (GCA) and selection of female parents

Because of the data loss in survival and growth traits for many combinations which
had less than 30 plants when planted, the data for hybrids planted and female parental
controls in these indexes were classified into three groups (the MA hybrid, the MV
hybrid and the female open-pollinated control). Only the data for the three groups from
the same female parents were used to calculate the GCAs of the 10 female parents.

GCA analysis of different female parents showed that the GCAs of some female
parents were high only in one or some special indexes, which can be selected for specific breeding aims (Figure 4 and Table S1 ~ 2). For example, for the setting trait, female parents with the highest GCA were No. 7 (GCA = 7.8 % in SR index). For the seed traits, No. 8 had the highest GCA in the TGW index (18.95 g). There were also excellent female parents with high GCAs in most indexes of one or several traits (Figure 4 and Table S1 ~ 2). For example, the GCAs in the growth indexes of No. 15 were the highest (26.94 ~ 43.70 cm in above 6-yr TH indexes, 5.89 mm and 10.89 mm in 7- and 9-yr GD indexes, 5.03 mm in DBH index and 870.13 cm$^3$ in volume index), the GCAs in the SER index (3.4 %), the survival indexes (6.2 % and 9.7 % in the 7 and 9-yr survival indexes) and growth indexes (16.66 ~ 41.49 cm in above 6-yr TH indexes, 703.57 cm$^3$ in volume index and 0.18 in ST index) of No. 16 were high, and the GCAs in the $N_A$ index (5.44), the survival indexes (6.0 %, 5.2 % and 4.0 % in the 3-, 7- and 9-yr survival rate indexes) and growth indexes (18.20 ~ 24.02 cm in above 6-yr TH indexes and 452.69 cm$^3$ in volume index) of No. 17 were high, and all 3 female parents selected were excellent.

3.5 Genetic analysis and selection of F1s in growth traits and the heterosis analysis of selected F1s

In the section of genetic analysis, estimates of female and male heritabilities ($h^2$), the proportion of dominance ($d^2$), the ratio of dominance to additive variance ($\sigma_A^2/\sigma_D^2$), the variation extent and the phenotypic CV for MA and MV hybrids in growth indexes were all conducted.

For MA and MV hybrids in growth indexes, the female heritabilities ranged from
0.05 ~ 0.30 and 0.11 ~ 0.40, and the male heritabilities ranged from 0.07 ~ 0.19 and
0.06 ~ 0.11. The female heritabilities in the 9-yr TH, GD, DBH, volume and ST indexes
were 0.30, 0.26, 017, 0.12, 0.10 for the MA hybrid and 0.29, 0.11, 0.24, 0.27, 0.24 for
the MV hybrid. The male heritabilities in the 9-yr TH, GD, DBH, volume and ST
indexes were 0.14, 0.15, 0.15, 0.14, 0.08 for the MA hybrid and 0.06, 0.06, 0.05, 0.04,
0.11 for the MV hybrid. For the same index of MV, the female heritabilities were higher
than the male heritabilities. The \( \sigma^2_D/\sigma^2_A \) ranged from 0.22 ~ 2.00 (Table 3 and 4). The maximums
of MA and MV were 550 cm and 615 cm for the 9-yr TH index, 101.73 mm and 102.75
mm for the GD index, 93.89 mm and 96.99 mm for the DBH index, 9,039.22 cm³ and
9,128.79 cm³ for the volume index, and 4 and 4 for the ST index. The CVs of the TH
indexes in different ages of MA and MV ranged from 22.0 % ~ 47.2 % and 22.8 % ~
51.7 %, respectively. The CVs of the GD indexes in different ages ranged from 25.4 %
~ 32.3 % and 26.0 % ~ 32.4 %. The CVs of the 9-yr DBH, V and ST indexes of MA
were 30.8 %, 75.3 % and 35.5 %, respectively. The CVs of MV in these indexes were
30.7 %, 69.6 % and 38.3 %, respectively (Table 3 and 4).

Upon ranking the CSI results of the different combinations of the two kinds of
hybrid progenies (Table 5 and 6), 7 superior combinations were selected in total
according to a 10 % selection rate. 3 hybrid combinations of the MA (D94, D70 and
D100) and 4 hybrid combinations of MV (D103, D116, D105 and D104) were selected.
In the above 6-yr growth indexes, except for the ST index, the 7 hybrid
combinations were significantly higher than the double parents (the HHP reached 7.5 %
The HHPs of the above 6-yr growth indexes for the 3 hybrid combinations selected from MA were as follows: TH indexes = 11.4 % ~ 32.8 %, GD indexes = 17.2 % ~ 37.9 %, DBH index = 12.2 % ~ 23.6 %, and V index = 39.1 % ~ 59.1 %. Among the 3 hybrid combinations, D94 was the highest, with a volume index HHP of 57.8 %.

