Associations of SNPs and phenotypic variables of breeding value in poplars

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Abstract

Evaluations of the growth characters of a set of poplar cultivars including members of the sections Aigeiros and Tacamahaca as well as intra- and intersectional hybrids were made in experimental fields situated nearby the breeding station in Hann. Münden (Germany). The SNP character of each cultivar was determined by resequencing of five candidate genes [cinnamyl alcohol dehydrogenase-like (cad-like, gibberellic acid 20-oxidase (GA20ox), C-repeat binding factor 1 (cbf1), teosinte branched-like1 (tb1), phytochrome B2 (phyB2) and clavata1 (clv1)]. The data from Sanger sequencing (both directions) of the PCR products allowed the creation of a consensus sequence using the software CodonCodeAligner 3.7.1. Heterozygous alleles could be simply recognized as single nucleotide overlays. Using the internet-based program SNPStats (Sole et al. 2006) and the R-Package: SNPassoc [R, version 2.14.1., (R development core team 2011)] significant associations of SNPs with phenotypic characters like height and diameter growth, crown shape and density of branches, became obvious. Since the statistic model used compares genotypes heterozygous and homozygous for the variant allele to the genotypes homozygous for the most frequent allele different modes of inheritance are considered for each SNP. The SNP markers described will allow the genotyping of future breeding populations.

Key words: poplars, SNPs, clavata1, teosinte branched-like1, phytochrome B2

Zusammenfassung

Assoziationen von SPNs und phänotypischen Merkmalen in der Pappelzüchtung

Bewertungen des Wachstumscharakters einer Reihe von Pappel-Sorten aus den Sektionen Aigeiros und Tacamahaca einschließlich intra- und intersektionaler Hybriden wurden im Kamp der Zuchtstation in Hann. Münden gemacht. Der SNP-Charakter jeder Sorte wurde durch Resequenzierung von fünf Kandidaten-Genen bestimmt, [cinnamyl alcohol dehydrogenase-like (cad-like, gibberellic acid 20-oxidase (GA20ox), C-repeat binding factor 1 (cbf1), teosinte branched-like1 (tb1), phytochrome B2 (phyB2) and clavata1 (clv1)]. Die Daten aus der Sanger-Sequenzierung der PCR-Produkte (beide Richtungen) erlaubte die Erstellung einer Konsensus-Sequenz mit Hilfe der Software CodonCodeAligner 3.7.1. Heterozygote Allele waren als Einzel-Nukleotid-Überlagerungen einfach zu erkennen. Mit Hilfe des Internet-basierten Programm SNPStats (Sole et al. 2006), und des R-Paketes: SNPassoc [R, Version 2.14.1, (R development core team 2011)] wurden signifikante Assoziationen von SNPs mit phänotypischen Merkmalen wie Höhen- und Durchmesserwachstum, Kronenform und Beastungsdichte offensichtlich. Die charakterisierten SNP-Marker erlauben die Genotypisierung bestehender und künftiger Zuchtpopulationen.

Schlüsselworte: Pappeln, SNPs, clavata1, teosinte branched-like1, phytochrome B2

Introduction

Since the genome of a black cottonwood was sequenced in 2004 and became available in public domains numerous attempts have been made in order to assign elements of function to the genes of poplar species. Here the results of a candidate gene approach is reported which could be helpful in breeding of poplars.

Materials and Methods

Evaluations of the growth characters of a set of poplar cultivars including members of the sections Aigeiros and Tacamahaca as well as intra- and intersectional hybrids were made in experimental fields situated nearby the breeding station in Hann. Münden and documented by Fröhlich and Grosscurth (1973) for the first time. The scoring of growth characters of even aged clones [1 (best) to 5, average 3] growing on adventitious roots included height and diameter stem growth, crown shape (fastigiate to broad), as well as density of branches (Fig. 1). In order to harmonize the scoring of the different characters, we reduced the scoring levels to 3. The SNP character of each cultivar was determined by resequencing of five candidate genes (Table 1). The data from Sanger sequencing (both directions) of the PCR products allowed the creation of a consensus sequence using the software CodonCodeAligner 3.7.1 Heterozygous alleles could be simply recognized as single nucleotide overlays.

