

# QTL for yield in bioenergy *Populus*: identifying G×E interactions from growth at three contrasting sites

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**Abstract** *Populus* is a genus of fast growing trees that may be suitable as a bioenergy crop grown in short rotation, but understanding the genetic nature of yield and genotype interactions with the environment is critical in developing new high-yield genotypes for wide-scale planting. In the present study, 210 genotypes from an F<sub>2</sub> population (Family 331; POP1) derived from a cross between *Populus trichocarpa* 93-968 and *P. deltoides* ILL-129 were grown in southern UK, central France and northern Italy. The performance of POP1, based upon first- and second-year main stem traits and biomass production, improved from northern to southern Europe. Trees at the Italian site produced the highest mean biomass ranging from 0.77 to 18.06 oven-dried tonnes (ODT) ha<sup>-1</sup> year<sup>-1</sup>, and the UK

site produced the lowest mean biomass ranging from 0.18 to 10.31 ODT ha<sup>-1</sup> year<sup>-1</sup>. Significant genotype×environment interactions were seen despite heritability values across sites being moderate to high. Using a pseudo-testcross analysis, 37 quantitative trait loci (QTL) were identified for the maternal parent and 45 for the paternal parent for eight stem and biomass traits across the three sites. High genetic correlations between traits suggested that collocating QTL could be inferred as a single pleiotropic QTL, reducing the number of unique QTL to 23 and 24 for the maternal and paternal parent, respectively. Additive genetic effects were seen to differ significantly for eight QTL on the maternal map and 20 on the paternal map across sites. An additive main effects and multiplicative interaction analysis was carried out to obtain stability parameters for each trait. These parameters were mapped as QTL, and collocation to trait QTL was accessed. Two of the eight stability QTL collocate to trait QTL on the maternal map, and 8 of the 20 stability QTL collocate to trait QTL on the paternal map, suggesting that a regulatory gene model is prevalent over an allele sensitivity model for stem trait stability across these environments.

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## Introduction

Hardwood trees provide a sustainable source of carbon-neutral bioenergy able to combat the effects of rising atmospheric CO<sub>2</sub> and other greenhouse gases (Cannell 2003; Tuskan 1998; El Bassam 1998; Sims et al. 2006). *Populus* hybrids are superior hardwood trees for biomass production (Heilman et al. 1994; Rae et al. 2004) with high

photosynthetic capacity (Barigah et al. 1994), rapid juvenile growth (Bergez et al. 1989) and a high proportion of woody content in the first growing season (Hansen 1991), producing yields of up to 35 oven-dried tonnes per hectare per year ( $\text{ODT ha}^{-1} \text{ year}^{-1}$ ; Scarascia-Mugnozza et al. 1997). The discrepancy between such potential yields seen in small scale experiments and yields in large commercial plots is often great, so there is a requirement to better understand the genetic nature and environmental input for yield, enabling breeding programmes to benefit from marker-assisted selection and other biotechnological approaches (Boerjan 2005).

*Populus* is now well recognised as the ‘model’ forest tree (Brunner et al. 2004; Taylor 2002; Wullschleger et al. 2002). It has several advantages as a model system, including the availability of several linkage maps and pedigrees, transformation systems and a large genomic resource including expressed sequence tag collections and *Populus* microarrays (Andersson et al. 2004). Moreover, the release of the *Populus* genome sequence was the first for a tree species (<http://genome.jgi-psf.org/Poptr1/Poptr1.home.html>). Not only does this facilitate significant advances in our understanding of economically important traits unique to woody plants (such as dormancy and wood formation), it also increases our ability to answer fundamental questions in forest ecology and evolution.

Improving the commercial biomass yield of hybrid *Populus* is an essential requirement for an economically viable alternative to other land uses (Updegraff et al. 2004), and yet these technologies have yet to be fully integrated into most breeding programmes, although their potential is significant (Boerjan 2005; Neale and Savolainen 2004). Tree height, basal area and number of sylleptic branches are known to be good indicators of final biomass yield in *Populus* (Madgwick 1971; Pontailier et al. 1997; Scarascia-Mugnozza et al. 1997; Heilman and Xie 1993; Rae et al. 2004) as is the ability of the main stem to undergo rapid initial growth with the development of large leaf area in a reasonably short time (Van Volkenburgh and Taylor 1996; Robinson et al. 2004).

Sylleptic branches (late season branches produced from current year buds without a period of dormancy) are important, as they possess 50–100% greater leaf area than the leaves on the main stem, allow optimised light interception and inhibit competition by weeds (Scarascia-Mugnozza 1991; Wu and Stettler 1998). Production of sylleptic branches in many species has been shown to be highly variable under differing environmental conditions (Costes and Guedon 2002). Influences such as solar radiation, water availability, nutritional level and competition may have a large effect on final biomass production and could potentially be of more importance than genotypic effects. Identification of the genetic components for

biomass-related traits and their environmental interactions will be important for the production of high-yielding genotypes. Quantitative variation of such complex traits can be dissected by mapping quantitative trait loci (QTL). QTL mapping is a powerful tool to understand the genetic control of complex traits such as biomass and has been used to look at a number of traits in *Populus* such as rust resistance (Newcombe et al. 1996; Jorge et al. 2005), canopy structure (Wu and Stettler 1998), leaf traits (Wu et al. 1997; Ferris et al. 2002; Rae et al. 2006), stem growth (Bradshaw and Stettler 1995; Wullschleger et al. 2005), growth rates (Wu et al. 2003), bud dormancy (Frewen et al. 2000) and metabolites (Morreel et al. 2006). QTL have also been mapped in the closely related genera, *Salix*, for growth-related traits (Tsarouhas et al. 2002), bud dormancy (Tsarouhas et al. 2003, 2004) and pest tolerance (Ronnberg-Wastljung et al. 2006).