For the 4 hybrid combinations selected from MV, the HHPs were 7.5 % ~ 31.9 % for the TH indexes, 19.9 % ~ 39.2 % for the GD indexes, 15.5 % ~ 37.4 % for the DBH index and 61.0 % - 112.5 % for the volume index. Among the 4 combinations, the HHPs in growth indexes of D103 were most significant, with an HHP in the volume index of 112.5 % (Table 7).

### 3.6 Simulation of growth curve in TH indexes of F1 and FP progenies

Regression analysis methods are often used to reveal the connection between variables with causal relationships where the known data are used to establish regression equations, which are used for predicting dependent variables from independent variables. Usually, it takes approximately 20 ~ 30 years before sexual maturity for *F. mandshurica* (Zhu, 2016) and the F1 and female parental progenies grow fast during the young stage (< 15 A). The correlation analysis of the 9-yr growth indexes of F1 and female parental progenies were conducted by the Pearson method and the bilateral test, showing that the growth indexes of F1 and female parental progenies were significantly (P < 0.01) and positively correlated and that the correlation coefficients between the TH index and the DBH, V, GD indexes were 0.781 ~ 0.811, 0.751 ~ 0.818 and 0.798 ~ 0.815, respectively. Therefore, the TH indexes can represent the growth traits of F1 and female parental progenies to some extent. Then, six years
data (< 10 A) of the TH indexes of the hybrids and the female parental control were subjected to 14 kinds of curve equations to understand the growth trends in young stage and predict TH indexes heterosis in future years (10 ~ 15 A) (Table S3).

The results of the F-test showed that the curve equations of the hybrids and the female parents with the highest degree of fitness all were the power function curve without constant term, whose equation was \( y = x^a \). The \( R^2 \) values were 0.979, 0.981 and 0.980 respectively, and the fitness was significant (\( P < 0.01 \)). Therefore, the TH index (\( Y \)) and tree age (\( x \)) corresponding to the curve equations of the two kinds of hybrids and the female parental control were as follows (Table 8):

\[
Y_{MA} = x^{2.811 \pm 0.006}; \quad Y_{MV} = x^{2.812 \pm 0.005}; \quad Y_M = x^{2.779 \pm 0.005}
\]

However, when the regression analysis of the male parental controls conducted by the same curve equation (\( y = x^a \)), the \( R^2 \) values were only 0.724 for A and 0.563 for V (Table 8). From this, we can infer that the growth trends in TH index of the hybrids were influenced strongly by \( F. mandshurica \), rather than their male parents.

To predict the TH heterosis of the two kinds of hybrids to the female parents, by conversion, another two equations were obtained as follows:

\[
Y_{MA}/Y_M = x^{0.032}; \quad Y_{MV}/Y_M = x^{0.033}
\]

The equations show that when \( x \) is 10 ~ 15, the values of \( Y_{MA}/Y_M \) and \( Y_{MV}/Y_M \) are 1.077 ~ 1.091 and 1.079 ~ 1.093, which indicates that the 10- ~ 15-yr heterosis in the TH indexes of the F1 progenies will reach to 7.7 % ~ 9.1 % and 7.9 % ~ 9.3 %, compared to the female parents (Figure 5).
4. Discussion

The tree species *Fraxinus americana* and *Fraxinus velutina* are important fast-growing timber tree species with special virtues of resistance. However, when *F. americana* and *F. velutina* were introduced into Heilongjiang province directly in 2006a, they exhibited shoot blight and poor trunk formation because of the cold damage caused by different growth rhythms, which lowered the timber values of the two tree species (Li 2009). By introducing the pollen of *F. americana* and *F. velutina*, interspecific F1 hybrids with *Fraxinus mandshurica* were obtained. In the adaptability and growth traits investigation, the F1 progenies inherited the cold resistance of *F. mandshurica* (female parents) and the fast growth of *F. americana* and *F. velutina* (male parents), which adapted and grew better in Heilongjiang province. Thus, the problem of poor cold-adaptation of the two tree species of *Fraxinus* when introduced to Northeast China was successfully solved by interspecific hybridization, which also provides an effective breeding strategy for tree genetic improvement.

HVEF treatment to the seeds increased seed activity and promoted seed germination in many plants (Cramariuc et al. 2005; Wang et al. 2009; Yang and Shen 2011; Patwardhan and Gandhare 2013). In our study, the HVEF treatment to pollen also helped to improve the seed vitalities (>10 % higher SER index of the seeds to the control groups). Apart from this, it enhanced the pollen affinity and reduced interspecific hybridization incompatibility (including obtaining a higher setting rate and more seeds and seedlings). Interestingly, HVEF treatment to the pollen significantly promoted the 1-yr growth traits (higher TH index and GD index) of the F1s of *Fraxinus*
(Wan 2007). Several reports have also demonstrated that HVEF treatment to seeds enhances the growth of the 1-yr seedlings (Yang and Shen 2011; Patwardhan and Gandhare 2013). However, in the 3-, 7- and 9-yr growth indexes of *Fraxinus* F1s in this study, the effects of the HVEF treatment were significantly reduced, which may indicate that the function of HVEF treatment is effective only to seed development and the early growth of seedlings.