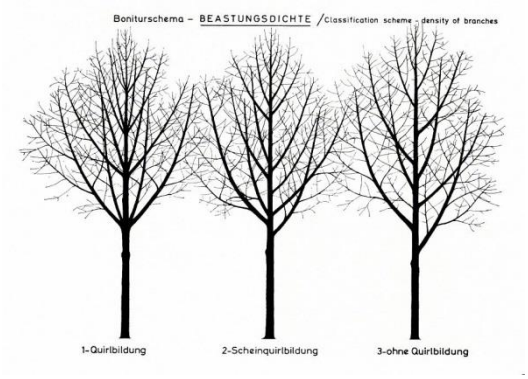

Fig. 1: Scoring of the density of branches

Table 1: Nucleotide-Polymorphisms (SNPs) in the candidate genes cinnamyl alcohol dehydrogenase-like (cad-like), gibberellic acid 20-oxidase (GA20ox), C-repeat binding factor 1 (cbf1), teosinte branched-like1 (tb1), phytochrome B2 (phyB2) and clavata1 (clv1).

Gene	LG	PCR-fragment size db [bp]	Aligned [bp]	Position from Start to	SNPs total	Single-tons	SNPs in Exons	Amino-acidex-change nonsyn.	SNPs in Introns	SNPs in 5'-UTR	In-dels	Amples [n]
CAD-L.	9	517	511	2 - 512	40	4	7	3	32	-	6	70
GA20ox	15	562	560	9 - 567	32	5	32	10	-	-	-	69
CBF1	1	666	659	37 696	58	5	58	20	-	-	-	70
TB1	8	650	648	43 - 691	28	2	28	20	-	-	3	73
PHYB2	10	913	827	-42 - 779	15	3	13	5	-	2	-	64
CLV1	13	1013	989	-106 - 883	89	23	71	42	-	7	1	74
Sum		4321	4194		262	42	209	100	32	9	10	420

Results

Within members of the poplar sections Aigeiros and Tacamahaca inclusively inter- and intrasectional hybrids PCR products of 5 candidate genes (Table 1) were sequenced and SNPs of exons, introns and the 5-UTR-region identified. Using the internet-based program SNPStats (Sole et al. 2006) and the R-Package: SNPassoc [R, version 2.14.1., (R development core team 2011)] significant associations of SNPs with phenotypic characters of breeding value became obvious. Since the statistic model used compares genotypes heterozygous and homozygous for the variant allele to the genotypes homozygous for the most frequent allele different modes of inheritance are considered for each SNP (Tables 2 and 3). The Akaike (AIC)-value and the Bayesian information criterion (BIC) should be lowest for the mode of inheritance that best fits the data. Significant associations became obvious for, diameter growth with SNP 453 of phytochrome B2 (Table 2) and the density of branches with SNP 431 of teosinte branched-like1 (Table 3). Figure 2 illustrates the mean values of the phenotypic response of height growth (Fig. 2A) with SNP 143 and crown shape (Fig. 2B) with SNP 387 of clavata1 -gene accounted for the codominant mode of inheritance.

Table 2: Association of diameter growth with SNP 453 of phytochome B2

X453 association with response diameter.growth (n=64, crude analysis)							
Model	**Genotype**	**n**	**Response mean (s.e.)**	**Difference (95% CI)**	**P-value**	**AIC**	**BIC**
Codominant	C/C	50	2.4 (0.11)	0.00			
	C/T	6	1.5 (0.22)	-0.90 (-1.53 - -0.27)	3e-04	148	156.6
	T/T	8	1.38 (0.18)	-1.03 (-1.58 - -0.47)			
Dominant	C/C	50	2.4 (0.11)	0.00	<0.0001	146.1	152.5
	C/T-T/T	14	1.43 (0.14)	-0.97 (-1.41 - -0.54)			
Recessive	C/C-C/T	56	2.3 (0.11)	0.00	0.0025	153.8	160.3
	T/T	8	1.38 (0.18)	-0.93 (-1.51 - -0.35)			
Overdominant	C/C-T/T	58	2.26 (0.11)	0.00	0.033	158.5	165
	C/T	6	1.5 (0.22)	-0.76 (-1.44 - -0.08)			
Log-additive	---	---	---	-0.56 (-0.83 - -0.30)	1e-04	147.4	153.8

Table 3: Association of the density of branches with SNP 431 of teosinte branched-like1