However, an understanding of the genetic control of the differential expression of phenotypic traits across environments or genotype  $\times$  environment ( $G \times E$ ) interaction is of great importance for quantitative genetics and its applications in breeding, conservation and theory of evolution. Changes in soil–water relations (Zhang et al. 2005; Bungart and Huttl 2004), cold hardiness (McCamant and Black 2000), soil type (Crow and Houston 2004; Li and Wu 1997) and latitude (Ikuko and Masahiko 2001) have all been shown to have an effect on biomass traits. The majority of  $G \times E$  interactions affect traits with low heritability normally at the individual level (Lin and Togashi 2002). Understanding the interaction between the genetic material present in a population and the environment in which that population is grown allows breeders to achieve impact from their breeding programs (Basford and Cooper 1998). Whether the breeding aims are to select genotypes best suited for a particular environment or to identify genotypes stable across several environments, it is necessary to understand the genetic nature of the  $G \times E$  interactions.

$G \times E$  stability statistics can be used to assess the performance of a pedigree by providing estimates of each genotype’s contribution to the  $G \times E$  complex. One of the most important questions of a stability parameter is whether it is genetic (Lin and Binns 1991). It can be argued that there are two possible genetic mechanisms underpinning stability (Via et al. 1995; Emebiri and Moody 2006): the allelic sensitivity model, which implies that the gene is regulated itself in direct response to the environment by the activation of different alleles in different environments, and the gene regulation model where one or more regulatory loci are under the direct influence of the environment and the gene for the trait of interest is switched on or off by the regulatory gene. The collocation of QTL exhibiting  $G \times E$  interactions and QTL for stability parameters would support

the allelic sensitivity model, whereas QTL for stability parameters detected in regions other than those for the trait would support the regulatory model (Kraakman et al. 2004; Emebiri and Moody 2006).

This present study aims to explore the genetic control of G×E interactions affecting biomass production at three contrasting European sites. The F<sub>2</sub> family (Family 331; POP1) was grown in three contrasting climates across Europe, ranging from the relatively wet and cool UK site to the warmer drier Italian site. The grandparents of this population, both economically important species in their own right (Dickmann and Stuart 1983; Cervera et al. 2001), differ significantly in their phenotype including leaf morphology (Ferris et al. 2002) and in the production of sylleptic branches (Cline and Dong-il 2002).

A QTL mapping method was used to identify traits that exhibit additive genetic effects that differ between sites. An advantage that simultaneous treatment of data from multiple environments provides is an increased statistical power of QTL detection and accuracy of the estimates of QTL position and effect (Jansen et al. 1995; Korol et al. 1998).

In addition, an additive main effects and multiplicative interaction (AMMI) model (Gauch 1992) was used to generate stability statistics, which can be mapped as QTL. Comparison of QTL location from these two methods was used to discuss the model for the genetic control of G×E interaction.

## Materials and methods

### Location and planting of the F<sub>2</sub> pedigree POP1

An F<sub>2</sub> population was created from a cross between the *P. trichocarpa* clone 93-968 from the western Washington wetlands and the *P. deltoides* clone ILL-129 from the relatively dry area of central Illinois (Bradshaw and Stettler 1995). Grandparents, parental F<sub>1</sub>s and 210 F<sub>2</sub> genotypes were planted using a randomised block design, with six complete blocks at each location, in April 2003 at three sites across Europe: southern UK, central France and northern Italy. Location, elevation and climate of the three

sites are summarised in Table 1. Cuttings were planted at intervals of 0.75 m in rows 2 m apart with a double border row of commercial varieties to minimise edge effects. The three sites were irrigated, weeded and treated with pesticides as necessary during the two growing seasons.

### Measurements of stem traits

First- and second-year maximum stem height was measured to the nearest centimetre using a measuring pole. Stem circumference was measured to the nearest millimetre at 22 cm above ground level in the first year and at 1 m in the second year. Stem volume index was calculated using stem height and circumference in both years, assuming a conical shape (Pontailier et al. 1997). The number of sylleptic branches on the main stem was counted at the end of the first year.

### Biomass

Biomass samples were collected at all sites at the end of the second growing season. Three replicates of a representative subset of 20 genotypes plus the parents and grandparents were selected from the population at each site independently. Genotypes were selected to be representative for growth and branching habit within the pedigree, using sampling optimisation ( $n=10,000$ ) with the following criterion:

$$C = [\text{Var}_s(\text{circ}) - \text{Var}_p(\text{circ})]^2 + [\text{Var}_s(\text{no syl}) - \text{Var}_p(\text{no syl})]^2 + [\text{Cov}_s(\text{circ} - \text{no syl}) - \text{Cov}_p(\text{circ} - \text{no syl})]^2,$$

where “circ” is the stem circumference at 1 m above ground level measured at the end of the second growing season and “no syl” is the number of sylleptic branches counted at the end of the first growing season; “Var<sub>s</sub>” is the variance of the sample, and “Var<sub>p</sub>” is the variance of the population. Three replicates of each genotype were selected as those with values closest to the mean in terms in stem circumference for that genotype. The total fresh weight of each individual

**Table 1** Location, elevation and climatic characteristics of the three experimental sites in Europe

Site	Latitude	Longitude	Elevation (m)	Annual average temperature (°C)	Annual rainfall (mm)	Solar radiation (MJ m <sup>-2</sup> d <sup>-1</sup> )
Headley (UK)	51°07'N	0°50'W	60	10.9	470.9	10.7
Ardon (France)	47°46'N	1°52'E	110	11.7	685.0	–
Cavallermaggiore (Italy)	44°42'N	7°40'E	285	12.9	729.3	13.7

Climatic conditions were obtained in 2003 at nearby meteorological stations (Bra and Villanova Solaro, Italy; Ardon, France; Headley, UK).