Ash from continental Europe has been introduced to stock plantations of Ireland in the 1990s, but after a few years, the hybrids of *Fraxinus excelsior* with *Fraxinus angustifolia* often exhibited poor stem form (Thomasset et al. 2011). In this study, MA and MV hybrids had better stem form than their parental controls. Apart from this, the adaptability and growth traits analysis showed that the adaptability and growth heterosis of the F1 progenies were significant in different years. The growth heterosis values for the 7 hybrid combinations selected were significant (the HHPs of the volume index were 39.1 % ~ 112.5 %). In addition to these results in our findings, F1 progenies with the drought resistance and salt tolerance traits were obtained in recent years. 3 hybrid combinations with HVEF treatment to pollen of MV (D78, D48 and D67) had the highest salt tolerance, and we found that the changes in DNA methylation under salt stress had a strong correlation with the resistance to salt heterosis of MV (Zeng et al. 2015). After conducting a comprehensive evaluation, the 39 *Fraxinus* hybrids were sorted with respect to drought resistance as well. The combination D110 of MA turned out to be the most drought resistant hybrid combination (Dong 2012), and further study revealed that the higher expression of rhythmic genes in the hybrids of the MA than
their female parents was one of the main reasons for the drought resistance mechanism (He et al. 2015). In the future, more works will focus on the identification of wood qualities and properties, as well as asexual reproduction and clone testing of the excellent F1s selected.

In summary, in this article, protocols for the interspecific hybridization of *F. mandshurica × F. americana* and *F. mandshurica × F. velutina*, subsequent adaptability and growth heterosis, and the selection effects of F1s were systematically presented. Additionally, appropriate HVEF treatment to pollen was carried out to eliminate hybridization incompatibility and the effects were significant. Finally the growth trends in TH index of the hybrids and the female parent controls in Mao Ershan at young ages indicate that the hybrids will maintain a 7.7% to 9.3% height advantage over *F. mandshurica* through ages 10 to 15 years. These findings will accelerate the genetic breeding process of *Fraxinus* species.

**Acknowledgments**

This work was financially supported by the National Key Research and Development Program of China (No. 2017YF D0600605-01) and the National Natural Science Foundation of China (No. 31270697).

**Reference**


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doi: 10.1016/j.elstat.2009.05.004.


https://link.springer.com/article/10.1007%2FBF02913236


Forestry University, Harbin, P.R. (in Chinese).


674-678,736 (in Chinese). Available from:


Table 1. The design for the hybridization experiment.

<table>
<thead>
<tr>
<th>Male parents</th>
<th>Female parents</th>
<th>Xinjiang (A-1, 1<del>10) / Panjin (V-1,1</del>10)</th>
<th>Beijing (A-2,1<del>5) / Tianjin (V-2,1</del>6)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CK</td>
<td>1 2 3 4 5 6 7 8 9 10</td>
<td>1 2 3 4 5 6</td>
</tr>
<tr>
<td>No. 2</td>
<td>Y Y Y Y Y Y</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. 3</td>
<td>Y Y Y Y Y Y</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. 5</td>
<td>Y Y Y Y Y Y</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. 7</td>
<td>Y Y Y Y Y Y</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. 8</td>
<td>Y Y Y Y Y Y</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. 6</td>
<td>Y Y Y Y Y Y</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. 11</td>
<td>Y Y Y Y Y Y</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. 13</td>
<td>Y Y Y Y Y Y</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. 18</td>
<td>Y Y Y Y Y Y</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. 19</td>
<td>Y Y Y Y Y Y</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. 10</td>
<td>Y Y Y Y Y Y</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. 14</td>
<td>Y Y Y Y Y Y</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. 15</td>
<td>Y Y Y Y Y Y</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. 16</td>
<td>Y Y Y Y Y Y</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. 17</td>
<td>Y Y Y Y Y Y</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: “Y” was the hybrid combination designed to be conducted.
Table 2. The setting and seed trait of F1s and female parents.

