

Aspen hybridization: Parents' compatibility and seedlings' growth

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Abstract

First results of new trembling aspen (*Populus tremula* L.) special factorial crosses are presented. At first, productive parent trees were selected: five maternal and five paternal trees without symptoms of heart rot and one tree with fruiting bodies of this fungal attack. The results of phenotypic and genotypic analyses of parent trees, their compatibility, and growth performance are presented. The analysis of the hybrid seedlings' survival and their growth performance on open ground are shown. Estimates of general and specific combining abilities of the parent trees were carried out and potentially best hybrid families and seedlings were selected.

Key words: aspen, compatibility, hybrids, success indexes of hybridization, rooting, seedlings' survival and growth, general and specific combining ability, microsatellite markers.

Introduction

Trembling Aspen (*Populus tremula* L.) and quaking aspen (*P. tremuloides* L.) are widely spread deciduous tree species of northern Eurasia and North America respectively. They are valuable species known for a broad range of uses. Aspen is characterized by rapid growth and the ability to recover vacant spaces. But over a long period, many of its best stands and trees have been cut down by selective logging and excessive fellings. Its further regeneration derived in many cases from heart rot affected plants. To improve its gene pool, there are different methods of breeding. Among them hybridization occupies a prominent place.

Intraspecific and interspecific aspen hybridization in Europe has been engaging researchers from different countries: G. Melchior, H. Seitz, 1966; O. Mohrdiek, 1977; M. Hofmann, 2005

(Germany); H. Johnsson, 1976 (Sweden); Y. Smilga, 1986 (Latvia). The aspen hybridization work in Germany was summarized by von Wühlisch (2006).

In Russia, aspen hybridization was accomplished by A. Albensky (1959), P. Bogdanov (1965), A. Yablokov (1956, 1963), S. Ivannikov (1959), N. Konovalov (1963), and others.

The beginning of aspen hybridization in the Central Chernozem region was initiated in the 50-ies of the last century by M. M. Veresin of the Voronezh Forest Technical Institute. He crossed aspen by white poplar and white poplar with aspen. He generated dozens of interspecific hybrids, significantly exceeding their parent's growth performance and productivity.

In the mid 80-ies of the 20th century, local aspen crosses with other ecotypes of *P. tremula* L. and with *P. alba* L. were continued by Professor A. P. Tsarev and the senior researcher of the Central Research Institute of Forest Genetics and Breeding V. P. Petrukhnov (A. Tsarev, 1976, 2013b; V. Petrukhnov, 1988). They generated hundreds of interspecific hybrids. Some of them recently received the rank of "variety" legalized by patents and copyright certificates. There are such varieties as 'Bolide' and 'Veduga' selected by A. P. Tsarev. However, to improve the productivity, fungus resistance, and to increase the diversity of forms and varieties of the genus *Populus* in general and aspens in particular, more new genotypes are required. To obtain new genotypes a broad front of research is required to estimate the compatibility of the different parents and the growth performance of the obtained hybrid offspring.

Aspen cross breeding work and hybrid analysis were initiated 2016 in the framework of the international project "MaRussiA" (A. Tsarev, 2016; R. Tsareva et al. 2016).

The aim of this work is to study the crossing compatibility of different aspen genotypes on the basis of their progenies, as well as the survival and growth analysis of 1-year hybrid seedlings obtained in factorial crosses between six females and five

males. To study the relatedness among the parent trees, they were characterized by 34 microsatellite (SSR) markers located on 18 of the 19 chromosomes.

Material and Methods

Plant material

The original parental material for hybridization was received from Semiluksky tremuletum, created by V. Petrukhnov in 1973, and from Semiluksky poplar and aspen hybrids' collection № 1, created by A. Tsarev in 1978. The geographical coordinates are 51° 42' North and 38°57' East.

On these experimental test sites, 11 clones of aspen, six female and five male trees were selected for hybridization, including five aspen clones from Training and Experimental Forestry of Voronezh State Forest Technical University (VSFTU – Voronezh), one American aspen (*P. tremuloides*) from Latvia, one form from the Botanical Garden of Voronezh State University (VSU – Voronezh), one aspen clone from Valuiky (Belgorod region) and 3 hybrids obtained earlier (1978) from the crossing of Voronezh' local white poplar with aspen from Cornetu Experimental Station (Romania) – *P. alba* × *P. tremula*. In addition, a male aspen form (aspen 'local') was selected in the Forest-Park section of All-Russian Research Institute of Forest Genetics, Breeding and Biotechnology (Voronezh) - (Table 1). All trees were selected for their high growth performance and resistance to heart rot attack by *Fomes igniarius* Fr. to study the heredity of disease resistance, also one infected tree was included.

