SNPs of the Clavata1-Gene exhibit associations with growth characteristics of willows (*Salix spp.*)

Karl Gebhardt[1], Marion Hoffmann[1], Birgit Ziegenhagen[2], Sascha Liepelt[2] Alwin Janßen[1]

[1]Nordwestdeutsche Forstliche Versuchsanstalt (NW-FVA), Hann. Münden
[2]Philipps-Universität Marburg, Marburg

Abstract

Like microsatellites that can be amplified in members of the different sections as well as in hybrids, SNPs of candidate genes may exist that can be detected in the genes of distantly related species and hybrids. Some of these SNPs can be associated with certain phenotypes and may be indicative of variation important for adaptation. The object of this study was to identify SNP markers that will explain a large proportion of the heritable variation of phenotypes and could be useful for marker assisted selection of willows. The SNP character of willows was determined by resequencing of the candidate gene CLAVATA1 (CLV1). Using the acquired position information of 12 SNPs and the KASP technology it was possible to genotype 30 cultivars of known growth character evaluated after 3 growing seasons in short rotation. Two SNPs of the CLV1 gene exhibited significant associations with fresh weight and the number of axillary shoots of willow cultivars.

Key words: CLAVATA1, SNP associations, willow cultivars, short rotation

Zusammenfassung

SNPs des Clavat1-Gens zeigen Assoziationen mit Wachstumsmerkmalen bei Weide (*Salix* spp.)

Wie Mikrosatelliten, die in Individuen unterschiedlicher Sektionen sowie in Hybriden amplifiziert werden können, existieren SNPs von Kandidatengenen auch in entfernt verwandten Arten und Hybriden. Einige dieser SNPs können mit bestimmten Phänotypen assoziiert und somit anpassungsrelevant sein. Das Ziel dieser Studie war es, SNP-Marker, die einen großen Anteil der vererbbaren Variation der Phänotypen erklären, zu identifizieren um sie künftig für markergestützte Selektion von Weiden-Kreuzungsprodukten nutzen zu können. Der SNP-Charakter der Weiden wurde durch Resequenzierung des Kandidatengens CLAVATA1 (CLV1) bestimmt. Mit der gewonnenen Positionsinformation konnte für 12 SNPs mit Hilfe der KASP-Technologie die Genotypisierung von 30 Sorten erfolgen deren Wuchseigenschaften im Kurzumtrieb nach drei Vegetationsperioden ermittelt wurden. Für zwei SNPs des CLV1-Gens konnten signifikante Assoziationen mit dem Frischgewicht und der Anzahl gebildeter Achselsprosse der Weidensorten nachgewiesen werden.

Schlüsselworte: CLAVATA1, SNP-Assoziationen, Weidensorten, Kurzumtrieb

Introduction

Like microsatellites that can be amplified in members of the different sections as well as in intra- and intersectional hybrids SNPs of candidate genes may exist that can be detected in the genes of distantly related species and hybrids. Some of these SNPs may be indicative of variation important for adaptation and can be associated with certain phenotypes. The object of this study was to identify SNP markers that will explain a large proportion of the heritable variation of phenotypes and could be useful for marker assisted selection of willows. A candidate gene approach including the

CLAVATA1 (CLV1) gene was chosen. As summarized by BUSH (2008) the CLV1-Gene has been postulated to either inhibit proliferation of undifferentiated cells at the meristem or promote the transition of these cells toward differentiation. By the effort of CLARK et al. (1997) it became obvious that CLV1 encodes a putative receptor kinase. When it was downregulated in *Arabidopsis*, a significant increase in secondary growth was observed.