<table>
<thead>
<tr>
<th>Index</th>
<th>Female parents</th>
<th>MA-1</th>
<th>MA-2</th>
<th>MV-1</th>
<th>MV-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>SR / %</td>
<td>64.2 ± 13.8 a</td>
<td>68.4 ± 12.3 a</td>
<td>67.3 ± 16.4 a</td>
<td>65.6 ± 15.2 a</td>
<td>67.9 ± 12.1 a</td>
</tr>
<tr>
<td>N_p</td>
<td>6.33 ± 4.34 a</td>
<td>5.45 ± 3.43 a</td>
<td>6.40 ± 4.02 a</td>
<td>6.39 ± 4.97 a</td>
<td>5.76 ± 4.23 a</td>
</tr>
<tr>
<td>TGW / g</td>
<td>60.92 ± 10.23 a</td>
<td>67.49 ± 12.07 a</td>
<td>62.27 ± 5.30 a</td>
<td>65.97 ± 7.63 a</td>
<td>68.14 ± 11.46 a</td>
</tr>
<tr>
<td>SL / mm</td>
<td>30.53 ± 1.12 a</td>
<td>30.11 ± 2.92 a</td>
<td>31.27 ± 2.67 a</td>
<td>31.59 ± 2.01 a</td>
<td>30.59 ± 2.75 a</td>
</tr>
<tr>
<td>SW / mm</td>
<td>6.91 ± 1.01 b</td>
<td>7.66 ± 0.77 a</td>
<td>6.77 ± 0.45 b</td>
<td>7.24 ± 0.63 ab</td>
<td>7.68 ± 0.71 a</td>
</tr>
<tr>
<td>N_{NA}</td>
<td>470.02 ± 201.77 a</td>
<td>392.91 ± 169.96 a</td>
<td>462.69 ± 120.98 a</td>
<td>457.77 ± 177.54 a</td>
<td>423.05 ± 270.68 a</td>
</tr>
<tr>
<td>N_{NB}</td>
<td>89.71 ± 22.19 c</td>
<td>145.5 ± 48.87 b</td>
<td>272.48 ± 85.94 ab</td>
<td>200.09 ± 72.3 ab</td>
<td>301.79 ± 85.25 a</td>
</tr>
<tr>
<td>SER / %</td>
<td>15.7 ± 12.0 b</td>
<td>31.6 ± 16.4 ab</td>
<td>40.8 ± 13.8 a</td>
<td>37.5 ± 19.8 a</td>
<td>43.8 ± 16.3 a</td>
</tr>
</tbody>
</table>

Note: Data are mean ± SE from >3 independent replicates. The different normal letters indicate significant difference at 0.05 level (lowercase letters) tested with Duncan and determined by one-way analysis of variance (ANOVA). MA-1 (male parents from Xinjiang provenance of MA), MA-2 (male parents from Beijing provenance of MA), MV-1 (male parents from Panjin provenance of MV), MV-2 (male parents from Tianjin provenance of MV), SR (seed setting rate), N_p (number of seeds per inflorescence), TGW (thousand-grain weight), SL (seed length), SW (seed width), N_{NA} (number of seeds per 100 inflorescences), N_{NB} (number of seedlings per 100 inflorescences) and SER (seedlings emergence rate). The same is below.
Table 3. Estimates of heritability ($h^2$), the proportion of dominance ($d^2$), the ratio of dominance to additive variance ($\sigma^2_D/\sigma^2_A$), the variation extent and the phenotypic CV for the MA hybrid.

<table>
<thead>
<tr>
<th>Index</th>
<th>Heritability</th>
<th>Proportion of dominance ($d^2$)</th>
<th>$\sigma^2_D/\sigma^2_A$</th>
<th>Variation extent</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>female $h^2$</td>
<td>male $h^2$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TH-2A</td>
<td>0.07</td>
<td>0.07</td>
<td>0.14</td>
<td>2.00</td>
<td>47.2</td>
</tr>
<tr>
<td>TH-3A</td>
<td>0.05</td>
<td>0.16</td>
<td>0.12</td>
<td>1.14</td>
<td>33.3</td>
</tr>
<tr>
<td>TH-6A</td>
<td>0.15</td>
<td>0.16</td>
<td>0.06</td>
<td>0.36</td>
<td>31.1</td>
</tr>
<tr>
<td>TH-7A</td>
<td>0.22</td>
<td>0.14</td>
<td>0.04</td>
<td>0.24</td>
<td>27.7</td>
</tr>
<tr>
<td>TH-8A</td>
<td>0.20</td>
<td>0.16</td>
<td>0.09</td>
<td>0.48</td>
<td>22.0</td>
</tr>
<tr>
<td>TH-9A</td>
<td>0.30</td>
<td>0.14</td>
<td>0.07</td>
<td>0.31</td>
<td>22.3</td>
</tr>
<tr>
<td>GD-3A</td>
<td>0.03</td>
<td>0.19</td>
<td>0.08</td>
<td>0.70</td>
<td>25.4</td>
</tr>
<tr>
<td>GD-7A</td>
<td>0.15</td>
<td>0.12</td>
<td>0.12</td>
<td>0.91</td>
<td>32.3</td>
</tr>
<tr>
<td>GD-9A</td>
<td>0.26</td>
<td>0.15</td>
<td>0.11</td>
<td>0.53</td>
<td>30.7</td>
</tr>
<tr>
<td>DBH-9A</td>
<td>0.17</td>
<td>0.15</td>
<td>0.07</td>
<td>0.44</td>
<td>30.8</td>
</tr>
<tr>
<td>Volume-9A</td>
<td>0.12</td>
<td>0.14</td>
<td>0.06</td>
<td>0.49</td>
<td>75.3</td>
</tr>
<tr>
<td>ST-9A</td>
<td>0.10</td>
<td>0.08</td>
<td>0.05</td>
<td>0.55</td>
<td>35.5</td>
</tr>
</tbody>
</table>
Table 4. Estimates of heritability ($h^2$), the proportion of dominance ($d^2$), the ratio of dominance to additive variance ($\sigma^2_D/\sigma^2_A$), the variation extent and the phenotypic CV for the MV hybrid.