Table 1
Parents' forms, origin, and taxonomic characteristics

Sex	Code	Aspen name/ species	Origin*	Inventory characteristics of the parent trees			Remarks
				age, years	diameter, cm	height, m	
♀	02.01	<i>Populus tremula</i> ISFTU	1	43	54	25.5	green bark
	10.03	<i>Populus tremula</i> ISFTU	1	43	50	33.0	
	15.01	<i>Populus tremula</i> ISU	3	43	33	30.5	triploid**
	18.02	<i>Populus tremula</i> Valuiky	4	43	39	29.0	
	23.05	<i>Populus tremula</i> ISFTU	1	43	43	30.0	bronze bark
	45.01	hybrid family 78.B.35 (<i>P. alba</i> × <i>P. tremula</i>)	5	36	31	20.5	hybrid obtained by A. P. Tsarev
♂	07.02	aspen _{ISFTU}	1	43	49	29.0	
	08.02	aspen _{ISFTU}	1	43	43	30.0	bronze bark
	32.03	<i>P. tremuloides</i>	2	43	43	32.0	
	45.03	hybrid family 78.B.35 (<i>P. alba</i> × <i>P. tremula</i>)	5	36	40	19.5	hybrid obtained by A. P. Tsarev
	48.02	hybrid family 78.B.35 (<i>P. alba</i> × <i>P. tremula</i>)	5	36	31	20.0	hybrid obtained by A. P. Tsarev***
	asp. 'local'	aspen local	6	no data	21	12.0	green bark

Comments:

*1 – Training and Experimental Forestry of Voronezh State Forest Technical University; 2 – Latvia; 3 – Botanical Garden of Voronezh State University; 4 – Valuiky Forestry of Belgorod region; 5 – Semiluksky experiments site of Voronezh region (hybrid *P. alba* from Voronezh × *P. tremula* from Romania); 6 – Forest-Park section of All-Russian Research Institute of Forest Genetics, Breeding and Biotechnology.

**Caryology analysis was carried out in Genetic laboratory of Voronezh Research Institute of Forest Genetics, Breeding and Biotechnology by E. Shabanova.

***None of the parents show any fungus conks, except 48.02, which has one fungus conk.

The age of the parent trees was 36 and 43 years. Their average height and diameter ranged from 20 to 33 m and from 31 to 54 cm respectively (Table 1).

The harvesting of the branches for hybridizations was carried out by climbers (Figure 1). Branches of female trees had a length of 2-3 m and of male trees – 1-2 m.



Figure 1

The branch collection for hybridization (♂ tree 32-03). Semiluksky tremuletum. Voronezh region. 15.03.2016. Photo courtesy by A. P. Tsarev.

The branches were harvested on March 12, 2016 when the buds increased in size due to rising temperatures. Harvested branches were installed in prepared buckets with a nutrient solution of Knop. The composition of the nutrient solution was: $Ca(NO_3)_2$ – 10 g, KH_2PO_4 – 2.5 g, $MgSO_4$ – 2.5 g, KNO_3 – 2.5 g, KCl – 1.25 g, $FeCl_3$ – 0.125 g in 10 liters of water. When the solution became turbid (2-3 days) it was replaced.

To avoid mixing pollinators the branches from only one of the male parent trees and from all female parent trees were established per laboratory room. The mature pollen was stored in the refrigerator for 3 to 7 days until the female catkins opened. Pollination was carried out with squirrel hair brushes repeated 2-3 times. The numbers of catkins per female branch differed from 11 to 25 but for maturation of seeds and better comparability of crossing success only five each were left.

Thirty crosses were performed in 2016, in addition, a single cross *P. tremula* 15.01×*P. tremula* 'local' was made.

The hybrid seedlings were planted in the Forest-Park section of All-Russian Research Institute of Forest Genetics, Breeding and Biotechnology (Voronezh). The geographical coordinates are 51°43.516' North and 39°11.772' East. The soil is a gray forest sandy-loam. The mean yearly temperature is +6.9°C, the maximum temperature is +40.5°C, and the minimum temperature is -36°C. Mean yearly precipitation is 579 mm. In 2016 these data were: mean yearly temperature +7.1°C, maximum temperature +38.0°C, minimum temperature -42°C; and mean precipitation 505 mm. The seedlings of the hybrid families were planted separately in garden beds without replications. The distance between seedlings was about 20 × 10 cm. In the following year the seedlings were planted in permanent field sites. The data was processed with Microsoft Excel program.

