Theor Appl Genet (2005) 110: 537–549
DOI 10.1007/s00122-004-1866-7

ORIGINAL PAPER

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QTL analyses of drought tolerance and growth for a *Salix dasyclados* × *Salix viminalis* hybrid in contrasting water regimes

Received: 7 January 2004 / Accepted: 28 October 2004 / Published online: 24 December 2004

Abstract Quantitative trait loci (QTL) for growth traits and water-use efficiency have been identified in two water regimes (normal and drought-treated) and for a treatment index. A tetraploid hybrid F_2 population originating from a cross between a *Salix dasyclados* clone (SW901290) and a *Salix viminalis* clone ('Jorunn') was used in the study. The growth response of each individual including both above and below ground dry-matter production (i.e. shoot length, shoot diameter, aboveground and root dry weight, internode length, root dry weight/total dry weight, relative growth rate and leaf nitrogen content) was analysed in a replicated block experiment with two water treatments. A composite interval mapping approach was used to estimate number of QTL, the magnitude of the QTL and their position on genetic linkage maps. QTL specific for each treatment and for the treatment index were found, but QTL common across the treatments and the treatment index were also detected. Each QTL explained from 8% to 29% of the phenotypic variation, depending on trait and treatment. Clusters of QTL for different traits were mapped close to each other at several linkage groups, indicating either a common genetic base or tightly linked QTL. Common QTL identified between treatments and treatment index in the complex trait dry weight can be useful tools in the breeding and selection for drought stress tolerance in *Salix*.

Communicated by D.B. Neale

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Introduction

Drought is a major limiting factor in agriculture and considered as the most important cause of yield reduction in crop plants (Boyer 1982). For biomass production of *Salix* grown in Sweden, water is identified as one of the most growth-limiting factors (Lindroth and Cienciala 1996). The possible interactions between drought-stressed trees and susceptibility to insect attacks (e.g. Mattson and Hack 1987, but see Koricheva et al. 1998; Huberty and Denno 2004) suggest the importance of selection of drought-tolerant plant material. Therefore, a selection of plant material with high water-use efficiency, which minimizes impacts of drought, should increase the long-term productivity, especially in areas with periodically low water availability and/or during years with low precipitation.

Breeding programmes with the aim of increasing biomass production by phenotypic screening and recurrent selection have been developed for *Salix dasyclados*, *Salix schwerinii* and *Salix viminalis* (Gullberg 1993; Åhman and Larsson 1994). An early selection of interesting genotypes would make the breeding process more efficient, especially in systems with long generation times such as forest trees. Identification of quantitative trait loci (QTL) on genetic linkage maps constructed from molecular markers will compliment classical breeding and allow early, indirect marker-aided selection of the phenotype in breeding programs (Tauer and Hallgren 1992). Different molecular markers such as AFLP, RFLP and microsatellites have been used for the construction of genetic linkage maps and for the study of the genetic architecture in many forest tree species (Bradshaw et al. 1994; Grattapaglia and Sederoff 1994; Paglia et al. 1998; Arcade et al. 2000; Cervera et al.

2001). In *S. viminalis* and the hybrids *S. viminalis* × *S. schwerinii* and *S. dasyclados* × *S. viminalis* linkage maps, using AFLP, RFLP and microsatellite markers have recently been developed (Hanley et al. 2002; Tsarouhas et al. 2002; Rönnberg-Wästljung et al. 2003).

Mapping QTL has been possible through the association between marker loci and the phenotypic variation found in the segregating population under study. QTL can be further studied in terms of the magnitude of their effects on the phenotype, the parental origins of the favourable QTL alleles and the relationships among QTL underlying different physiological processes. Reports on the inheritance of quantitative traits in interspecific hybrids of forest trees support the existence of QTL controlling large proportions of the total genetic variation (Bradshaw and Stettler 1995; Wu et al. 1997; Marques et al. 1999). QTL mapping in forest trees has been used for detecting regions of the genome controlling different growth traits (Kaya et al. 1999) as well as for regions controlling resistance traits (Li and Yeh 2002; Newcombe and Bradshaw 1996; Newcombe et al. 1996) and tolerance traits (Tauer and Hallgren 1992; Byrne et al. 1997a, Jermstad et al. 2001; Yazdani et al. 2003). QTL affecting growth traits, i.e. shoot height, shoot diameter and number of shoots, have also been mapped in *Salix* (Tsarouhas et al. 2002).

Elucidation of the genetic structure of growth and water-use efficiency traits (WUE) in *Salix* would help direct the breeding efforts for short-rotation biomass production. Plant responses to water stress have been studied by means of growth, nutrient and water economy of various *Salix* clones (e.g. Weih 2001; Weih and Nordh 2002). The aim of the present study is to identify and localize QTL for these traits, quantify the effect of the QTL and to evaluate common QTL in two contrasting treatments, i.e. well watered and drought-stressed. For this purpose we use a hybrid tetraploid F_2 population of *S. dasyclados* × *S. viminalis*, segregating for growth and water-use efficiency. We base our study on a previously developed genetic linkage map of *S. dasyclados* × *S. viminalis*, constructed with polymorphic AFLP markers segregating in the F_2 family studied (Rönnberg-Wästljung et al. 2003). The identification of genetic factors involved in plant responses to drought stress will provide important information for the breeding of highly drought-tolerant plant material.

Material and methods

Plant material

The genus *Salix* consists of approximately 300 species distributed in the northern temperate to the arctic regions, with only a few species found in the tropics (Larsson and Bremer 1991; Skvortsov 1968). *Salix* is a variable genus with species of small low shrubs and others with large trees over 10 m in height. The two species *S. viminalis* and *S. dasyclados* have high biomass production, are easy to propagate vegetatively and resprout from the stump after harvest, which has made them excellent candidates in short rotation energy production systems (Verwijst 2001).

The mapping pedigree was initiated in 1996 by crossing the female diploid *S. viminalis* (clone: 'Jorunn') to the male hexaploid *S. dasyclados* (clone: SW901290). Two randomly chosen individuals of the tetraploid F_1 progeny were crossed in 1998 to produce the F_2 mapping pedigree used in the present study. The grandparental clones were initially selected for their different response to frost, with the clone SW901290 being highly frost tolerant, while clone 'Jorunn' is frost sensitive. The *S. viminalis* clone 'Jorunn' is a result of breeding and selection for high productivity (Svalöv Weibull AB), while the clone SW901290 was collected in a natural population in Russia. Growth and other traits such as susceptibility to different insect and fungal pests have also been shown to segregate in the F_2 population. The grandparents, parents and F_2 pedigree were vegetatively propagated, and cuttings of each clone were used for the experiment.