<table>
<thead>
<tr>
<th>Index</th>
<th>Heritability</th>
<th>Proportion of dominance ($d^2$)</th>
<th>$\sigma^2_D/\sigma^2_A$</th>
<th>Variation extent</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>female $h^2$</td>
<td>male $h^2$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TH-2A</td>
<td>0.11</td>
<td>0.06</td>
<td>0.05</td>
<td>0.58</td>
<td>4 ~ 64 cm</td>
</tr>
<tr>
<td>TH-3A</td>
<td>0.15</td>
<td>0.11</td>
<td>0.09</td>
<td>0.72</td>
<td>8 ~ 139 cm</td>
</tr>
<tr>
<td>TH-6A</td>
<td>0.31</td>
<td>0.09</td>
<td>0.06</td>
<td>0.28</td>
<td>50 ~ 305 cm</td>
</tr>
<tr>
<td>TH-7A</td>
<td>0.40</td>
<td>0.08</td>
<td>0.05</td>
<td>0.22</td>
<td>102 ~ 404 cm</td>
</tr>
<tr>
<td>TH-8A</td>
<td>0.31</td>
<td>0.07</td>
<td>0.08</td>
<td>0.40</td>
<td>124 ~ 490 cm</td>
</tr>
<tr>
<td>TH-9A</td>
<td>0.29</td>
<td>0.06</td>
<td>0.07</td>
<td>0.39</td>
<td>150 ~ 615 cm</td>
</tr>
<tr>
<td>GD-3A</td>
<td>0.11</td>
<td>0.05</td>
<td>0.08</td>
<td>1.04</td>
<td>1.12 ~ 26.31 mm</td>
</tr>
<tr>
<td>GD-7A</td>
<td>0.28</td>
<td>0.08</td>
<td>0.06</td>
<td>0.33</td>
<td>9.17 ~ 72.61 mm</td>
</tr>
<tr>
<td>GD-9A</td>
<td>0.11</td>
<td>0.06</td>
<td>0.08</td>
<td>0.95</td>
<td>12.46 ~ 102.75 mm</td>
</tr>
<tr>
<td>DBH-9A</td>
<td>0.24</td>
<td>0.05</td>
<td>0.07</td>
<td>0.47</td>
<td>12.21 ~ 96.99 mm</td>
</tr>
<tr>
<td>Volume-9A</td>
<td>0.27</td>
<td>0.04</td>
<td>0.07</td>
<td>0.43</td>
<td>20.99 ~ 9,128.79 cm3</td>
</tr>
<tr>
<td>ST-9A</td>
<td>0.24</td>
<td>0.11</td>
<td>0.05</td>
<td>0.26</td>
<td>1 ~ 4</td>
</tr>
</tbody>
</table>
Table 5. The ranks of comprehensive selection index (CSI) in growth traits for the
MA hybrid.

