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Improving the productivity, resistance, and adaptability in poplar – development of genetic markers for aspen ("MaRussiA")

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Abstract

The joint German-Russian co-operation project with the acronym "MaRussiA" focuses on the improvement of poplar in eastern and western Europe (Russia respectively Germany), where the aspen species *Populus tremula* is indigenous and distributed widely. Background and objective of the joint research project is the development of genetic markers for marker-assisted selections (MAS). Such markers would represent major advances in forest tree breeding e.g. for accelerating the breeding process. Different full-sib families were provided by the Russian partners, and those with the highest progeny numbers were chosen for the development of genetic maps. Several hundreds of microsatellite (SSR) markers were tested in the parents for heterozygosity and mapping ability, and 30-40 SSR markers were successfully selected covering all 19 chromosomes. The available full-sib progenies will be tested in field trials to phenotype productivity and resistance characters, e.g. heart rot and drought. Segregating populations will be identified and QTL analyses for the phenotyped traits will be performed. Based on the results obtained, gene markers for a marker-based selection will be developed to intensify poplar / aspen breeding in Russia.

Key words: Microsatellite marker, genetic map, poplar breeding, heart rot disease, drought

"MaRussiA" participating institutions

The "MaRussiA" consortium consists by the Russian partners St. Petersburg Forest Technical University, St. Petersburg (A. ZHIGUNOV, E. POTOKINA, M. VISHNEVSKAYA), Saint-Petersburg Forestry Research Institute, St. Petersburg (D. SHABUNIN), All-Russian Institute of Forest Genetics, Breeding and Biotechnology, Voronezh (A. TSAREV), and Moscow State Forest University, Moscow (OXANA CHERNISHENKO), and the German partner Thuenen-Institute of Forest Genetics, Grosshansdorf (G. VON WUEHLISCH, M. FLADUNG), being the coordinator of this project.

General and specific project aims

The general aim of the German-Russian co-operation "MaRussiA" project is to raise the productivity of the indigenous and widely distributed poplar species aspen (*Populus tremula*) by enhancing tolerance against biotic and abiotic stresses. In economically active target regions of St. Petersburg and Moscow, wood is intensively used and the efficiency of wood production needs to be raised. Large areas of abandoned land can be put into use by producing wood, a renewable resource forecast to be required more intensely in future. Classical tree breeding strategies are establishment and conservation of genetic resources, germplasm exchange, hybridisation, selections and field testing of elite material. However, to intensify and accelerate the breeding process, also modern technologies e.g. development of genetic markers for clone identification and marker-assisted selections (MAS) need to be considered (SCHROEDER and FLADUNG 2010, FLADUNG and GEBHARDT 2010).

Focus of any breeding activity is on the end product, the wood produced and its quality, which are to be investigated. Within this joint co-operation project, poplar and aspen reproductive materials for cross breeding by a jointly developed factorial crossing scheme are to be exchanged. The produced progenies will be shared among the partners for further testing in field trials and in the lab. In the field, productivity and resistance characters, e.g. heart rot and drought, are phenotyped by the Russian partners in the segregating populations, and QTLs will be identified. The German partner will test as many segregating microsatellite (simple sequence repeats, SSR) and single nucleotide polymorphism (SNP) markers as possible in the same progeny populations. Identified QTLs will be mapped on the respective genetic maps and association studies with SSR- and/or SNP-markers will be performed. An already available, with SSR- and SNP-markers highly saturated *P. tremula × P. tremuloides* map (Brauna11 × Tur141; PAKULL et al. 2009, 2011) will serve as reference map. SSR- and/or SNP-markers linked to QTLs will be used to screen already available *P. tremula* and *P. tremuloides* total genomic sequences to identify putative candidate genes.

WP 1 Crossings and establishment of F1 populations

In 2015, leaf material from 20 different aspen clones, used for crossings, was provided by Russian partner (A. TSAREV [PETROZAVODSK and VORONEZH, Russia]) (TSAREV et al. 2016, 2018) (Table 1). The progenies were established and planted in the field. In Table 2, five male and five female individuals with the numbers of the respective offspring are shown. From these, progenies having different mothers (05-02, 15-04, 25-05), but the same father (here: 08-02) with 62, 61 and 120 individuals, respectively, were selected for further molecular analyses.