Linkage-map construction

The linkage map is based on segregation data generated from an AFLP (Vos et al. 1995) analysis, which was performed as described by Tsarouhas et al. (2002). The linkage map is based on 92 F_2 progeny and reported by Rönnberg-Wästljung et al. (2003).

Construction of linkage maps on polyploids is more complicated than those done, for example, on diploids. In polyploids, a large number of genotypes are expected to be found in the segregating population, if the genome constitution is unknown (i.e. allo- or auto-polyploid), it is therefore difficult to determine the pattern of inheritance. One approach to avoid such difficulties in mapping polyploids is to use only those single-dose markers that segregate 1:1 (Wu et al. 1992). In this study, the hexaploid origin of the grandparental clone SW901290 is not known, but the high proportion of markers segregating as single-dose 1:1 or 3:1 in the tetraploid F_2 mapping-population indicates a bivalent pairing of chromosomes during meiosis (Rönnberg-Wästljung et al. 2003). For the QTL analysis in this study, the single-dose markers segregating 1:1 in the F_2 population were selected. Thus, two parental maps were constructed and used for the QTL analysis: a female map consisting of 147 markers divided into 20 major groups, eight triplets and seven doublets and a male map that consists of 168 markers distributed over 23 major groups, nine triplets and 13 doublets. The expected number of linkage groups in the two parental maps of this tetraploid hybrid is 38, since the haploid chromosome number in *Salix* is 19 (Larsson and Bremer 1991). The large number of small linkage groups indicates unmapped gaps on the *Salix* chromosomes, which is also indicated by the estimated genome coverage of 43% and 50% for the male and the female map, respectively (Rönnberg-Wästljung et al. 2003).

Greenhouse experiment

In the spring of 2001, a greenhouse experiment with two water regimes was conducted. All F_2 individuals were included in the experiment together with the grandparents and the F_1 parents, that is, in total 96 clones were grown in each of 25 blocks. Cutting material was used to replicate each clone ten times for each experimental treatment: ten blocks in the drought treatment and ten blocks in the well-watered treatment (1,920 plants in total in the final harvest). Five additional blocks were also planted for an initial harvest to assess plant biomass before irrigation treatments were implemented (480 plants in total in the initial harvest). The clones were randomly positioned within the blocks. The cuttings were planted in 1 litre plastic pots, the bottom dressed with a fibre cloth to prevent the roots from growing outside the pot. The soil consisted of 20% clay and 80% peat and contained 180 g N, 100 g P, and 210 g K per m^3, plus micronutrients. All 25 replicates were grown for 5 weeks in a greenhouse at ambient light (photoperiod >12 h) with no additional nutrients, but watered to field capacity of the substrate. After 5 weeks, the five blocks for the initial biomass estimates were harvested. At this time, ten blocks were assigned to the drought treatment, while the remaining ten blocks were maintained as the well-watered treatment. In the drought treatment the plants were kept just above the wilting stage and given as little water as possible without allowing for leaf necrosis. In each of the drought-treated and well-watered blocks, an extra pot with a plastic bag was randomly placed among the plants. After each watering application, the amount of water in the plastic bag was measured and an estimate of the amount of water given to the plants was achieved. At the end of the treatment period, the drought-treated plants had been supplied with approximately 55% of the water given to the well-watered plants. In both treatments each plant received a low level of fertilization at 6 mg N per week. After 3 weeks of growth, the nutrient supply was doubled by applying the same amount of nutrients twice a week to account for the increased nutrient demands of the plants. After 4 weeks of drought treatment, the final harvest started and pairs of two blocks, one from each treatment, were harvested successively during a period of 3 weeks.

Phenotypic measurements

At the harvests, both above- and belowground productions were measured. The aboveground production was separated in leaf and shoot dry-matter production fractions. In addition to the dry-matter production, the numbers of shoots on the plant, numbers of buds and leaves on the shoots were counted, and the height of each shoot and diameter at the base of each shoot was measured.

At the final harvest sylleptic shoots had appeared on the main shoots, which were counted and kept in a separate dry-matter fraction. Before the final harvest, three fully developed leaves at the top of the plant, from all individuals in six blocks (three from each treatment), were sampled for leaf area measurements and analysis of nitrogen content and stable carbon isotopes. Internode length was estimated by dividing the number of buds and leaves by shoot height, and root fraction was calculated as the ratio between root dry matter and total plant dry matter.

To estimate the C-isotope composition and the N concentration in leaf samples, the leaves were milled and analysed with an ANCA-MS (Europe Scientific, Crewe, UK) by Waikato Stable Isotope Unit, Hamilton, New Zealand. C-isotope ratio of leaf samples ($\delta^{13}C_{sample}$) is expressed as

$$\delta^{13}C_{sample} = (R_{sample}/R_{PDB} - 1) \times 1,000,$$

where R_{sample} and R_{PDB} are the $^{13}C/^{12}C$ molar abundance ratios of the leaf material and the PeeDee Belemnite standard (Craig 1957). The $\delta^{13}C$ signature has been shown to strongly correlate with the intrinsic WUE by means of the CO_2 assimilation per unit of water used (Farquhar and Richards 1984; Farquhar et al. 1989; Condon and Richards 1992).

Plant growth and biomass allocation were analysed using classical growth analysis (Weih and Nordh 2002), based on biomass changes between two consecutive harvests. Thus, relative growth rate for the whole plant, including roots (RGRp, g g^{-1} $week^{-1}$), was calculated as the biomass increment between the initial and final harvest per unit of time.

A treatment index was estimated for each trait and individual (i.e. clone) by dividing the mean value in the drought-stress treatment by the mean value in the well-watered treatment.

For this study, ten different traits were selected: start weight, shoot length (shl), shoot diameter (shd), aboveground dry weight (dwt), root dry weight (rdwt), leaf $\delta^{13}C$ ($\delta^{13}C$), internode length (node), RGRp, leaf N content (LNC) and root fraction (rfrac), i.e. rdwt divided by the sum of dwt and rdwt. The traits are presented for the well-watered treatment, the drought-stress treatment and for the treatment index.