DNA isolation, microsatellite (SSR) loci and PCR reaction

Clones listed in Table 1 (with exception of aspen 'local') were used for genotyping with 34 microsatellite (SSR) markers located on 18 of the 19 poplar chromosomes (7, 6, 5 chromosomes with 1, 2, 3 SSR marker, respectively, Table 2). Genomic DNA was extracted from leaves by the standard ATMB protocol of Dumolin et al. (1995) and stored at -20°C as stock. Aliquots were diluted to 10 ng/μL and stored at 4°C until use.

Table 2
Microsatellite markers used for genotyping of clones listed in Table 1.

SSR marker	Motif*	LG number**	Forward	Reverse
GCPM 1108-1	TC	7	CAGTGATTTAGCCGAAAAC	GGAGAACGAGAAATGATAGC
GCPM 124	CAC	1	TTTGAGCCTTCAACTACCA	TGCTTCCCTTAGTCACAC
GCPM 134-2	ATTT	3	AGGCATCAAACTAATTGGA	GAATCCAAAACAAACATCC
GCPM 1809	TTA	4	TACAAATGCTAATTACCC	AATTAGCAATCAGCATCTGC
GCPM 1831	AG	6	CAGCTAAACAGCTTCCAAGT	CAGATGGAAAATACGGAGAC
GCPM 2081	TC	14	ACAAAATTTGCAAGAAAG	TTCTGTTCATGAGAGTGGAA
GCPM 2167	AG	13	TTTGCTAGGAACAAAAAC	TGATTTGCATGAATCTTTGG
GCPM 2768	GA	2	TTATTTGGATCCTGAAATGG	GATGGTTCGGTATGTGAGTT
GCPM 3261	AAT	16	TGAAGCCCTCACTACTCATT	CCCAATCTTTTGTATTG
GCPM 3362	CTT	8	TGAACCAATACCATGTCAAA	AATCTTCAATGCCAGAAAA
ORPM 1031	n. k.	1	ATGTTTCGATTTGGAATGG	GGCTTGGACTAGAGATGATG
ORPM 190	[TG]7*	6	CCCTGGTTTTCTCTCTGG	CCAGATTTGGACTTGGGATC
ORPM 202	[TAA]5	8	TCGCAAAAGATTTCTCCAGT	TTCAAATCCCGTAATGCTC
ORPM 30	[TC]9	3	ATGTCACACCCAGATGACA	CCGGCTTCATTAAGAGTTGG
ORPM 489	[TC]12	18	CTCTAATGGCCGGTCACT	AATTCAAATCCCGAAAAGC
PMGC 108	CTT	12	TGCAGGTGATGTCATCACC	AACCGAATCCATCGCTCACC
PMGC 2020	GA	4	TAAGGCTCTGTTTGTAGTCA	GAGATCTAATAAAGAAAGTCTC
PMGC 2088	GA	2	TCACAAAAGGTTAAGCACTCG	CAGTACTCAGCTGCAGTCC
PMGC 2419	GA	10	TTTCCCTGTCATGGGCACTG	CATTGGAGACAGCTAATCAGC
PMGC 2531	GA	11	TAAGAGAATTTGGAGAGCAAC	TTTTATCTTTTCCAGTTGTCTAC
PMGC 2550	GA	1	AGGTTACAACTTTGTTAGC	GAACAACTCTCACTGTGGCTC
PMGC 2607	GA	8	TTAAAGGGTGGTCTGCAAGC	CTTCTTGCACCTCTGTTTGG
PMGC 2658	GA	13	GCCTTGAATACCATGAGCG	ACCTTCAGTAGATCAGTTAGTG
PMGC 2826	GA	4	GCTCTTTAGCGGACATGCATC	GTCAAGAACTGTACAGTAACC
PMGC 2880	n. k.	18	TCTGGGATATGGGTTTGC	GCCAGCACACTCATGTTAG
PMGC 420	GA	14	ATGGATGAGAATGCTTGTG	ACTGGCACACTCTTAACTGG
PMGC 433	GA	19	GCAGATTGTAGAAATAAAG	AAGGGGTCTATTATCCACG
PMGC 648	GA	17	GAAAGAATGAGTATCATG	ATAAATCTCTCTGTGATTTC
PTR 2	TGG	9	AAGAAGAACTGCAAGATGAAGA	ACTGACAAAACCCCTAATCAACA
PTR 5	n. k.	11	CTTCTCGAGTATAAATAAACA	TCACATCACCTCTCAGTTTCGC
PTR 7	n. k.	12	ATTTGATGCTCTCTCTCAGT	TATTTTCATTTTCCCTTGTCTT
WPMS 5	GT	12	TTCTTTTCAACTGCCTAATCT	TGATCCAATAACAGACAGAACA
WPMS 14	TTCTTG	5	CAGCCGACGCACTGAGAAATC	GCCTGTGAGAAAGACTGCCTTGC
WPMS 20	n. k.	13	GTGCGCACATCTATGACTATCG	ATCTTGTAAATCTCCGGGCACTC