Materials and Methods

Evaluations of the growth characters of 30 willow cultivars were made 3 years after the establishment of a field trial close to the breeding station in Hann. Münden (distance of planted cuttings: 0.5 x 1.5 m). The measurements of growth characters of regularly 24 ramets/clone (total: 720) included the number of axillary shoots/ramet, the height [m] and the fresh weight [kg] of the total shoot mass per ramet (Fig. 1). The SNP character of willows was determined by resequencing of the candidate gene CLAVATA1 (CLV1) described for poplars by Bush (2008). The data from Sanger sequencing (both directions) of the PCR product (915 bp) (Tab. 1), allowed the creation of a consensus sequence using the software CodonCode Aligner 3.7.1. Heterozygous alleles could be simply recognized as single nucleotide overlays. Providing the positional information of 12 SNPs to the company LGC Genomics (http://www.lgcgenomics.com/) the SNP genotyping of the cultivars was performed using the KASP technology. In order to analyze associations the internet based program SNPStats (Sole et al. 2006) and the R-Package: SNPassoc [R, version 2.14.1., (R development core team, 2011)] were used. This model compares genotypes heterozygous and homozygous for the variant allele to the genotypes homozygous for the most frequent allele with respect to different modes of inheritance.

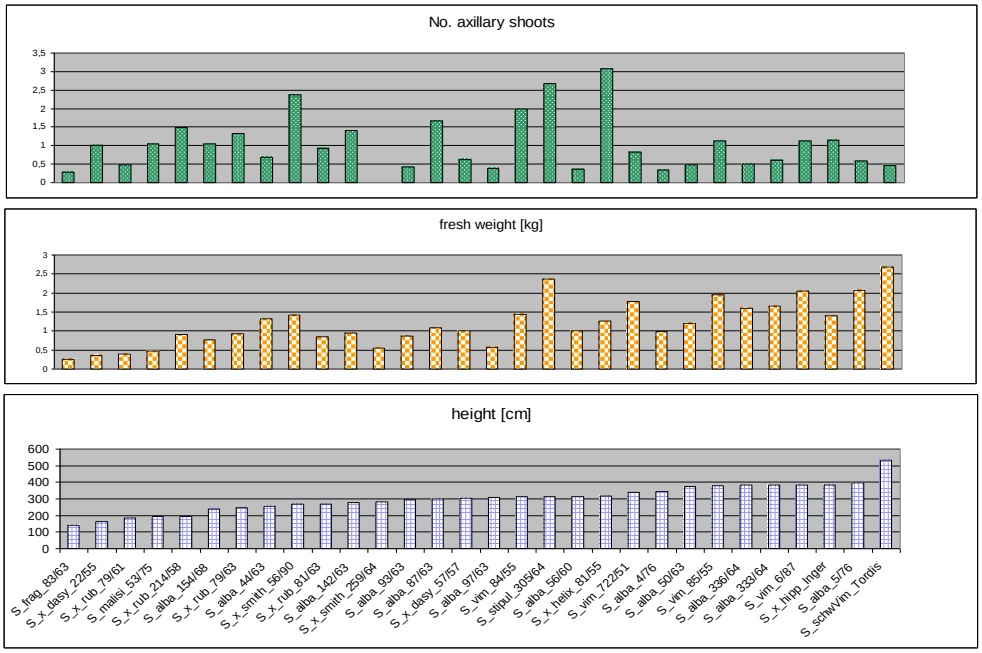

Fig. 1: Growth characters of 30 cultivars in a short rotation field test, age 3

Table 1: Nukleotide-Polymorphisms (SNPs) in CLV1 (P.trichocarpa: XM_002319628)

Gene / Identification	Function	Literature	Sequence	Coding		Noncoding		Total
				syn.	nonsyn.	5'UTR	Intron	
Clavata1 (CLV1), (AT1G75820)	Receptor kinase, (secundary) growth regulation	Bush (2008)	915 bp	9	10	2	0	21