X431 association with response density.of.branches (n=73, crude analysis)							
Model	**Genotype**	**n**	**Response mean (s.e.)**	**Difference (95% CI)**	**P-value**	**AIC**	**BIC**
Codominant	G/G	28	2.11 (0.18)	0.00			
	A/G	39	1.51 (0.12)	-0.59 (-0.98 - -0.21)	0.0016	178.9	188.1
	A/A	6	1 (0)	-1.11 (-1.81 - -0.40)			
Dominant	G/G	28	2.11 (0.18)	0.00	0.001	179.1	186
	A/G-A/A	45	1.44 (0.1)	-0.66 (-1.04 - -0.28)			
Recessive	G/G-A/G	67	1.76 (0.11)	0.00	0.037	185.8	192.7
	A/A	6	1 (0)	-0.76 (-1.46 - -0.06)			
Overdominant	G/G-A/A	34	1.91 (0.17)	0.00	0.048	186.2	193.1
	A/G	39	1.51 (0.12)	-0.40 (-0.79 - -0.01)			
Log-additive	---	---	---	-0.57 (-0.87 - -0.27)	3e-04	176.9	183.8

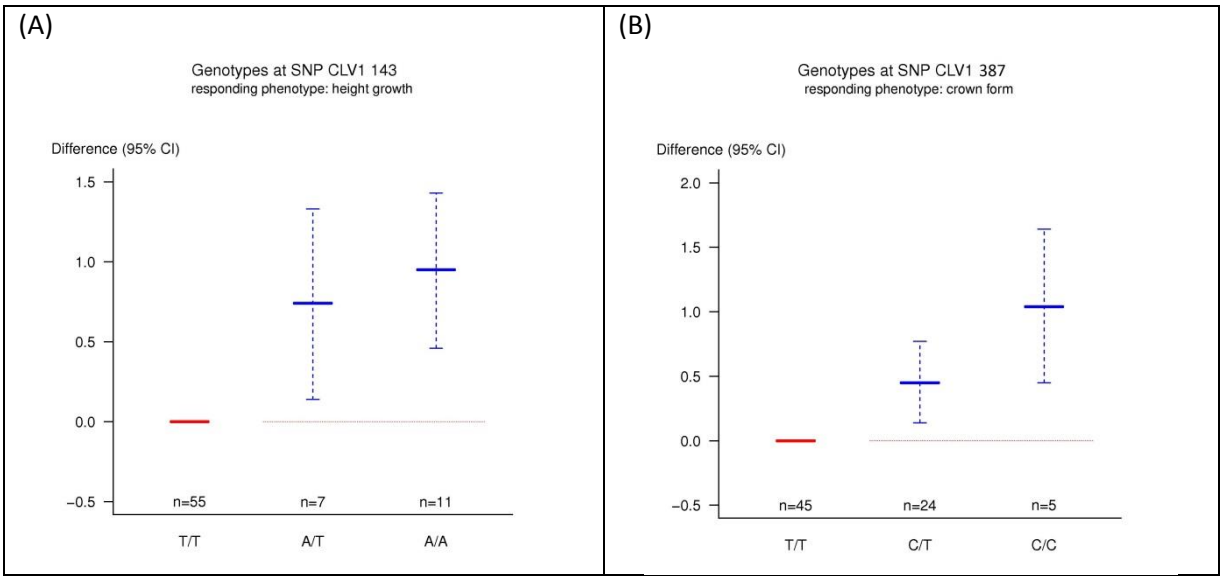

Fig. 2: Differences of the response means for height growth (A) and crown form (B) of the genotypes of two SNPs of the clavata1-gene by codominant mode of inheritance: (A) Response height growth with SNP 143, and (B) response crown form with SNP 387

Conclusion

With the information gained from resequencing candidate genes of phenotypically characterized cultivars it was possible to identify significant associations of SNPs with phenotypic characters of high breeding value. The SNP markers described will allow the genotyping of future breeding populations. With the use of genotyping platforms now available the genotyping for breeding purposes will become more attractive and cost efficient. Crucial to the success of genotyping will be next the underlying biological evidence also the robustness of SNP markers. This should be secured by clarifying the inheritance of each marker, using population studies respectively genetic epidemiology studies with different populations and progenies.

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