*n. k.=not known; **based on *P. trichocarpa* v3.0
(https://phytozome.jgi.doe.gov/pz/portal.html#Info?alias=Org_Ptrichocarpa)

Different sources were screened to select the SSR markers for amplification in the clones listed in Table 1: (i) sequences of *Populus trichocarpa* SSR primers, released by the "International Populus Genome Consortium", (ii) GCPM and PMGC primers published at http://www.ornl.gov/sci/ipgc/ssr_resource.htm, (iii) ORPM primers by Tuskan et al. (2004), (iv) WPMS primers by van der Schoot et al. (2000) and Smulders et al. (2001), (vi) PTR primers by Dayanandan et al. (1998) and Rahman et al. (2000) (see also Schroeder and Fladung 2010, Bruegmann and Fladung 2013).

For the PCR reaction, 0.5 μL forward and reverse primers with a concentration of 5 μM each were mixed to 2.5 μL 10×reaction buffer, 2.0 μL MgCl₂ (25 μM), 0.5 μL dNTP-Mix (5 mM), 0.5 μL dimethyl sulfoxide (2 %), 0.2 μL DNA polymerase (5 U/μL) and 1.0 μL DNA (100 ng/μL). The PCR mix was filled up to 25 μL with water.

The PCR program was started with an initial denaturation for 4 min at 94°C, followed by thirty-five cycles comprising denaturation at 94°C (30 s), a variable annealing temperature (45 s), and 72°C (60 s). The reaction was completed by a final elongation step for 5 min at 72°C. Annealing temperatures were calculated on the basis of the primer sequences as recommended by the supplier (Eurofins, Ebersberg, Germany). For checking of unimorphic amplification, five μL of each PCR product were separated on 1.2 % agarose gels (120 V, 1:20 h) and stained with the DNA fluorescence additive Roti-Safe Gel Stain (Carl Roth, Karlsruhe, Germany).

Fragment Length Analysis and similarity test

An own PCR reaction was executed for fragment length analysis by replacing the dNTP-Mix with 1.5 μL Cy5-labelled nucleotides. An ALF Express II (Amersham Pharmacia Biotech, Vienna, Austria) was used for electrophoresis. Obtained data were further processed by the ALF Win Fragmentanalyser 1.02 (Amersham Pharmacia Biotech, Vienna, Austria).

Similarity test were performed with the GDA_NT program (vs. 2, Degen 2008). Results were visualized with the PAST-program (Hammer et al. 2001).

Results

Factorial crosses

The three crosses 02.01×07.02, 02.01×aspen 'local' and 45.01×07.02 gave no seeds. Seeds from two crosses were obtained, but they did not germinate (02.01×32.03 and 45.01×32.03). Six crosses, in which the female aspen individuals (02.01, 10.03, 23.05, and triploid 15.01) were pollinated with two male individuals (aspen 'local' and hybrid № 48.02), revealed infections with aspen polypore (*Fomes igniarius* Fr.). In these catkins, seeds were obtained, but the germinants lacked vitality.

The maternal individuals 02.01, 15.01 and 23.05 produced a low number of seeds or they were not viable. The maximum

yield of seeds in the maternal line was observed in combination 10.03x08.02. Female aspen individuals 45.01 and 23.05 were selectively successful in mating.

Among the paternal crossing partners good results were derived from trees 08.02 and 45.03. The male aspen tree 'local' showed little success in mating. The remaining males were selectively successful, depending on the crossing partner. The overall hybridization success was scored as follows:

- 0 – negative result, including:
 - 0₁ – pollination not successful;
 - 0₂ – the obtained seeds were not viable;
 - 0₃ – sprouts were developing, but proved to be lacking vitality.

- 1 – a very low number (1 – 10) of seedlings was obtained.
 - 2 – the number of seedlings was low (11 to 50).
 - 3 – the number of seedlings was moderate (51 to 100).
 - 4 – the number of seedlings was high (101 to 200).
 - 5 – the number of seedlings was very high (over 200).
- Using this scale, the obtained results are presented in Table 3.