Results and Discussion

A genetically diverse number of cloned cultivars (Fig. 1) differed significantly in no. of axillary shoots, freshweight and mean height growth after 3 vegetation periods in the field. About one third of the Gene CLV1 including the 5'-UTR-Region was successfully resequenced and exhibited 19 SNPs in the exon and two in the noncoding regions. The clustering of the SNPs as demonstrated in Figure 2 revealed a number of so called tag-SNPs representing significant allelic effects. In order to assess the association between the SNP polymorphisms and the quantitative growth characters we used the internet based program SNPStats which applies a chi-square test and gives an estimation of the odds ratio for each genotype with respect to the most frequent (reference) genotype and the expected mode of inheritance (5 modes are defined). Table 2 (association of SNP 300 of CLV1 with fresh weight (kg) per ramet) and Table 3 (association of SNP 147 of CLV1 with the number of axillary shoots per ramet) show the response means of the different genotypes with respect to the mode of inheritance, the calculated differences, p-values, the Akaike (AIC)-value and the Bayesian information criterion (BIC) which are lowest for the inheritance model that best fits the data. In addition the differences of the response means and their 95 % confidence intervals are viualized in Figure 3. The two significant associations demonstrated should be confirmed with a higher number of individuals and after short rotation has progressed further. The genotyping platforms which are nowadays available can make the use of SNP markers for breeding purposes more attractive and cost efficient.

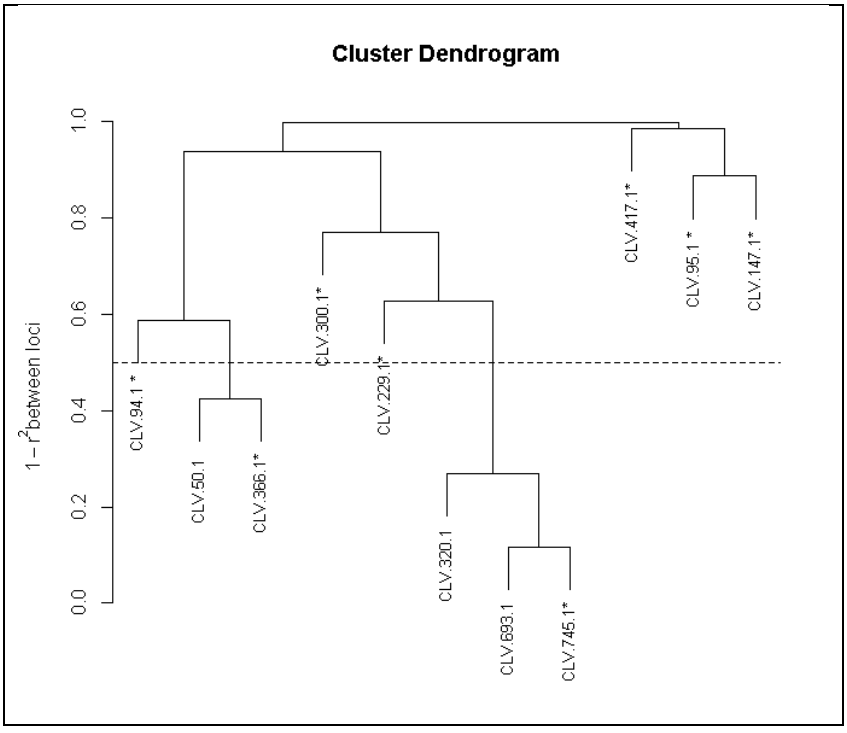

Fig. 2: Clustering of selected SNPs of the candidate gene CLAVATA1 (CLV1) from R-Package SNPClust; Tag-SNPs are marked with an asteriks (*)

Table 2: Association of SNP 300 of CLV1 with fresh weight (kg) per ramet

CLV.300.1 association with response fresh.weight..kg. (n=27, crude analysis)							
Model	**Genotype**	**n**	**Response mean (s.e.)**	**Difference (95% CI)**	**P-value**	**AIC**	**BIC**
Codominant	A/A	19	1 (0.11)	0.00			
	A/G	4	1.69 (0.33)	**0.70 (0.18 - 1.21)**	8e-04	41.5	46.7
	G/G	4	2.04 (0.12)	**1.04 (0.52 - 1.55)**			
Dominant	A/A	19	1 (0.11)	0.00	2e-04	40.6	44.5
	A/G-G/G	8	1.87 (0.18)	**0.87 (0.47 - 1.26)**			
Recessive	A/A-A/G	23	1.12 (0.12)	0.00	0.0039	46.4	50.3
	G/G	4	2.04 (0.12)	**0.92 (0.35 - 1.48)**			
Overdominant	A/A-G/G	23	1.18 (0.12)	0.00	0.13	53	56.9
	A/G	4	1.69 (0.33)	0.51 (-0.12 - 1.15)			
Log-additive	---	---	---	**0.55 (0.30 - 0.79)**	2e-04	40	43.8