in the subset was scored and samples of first-year stem growth, second-year stem growth and branch were taken, weighed in the field, oven dried at 105°C until constant weight was reached and re-weighed to calculate fresh-to-dry mass ratios. The total tree fresh weight and the fresh-to-dry mass ratios for stem and branch samples were used to calculate total tree dry mass. Using the population subset, linear and non-linear regressions were carried out to find the best equation to estimate the total tree dry mass of the whole pedigree. Stepwise multiple regression showed there to be no advantage in including height or number of sylleptic branches in the equation. A cubic model using circumference of the second year gave best fits at all three sites:

$$\begin{aligned} \text{UK Dry mass} &= 893.427 - (29.757 \times \text{circ}) \\ &+ (0.3803 \times \text{circ}^2) \\ &- (0.0008 \times \text{circ}^3), R^2 = 0.950 \end{aligned}$$

$$\begin{aligned} \text{French Dry mass} &= 526.514 - (16.301 \times \text{circ}) \\ &+ (0.2592 \times \text{circ}^2) \\ &- (0.0003 \times \text{circ}^3), R^2 = 0.975 \end{aligned}$$

$$\begin{aligned} \text{Italian Dry mass} &= 2727.63 - (68.796 \times \text{circ}) \\ &+ (0.6196 \times \text{circ}^2) \\ &- (0.001 \times \text{circ}^3), R^2 = 0.971 \end{aligned}$$

These equations were then used to calculate dry mass for the whole pedigree. The dry mass was converted to an estimated ODT ha<sup>-1</sup> year<sup>-1</sup> taking into account the planting density of 6,667 trees ha<sup>-1</sup> and percentage of mortality at each site.

#### Data analysis

Data management and statistical analyses were performed with R software (version 2.0.1, A Language and Environment Copyright, 2004). Micro-environmental effects within field sites were minimised using Papadakis spatial correction (Papadakis 1984), based on a 7×3 grid on individual data. Lines for which there were less than three replicates were removed from all further analysis. Analysis of variance (ANOVA) were carried out for all traits scored

at each site using R-script and the following model of ANOVA:

$$Y_{ij} = \mu + B_i + G_j + \varepsilon_{ij}$$

where  $\mu$  is the general mean,  $B_i$  is the effect of block  $i$  considered as fixed after correction with Papadakis,  $G_j$  is the effect of genotype  $j$  considered as random and  $\varepsilon_{ij}$  is the error. No significant block effects were seen.

One-way ANOVA was carried out to test for significant variation between grandparents and F<sub>1</sub> parents at each site, and Tukey post-hoc analyses was carried out to identify which of the parents and grandparents portrayed significant variation (Table 2, a–c).

Between sites, comparison was tested using two-way ANOVA:

$$Y'_{jkl} = \mu + G_j + S_k + G \times S_{jk} + \varepsilon_{jkl}$$

where  $Y'_{jkl}$  are individual values adjusted for micro-environmental effects using Papadakis's spatial correction,  $\mu$  is the general mean,  $G_j$  is the genotype effect (random),  $S_k$  is the site effect (random),  $G \times S_{jk}$  is the genotype by site interaction (random) and  $\varepsilon_{jkl}$  is the error.

Variance components were calculated by equating observed mean squares to expected mean squares in a random model (Henderson 1953), and individual broad-sense heritability ( $h^2$ ) and genotype heritability ( $h_c^2$ ) were calculated for each trait (1) at each site using  $h^2 = \frac{V_G}{V_G + V_E}$  and  $h_c^2 = \frac{V_G}{V_G + (V_E/r)}$ , and (2) across sites as  $h^2 = \frac{V_G}{V_G + V_S + V_{G \times S} + V_E}$  and  $h_c^2 = \frac{V_G}{V_G + V_S + V_{G \times S} + (V_E/r)}$  where  $V_G$  is the genetic variance,  $V_E$  is the residual variance,  $V_S$  is the site variance,  $V_{G \times S}$  is the genotype by site interaction variance and  $r$  is the average number of replicates. Standard errors of heritability were calculated using the method suggested by Singh et al. (1993). Changes in genotype ranking were tested for using the Spearman rank coefficient on genotype means at each site.

Genetic correlations between traits ( $r_g$ ) were calculated from the variance–covariance matrices obtained from the multivariate ANOVA as  $r_g = \frac{\text{Cov}_{G(x,y)}}{\sqrt{(\sigma_{G(x)}^2 \times \sigma_{G(y)}^2)}}$ , where  $\text{Cov}_{G(x,y)}$  is genetic covariance between traits  $x$  and  $y$ , estimated by equating the mean co-products with their expected values according to the Henderson III procedure.

#### QTL analysis

The genotype means for each site were used to map QTL. A linkage map for this pedigree has been produced by Tuskan (personal communication) consisting of 91 simple sequence repeats (SSRs) genotyped on 350 individuals and 92 fully informative amplified fragment length polymorphisms genotyped on 165 individuals. The total map distance was 1,453.1 cM with an average of 8 cM between markers.

**Table 2** Growth characteristics for the *P. trichocarpa* 93-968 and *P. deltooides* ILL-129 and F<sub>1</sub> parents grown in the (a) UK, (b) France and (c) Italy