Statistical analyses and QTL identification

Analysis of variance was performed with the GLM procedure in SAS (SAS Institute 1996), including treatments and blocks in the model according to the following equation:

$$Y_{ijkl} = \mu + T_i + B_{j(i)} + C_k + TC_{(ik)} + e_{ijkl} \quad (1)$$

where Y_{ijkl} is the phenotypic value for the kth clone in the jth block in the ith treatment, μ is the overall mean, T_i and $B_{j(i)}$ are the fixed effects of the ith treatment and the jth block, C_k is the random effect of the kth clone, $TC_{(ik)}$ is the random effect of the

interaction between the treatment and the clone and e_{ijkl} is the residual error.

The trait RGRp was analysed with model 1 by excluding the effect of block ($B_{j(i)}$), since the estimation of this trait is based on mean values over all blocks.

Analysis of variance was also performed for each experimental treatment separately using the same procedure as above. The variance components were estimated using the REML method in the VARCOMP procedure applying the statistical model:

$$Y_{ijk} = \mu + B_i + C_j + e_{ijk} \quad (2)$$

where Y_{ijk} is the phenotypic value for the jth clone in the ith block, μ is the overall mean, B_i is the fixed effect of the ith block, C_j is the random effect of the jth clone and e_{ijk} is the residual error.

Clone mean heritabilities were estimated for the traits in each experimental treatment with the formula:

$$H_c^2 = \frac{\sigma_c^2}{\sigma_c^2 + (\sigma_e^2/b_c)} \quad (3)$$

where b_c is the coefficient for $\sigma^2{}_c$ from the clone expected mean square in the RANDOM statement of PROC GLM.

Pairwise comparison among grandparents and F_1 parents were done for the traits in each treatment, using model 2 and a t-test option in the LSMEANS statement in SAS.

Pearson correlation coefficients were estimated among corresponding traits in the two water regimes and the treatment index using mean values for each clone (PROC CORR, SAS).

QTL analysis was performed by composite interval mapping (CIM) conducted with the program QTL Cartographer, version 1.15 (Basten et al. 2001), using mean values for each F_2 clone in each water treatment and for the treatment index of all traits. Traits showing a non-normal distribution were transformed and re-analysed (Table 1). For CIM, model 6 in the Zmapqtl program module was used for detecting QTL and their effects. The genome was scanned at 2-cM intervals, with a window size of 10 cM. Up to ten background markers were used as cofactors in the CIM analysis identified with the program module Srmapqtl (model 6). A series of 1,000 permutations were performed to determine the genome-wide significance level at $P < 0.05$ for each trait and linkage map. To declare a putative QTL, the threshold was set to a genome-wide significance level of 0.05. The significances are presented as LOD values, where LOD is the logarithm of the odds ratio. Additive effects of the detected QTL were estimated by using the Zmapqtl program. The proportion of phenotypic variation explained by each significant marker was estimated as the coefficient of determination (R^2) at the peak QTL position estimated by QTL Cartographer. Separate QTL analyses were carried out for each parent under the backcross model (Grattapaglia and Sederoff 1994).

Table 1 Transformation of data not showing normal frequency distribution in well-watered (*w*), drought-stress (*d*) treatments and treatment index (*d*/*w*)

Trait[a]	Treatment/index	Normality (yes/no)	Transformation[b]
start weight	w	Yes	–
shl	w	Yes	–
	d	No	Log
	d/w	No	Log
shd	w	No	–
	d	No	–
	d/w	No	–
dwt	w	Yes	–
	d	Yes	–
	d/w	No	–
rdwt	w	Yes	–
	d	No	Log
	d/w	No	Log
δ^{13}C	w	Yes	–
	d	Yes	–
	d/w	Yes	–
node	w	Yes	–
	d	Yes	–
	d/w	Yes	–
rfrac	w	Yes	–
	d	Yes	–
	d/w	No	Log
RGRp	w	No	Cosine
	d	No	Log
	d/w	No	–
LNC	w	Yes	–
	d	Yes	–
	d/w	Yes	–

[a] *shl* shoot length, *shd* shoot diameter, *dwt* aboveground dry weight, *rdwt* root dry weight, $\delta^{13}C$ leaf δ^{13}C, internode length *node*, *rfrac* rootdwt/total dry weight, *RGRp* relative growth rate for whole plant (roots included), *LNC* leaf nitrogen content

[b] *Dash* indicates when no transformation was needed or when no transformation improved the distribution of the data

Results

Phenotypic variation

All traits showed a large phenotypic variation in the F_2 population (model 1; clone effect for RGRp: $P < 0.05$, all other traits: $P < 0.001$, Table 2; Fig. 1). The frequency distributions for some of the traits are presented in Fig. 1. Mean values for grandparents, parents and F_2 as well as clonal mean broad-sense heritabilities are reported in Table 2. The differences between the grandparents and the parents in the well-watered and drought treatments were tested (Table 2). The *S. dasyclados* grandparent showed significantly lower height growth and total dwt compared with the *S. viminalis* grandparent, independent of the water treatment. However, the *S. dasyclados* grandparent showed a larger node, especially in the drought stress and a higher rfrac in the well-watered treatment as compared with *S. viminalis*. In the well-watered environment, the δ^{13}C-isotope signature was significantly lower, i.e. more negative for the *S. dasyclados* grandparent compared to the *S. viminalis*

Table 2 Mean values for phenological traits recorded on grandparents ('Jorunn', SW901290), F1 parents (SW950182, SW950185) and F_2 population as well as broad-sense heritabilities (H^2_c) in w and d treatments, as well as the corresponding d/w