Table 3
The aspen scheme and indices of their success in 2016

♀ \ ♂	07.02	08.02	32.03	45.03	48.02	Aspen local	Total crossings
02.01	0 ₁	2	0 ₂	0 ₃	0 ₃	0 ₁	6
10.03	2	5	3	4	4	0 ₃	6
18.02	1	4	4	4	3	2	6
23.05	3	3	3	3	0 ₃	0 ₃	6
45.01	0 ₁	4	0 ₂	1	3	-	5
15/01	-	-	-	-	-	0 ₃	1
Total crossings	5	5	5	5	5	5	30

Note: a dash (-) indicates that hybridization was not performed;
 0 – negative result, including:
 0₁ – pollination not successful;
 0₂ – the obtained seeds were not viable;
 0₃ – sprouts were developing, but proved to be lacking vitality;
 1 – a very low number (1 – 10) of seedlings was obtained;
 2 – the number of seedlings was low (11 to 50);
 3 – the number of seedlings was moderate (51 to 100);
 4 – the number of seedlings was high (101 to 200);
 5 – the number of seedlings was very high (over 200).

Compatibility evaluation of the parent pairs can be determined not only by the output of viable seeds and their germination, but also by the survival of seedlings grown in pots and in open ground.

Hybrid seeds produced in the spring of 2016 were sown in growing boxes, where their germinating power and survival was recorded. During the first month 8-83 % mortality was observed, and some progenies were lost completely. The reason for this was weak germinant power and a fungal contamination. For fungus control, the substrate was repeatedly treated with a fitosporin solution.

After the seedlings had been in the seed beds for 2.5 months a total of 937 were transplanted to open ground with a shelter by film cover. The subsequent total rooting rate amounted to 77.5 % (Figure 2).

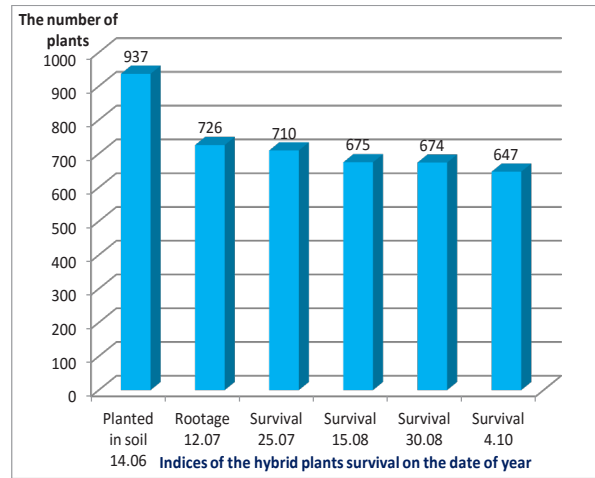


Figure 2
Rooting and survival dynamics of the 1-year-old aspen hybrid seedlings during 2016

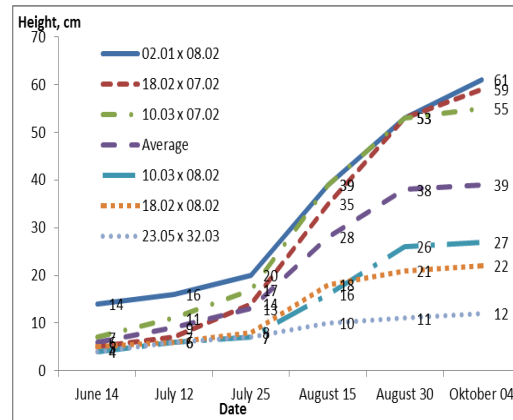


Figure 3
The dynamics of height growth of aspen hybrid seedlings: the fastest growing hybrid families (upper three lines), the slowest growing hybrid families (lower three lines) and the average of all hybrid plants (middle line).

The survival at the end of the vegetation period (04.10.2016) was 69.1 %. By the end of the first year 647 hybrid seedlings from 19 families remained.

The height growth dynamics of the three most rapidly growing and the three slowest-growing families, and average height of the entire set of aspen hybrid seedlings are represented in Figure 3.

As can be seen from Figure 3, the height of the plants varied very little over one month after transplantation, afterwards the seedlings started to gain height vigorously until the end of August, when growth slowed down to the end of the vegetation period. The mean height of most rapidly growing progeny was 58.3 cm and the lowest one was 20.3 cm. The difference between them was 38 cm or 187 % above the smallest progeny.