	Trait	<i>P. trichocarpa</i>	<i>P. deltooides</i>	53–246	53–242	Significant differences
<b>(a) UK</b>						
Year 1	Height (cm)	208.46 (15.18) <sup>a</sup>	191.21 (40.39) <sup>a</sup>	211.52 (13.59) <sup>a</sup>	264.61 (6.66) <sup>b</sup>	***
	Basal circumference (mm)	71.65 (7.9) <sup>c</sup>	54.18 (11.32) <sup>a</sup>	70.16 (8.38) <sup>c</sup>	87.15 (7.23) <sup>b</sup>	***
	No. sylleptic branches	34.094 (0.762) <sup>c</sup>	9.004 (5.108) <sup>a</sup>	24.262 (4.15) <sup>b</sup>	24.752 (2.855) <sup>b</sup>	***
	Stem volume (cm <sup>3</sup> )	298.4 (83.2) <sup>a</sup>	172.9 (90.5) <sup>a</sup>	306.2 (101.6) <sup>a</sup>	571.6 (138.6) <sup>b</sup>	***
Year 2	Height	517.17 (41.33) <sup>b</sup>	400.1 (64.26) <sup>a</sup>	436.78 (21.59) <sup>a</sup>	507.44 (35.92) <sup>b</sup>	**
	Circumference at 1 m	148.15 (10.54) <sup>b</sup>	111.36 (15.95) <sup>a</sup>	125.08 (11.35) <sup>a</sup>	159.11 (10.34) <sup>b</sup>	***
	Stem volume	4622 (896) <sup>b</sup>	2378 (827) <sup>a</sup>	3183 (648) <sup>a</sup>	5211 (851) <sup>b</sup>	***
	Biomass (ODT ha <sup>-1</sup> y <sup>-1</sup> )	2.205 (0.369) <sup>ab</sup>	1.1957 (0.4667) <sup>a</sup>	1.6046 (0.3474) <sup>a</sup>	2.505 (0.409) <sup>b</sup>	***
<b>(b) France</b>						
Year 1	Height	254.5 (44.22)	252.5 (38.37)	238.5 (34.9)	263.2 (32.94)	ns
	Basal circumference	88.91 (19.02)	75.42 (14.22)	81.22 (16.65)	82.92 (13.33)	ns
	No. sylleptic branches	38.833 (1.941) <sup>c</sup>	11 (8.042) <sup>a</sup>	29 (5.329) <sup>b</sup>	16.4 (3.05) <sup>a</sup>	***
	Stem volume	586 (392.1)	455.5 (290.8)	464.7 (240.3)	522.8 (197.2)	ns
Year 2	Height	581 (71.31) <sup>a</sup>	511.75 (79.71) <sup>a</sup>	505 (71.3) <sup>a</sup>	627.8 (56.3) <sup>b</sup>	*
	Circumference at 1 m	155.83 (22.02) <sup>ab</sup>	122 (23.76) <sup>a</sup>	142.67 (25.65) <sup>ab</sup>	167.6 (17.69) <sup>b</sup>	*
	Stem volume	5652 (2022)	3291 (1571)	4418 (1765)	6744 (1845)	ns
	Biomass	3.192 (0.96)	1.9124 (0.843)	2.6753 (1.0401)	3.689 (0.816)	ns
<b>(c) Italy</b>						
Year 1	Height	176.91 (45.6) <sup>a</sup>	221.86 (44.93) <sup>ab</sup>	215.39 (25.68) <sup>a</sup>	285.27 (44.28) <sup>b</sup>	**
	Basal circumference	64.08 (14.14) <sup>a</sup>	77.61 (10.11) <sup>ab</sup>	81.49 (17.52) <sup>ab</sup>	100.82 (20.46) <sup>b</sup>	*
	No. sylleptic branches	35.139 (4.616) <sup>c</sup>	15.755 (5.198) <sup>a</sup>	28.026 (7.069) <sup>bc</sup>	18.606 (6.645) <sup>ab</sup>	***
	Stem volume	221 (191.8) <sup>a</sup>	357.8 (187.7) <sup>ab</sup>	396.8 (191.5) <sup>ab</sup>	792.7 (403.9) <sup>b</sup>	*
Year 2	Height	612.03 (151.65) <sup>a</sup>	644.73 (54.22) <sup>a</sup>	667.09 (43.67) <sup>a</sup>	823.32 (43.84) <sup>b</sup>	**
	Circumference at 1 m	127.92 (44.96) <sup>a</sup>	166.55 (18.68) <sup>a</sup>	169.07 (19.53) <sup>a</sup>	224.89 (33.85) <sup>b</sup>	**
	Stem volume	4449 (4034) <sup>a</sup>	6911 (2136) <sup>a</sup>	7303 (1768) <sup>a</sup>	14769 (4605) <sup>b</sup>	**
	Biomass	2.336 (2.112) <sup>a</sup>	3.9259 (1.1636) <sup>a</sup>	4.0906 (1.0506) <sup>a</sup>	7.184 (1.788) <sup>b</sup>	**

Tukey's post-hoc test identified significant differences between genotypes and are denoted by superscript letters. Numbers in parenthesis are for standard errors.

Significant differences between genotypes are indicated where \* $p < 0.05$ , \*\* $p < 0.01$  and \*\*\* $p < 0.001$ .

QTL were identified with multiQTL software (<http://www.multiQTL.com>), for the parental maps separately using a pseudo-testcross analysis (Grattapaglia and Sederoff 1994). Single trait analysis was performed using first the interval mapping approach followed by multiple interval mapping (MIM). The combination of MIM with the multiple environment approach increases the accuracy of the estimated QTL position.

The entire genome was scanned for QTL assuming a single model, i.e. one QTL per linkage group (LG). Permutation tests comparing hypotheses H<sub>1</sub> (a single QTL present on the LG) and H<sub>0</sub> (no QTL on the LG) were run to obtain chromosome-wide statistical significance. In a second step, the genome was scanned for QTL assuming a two-linked QTL model. Permutation tests were carried out to compare the hypothesis H<sub>2</sub> (two-linked QTL on the LG) to H<sub>0</sub>. Where the probability of H<sub>2</sub> vs H<sub>0</sub> was less than 0.05, further permutation tests were run to compare the hypotheses H<sub>2</sub> vs H<sub>1</sub> to ensure that the two-linked QTL model fitted the data better than a single QTL model. In all cases, chromosome-wide permutation tests with 1,000 iterations were carried out to determine significance thresholds (Churchill and Doerge 1994).

The option 'marker restoration' was used to reduce the effect of missing information. In a final step, MIM was

performed, to ensure that all single and two-linked QTL identified in the single interval analysis were significant when multiple intervals were analysed. For the remaining significant QTL, again, all necessary permutations were run, and bootstrap analysis was performed to estimate the 95% confidence interval for the additive genetic effect.

Differences between additive genetic components at the different sites were tested by creating sub-models with the additive genetic components set as equal at all sites. Permutation tests (1,000 runs) were carried out to compare the sub-model to the original model where the additive genetic components were estimated separately for each site. Where models were seen to differ significantly the genetic components of the mapped QTL were assumed to differ across sites.