Trait	Treatment/ index	'Jorunn' (different from 901290)[a]	SW901290	SW950182 (different from 950185)[a]	SW950185	F_2 (std)	H^2_c
start weight (g)	w	3.1(*)	1. 8	2.0 (ns)	1.8	3.8 (0.64)	0.82
shl (cm)	w	131.2(***)	60.9	86.1(*)	108.3	78.2 (19.9)	0.91
	d	101.6(***)	53.3	54.6(***)	80.5	59.6 (11.3)	0.89
	d/w	0.77	0.87	0.63	0.74	0.78 (.13)	–
shd (mm)	w	6.61 (ns)	6.6	6.6 (ns)	7.1	6.4 (.82)	0.88
	d	5.98 (ns)	6.3	5.9(*)	6.7	6.1 (.56)	0.85
	d/w	0.90	0.96	0.90	0.95	.96 (.08)	-
dwt (g)	w	14.08(**)	8.5	11.4 (ns)	11.1	9.0 (2.7)	0.98
	d	9.83(*)	6.4	6.7 (ns)	7.9	6.7 (1.3)	0.85
	d/w	0.70	0.76	0.59	0.71	0.79 (.20)	–
rdwt (g)	w	3.82 (ns)	3.5	4.6(*)	2.3	2.7 (1.0)	0.83
	d	2.31 (ns)	2.3	2.4 (ns)	2.3	2.2 (.72)	0.74
	d/w	0.60	0.66	0.52	1.0	0.88 (.27)	–
$\delta^{13}C$	w	−28.20(*)	−30.13	−29.39 (ns)	−28.22	−29.19 (.95)	0.77
	d	−26.44 (ns)	−26.63	−27.80 (ns)	−25.97	−27.18 (1.2)	0.66
	d/w	0.94	0.88	0.94	0.92	0.93 (.02)	–
node (mm)	w	12.5 (ns)	12.8	12.7 (ns)	12.6	12.0 (1.8)	0.91
	d	10.0(**)	13.1	11.3 (ns)	11.2	10.9 (1.5)	0.87
	d/w	0.80	1.02	0.89	0.89	0.92 (.08)	–
rootfrac	w	0.20(*)	0.31	0.29(**)	0.17	0.23 (.05)	0.73
	d	0.20(**)	0.25	0.26 (ns)	0.22	0.24 (.05)	0.77
	d/w	1.02	0.82	0.88	1.26	1.06 (.19)	–
LNC (%)	w	2.49 (ns)	1.99	1.91(*)	2.24	2.28 (.37)	0.66
	d	2.56 (ns)	2.95	2.48 (ns)	2.98	2.62 (.31)	0.34
	d/w	1.03	1.48	1.30	1.33	1.16 (.17)	–
RGRp (g g^{-1} $week^{-1}$)	w	0.31	0.33	0.36	0.36	0.32 (.05)	–
	d	0.24	0.27	0.24	0.31	0.28 (.05)	–
	d/w	0.77	0.82	0.67	0.86	0.86 (.14)	–

[a]Test of difference (*t*-test) between grandparents and parents: ***$P < 0.001$, **$0.001 < P < 0.01$, *$0.01 < P < 0.05$
ns Non-significant

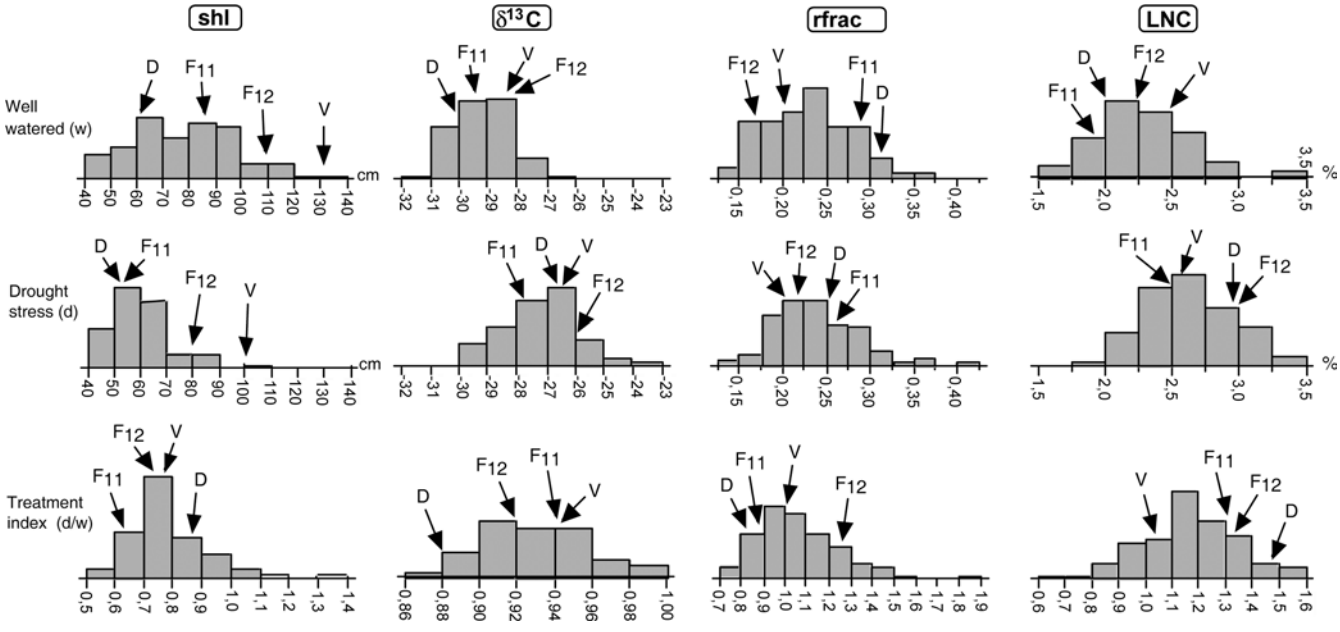


Fig. 1 Frequency distributions of phenological traits of *Salix* grown in different treatments and for treatment indices. Mean values for parents and grandparents are indicated. *S. dasyclados* grandparent (*D*) , *S. viminalis* grandparent (*V*), SW950182 parent (F_{11}), SW950185 parent (F_{12})

grandparent, indicating a lower WUE (e.g. Farquhar and Richards 1984). No heterosis effects in the F_1 parents compared to the grandparents were observed (Fig. 1; Table 2). For all traits the drought treatment showed significant effects (rfrac: $P < 0.01$, all other traits: $P < 0.001$) by means of less growth, higher LNC, higher

Table 3 Quantitative trait loci (QTL) detected for growth and water-use efficiency ($\delta^{13}C$) traits in two different water regimes and for treatment indices