Table 3: Association of SNP 147 of CLV1 with the number of axillary shoots per ramet

CLV.147.1 association with response No..axillary.shoots (n=28, crude analysis)							
Model	**Genotype**	**n**	**Response mean (s.e.)**	**Difference (95% CI)**	**P-value**	**AIC**	**BIC**
Codominant	G/G	25	0.83 (0.11)	0.00			
	G/C	1	2 (0)	**1.17 (0.12 - 2.23)**	<0.0001	48.6	53.9
	C/C	2	2.88 (0.21)	**2.05 (1.29 - 2.81)**			
Dominant	G/G	25	0.83 (0.11)	0.00	<0.0001	48.5	52.5
	G/C-C/C	3	2.58 (0.32)	**1.76 (1.12 - 2.40)**			
Recessive	G/G-G/C	26	0.87 (0.11)	0.00	1e-04	51.4	55.4
	C/C	2	2.88 (0.21)	**2.00 (1.19 - 2.82)**			
Overdominant	G/G-C/C	27	0.98 (0.15)	0.00	0.19	67.5	71.5
	G/C	1	2 (0)	1.02 (-0.48 - 2.53)			
Log-additive	---	---	---	**1.04 (0.68 - 1.40)**	<0.0001	46.7	50.6

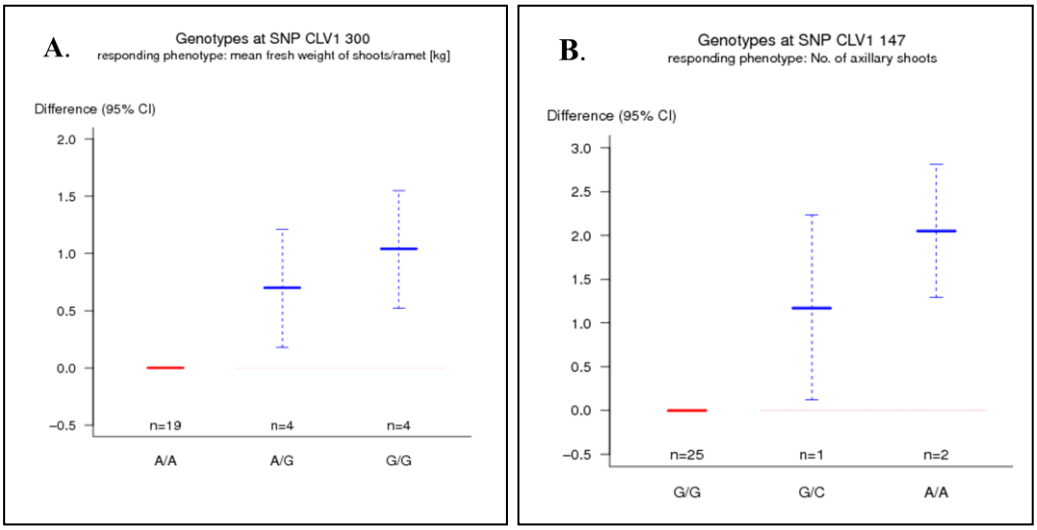

Fig. 3: Differences of the response means of fresh weight (A) and number of axillary shoots (B) for two SNP genotypes: (A) CLV1_300 fresh weight / ramet, and (B) CLV1_147 No. of axillary shoots / ramet

Acknowledgement

For technical assistance in the lab we are grateful to Mrs. Müller (NW-FVA). For advices on the statistical analysis we thank Dr. Egbert Schoenfelder. The underlying project of this report was

financially supported by the German Federal Minister of Food and Agriculture (FKZ: 22013709 PT-FNR). The authors take full responsibility for the content.

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Korrespondierender Autor.
Dr. Karl Gebhardt
Nordwestdeutsche Forstliche Versuchsanstalt (NW-FVA)
Abteilung Waldgenressourcen
Prof.-Oelkers-Str. 6
34346 Hann. Muenden
karl.gebhardt@nw-fva.de