#### AMMI model

To estimate the genetic variation of the stability parameters through a multi-parametric approach, the AMMI analysis (Mandel 1969; Gauch 1992) was performed. The goal of the analysis was to summarise the interaction sum of squares (SS) with a few singular value decomposition (SVD) axes, leaving a reduced model with residuals

containing mostly noise. Only the early interaction axes have a biological interpretation (Gauch 1992). The maximum number of axes is the number of sites minus one, i.e. two in this case. The AMMI model with two axes was:

$$E(Y'_{jk}) = \mu + \alpha_j + \beta_k + \theta_1 \gamma_{j1} \delta_{k1} + \theta_2 \gamma_{j2} \delta_{k2},$$

where  $Y'_{jk}$  is the spatially corrected trait score of genotype  $j$  in site  $k$ ,  $\mu$  is the grand mean,  $\alpha_j$  are genotype mean deviations (mean minus the grand mean),  $\beta_k$  are the site mean deviations,  $\theta_n$  is the singular value for SVD axis  $n$ ,  $\gamma_{jn}$  is the genotypic interaction parameter (score) that measures sensitivity to hypothetical site factor denoted by  $\delta_{kn}$  and  $\delta_{kn}$  is the site interaction parameter (score) that measures sensitivity to hypothetical genotypic factor denoted by  $\gamma_{jn}$ . The eigenvalue for a given SVD axis is the SS retained by that axis, and it equals the square of the singular value  $\theta^2$ . The sum of the eigenvalues  $\sum \theta^2$  for the  $N$  axes, plus the residual SS for a reduced model, is equal to the  $G \times S$  interaction SS. Thus, the interaction SS is partitioned by SVD into interaction axes SS and associated degrees of freedom (Gauch 1992), allowing significance testing of the SVD axes. Those that showed  $p$  values above 0.05 were declared noise and removed from further analysis. The units for  $\mu$ ,  $\alpha$ ,  $\beta$  and  $\theta$  are in the same units as the response  $Y$ . The singular vectors for genotype and site are dimensionless. The genotype interaction scores ( $\gamma_{jn}$ ) and the site interaction scores ( $\delta_{kn}$ ) are in units which are square roots of the unit for  $Y$ .

The greater the deviation of a principal component of a genotype from zero, the less stable is the genotype across sites. QTL for stability were mapped using the same MIM method as used for stem traits.

The genotype data for SSRs were converted into the format for use in MAPMAKER-EXP, and links between these SSRs and restriction fragment length polymorphisms (RFLPs) previously mapped by Bradshaw et al. (1994) for this population were found so that comparisons to QTL cited in literature could be compared to those mapped in this study.

## Results

### Grandparent and parental characteristics

Significant differences were seen between the grandparents and parents for all traits in the UK (Table 2, a) and Italy (Table 2, c), while at the French site, only the number of sylleptic branches, second-year height and circumference showed significant variation (Table 2, b).

Throughout the two growing seasons, the *P. trichocarpa* grandparent showed significantly superior stem growth in the UK and French sites compared to *P. deltooides*. Conversely, the Italian site showed a general trend for

superior growth in *P. deltooides* throughout the growing seasons. The maternal parent, clone 53-246, showed very similar results to the *P. deltooides* grandparent at all sites for stem growth traits, while the paternal parent, clone 53-242, showed heterosis as compared to the grandparents at all sites, with the exception of first-year circumference at the French site. Of particular notice is the increased stem growth of this parent at the Italian site especially in the second growing season. The number of sylleptic branches was highest in the *P. trichocarpa* grandparent, lowest in the *P. deltooides* grandparent and intermediate in the F<sub>1</sub> parent at all sites. A slight significant increase was seen in a number of sylleptic branches between the UK and Italian site in the *P. deltooides* grandparent, and a decrease was seen in the paternal parent.

### F<sub>2</sub> population (POP1)

The highest percentage of mortality occurred at the Italian field site with 11.6% of genotypes no longer represented at the end of the second year of growth. The greatest mortality occurred during the first growing season, possibly because of poor rooting ability in the drier conditions at this site. The lowest values for stem and branch traits were seen across the 2 years at the UK field site (Table 3). The highest stem traits in year 1 were seen at the French site, while the highest number of sylleptic branches and second-year stem traits were seen in Italy.

All stem and branch traits scored in the F<sub>2</sub> population at the end of both growing seasons showed significant variation for genotypes, sites and  $G \times S$  interactions across the three sites (Table 3). In the case of first-year stem traits, the residual variation explained nearly half of the total variation with the genetic variation explaining approximately a third (between 31.2 and 32.2%). Environmental differences between sites explained between 12.9 and 23.2% of the total variation, and the  $G \times S$  interaction explained between 6.5 and 8.6%. The variation in number of sylleptic branches showed a slightly different behaviour to other first-year stem traits in that the genetic variation explained 43.5% of the phenotypic variation. The variation between sites was relatively low (2.7%), and the percentage of variation explained by the  $G \times S$  interaction was higher than that for other first-year stem traits.

In the second year of growth, the variation assigned to the genotypes was higher than in the first year, and with the exception of second-year stem height, the variation because of sites were reduced, but the  $G \times S$  interaction was increased.

Spearman's rank analysis showed there to be a high correlation between biomass scores at the French and UK site (0.74), but a lower correlation of the Italian site with the UK (0.58) and the French site (0.54) suggesting that the

**Table 3** Growth characteristics for the F<sub>2</sub> population (POP1) grown in the UK, France and Italy

Year	Trait	UK	France	Italy	Percentage of phenotypic variation explained			
					Genotype	Site	Interaction	Residual
Year 1	Height	159.54 (1.362)	196.71 (1.412)	151.47 (1.933)	31.24***	23.22***	7.24***	38.30
	Circumference at 22 cm	51.5 (0.466)	62.57 (0.488)	57.22 (0.7162)	32.18***	12.90***	8.62***	46.30
	No. sylleptic branches	17.55 (0.303)	18.93 (0.3128)	21.28 (0.373)	43.46***	2.71***	10.05***	43.78
	Stem volume	145.8 (3.645)	249.7 (5.516)	174.4 (6.862)	32.14***	13.82***	6.46***	47.58
	Percentage mortality	9.2	15.4	47.4				
Year 2	Height	318 (2.555)	373.5 (2.838)	450.9 (5.241)	43.85***	19.04***	9.55***	27.56
	Circumference at 1 m	83.79 (0.872)	90.31 (0.868)	100.44 (1.626)	48.16***	2.47***	12.99***	36.38
	Stem volume	1535 (32.989)	1785 (39.488)	2647 (111.949)	42.22***	4.29***	21.24***	32.25
	Biomass	0.7542 (0.016)	1.1004 (0.022)	1.5224 (0.0565)	39.58***	9.74***	20.18***	30.50
	Percentage mortality	9.2	15.4	50.0				

The percentage phenotypic variation explained by each level in a two-way ANOVA are denoted and significant variation is indicated (\* $p \leq 0.05$ , \*\* $p \leq 0.01$  and \*\*\* $p \leq 0.001$ ). Numbers in parenthesis are for standard errors. Where height is in cm, circumference in mm, stem volume, cm<sup>3</sup> and biomass ODT ha<sup>-1</sup> y<sup>-1</sup>.