Table 1: Leaf material from 20 different aspen clones (kindly provided by A. Tsarev [Petrozavodsk and Voronezh, Russia]) collected in the Central Chernozem region (Russia). In addition to the clone name (Index), the gender, the local collection and origin, and the "form of life" are given. In red: gender information not provided, newly determined.

Number	Index	Gender	Local of collection	Origin	Life form
Parents of hybrids-frozen and alcohol transport, collected in May 2015					
1	07-02	male	Semiluksky tremuletum	Voronezh	tree
2	07-04	male	-"-	-"-	-"-
3	25-05	female	-"-	-"-	-"-
4	05-02	female	-"-	Latvia	-"-
5	06-04	female	-"-	Latvia	-"-
6	15-04	female	-"-	Voronezh	-"-
7	08-02	male	-"-	-"-	-"-
8	22-05	female	-"-	-"-	-"-
9	23-03	male	-"-	Saval (Voronezh)	-"-
10	20-04	male	-"-	Oboyan (Kursky)	-"-
Leaves collected in natural forests and tremuletum and put in envelops in July 2015					
11	W-1	male	Voronezh suburb	Voronezh	tree
12 (15)	17-05	male	Semiluksky tremuletum	Gubkino (Belgorod)	-"-
13 (16)	02-01	female - male	-"-	Voronezh	-"-
14 (17)	15-01	female	-"-	-"-	-"-
15 (18)	18-04	male	-"-	Valuyky (Belgorod)	-"-
16	W-2	female	Voronezh suburb	Voronezh	little tree
17	W-3	male	-"-	-"-	tree
18	W-4	male	-"-	-"-	little tree
19	W-5	female	-"-	-"-	root shoot
20	W-6	"supermale"	-"-	-"-	-"-

Table 2: Already available progenies of five female and five male parents. Parents of the grey tagged progenies, namely 05-02, 15-04, 25-05 (female) and 08-02 (male), were selected for microsatellite analyses.

♀	♂ 07-02	07-04	08-02	20-04	23-03	∑
05-02	24	-	62	66	5	157
06-04	8	1	7	1	-	17
15-04	2	6	61	2	48	119
22-05	1	-	-	-	34	35
25-05	34	1	120	15	32	202
∑	69	8	250	84	119	530

WP 2 Development and use of molecular markers

The microsatellite markers tested were either selected from (*i*) *Populus*-database http://www.ornl.gov/sci/ipgc/ssr_resource.htm; YIN et al. 2009) or (*ii*) developed in own work (BRÜGMANN and FLADUNG 2013, SCHRÖDER and FLADUNG 2010). All selected microsatellite markers were tested in the parental clones first for amplificability in PCR reactions and later for polymorphism on ALF DNA

Automated Sequencer (Amersham Pharmacia Biotech, Buckinghamshire, UK). Overall, about 270 microsatellite markers turned out to be useful. Second, about 60 microsatellite markers could successfully be transferred to the ABI3730 Capillary sequencer (Applied Biosystems, Foster City, CA, USA; SCHROEDER et al. 2017). In different sets, four to five microsatellites could be combined and analysed simultaneously in one run ("multiplex analysis"). The aim is to amplify one to four microsatellite markers for each of the 19 poplar chromosomes.

Gender determination in different poplar clones

As first molecular investigation, gender determination was carried out in plant material from Table 1 according to PAKULL et al. (2015). The results are shown in Figure 1. With exception of sample 02-01, which is declared as female in the sample delivery protocol but clearly revealing the male-specific band in the PCR (yellow in Figure 1), the gender of all other genotypes is confirmed. However, the Russian side confirmed that sample 02-01 is actually female. Further molecular analyses of 02-01 have shown that this genotype is not a pure *P. tremula* but an unknown species hybrid. As the PAKULL et al. (2015) procedure for gender determination is exclusively in aspen (*P. tremula* and *P. tremuloides*) as well as their hybrids, the presence of an unknown species hybrid could explain the discrepancy between Russian declarations (female) and molecular analysis (male).

For six clones (W1 to W6), gender was newly determined (red in Table 1) as no respective information was given in the sample delivery protocol. Three clones turned out to be male (W1, W3, W4) and two female (W2, W5). For clone W6, a "supermale" status could be determined as no female allele could be PCR-amplified.