<table>
<thead>
<tr>
<th>HC</th>
<th>CSI</th>
<th>Female parent</th>
<th>Male parent</th>
<th>HC</th>
<th>CSI</th>
<th>Female parent</th>
<th>Male parent</th>
</tr>
</thead>
<tbody>
<tr>
<td>D94</td>
<td>0.95</td>
<td>No.15 BJ-2</td>
<td></td>
<td>D115</td>
<td>0.47</td>
<td>No.17 BJ-3</td>
<td></td>
</tr>
<tr>
<td>D70</td>
<td>0.90</td>
<td>No.7 XJ-1</td>
<td></td>
<td>D69</td>
<td>0.47</td>
<td>No.7 XJ-2</td>
<td></td>
</tr>
<tr>
<td>D100</td>
<td>0.78</td>
<td>No.16 BJ-3</td>
<td></td>
<td>D33</td>
<td>0.44</td>
<td>No.6 XJ-7</td>
<td></td>
</tr>
<tr>
<td>D128</td>
<td>0.77</td>
<td>No.18 XJ-10</td>
<td></td>
<td>D2</td>
<td>0.43</td>
<td>No.3 XJ-CK</td>
<td></td>
</tr>
<tr>
<td>D110</td>
<td>0.75</td>
<td>No.17 BJ-4</td>
<td></td>
<td>D45</td>
<td>0.39</td>
<td>No.2 XJ-3</td>
<td></td>
</tr>
<tr>
<td>D126</td>
<td>0.73</td>
<td>No.18 XJ-6</td>
<td></td>
<td>D133</td>
<td>0.39</td>
<td>No.19 XJ-8</td>
<td></td>
</tr>
<tr>
<td>D74</td>
<td>0.73</td>
<td>No.7 XJ-CK</td>
<td></td>
<td>D36</td>
<td>0.38</td>
<td>No.6 XJ-10</td>
<td></td>
</tr>
<tr>
<td>D49</td>
<td>0.63</td>
<td>No.2 XJ-1</td>
<td></td>
<td>D134</td>
<td>0.35</td>
<td>No.19 XJ-7</td>
<td></td>
</tr>
<tr>
<td>D72</td>
<td>0.62</td>
<td>No.7 XJ-3</td>
<td></td>
<td>D12</td>
<td>0.34</td>
<td>No.8 XJ-2</td>
<td></td>
</tr>
<tr>
<td>D34</td>
<td>0.59</td>
<td>No.6 XJ-8</td>
<td></td>
<td>D131</td>
<td>0.33</td>
<td>No.19 XJ-10</td>
<td></td>
</tr>
<tr>
<td>D1</td>
<td>0.57</td>
<td>No.3 XJ-2</td>
<td></td>
<td>D10</td>
<td>0.31</td>
<td>No.8 XJ-4</td>
<td></td>
</tr>
<tr>
<td>D43</td>
<td>0.52</td>
<td>No.2 XJ-CK</td>
<td></td>
<td>D86</td>
<td>0.28</td>
<td>No.13 XJ-8</td>
<td></td>
</tr>
<tr>
<td>D32</td>
<td>0.51</td>
<td>No.6 XJ-6</td>
<td></td>
<td>D9</td>
<td>0.13</td>
<td>No.8 XJ-5</td>
<td></td>
</tr>
<tr>
<td>D71</td>
<td>0.50</td>
<td>No.7 XJ-3</td>
<td></td>
<td>D132</td>
<td>0.12</td>
<td>No.19 XJ-9</td>
<td></td>
</tr>
<tr>
<td>D73</td>
<td>0.49</td>
<td>No.7 XJ-4</td>
<td></td>
<td>D11</td>
<td>0.10</td>
<td>No.8 XJ-3</td>
<td></td>
</tr>
<tr>
<td>D13</td>
<td>0.48</td>
<td>No.8 XJ-CK</td>
<td></td>
<td>D26</td>
<td>0.10</td>
<td>No.11 XJ-10</td>
<td></td>
</tr>
</tbody>
</table>

Note: XJ-CK: the pollen was not treated by the high voltage electrostatic field from Xinjiang provenance.
Table 6. The ranks of comprehensive selection index (CSI) in growth traits for the MV hybrid.

<table>
<thead>
<tr>
<th>HC</th>
<th>CSI</th>
<th>Female parent</th>
<th>Male parent</th>
<th>HC</th>
<th>CSI</th>
<th>Female parent</th>
<th>Male parent</th>
</tr>
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<tbody>
<tr>
<td>D103</td>
<td>1.00</td>
<td>No.16 TJ-2</td>
<td>D122 0.48</td>
<td>D122</td>
<td>0.48</td>
<td>No.18 PJ-9</td>
<td>D80 0.29</td>
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<tr>
<td>D105</td>
<td>0.79</td>
<td>No.16 TJ-4</td>
<td>D106 0.47</td>
<td>D106</td>
<td>0.47</td>
<td>No.7 PJ-1</td>
<td>D117 0.28</td>
</tr>
<tr>
<td>D116</td>
<td>0.77</td>
<td>No.17 TJ-4</td>
<td>D79 0.44</td>
<td>D79</td>
<td>0.44</td>
<td>No.9 TJ-6</td>
<td>D82 0.26</td>
</tr>
<tr>
<td>D104</td>
<td>0.70</td>
<td>No.16 TJ-3</td>
<td>D107 0.42</td>
<td>D107</td>
<td>0.42</td>
<td>No.11 PJ-10</td>
<td>D84 0.24</td>
</tr>
<tr>
<td>D102</td>
<td>0.69</td>
<td>No.16 TJ-1</td>
<td>D38 0.41</td>
<td>D38</td>
<td>0.41</td>
<td>No.6 TJ-6</td>
<td>D55 0.24</td>
</tr>
<tr>
<td>D108</td>
<td>0.67</td>
<td>No.17 TJ-2</td>
<td>D56 0.41</td>
<td>D56</td>
<td>0.41</td>
<td>No.5 PJ-1</td>
<td>D91 0.21</td>
</tr>
<tr>
<td>D64</td>
<td>0.64</td>
<td>No.7 PJ-2</td>
<td>D41 0.37</td>
<td>D41</td>
<td>0.37</td>
<td>No.6 PJ-9</td>
<td>D5 0.20</td>
</tr>
<tr>
<td>D67</td>
<td>0.63</td>
<td>No.7 PJ-1</td>
<td>D46 0.36</td>
<td>D46</td>
<td>0.36</td>
<td>No.2 PJ-CK</td>
<td>D53 0.16</td>
</tr>
<tr>
<td>D78</td>
<td>0.61</td>
<td>No.7 PJ-4</td>
<td>D4 0.35</td>
<td>D4</td>
<td>0.35</td>
<td>No.3 PJ-3</td>
<td>D3 0.13</td>
</tr>
<tr>
<td>D95</td>
<td>0.61</td>
<td>No.15 TJ-3</td>
<td>D44 0.34</td>
<td>D44</td>
<td>0.34</td>
<td>No.2 PJ-2</td>
<td>D48 0.12</td>
</tr>
<tr>
<td>D111</td>
<td>0.57</td>
<td>No.17 PJ-5</td>
<td>D81 0.32</td>
<td>D81</td>
<td>0.32</td>
<td>No.9 TJ-2</td>
<td>D51 0.11</td>
</tr>
<tr>
<td>D66</td>
<td>0.57</td>
<td>No.7 PJ-CK</td>
<td>D7 0.30</td>
<td>D7</td>
<td>0.30</td>
<td>No.8 PJ-4</td>
<td>D20 0.09</td>
</tr>
<tr>
<td>D112</td>
<td>0.54</td>
<td>No.17 TJ-CK</td>
<td>D28 0.29</td>
<td>D28</td>
<td>0.29</td>
<td>No.6 PJ-10</td>
<td>D21 0.06</td>
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<tr>
<td>D63</td>
<td>0.49</td>
<td>No.7 PJ-3</td>
<td>D54 0.29</td>
<td>D54</td>
<td>0.29</td>
<td>No.5 PJ-4</td>
<td>D19 0.05</td>
</tr>
</tbody>
</table>