G×E interactions were due not only to magnitude differences but to changes in rank order of genotypes at the Italian site when compared to the UK and French sites. This is backed up by the behaviour of the grandparents at the different sites; that is, *P. deltoides* ILL-129 consistently showed greater stem volume and biomass than *P. trichocarpa* 93-968 at the two more northerly sites, but the opposite was true at the Italian site.

It can be seen from Table 4 that both individual and genotypic heritabilities were moderate to high for all traits when calculated for each site separately. As expected, individual heritability calculated across sites was slightly reduced, but strong genotype heritabilities were still apparent. As expected from the proportions of phenotypic variation explained, heritability was higher in second-year than first-year growth traits (Fig. 1).

Genetic correlations

With the exception of sylleptic branching, genetic correlations were seen to be high between stem traits and biomass

over the 2 years, ranging between 0.85 and 0.99 (Table 5). Moreover, genetic and residual correlations were comparable (data not shown), suggesting a possible pleiotropic control of these traits, and QTL for the correlated traits would be expected to co-locate. Genetic correlations between sylleptic branching and the other stem traits, however, were lower, ranging between 0.25 (height year 1) and 0.50 (circumference year 1).

QTL analysis

For the eight stem and biomass traits, 37 QTL were mapped based on the maternal linkage map (Table 6), and 45 were mapped based on the paternal map (Table 7). The percentage variance explained (PEV) for individual QTL ranged from 0.3 to 24.1%, with a mean of 6.3%. Total PEV per trait ranged from 3.9 to 75.3% suggesting that there are more genetic factors segregating in this pedigree that remained undetected in this analysis.

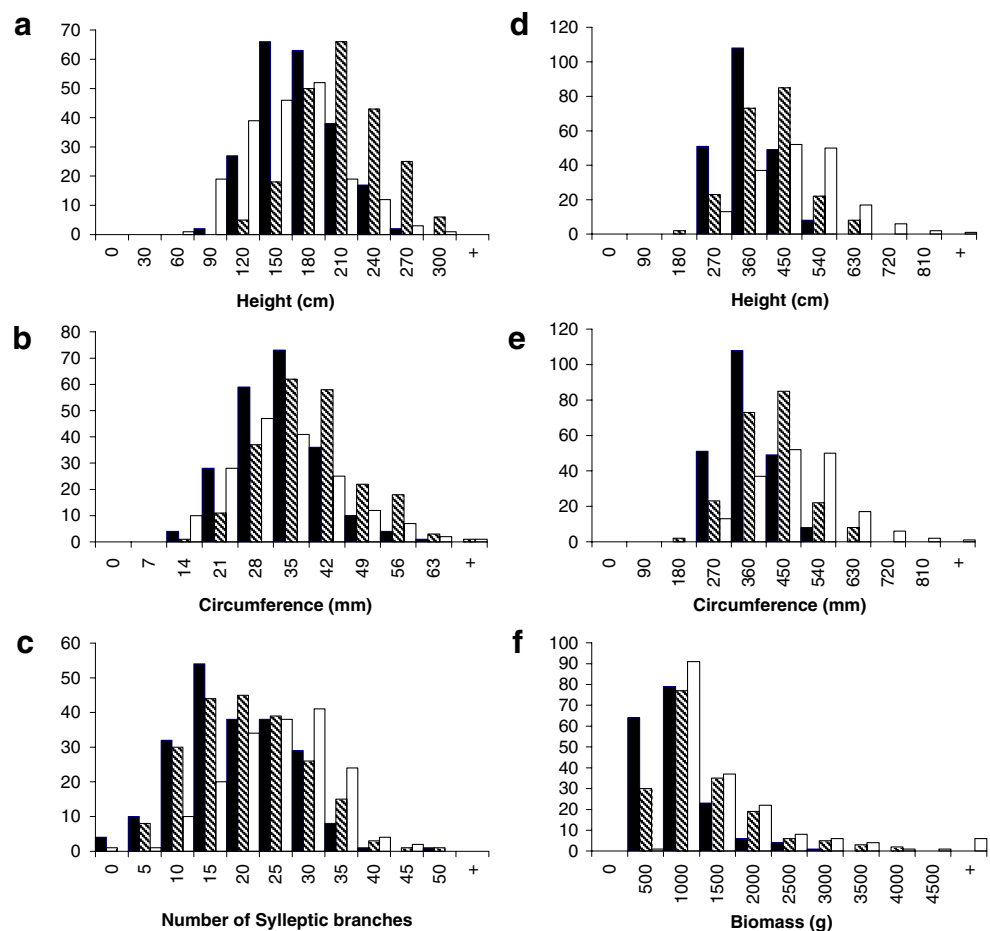
Tables 6 and 7 show the genetic parameters for the QTL detected on the maternal and paternal map, respectively.