In 2016, the Russian partners provided leaf material from two additional progenies, namely 10-03 × 08-02 with 127 individuals and 18-02 × 08-02 with 90 individuals. Also for these parental individuals, all available microsatellite markers were tested for being polymorphic in the respective parental individuals. In addition, all F_1 individuals were included in molecular analyses.

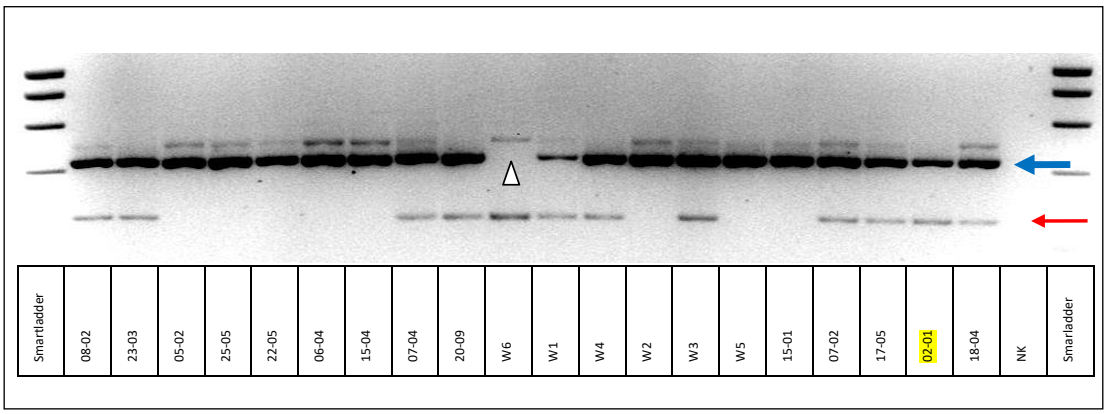

Figure 1: Gender check for the genotypes listed in Table 1. The band indicated by the red arrow is formed in the PCR only by male genotypes. The yellow-labelled clone 02-01 shows the male-specific band, but is declared as female in Table 1. The black triangle in W6 indicates the missing female band (blue arrow), thus this clone is possibly a "supermale".

Analysis of 05-02, 15-04, 25-05 (female) and 08-02 (male)

The three female parents, namely 05-02, 15-04, 25-05, as well as the one male genotype 08-02 were intensively characterized by microsatellite markers (Table 3). In total, 32 microsatellite markers qualified to be applicable for further molecular studies. All microsatellite markers were also tested in *P. tremula* Brauna11 (female) and *P. tremuloides* Tur141 (male) being parents of a progeny of about 120 F_1-individuals. The genetic map of this offspring has already been published (PAKULL et al. 2009).

Table 3: Extract from the results table of the molecular analysis of the Russian parents 05-02, 15-04, 25-05 (female), and 08-02 (male), compared to Brauna11 and Tur141 (PAKULL et al. 2009), by a total of more than 120 microsatellites (primer). Green and yellow-green tagged primer: applicable, red tagged primer: not applicable.