Trait	Chrom.	AFLP marker to the left of LOD peak (origin D/V)[a]	w treatment				d treatment				d/w			
			Position (cM)	Add. effect	LOD[b]	R^2	Position (cM)	Add. effect	LOD[b]	R^2	Position (cM)	Add. effect	LOD[b]	R^2
start weight	k	F3113 (D)	3.3	−0.48	2.9	0.13								
	Triplet	M3510 (V)	6.0	0.53	3.7	0.13								
shl0,04	a	F5715 (D)	49.3	15.2	5.2	0.12	49.3	7.6	4.0 (3.3)	0.10				
	i	F7112 (D)	31.8	−14.6	5.0	0.12	21.8	−8.0	4.6 (3.0)	0.11				
	i	F204 (D)									40.5	0.12	5.6 (8.3)	0.20
	o	F379 (D)	60.3	−14.4	3.9	0.08								
	t	F248 (D)	41.2	−14.7	4.3	0.12								
	Doublet	F5012 (D)									0	−0.1	3.7 (4.1)	0.12
	I	M247 (D)	0	−15.0	3.8	0.12					2.0	0.12	4.6 (4.4)	0.17
	D	M911 (D)					0	13.4	4.9 (7.0)	0.13				
	G	M5510 (D)									0	−0.1	2.8 (3.0)	0.09
	P	M622 (D)					62.3	8.4	3.8 (2.0)	0.1				
	P	M5810 (D)									19.6	0.08	3.0 (3.7)	0.12
	Y	M292 (D)									16.0	−0.9	3.2 (3.3)	0.11
shd	o	F486 (D)	49.5	−0.53	3.4	0.11								
	o	F453 (D)									0	0.07	4.3	0.15
	III	M3012 (D)					8.0	0.45	4.5	0.13				
	B	M711 (D)					0	0.45	5.4	0.14				
	K	M493 (V)									18.6	0.07	3.8	0.12
	K	M725 (V)	29.9	−0.77	5.0	0.16	29.9	−0.49	5.2	0.14				
	N	M566 (D)	39.8	−0.74	5.2	0.16					41.8	0.08	5.3	0.19
	R	M607 (D)					31.0	0.36	3.5	0.09				
	Triplet	M557 (D)					7.0	−0.35	3.2	0.08				
dwt	II	F1010 (D)									11.0	0.14	3.9	0.11
	IV	F275 (D)									7.4	−0.13	3.4	0.10
	VII	F426 (D)									0	−0.15	3.5	0.10
	i	F7112 (D)					21.8	−0.10	4.3	0.13				
	i	F204 (D)	40.5	−2.0	4.3	0.13					38.5	0.13	3.2	0.09
	o	F453 (D)	12.0	−2.0	3.8	0.13					0	0.12	3.2	0.09
	o	F209 (D)					32.3	−0.91	3.9	0.12				
	r	F279 (D)									0	0.12	3.0	0.08
	t	F248 (D)	41.2	−2.2	3.6	0.10	41.2	−1.5	5.9	0.22				
	III	M1417 (D)					18.5	0.93	3.3	0.12				
	F	M4515 (D)					0	1.02	5.1	0.14				
	K	M725 (V)	29.9	−2.13	3.9	0.12	31.9	−0.96	3.6	0.11				
	Y	M292 (D)	16.0	1.76	3.1	0.10								
	Doublet	M397 (D)									0	0.20	4.8	0.18
	Doublet	M338 (V)					4.0	−0.87	3.6	0.10				
rdwt	n	F281 (D)	8.0	1.06	4.0	0.24								
	E	M1912 (D)									0	0.21	3.8 (2.2)	0.12
	Y	M292 (D)									16.0	−0.19	2.9 (4.8)	0.09
	Doublet	M397 (D)									0	0.22	5.5 (2.9)	0.12
	Triplet	M611 (D)									3.0	0.21	4.5 (3.0)	0.11
$\delta^{13}C$	III	F184 (D)	5.3	0.83	6.6	0.17	3.3	0.96	4.5	0.13				
	IV	F275 (D)					7.4	0.85	3.6	0.10				
	i	F7112 (D)	21.8	−1.29	7.3	0.18								
	n	F281 (D)									0	−0.02	3.7	0.11

	p	F185 (D)									32.7	0.02	3.5	0.11
	t	F248 (D)	41.2	−0.62	4.0	0.09								
	Y	M292 (D)	16.0	0.78	4.1	0.15								
	Doublet	M1412 (D)									28.0	−0.02	3.0	0.11
node	a	F5715 (D)	49.3	0.15	5.9	0.14	49.3	0.12	6.3	0.15				
	b	F686 (D)	33.8	0.11	4.3	0.09								
	d	F754 (D)	2.0	−0.10	3.7	0.08								
	i	F204 (D)					36.5	0.09	3.7	0.08	40.5	0.06	3.5	0.12
	m	F266 (V)	17.0	0.15	6.2	0.17								
	o	F379 (D)	64.3	−0.20	9.1	0.22	64.3	−0.10	5.2	0.12				
	B	M489 (D)									35.2	−0.06	3.9	0.15
rtfrac	n	F281 (D)	12.0	0.05	3.2	0.21								
	C	M1517 (V)					19.8	0.03	3.4	0.10				
	O	M653 (D)	26.0	−0.03	3.3	0.09								
	Doublet	M4212 (D)	0	−0.09	6.4	0.18	0	−0.10	5.1	0.14				
	Triplet	M3510 (V)	12.0	0.08	4.3	0.14	0	0.14	5.9	0.17				
RGRp	IV	F275 (D)									7.4	−0.11	4.0	0.12
	VI	F1420 (D)					22.4	0.03	3.5 (2.4)	0.09				
	VII	F426 (D)									0	−0.12	4.2	0.12
	i	F204 (D)									38.5	0.10	3.6	0.11
	k	F261 (D)					0	0.03	3.8 (-)	0.1				
	m	F266 (V)					11.0	0.04	4.6 (-)	0.14				
	o	F453 (D)					18.0	−0.04	5.2 (-)	0.09	0	0.13	5.8	0.18
	o	F379 (D)					64.3	0.04	3.2 (-)	0.12				
	q	F213 (V)					0	−0.04	4.0 (-)	0.13				
	IV	M1512 (D)									28.0	0.09	3.0	0.09
	J	M5018 (D)	43.3	0.04	3.1 (-)	0.11								
	Y	M292 (D)									16.0	−0.10	3.5	0.11
	Doublet	M397 (D)									2.0	0.12	5.3	0.17
	Triplet	M367 (D)									0	−0.11	3.9	0.12
	Triplet	M203 (D)	0	−0.04	4.3 (3.0)	0.15								
LNC	t	F2510 (D)	38.9	0.44	6.1	0.29								
	t	F248 (D)									41.2	−0.12	3.1	0.12
	B	M489 (D)	23.2	−0.23	3.4	0.1								
	O	M588 (D)	0	−0.28	5.0	0.13								
	Doublet	M338 (V)	4.0	0.22	3.1	0.08								
	Triplet[c]	M203 (D)	0	0.28	4.6	0.11								
	Triplet[c]	M557 (D)					13.0	0.25	4.5	0.14				
	Triplet	M249 (D)					29.5	0.27	3.5	0.11				