The numbers of the surviving plants of the hybrid families at the end of the vegetation period are given in Table. 4.

Table 4
Seedlings heights at the end of the first year(04.10.2016)

№ of the set	Parental pairs	Number n	Average \bar{X} maximum value cm	Standard error, cm $S_{\bar{X}}$	% of the total average	Variability factor, %
1	02.01×08.02	18	$\frac{61.2}{95}$	5.65	181	39
2	10.03×07.02	7	$\frac{55.4}{81}$	6.17	164	32
3	10.03×08.02	175	$\frac{27.8}{74}$	1.28	82	61
4	10.03×32.03	12	$\frac{47.8}{88}$	6.17	141	45
5	10.03×45.03	65	$\frac{36.9}{85}$	2.58	109	57
6	10.03×48.02	25	$\frac{38.9}{65}$	3.03	115	40
7	18.02×07.02	2	$\frac{58.5}{67}$	8.50	173	21
8	18.02×08.02	91	$\frac{23.8}{58}$	1.29	70	52
9	18.02×32.03	58	$\frac{37.4}{67}$	1.89	110	38
10	18.02×45.03	44	$\frac{37.3}{89}$	2.31	110	41
11	18.02×48.02	15	$\frac{33.6}{51}$	2.62	99	30
12	18.02×aspen 'local'	7	$\frac{47.9}{70}$	5.36	141	30
13	23.05×07.02	9	$\frac{29.3}{50}$	4.26	87	44
14	23.05×08.02	24	$\frac{42.0}{71}$	3.15	124	37
15	23.05×32.03	4	$\frac{11.3}{18}$	3.12	33	55
16	23.05×45.03	25	$\frac{37.3}{61}$	3.00	110	40
17	45.01×08.02	32	$\frac{45.1}{71}$	2.73	133	34
18	45.01×45.03	3	$\frac{47.3}{68}$	1.27	141	37
19	45.01×48.02	31	$\frac{27.4}{50}$	2.07	81	42
Sum and total average		647	$\frac{33.9}{95}$	0.71	100	53.5

From the data of Table 4 it can be seen that the number of hybrid seedlings surviving to the end of the vegetation period from different families ranged from 2 to 175 and averaged 34 individuals per family. The average height of the families ranged from 11 to 61 cm with an average across the population of 33.9 cm. The percentage of variation compared to the average of the aggregate ranged from 33 % (family 23.05×32.03) to 181 % (family 02.01×08.02). That is, there are families that exceed the average value by 1.8 times and the difference between the best and worst families can reach 5.5 times. The coefficient of variability ranged from 21 (family 18.02×07.02) to 61 % (family 10.03×08.02). Exceeding the maximum heights above the average values in individual families ranged from 15 % (18.02×07.02) to 166 % (10.03×08.02).

The number of surviving hybrid seedlings and their heights in every factorial hybrid family are shown in Table 5. The data of Tables 4 and 5 show that among hybrid families the best performance for height was noted in the family 02.01×08.02, whose average height was 61.2 cm with a maximum of 95 cm (Figure 4).

Table 5
Survival and average height of one-year old hybrid seedlings in aspen families on 04.10.2016 (number/cm)

♂ \ ♀	07.02	08.02	32.03	45.03	48.02	Aspen 'local'	Total:
02.01	0	$\frac{18}{61.2}$	0	0	0	0	$\frac{18}{61.2}$
10.03	$\frac{7}{55.4}$	$\frac{175}{27.8}$	$\frac{12}{47.8}$	$\frac{65}{36.9}$	$\frac{25}{38.9}$	0	$\frac{284}{32.4}$
18.02	$\frac{2}{58.5}$	$\frac{91}{23.8}$	$\frac{58}{37.4}$	$\frac{44}{37.3}$	$\frac{15}{33.6}$	$\frac{7}{47.9}$	$\frac{217}{32.0}$
23.05	$\frac{9}{29.3}$	$\frac{24}{42.0}$	$\frac{4}{11.3}$	$\frac{25}{37.3}$	0	0	$\frac{62}{36.3}$
45.01	0	$\frac{32}{45.1}$	0	$\frac{3}{47.7}$	$\frac{31}{27.4}$	-	$\frac{66}{36.9}$
15.01	-	-	-	-	-	0	0
Total:	$\frac{18}{42.7}$	$\frac{340}{31.1}$	$\frac{74}{37.7}$	$\frac{137}{37.9}$	$\frac{71}{32.8}$	$\frac{7}{47.9}$	$\frac{647}{33.9}$