Primer	LG	Motif	08-02 ♂		05-02 ♀		15-04 ♀		25-05 ♀		Tur141		Brauna 11	
GCPM1108-1	7	TC	162	198	196		198	202	194	198	198		198	202
GCPM124	1	CAC	197	212	206		206		197	206	210		196	
GCPM134-2	3	ATTT	225	239	227		233		227		224	228	240	244
GCPM1809	4	TTA	186		180	183	183		180	183	183	186	183	186
GCPM1831	6	AG	223	227	223		223	227	225	227	223	225	223	
GCPM2081	14	TC	204	206	194	198	202	206	202	204	204		194	
GCPM2167	13	AG	180	184	178	202	178	200	178	200	184		180	182
GCPM2768	2	GA	206	220	206	210	206	208	204	206	196	200	194	206
GCPM3261	16	AAT	190	193	187	193	187	196	190	196	193	196	187	196
GCPM3362	8	CTT	218		221		218	224	218	221	209	212	212	221
ORPM1031	1		146	158	152	170	143	146	146		131	146	146	188
ORPM190	6	[TG]7*	202		202	216	216		216		202		202	218
ORPM202	8	[TAA]5	193		193	196	193	196	196		196		193	196
ORPM30	3	[TC]9	222	232	222		236	238	230	236	230	236	230	
ORPM489	18	[TC]12	172	186	172	180	172	176	176		166		176	190
PMGC108	12	CTT	340	346	340		343	346	346		328	340	340	346
PMGC2020	4	GA	148	150	148	152	144	150	144	146	150	152	136	150
PMGC2088	2	GA	180	184	180	184	186		186	192	172	176	182	192
PMGC2419	10	GA	111	117	111	117	125	131	117	125	101	105	125	131
PMGC2531	11	GA	126	150	140	146	126	148	126		130	138	136	
PMGC2550	1	GA	150	156	150	156	156	162	156	162	150		156	162
PMGC2607	8	GA	143	161	143	153	143	169	153	169	161		143	
PMGC2658	13	GA	149	303	149		231		149		245		253	303
PMGC2826	4	GA	238	252	216	236	236	242	240	242	246	252	226	242
PMGC2880	18		174	0	202		176		174	194	170	198	202	
PMGC420	14	GA	101		101	105	101		103	105				
PMGC433	19	GA	197	207	197	201	197	209	197		205		197	
PMGC648	17	GA	184	208	194	198	198		196		188	214	198	226
PTR2	9	TGG	231		228	234	228		228		228	234	228	241
PTR5	11		268		268		268	278	268		270		262	268
PTR7	12		240		242	264	266		242	266	242		286	
WPMS05	12	GT	293	303	303		293		295		277	303	281	295
WPMS14	5	CGT	234	249	249		231	240	240	249	228	231	234	249
WPMS20	13	TTCTGG	245		245		239	245	221	245				

Analysis of families 10-03 (female) and 08-02 (male), and 18-02 (female) and 08-02 (male)

The results of the analyses for parents 10-03 (female) and 08-02 (male) as well as for some of the progeny F_1- individuals by testing more than 120 microsatellite markers are shown in Table 4. In total, 21 microsatellite markers revealed polymorphic in both parent genotypes. These markers were used to check whether all individuals actually belong to this progeny. In total, 5 individuals had to be excluded who had allelic combinations at 5 to 14 loci that could not be derived from both parents (Table 4).

For the second progeny (18-02 [female] and 08-02 [male]), 28 microsatellites revealed polymorphic in the two parent genotypes (data not shown). These markers were also used to check for individuals actually not belonging to this progeny. In this progeny, no individual had to be excluded on the basis of a non-derivable allele combination.

Table 4: Extract from the results table of the molecular analysis of the Russian parents 10-03 (female) and 08-02 (male), by a total of more than 120 microsatellites (primer). Green and yellow-green tagged primer: applicable, red tagged primer: not applicable. The yellow fields in the progeny individuals indicate allele combinations that cannot be derived from both parents.

Primer	LG	Motif	08-02 ♂		10-03_C ♀		01-01		01-02		01-112		01-113		01-114		01-115		01-116		01-117		01-118		01-119	
GCPM1108-1	7	TC	162	198	162	198	198		162		198		198		198		162	198	162	198	162		198		198	202
GCPM124	1	CAC	197	212	197	212	197	212	197		212		207	212	197	212	212		197		197		197	212	197	212
GCPM134-2	3	ATTT	225	239	227	239	239		225	239	225	239	227		239		227	239	225	239	239		225	227	227	
GCPM1831	6	AG	223	227	221	231	227	231	227	231	221	227	221	225	221	233	221	227	223	231	221	223	221	223	221	231
GCPM2081	14	TC	204	206	196	204	204		196	206	204	206	202	204	198	204	196	204	204		196	204	204		196	204
GCPM2167	13	AG	180	184	178	184	180	184	184		178	180	178		178		178	184	180	184	178	184	178	180	184	
GCPM2768	2	GA	206	220	206	220	206	220	206	220	220		206	220	206	212	206	220	220		206		206	220	206	
GCPM3261	16	AAT	190	193	190	193	190	193	190	193	193		190	193	187	190	190	193	190		193		190	193	190	193
ORPM1031	1		146	158	143	146	143	146	143	158	143	158	143		143	146	143	146	146		143	146	143	158	143	146
ORPM30	3	[TC]9	222	232	222	238	222	232	222	238	222	232	222	238	222		232	238	222		222		222		230	238
ORPM489	18	[TC]12	172	186	180	196	180	186	172	196	172	180	176	180	170	196	172	196	186	196	172	196	172	180	166	180
PMGC2020	4	GA	148	150	136	152	136	150	150	152	148	152	136	146	136	144	148	152	148	152	148	152	136	150	136	150
PMGC2088	2	GA	180	184	180	184	180	184	180	184	180	184	184		180	184	180	184	184		180	184	180	184	180	184
PMGC2419	10	GA	111	117	125		111	125	111	125	117	125	95	125	125		111	125	117	125	117	125	117	125	105	125
PMGC2531	11	GA	126	150	136	142	126	142	136	150	136	150	136		142		142	150	126	142	136	150	142	150	126	1136
PMGC2550	1	GA	150	156	150	162	150	156	150	156	150	156	162		150	164	156	162	150		150	162	150	162	150	
PMGC2658	13	GA	149	303	303		303		149		303		231	303	303		149	303	303		149		303		258	305
PMGC2826	4	GA	238	252	234	246	234	252	234	238	238	246	242	246	216	246	234	238	234	252	246	252	246	252	216	234
PMGC433	19	GA	197	207	197	201	197	207	197		197	207	197		197	205	197		197		197		197		197	201
PTR2	9	TGG	231		228	240	228	231	228	231	231	240	231	240	228		228	231	228	231	231	240	231	240	228	
PTR7	12		242		242	286	242	286	242		240	286	240	270	254	286	240		240		240	286	240	286	240	286