**Table 4** Individual ( $H^2$ ) and clonal ( $H_c^2$ ) broad-sense heritability values for the F<sub>2</sub> populations at the UK, French and Italian sites and overall individual and clonal heritability across sites

Year	Trait	UK		France		Italy		All	
		$H^2$	$H_c^2$	$H^2$	$H_c^2$	$H^2$	$H_c^2$	$H^2$	$H_c^2$
Year 1	Height	0.48 (0.046)	0.83 (0.022)	0.56 (0.043)	0.88 (0.017)	0.54 (0.054)	0.80 (0.029)	0.34 (0.019)	0.87 (0.010)
	Basal Circumference	0.40 (0.047)	0.78 (0.027)	0.50 (0.045)	0.85 (0.020)	0.46 (0.058)	0.75 (0.036)	0.35 (0.019)	0.88 (0.010)
	No. of sylleptic branches	0.58 (0.043)	0.88 (0.017)	0.58 (0.042)	0.89 (0.016)	0.53 (0.059)	0.79 (0.034)	0.47 (0.018)	0.92 (0.006)
	Stem volume	0.44 (0.047)	0.81 (0.025)	0.51 (0.045)	0.85 (0.020)	0.48 (0.057)	0.76 (0.035)	0.33 (0.019)	0.87 (0.010)
Year 2	Height	0.61 (0.041)	0.89 (0.015)	0.67 (0.037)	0.92 (0.012)	0.64 (0.049)	0.86 (0.023)	0.49 (0.017)	0.93 (0.006)
	Circumference at 1 m	0.58 (0.043)	0.88 (0.017)	0.63 (0.040)	0.90 (0.014)	0.62 (0.050)	0.85 (0.024)	0.52 (0.017)	0.94 (0.006)
	Stem volume	0.60 (0.042)	0.89 (0.016)	0.65 (0.038)	0.91 (0.013)	0.66 (0.047)	0.87 (0.022)	0.49 (0.017)	0.93 (0.006)
	Biomass	0.61 (0.041)	0.89 (0.015)	0.64 (0.039)	0.91 (0.013)	0.63 (0.049)	0.86 (0.023)	0.47 (0.018)	0.92 (0.007)

Numbers in parenthesis are for standard errors.

**Fig. 1** Frequency distributions for POP1 at the UK (*solid bars*), French (*hatched bars*) and Italian (*unshaded bars*) sites. **a–c** are for traits in the first growing season; **d–f** are for the second. **a, d** Height of tree in centimetres; **b, e** circumference at 1 m up height; **c** number of sylleptic branches; **f** Calculated biomass, grams per tree



The additive genetic effects with standard deviations are shown for each site, alongside an indication of the level of significance for the difference between this model and a QTL model with the additive genetic effect set as equal at all sites. It can be seen from this that for the maternal map, 8 of the 37 QTL mapped showed significant differences in effect across the sites, while 20 of the 45 QTL mapped on the paternal map showed significant differences across sites. For both parental analyses, QTL for the number of

sylleptic branches showed the highest proportion of QTL with differing additive genetic effects across sites.

Genetic correlations showed that stem height, circumference, volume and biomass are likely to be controlled by the same genetic regions; therefore, it is reasonable to assume that collocating QTL with the same direction of effect for these traits are pleiotropic. With this in mind, the number of unique QTL can be estimated. For example, height QTL in both years mapped to the first marker

**Table 5** Genetic correlations, between traits for the F<sub>2</sub> populations

		Year 1				Year 2			
		Height	Circumference	No. sylleptic branches	Stem volume	Height	Circumference	Stem volume	Biomass
Year 1	Height	–	0.849 (0.015)	0.287 (0.064)	0.915 (0.011)	0.926 (0.010)	0.887 (0.015)	0.849 (0.019)	0.851 (0.019)
	Circumference	0.849 (0.015)	–	0.501 (0.065)	0.969 (0.016)	0.870 (0.025)	0.964 (0.017)	0.919 (0.025)	0.929 (0.023)
	No. sylleptic branches	0.287 (0.064)	0.501 (0.065)	–	0.432 (0.057)	0.366 (0.060)	0.448 (0.056)	0.389 (0.059)	0.397 (0.059)
	Stem volume	0.915 (0.011)	0.969 (0.016)	0.432 (0.057)	–	0.923 (0.010)	0.976 (0.003)	0.975 (0.003)	0.980 (0.003)
Year 2	Height	0.926 (0.010)	0.870 (0.025)	0.366 (0.060)	0.923 (0.010)	–	0.947 (0.007)	0.917 (0.011)	0.914 (0.011)
	Circumference	0.887 (0.015)	0.964 (0.017)	0.448 (0.056)	0.976 (0.003)	0.947 (0.007)	–	0.962 (0.005)	0.969 (0.004)
	Stem volume	0.849 (0.019)	0.919 (0.025)	0.389 (0.059)	0.975 (0.003)	0.917 (0.011)	0.962 (0.005)	–	0.999 (0.000)
	Biomass	0.851 (0.019)	0.929 (0.023)	0.397 (0.059)	0.980 (0.003)	0.914 (0.011)	0.969 (0.004)	0.999 (0.000)	–

Numbers in parenthesis are for standard errors.