[a]Origin of marker: *Salix dasyclados* (*D*), *S. viminalis* (*V*)
[b]LOD values for transformed data are within *parentheses*, (–) not detected with transformed data
[c]Same triplet

Fig. 2 QTL for *Salix* found in the male map (**a**) and female map (**b**). Markers are indicated to the *left of the linkage groups*; see also Rönnberg-Wästljung et al. (2003) for a thorough map description. *shl* shoot length, *shd* shoot diameter, *dwt* aboveground dry weight, *rdwt* root dry weight, $\delta^{13}C$ leaf δ^{13}C, *node* internode length, *rfrac* rootdwt/total dry weight, *RGRp* relative growth rate for whole plant (roots included), *LNC* leaf nitrogen content

rfrac and a less negative δ^{13}C value. All traits except rfrac, RGRp, δ^{13}C and LNC also showed a significant genotype × treatment interaction effect ($P < 0.001$), indicating a difference among the F_2 clones in the responses to drought. For all traits except for δ^{13}C, there were F_2 clones with values ≥1 for the treatment index, which shows that some clones had a higher performance or performed similarly in the drought-stress treatment compared with the well-watered treatment (see examples in Fig. 1). In general the clones with a treatment index above 1 showed a low performance in the well-watered treatment in relation to the mean of all F_2 clones.

QTL identification

One to eight QTL were found for each trait, except for rdwt, under drought stress and for the treatment index of rfrac, where no QTL were found. Similar numbers of QTL were found in each of the two treatments and for the treatment index (Table 3). QTL identification differed between transformed and untransformed data only for the trait RGRp, indicating a possible false detection of QTL in this case (Table 3). LOD values for the detected QTL varied between 2.9 and 9.1 (Table 3). The QTL explained from 8% to 29% of the total phenotypic variation (Table 3). Most of the putative QTL alleles (84%) originated from the *S. dasyclados* grandparent (Table 3). Overlapping QTL were found for different traits, especially in the groups K and Y (male map) and i, o and t (female map); we interpret this to indicate a clustering of QTL (Fig. 2).

Consistency of QTL across different treatments

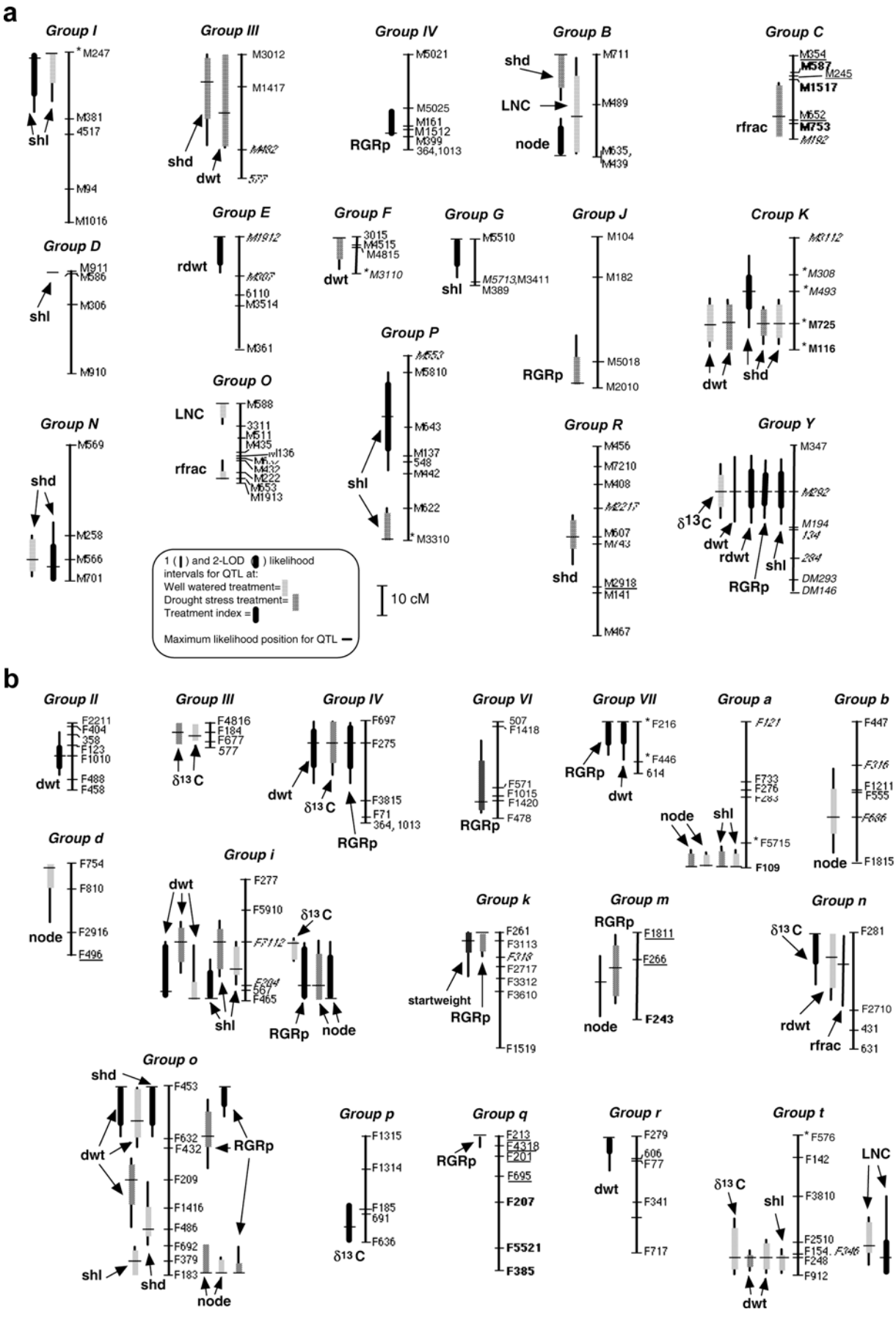
Twenty-one percent of the QTL identified were common for both treatments (Tables 3, 4). The number of common QTL varied among traits from zero (rdwt, RGRp) to 50% for dwt (Tables 3, 4). For dwt, four common QTL were found for the drought and well-watered treatments (Tables 3, 4; Fig. 2). For three of the traits (shl, shd and dwt) QTL in common for both treatments and treatment index were found (Table 3). Shl had a putative common QTL in group i (female map) for the two treatments and the treatment index. One locus was closest to the LOD peak for shl in the two different treatments, while another locus on the same linkage group was closest to the LOD peak for the treatment index, but the 2-LOD significance intervals overlapped between the two loci (Fig. 2). For the trait dwt the same QTL as for shl (in group i), and a similar overlap among the intervals of the loci was found for the two treatments and the treatment index (Table 3; Fig. 1). For the trait shd a QTL in common between the two treatments and the treatment index was found in group K (male map) (Table 3; Fig. 2). The LOD peak for the treatment index differed from the LOD peak for shd in the two treatments, but also in this case the 2-LOD significance interval overlapped (Fig. 2). For the traits node, RGRp and LNC common QTL between one of the treatments and the treatment index were found (Table 4).