WP3 *in vitro* propagation

Buds of selected aspen genotypes were successfully transferred to tissue culture for further propagation, including five *P. tremula* "davidiana" clones that are considered to be resistant to heart rot. The cultures were treated with three different mutagenic substances. The resulting callus tissues were sub-cultured and shoots could be regenerated. A total of 140 plants were obtained. The exact number of plants for the rooting experiments will be evaluated in the spring of 2018. These plants will be transferred to the field and studies on growth, lignin content and fibre length will be performed. Finally, resistance tests against heart rot will be conducted.

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References

BRUEGMANN T, FLADUNG M (2013): Potentials and limitations of the cross-species transfer of nuclear microsatellite marker in six species belonging to three sections of the genus *Populus* L. Tree Genetics & Genomes 9: 1413-1421.

FLADUNG M, GEBHARDT K (2010): Mit Smart-Breeding-Methoden neue Wege in der Forstpflanzenzüchtung gehen. Forst- und Holz 65: 37-40.

PAKULL B, GROPPE K, MECUCCI F, GAUDET M, SABATTI M, FLADUNG M (2011): Genetic mapping of linkage group XIX and identification of sex-linked SSR markers in a *Populus tremula* L. x *P. tremuloides* Michx. cross. Can J Forest Res 41: 245-253.

PAKULL B, KERSTEN B, LUENEBURG J, FLADUNG M (2015): A simple PCR-based marker to determine sex in aspen. Plant Biology 17: 256–261.

PAKULL B, GROPPE K, MEYER M, MARKUSSEN T, FLADUNG M (2009): Genetic linkage mapping in aspen (*Populus tremula* L. and *P. tremuloides* Michx.). Tree Genetics & Genomes 5: 505-515.

SCHROEDER H, FLADUNG M (2010): SSR and SNP markers for the identification of clones, hybrids and species within the genus *Populus*. Silvae Genetica 59: 257-262.

SCHROEDER H, KERSTEN B, FLADUNG M (2017): Development of Multiplexed Marker Sets to Identify the Most Relevant Poplar Species for Breeding. Forests 8: 492.

TSAREV A, WÜHLISCH G VON, TSAREVA R (2016): Hybridization of poplars in the central Chernozem region of russia. Silvae Genetica 65: 1-10.

TSAREV A, TSAREVA R, TSAREV V, FLADUNG M, VON WÜHLISCH G (2018): Aspen hybridization: Parents' compatibility and seedlings' growth. Silvae Genetica 67: 12-19.

YIN TM, ZHANG XY, GUNTER LE, LI SX, WULLSCHLEGER SD, HUANG MR, TUSKAN GA (2009): Microsatellite primer resource for *Populus* developed from the mapped sequence scaffolds of the Nisqually-1 genome. New Phytologist 181: 498-503.