Tests of similarity of the parents

To unravel the similarity of the different parents, in total 34 SSR loci were selected and amplified in 11 clones (with exception of aspen 'local', Table 1). The 34 SSR loci are located on 18 of the 19 poplar chromosomes, with 7, 6, 5 chromosomes harboring 1, 2, 3 SSR marker, respectively (Table 2). SSR marker analysis confirmed the triploid status of clone 15.01. Allele sizes of all 34 SSR loci were uploaded to the GDA_NT program (Degen 2008) and genetic differentiation on the basis of 33 SSR loci (PMGC 2880 was excluded because it could not be amplified in all of the clones) was determined by cluster analysis ("Unweighted Pair-Group Method using an Arithmetic average"; UPGMA). The used distance was the genetic distance of multilocus genotypes (Gregorius 1974).

Results of the similarity test were visualized with the PAST-program (Hammer et al. 2001) by using the "Monte Carlo"



Figure 4
The aspen hybrid progeny 02.01 × 08.02. The average height of the seedlings is 61.2 cm, maximum height – 95 cm. 04.10.2016. Photo courtesy by R. P. Tsareva.

randomization procedure (Raup and Crick 1979) (Figure 5). The dendrogram shows clearly that clones 45.01 and 45.03 are closely related, and the hybrids as well as the pure *P. tremula* cluster with each other. The clone 32.03 (*P. tremuloides*) takes a separate position, and the clone 02.01 seems not to be a pure *P. tremula* because it clusters close to clone 32.03, assuming that clone 02.01 is a hybrid of *P. tremuloides* with an unknown *Populus* species. Remarkable is also the isolated position of clone 23.05, clustering only with triploid 15.01.

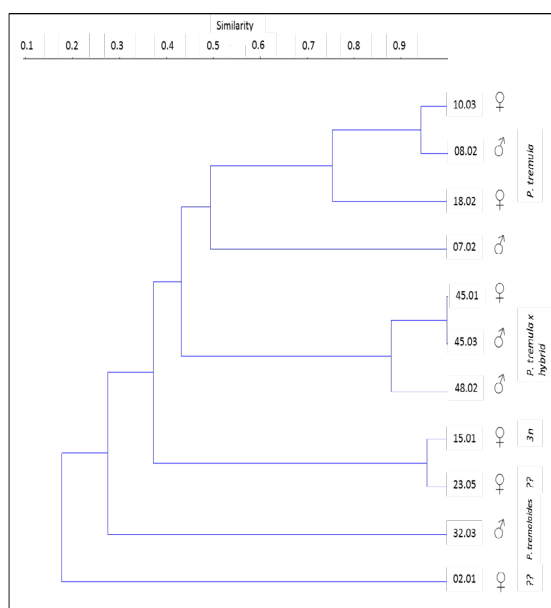


Figure 5
Dendrogram of the GDA_NT cluster analysis (Degen 2008) of the clones (Table 1) using the similarity measure following Raup and Crick (1979).

Discussion

Compatibility of parental pairs in crosses of various poplars sections, including section *Trepidae*, was shown by P. L. Bogdanov (1965). The same subject was discussed by other authors (A. S. Yablokov, 1963; R. P. Tsareva, 1996, 1997 et al.). The data presented shows that when crossing different genotypes, even of the same species, different results are obtained (Tables 4 and 5). The results of aspen hybridization received by M. Hoffmann (2005) have shown that the hybrids of *P. tremula* × *P. tremuloides* perform better than *P. tremula* × *P. tremula*. In the subsequent German studies (von Wühlisch, 2006, 2010, 2012) it is shown that the difference in diameter growth of the aspen hybrids from different crossing variants can reach significant values. So, if the average diameter of the hybrid families *P.*

tremuloides × *P. tremuloides* taken as 100 %, the hybrids of *P. tremula* × *P. tremula* were equal to 105 %, the hybrids of *P. tremula* × *P. tremuloides* 143 %, and the hybrids of *P. tremuloides* × *P. tremula* 161 %.