Discussion

Phenotypic growth responses of the plants in this study were similar to those found in other studies using *Salix* (e.g. Weih and Nordh 2002). Thus, reduced water availability affected biomass accumulation and allocation, LNC and intrinsic WUE by means of δ^{13}C. In addition, the treatment responses were in many cases affected by genotype (i.e. significant genotype × treatment interactions), suggesting that the growth performance of a given genotype in relation to other genotypes may vary among plantation sites (e.g. Weih and Nordh 2002; Rönnberg-Wästljung et al. 1994).

QTL identification

In this study, putative QTL for different growth components in *Salix*, including both above- and belowground biomass production and WUE (measured as δ^{13}C), have been discovered in two diverging water treatments and for a treatment index. Many QTL studies have been conducted to identify QTL related to drought and WUE in crop species, as, for example, in maize, soybean, rice and barley (Sari-Gorla et al. 1999; Specht et al. 2001; Teulat et al. 2001; Price et al. 2002), while similar studies in forest trees are sparse (Brendel et al. 2002). Quantitative traits such as height and diameter have been assumed to be under polygenic control where each gene contributes with a small and additive effect (Falconer 1989). Most of the QTL found in this study for the different growth traits each explained around 12% of the phenotypic variation, with a few exceptions explaining more than 20% of the variation (dwt, rdwt, LNC). This is in agreement with another recent study in *Salix*, where QTL associated with height and diameter growth in a field experiment explained between 14% and 22% of the phenotypic variation (Tsarouhas et al. 2002). Other QTL studies in forest trees also show major effects of single QTL for growth-related traits (Bradshaw and Stettler 1995; Byrne et al. 1997b, Wu et al. 1997; Kaya et al. 1999). In an F_2 population based on an interspecific cross in *Populus*, effects of single QTL for growth traits explained 24–33% of the phenotypic variation (Bradshaw and Stettler


a
Group I
Group III
Group IV
Group B
Group C
Group D
Group E
Group F
Group G
Group J
Croup K
Group N
Group O
Group P
Group R
Group Y
1 ( ) and 2-LOD ( ) likelihood intervals for QTL at:
Well watered treatment=
Drought stress treatment=
Treatment index =
Maximum likelihood position for QTL
10 cM
b
Group II
Group III
Group IV
Group VI
Group VII
Group a
Group b
Group d
Group i
Group k
Group m
Group n
Group o
Group p
Group q
Group r
Group t

1995). Furthermore, in a cross between two *Eucalyptus* species, one to six QTL explained 8–42% of the phenotypic variation in traits connected to vegetative propagation (Grattapaglia et al. 1995). An overestimation of the QTL effects in interspecific crosses, due to high linkage disequilibrium, could be one explanation for the relatively high major effects (Grattapaglia et al. 1995). In this study, however, on average three quarters of the genome in the F_2 population has a *S. dasyclados* origin due to the hexaploidy of the *S. dasyclados* grandparent, indicating that many of the QTL effects are estimated within species. A high heterozygosity in the undomesticated *S. dasyclados* grandparent apparently generated sufficient variation in the F_2 population to detect QTL. The relatively low number of individuals in this population negatively influences the accuracy of the calculated QTL effects and the power to detect QTL with small effects (Beavis 1994). This was, to some degree, compensated by the higher precision of the phenotypic screening due to vegetative propagation of each individual and a large number of replications. High-to-medium clonal broad-sense heritabilities (H^2) found in this F_2 population also facilitate the identification of QTL (Beavis 1994).

Table 4 Number of QTL in common among w, d treatments and d/w for each trait (*above diagonal*) and phenotypic correlation among traits (*below diagonal*)

Trait		Treatment		
		w	d	d/w
shl	w	**5**	2	2
	d	.85***	**4**	1
	d/w	−.70***	−.25*	**6**
shd	w	**3**	1	2
	d	.94***	**5**	1
	d/w	−.74***	−.28**	**3**
dwt	w	**5**	4	2
	d	.88***	**7**	2
	d/w	−.74***	−.42***	**7**
rdwt	w	**1**	0	0
	d	.68***	**0**	0
	d/w	−.62***	.08ns	**4**
δ^{13}C	w	**4**	1	0
	d	.76***	**2**	0
	d/w	−.06ns	−.69***	**3**
node	w	**5**	2	0
	d	.80***	**3**	1
	d/w	−.44***	.18ns	**2**
rfrac	w	**4**	2	0
	d	.60***	**3**	0
	d/w	−.35***	.49***	**0**
RGRp	w	**2**	0	0
	d	.66***	**6**	1
	d/w	−.45***	.33**	**8**
LNC	w	**5**	1	1
	d	.40***	**2**	0
	d/w	−.71***	.33**	**1**