Note: PJ / TJ-CK: the pollen was not treated by the high voltage electrostatic field from Panjin / Tianjin provenance.
Table 7. Heterosis (HHP) analysis of the growth traits for the excellent F1 selected.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>D70</td>
<td>18.3</td>
<td>11.4</td>
<td>17.3</td>
<td>19.3</td>
<td>37.9</td>
<td>20.5</td>
<td>23.6</td>
<td>59.1</td>
<td>-4.0</td>
</tr>
<tr>
<td>MA</td>
<td>31.9</td>
<td>28.5</td>
<td>22.9</td>
<td>21.4</td>
<td>30.2</td>
<td>41.4</td>
<td>25.6</td>
<td>57.8</td>
<td>12.2</td>
</tr>
<tr>
<td>D100</td>
<td>16.4</td>
<td>20.0</td>
<td>14.5</td>
<td>12.5</td>
<td>17.1</td>
<td>21.5</td>
<td>12.2</td>
<td>39.1</td>
<td>17.5</td>
</tr>
<tr>
<td>D103</td>
<td>7.5</td>
<td>11.5</td>
<td>22.3</td>
<td>20.7</td>
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<td>37.4</td>
<td>112.5</td>
<td>15.3</td>
</tr>
<tr>
<td>D104</td>
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<td>30.5</td>
<td>20.7</td>
<td>19.6</td>
<td>32.1</td>
<td>34.8</td>
<td>15.5</td>
<td>61.0</td>
<td>7.5</td>
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<tr>
<td>D105</td>
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<td>32.8</td>
<td>22.9</td>
<td>19.5</td>
<td>39.2</td>
<td>36.1</td>
<td>20.9</td>
<td>79.9</td>
<td>-0.6</td>
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<tr>
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<td>18.4</td>
<td>18.6</td>
<td>24.4</td>
<td>25.9</td>
<td>25.6</td>
<td>19.9</td>
<td>24.9</td>
<td>73.5</td>
<td>-1.4</td>
</tr>
</tbody>
</table>
Table 8. Variance analysis of the simulation equations (y = x ^ a) in tree height index for the hybrids and double parental progenies.

<table>
<thead>
<tr>
<th>Materials</th>
<th>F-test</th>
<th>R²</th>
<th>SD (a)</th>
<th>Curve equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>MA</td>
<td>P &lt; 0.01</td>
<td>0.979</td>
<td>0.006</td>
<td>y = x ^ 2.811</td>
</tr>
<tr>
<td>MV</td>
<td>P &lt; 0.01</td>
<td>0.981</td>
<td>0.005</td>
<td>y = x ^ 2.812</td>
</tr>
<tr>
<td>M</td>
<td>P &lt; 0.01</td>
<td>0.980</td>
<td>0.005</td>
<td>y = x ^ 2.779</td>
</tr>
<tr>
<td>A</td>
<td>P &lt; 0.01</td>
<td>0.724</td>
<td>0.013</td>
<td>y = x ^ 2.651</td>
</tr>
<tr>
<td>V</td>
<td>P &lt; 0.01</td>
<td>0.563</td>
<td>0.012</td>
<td>y = x ^ 2.602</td>
</tr>
</tbody>
</table>
Figure 1. The adaptability and growth traits of interspecific hybrid F1 and their double parental open-pollinated progenies of *Fraxinus*.