In our studies, even only within *P. tremula* the height difference between families was higher. If we compare the average height of the three best crosses and three worst ones (Figure 3), it can be seen that the difference between them amounted to 187 %. However, these results are not directly comparable, as the German data were gathered on adult plants (22 years old), and in our case on only one-year-old seedlings. But if we consider the results of hybridization within *P. tremula* L. by V. P. Petrukhnov, it can be seen that the difference between the average values of the stem volumes of the best hybrid families (*P. tremula* X₂ × *P. tremula local*– 0.50 m³) and of the worst one (*P. tremula* X₁ × *P. tremula local*– 0.34 m³) at age 29-years reached 47 %. At the same time, the difference between the average trunk volume of the best families of intraspecific hybrid (*P. tremula* X₂ × *P. tremula local*– 0.50 m³) and interspecific hybrid (*P. tremuloides* × *P. tremula local*– 0.42 m³) was 19 % in favor of the intraspecific crossing (A. Tsarev, 2013a).

This shows that the prediction of the hybrid quality remains difficult. Obviously, it is not connected only with botanical and systematic affiliation of the parent forms. For a final evaluation of the GCA and SCA (general and specific combining abilities) of the presently used parents it is too early yet. However, for a preliminary analysis of the basic material, which can be used in further studies, it seems to be quite appropriate. Therefore, the preliminary assessment of these indices on the hybrid offspring, being at the beginning of their ontogenesis has been conducted in this study.

The analysis of the present GCA (D. Falconer, 1989; B. Zobel and J. Talbert, 1984) shows that the one year old progenies obtained from the female aspen parents used in hybridization 23.05 (36.3 cm) and 45.01 (36.9 cm) performed above the experimental average of 33.9 cm. Among the male parents, highest GCA was observed in trees 32.03 (37.7 cm) and 45.03 (37.3 cm). While GCA from the mother tree 02.01 and a father– *P. tremula* 'local' were not studied because these parents had only one, albeit successful, alternative crossing (the average height of the seedlings with the participation of the first tree was 61.2 cm, and of the second – 47.9 cm). In other crossing combinations with these parents no seedlings were obtained.

Estimations of the SCA by height growth showed that the best result was obtained in the variant of crossing 02.01×08.02, with an average seedling height of 61.2 cm. The other crossings, in which the progenies distinctly exceeded the average (33.9 cm), are the combinations 18.02×07.02 (58.5 cm), 10.03×07.02 (55.4 cm), 18.02×aspen 'local' (47.9 cm), 10.03×32.03 (47.8 cm), 45.01×45.03 (47.6 cm).

Unfortunately, in these combinations, the number of surviving plants ranged from only 2 to 12, and in cross 02.01×08.02 only 18 seedlings were obtained. However, despite of the low representation, further studies of these plants should be considered appropriate, as in the hybridization practice there had been cases when only single hybrid plants gave a good result. This was the case in a hybrid of Professor M. M. Veresin 'E.s.–38'

('Voronezh Giant'). Tests in different regions of the Soviet Union and Russia proved the high performance of this hybrid (M. Veresin, 1974; A. Tsarev et al., 2014).

The maximum plant heights in individual families ranging from 15 % (18.02×07.02) to 166 % (10.03×08.02), show good prospects for selecting clones not only in the best families.

The cluster analysis revealed that clones of European aspen, hybrids and American aspen as well as the triploid clone form separate clusters each. Surprisingly, clone 02.01 clustered closest to the *P. tremuloides* clone, however with a large genetic distance, indicating that it is neither *P. tremula* nor a pure *P. tremuloides* clone, but possibly a hybrid with *P. tremuloides*. Clone 23.05 clustered closest to the triploid clone 15.01, indicating that this clone could also be a triploid *P. tremula* or an unknown hybrid. However, from SSR analysis, no triploid allele status could be detected in any of the 34 loci.

No correlations could be detected between genetic distance of the clones and success of crossings, seed set, germination rate, and growth performance of seedlings and plants. However, obvious are the isolated positions of clones 02.01 (unknown hybrid), 15.01 (triploid) and 23.05 (triploid or hybrid) which is in line with the observation of very low yield and viability of seeds.

Conclusions

- Different trees of the same species of *P. tremula* have different genetic features, different general combining abilities and by crossing show different specific combining abilities.
- Differences in average progeny plant heights can reach considerable magnitude, in the presented study 187 % and more.
- Analysis of 34 microsatellite markers located on 18 of the 19 chromosomes of the crossing parents revealed the genetic distances among each other and further information on the genotypes used, however, no direct relationship with any phenotypic characters.
- Genetic differences by intraspecific hybridization with *P. tremula* in the progeny are shown to exceed those by interspecific crosses with *P. tremuloides*.
- The main contribution to the reached phenotypic variation is caused by the specific genotypes of the parents.
- Within separate families, plants were selected which considerably exceeded the average plant heights of other full sibs. Their further testing will allow selections of ideotypes having practical value.

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