Untransformed data are used. Numbers in the *diagonal* (in *boldface*) represent number of QTL detected for each trait in each treatment or for the index

Significance levels: *$0.01 < P < 0.05$, **$0.001 < P < 0.01$, ***$P < 0.001$, *ns* Non-significant

The number of QTL detected for the different traits in the present study might have been underestimated due to incomplete genome coverage of the linkage maps for the tetraploid F_1 parents (Rönnberg-Wästljung et al. 2003). In addition, due to the tetraploidy in the F_1 parents, some of the QTL identified could be alleles of the same QTL but detected on homologous linkage groups. The dominant marker system and the use of markers segregating 1:1 in this study give no possibilities to identify homologous linkage groups. Fully informative markers (i.e. microsatellites) used for future QTL studies in this population will further improve the knowledge of inheritance of growth traits.

The use of single-dose markers segregating 1:1 in this F_2 population restricts the estimates of the quantitative effects of the marker alleles to additivity, since intralocus interactions (e.g. dominance) cannot be accounted for (Grattapaglia et al. 1995). Quantitative genetic studies in *Salix* have proved dominance and epistatic effects in *S. viminalis* for different growth traits (Rönnberg-Wästljung et al. 1994; Rönnberg-Wästljung 2001), and even though there is little evidence for heterosis in this population (which might result from dominance effects), it is probable to assume that such effects exist in this population. In QTL studies using co-dominant markers in *Populus*, dominance and over-dominance have been shown for leaf traits (Wu et al. 1997), for adventitious root and shoot regeneration (Han et al. 1994), as well as for growth, form and phenological traits (Bradshaw et al. 1994; Wu 1998).

In several cases in the present study, one QTL was found to be associated with different traits. Clusters of QTL influencing, for example, dwt, RGRp, δ^{13}C as well as shl and node were found. This probable pleiotropic effect of QTL indicates a common genetic base for these traits, even though this explanation could not be separated from the possibility that two tightly linked loci control several traits.

Consistency of QTL across treatments

In this study, common QTL showing significant effects in both the well-watered and drought treatments as well as QTL specific for one of the treatments were detected. Similar patterns, with both common and specific QTL in different water regimes, have been reported in crop species such as pearl millet (Yadav et al. 2002) and barley (Teulat et al. 2001). In maize (Frova et al. 1999), more than 50% of the QTL were found to be common in different water regimes. In the present study the number of common QTL varies among traits. For most of the traits where common QTL were identified, the effect of the QTL (R^2 value) was similar in the different treatments. One exception was the trait dwt, where 50% of the QTL were in common between the two treatments but one common QTL showed more than twice the effect in the drought-stress treatment compared to well-watered treatment. This suggests a differential control of

the expression of dwt in the well-watered versus drought-stress treatments.

Inconsistent effects of QTL over environments can explain the genetic component of a significant clone × treatment interaction (Beavis and Keim 1996). In this study, support for this suggestion came from rdwt, which was the only trait with significant clone × treatment interaction and no common QTL over treatments. An inconsistency in QTL effect as described above for the trait dwt, (drought treatment: $R^2 = 0.22$; well-watered treatment: $R^2 = 0.10$) might explain the significant clone × treatment interaction found for dwt. Other traits with significant clone × treatment interactions had common QTL with similar effects between treatments. Unidentified and/or identified QTL, specific for each treatment, probably have a larger impact on the trait as compared with the QTL in common over treatments, thus explaining the significant clone × treatment interaction. Another possible explanation might be that the estimate of the interaction is based on phenotypic values, composed of different genetic effects (additive, dominance and epistasis) and environmental effects, while the common QTL represent putative genes with additive effects for the trait at the two treatments. If effects other than additive are of main importance for the interaction, then the situation with significant interaction between clone and treatment together with common QTL with similar effects might occur.

For the traits shl, shd and dwt, common QTL existed for both treatments and for the treatment index, showing the reproducibility in identifying QTL across treatments and for drought tolerance. The treatment index in the present study on willow is interpreted to be a measure of drought tolerance. However, QTL in common between one of the treatments and the treatment index as well as specific QTL for the treatment index were also found. Percent QTL specific for treatment indices varied from 12% in the trait shd to 80% in rdwt, where almost all QTL identified were detected for the index. This suggests a complex picture of the genetics behind the responses to drought and to drought tolerance in *Salix*. Frova et al. (1999) as well as Sari-Gorla et al. (1999) suggested different genetic control of drought tolerance in maize compared to yield traits in various water regimes, since they found stable QTL across water regimes for yield components but specific and different QTL for drought tolerance.

Breeding implications

To be able to utilize molecular markers successfully in a breeding programme, the markers should be consistently found in various environments and show a large effect on the trait. High heritability of the trait as well as markers closely linked to the target gene will increase the possibilities for favourable selection (Quarrie 1996). In this study, QTL with significant effects on growth and WUE traits have been identified and mapped in different water regimes and for drought stress tolerance for the hybrid *S. dasyclados* × *S. viminalis*. QTL specific for each treatment and for the treatment index were found, but also QTL in common across treatments and in the treatment index were identified. Clusters of QTL for different traits were mapped at several linkage groups. Markers close to QTL common in different environments and to QTL with pleiotropic effects are good candidates for marker-assisted selection (Quarrie 1996). It appears from this study that markers linked to the large number of common QTL between treatments and treatment index identified for the complex trait dwt might be useful tools in the selection for drought-stress tolerance in *Salix*. Since this study is the first to our knowledge to identify QTL in a water-stress treatment and for drought tolerance in *Salix*, the QTL need to be verified in different genetic backgrounds. A larger mapping population would also increase the precision of the QTL mapping procedure.

Acknowledgements Technical assistance in the greenhouse with water treatments and harvest of plants by Eva-Marie Fryk, Barbro Johansson, Kristin-Sophie Mellsjö, Urban Pettersson, Christina Segerqvist and Ewa Winkler is highly appreciated. This study was supported by a grant from the Swedish National Energy Administration (project no. P13028-1).

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