Note: The survival rates (a), tree height indexes (b), ground diameter indexes (c), diameter at breast height index (DBH, d), volume index (e) and straightness index (f) of different hybrids and their parents. Data are mean ± SE from > 3 independent replicates. The different normal letters indicate significant difference at 0.05 level (lowercase letters) and 0.01 level (capital letters) tested with Duncan and determined by one-way analysis of variance (ANOVA).

Figure 2. The effects of HVEF treatment to pollen on pollen viability, setting of hybridization, seedlings emergence and seed traits of F1 progenies of *Fraxinus*.

Note: Pollen germination rates of three kinds of species of *Fraxinus* (Fam: *F. americana*, Fve: *F. velutina*, Fma: *F. mandshurica*, a), the seed setting rate (b), the number of seeds per inflorescence (c), the seedlings emergence rate (d), the number of seeds per 100 inflorescences (e), the number of seedlings per 100 inflorescences (f) and the seed traits (g ~ i) under high voltage electrostatic field (HVEF) treatment. Data are mean ± SE from > 3 independent replicates. The different normal letters indicate significant difference at 0.05 level (lowercase letters) and 0.01 level (capital letters) tested with Duncan and determined by one-way analysis of variance (ANOVA).
Figure 3. Effects of HVEF treatment to pollen on adaptability and growth traits of F1 progenies of *Fraxinus*.

Note: The survival rates in different years for MA(a) and MV(b), the tree height indexes in different years for MA(c) and MV(d) and the 9-yr straightness index for hybrids (e) under high voltage electrostatic field (HVEF) treatment. Data are mean ± SE from > 3 independent replicates. The different normal letters indicate significant difference at 0.05 level (lowercase letters) tested with Duncan and determined by one-way analysis of variance (ANOVA).

Figure 4. General combining ability (GCA) analysis of different female parents.


Figure 5. Curve models for the hybrid and female open-pollinated parental progenies.

Note: Curve models for the MA hybrid (a), the MV hybrid (b) and the female open-pollinated parental control (c). Solid lines represent the 1~9-yr tree height growth and dotted lines represent the predicted 10 ~ 15-yr tree height growth.
Figure 1. The adaptability and growth traits of interspecific hybrid F1 and their double parental open-pollinated progenies of Fraxinus.

Note: The survival rates (a), tree height indexes (b), ground diameter indexes (c), diameter at breast height index (DBH, d), volume index (e) and straightness index (f) of different hybrids and their parents. Data are mean ± SE from > 3 independent replicates. The different normal letters indicate significant difference at 0.05 level (lowercase letters) and 0.01 level (capital letters) tested with Duncan and determined by one-way analysis of variance (ANOVA).
Figure 2. The effects of HVEF treatment to pollen on pollen viability, setting of hybridization, seedlings emergence and seed traits of F1 progenies of Fraxinus.

Note: Pollen germination rates of three kinds of species of Fraxinus (Fam: F. americana, Fve: F. velutina, Fma: F. mandshurica, a), the seed setting rate (b), the number of seeds per inflorescence (c), the seedlings emergence rate (d), the number of seeds per 100 inflorescences (e), the number of seedlings per 100 inflorescences (f), and the seed traits (g ~ i) under high voltage electrostatic field (HVEF) treatment. Data are mean ± SE from > 3 independent replicates. The different normal letters indicate significant difference at 0.05 level (lowercase letters) and 0.01 level (capital letters) tested with Duncan and determined by one-way analysis of variance (ANOVA).
Figure 3. Effects of HVEF treatment to pollen on adaptability and growth traits of F1 progenies of Fraxinus. Note: The survival rates in different years for MA(a) and MV(b), the tree height indexes in different years for MA(c) and MV(d) and the 9-yr straightness index for hybrids (e) under high voltage electrostatic field (HVEF) treatment. Data are mean ± SE from > 3 independent replicates. The different normal letters indicate significant difference at 0.05 level (lowercase letters) tested with Duncan and determined by one-way analysis of variance (ANOVA).
Figure 4. General combining ability (GCA) analysis of different female parents.

Note: SR: setting rate. NP: number of seeds per inflorescence. TGW: thousand-grain weight. SL: seed length. SW: seed width. SER: seedlings emergence rate. SuR-3, 7, 9 A: 3-, 7-, 9-yr survival rate indexes. TH-3 ~ 9A: 3- ~ 9-yr tree height indexes. GD-3, 7, 9 A: 3-, 7-, 9-yr ground diameter indexes. DBH-9A: 9-yr diameter at breast height index. Volume-9A: 9-yr volume index. ST-9A: 9-yr straightness index. The data of the GCA in different indexes were transferred by the fuzzy mathematics.
Figure 5. Curve models for the hybrid and female open-pollinated parental progenies. Note: Curve models for the MA hybrid (a), the MV hybrid (b) and the female open-pollinated parental control (c). Solid lines represent the 1~9-yr tree height growth and dotted lines represent the predicted 10 ~ 15-yr tree height growth.