**Table 6** QTL identified from the maternal, 53–246, analysis

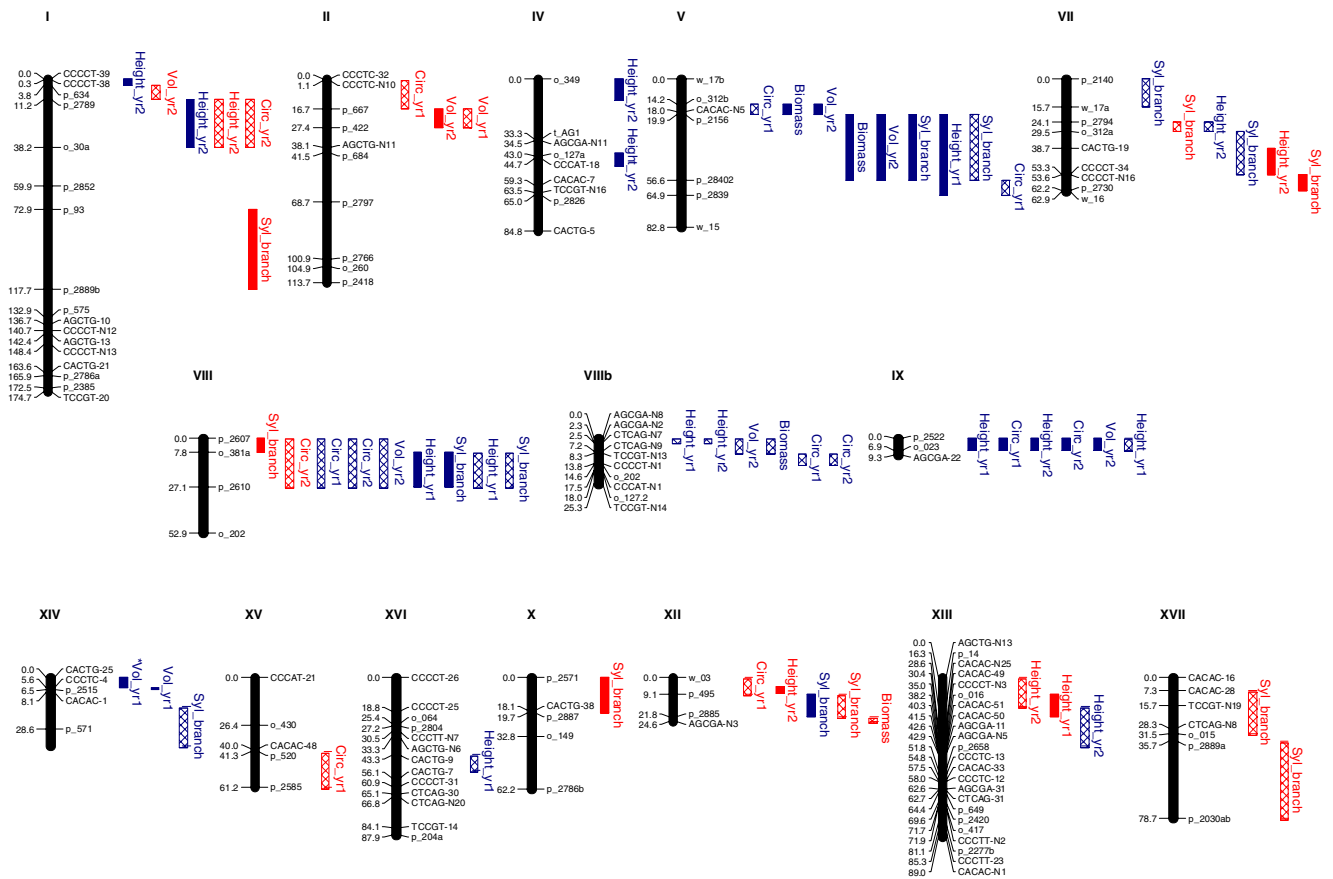
Year	Trait	LG	Marker interval	LOD	Additive genetic effect			<i>p</i> Value PEV		
					UK	France	Italy	UK	France	Italy
Year 1	Stem height	II	p_422–p_684	6.64	-10.9±5.37	-9.9±5.2	-17±6.61	0.031	0.024	0.04
	Stem height	III	o_203–CACAC-26	5.67	12.4±4.85	11.8±4.99	16.2±6.78	0.037	0.032	0.038
	Stem height	VI	p_2156–p_2839	4.08	9.48±5.21	8.1±6.55	7.52±6.13	0.025	0.021	0.011
	Stem height	Xb	CTCAG-34–o_049	9.28	-13.8±4.45	-19.5±4.22	-12.9±5.23	0.044	0.077	0.023
	Stem height	XIII	p_495–p_2885	7.85	5.43±26.8	7.03±24.9	17.1±33.5	***	0.119	0.125
	Stem height	XIII	p_2885–AGCGA-N3	–	-5.19±25.5	-13.2±23.7	-19.1±31.8	–	–	–
	Stem height	XVII	p_2889a–p_2030ab	5.36	-15.7±7.74	-14.3±8.27	-13.1±7.65	0.061	0.052	0.027
	Stem height	XVIII	p_2880–AGCGA-26	8.84	37.7±12.7	37.6±9.94	30.8±18.2	0.232	0.194	0.109
	Stem height	XVIII	AGCGA-26–o_028a	–	-37.6±12.3	-31.3±10.2	-28.8±18.5	–	–	–
	Stem circumference	II	p_667–p_422	4.13	-2.52±2.06	-4.91±2.03	-2.98±3.18	0.022	0.049	0.018
	Stem circumference	III	o_203–CACAC-26	5.15	3.74±2.14	6.09±2.68	5.75±3.23	0.038	0.076	0.04
	Stem circumference	X	p_2571–p_2887	4.11	-3.94±1.61	-4.43±1.86	0.687±2.85	0.038	0.04	0.008
	Stem circumference	XVII	p_2889a–p_2030ab	5.37	-5.58±2.62	-4.7±4.45	-4.29±3.48	0.074	0.071	0.028
	Stem circumference	XIX	o_276b–o_277	3.19	-2.3±1.98	-3.16±2.33	-3.74±2.87	0.019	0.027	0.021
	Stem Volume	III	CACAC-26–p_2696	4.67	28.8±13.6	58.7±21.2	52.8±25.7	0.039	0.071	0.021
	Stem Volume	VI	p_2156–p_2839	3.6	22.2±17.5	25.6±37.1	25.6±35.9	0.030	0.037	0.011
	Stem Volume	X	p_2571–p_2887	3.14	-23.7±11.6	-39.8±19.5	16.1±32.9	0.027	0.035	0.007
	No. sylleptic branches	I	p_93–p_2889b	11.6	-3.96±1.27	-4.74±1.95	-6.78±1.99	*	0.092	0.114
	No. sylleptic branches	III	o_203–CACAC-26	5.15	2.18±0.985	1.73±1.37	0.588±1.05	0.031	0.022	0.007
	No. sylleptic branches	IV	AGCGA-N11–o_127a	13.8	4.03±1.93	6.26±2.43	6.69±1.02	0.054	0.091	0.112
	No. sylleptic branches	IV	o_127a–TCCGT-N16	–	-4.15±1.87	-5.66±2.33	-5.71±1.13	–	–	–
	No. sylleptic branches	V	p_2156–p_2839	4.73	2.35±0.839	51.5±0.871	1.11±0.829	0.034	0.014	0.009
	No. sylleptic branches	VII	CCCCT-34–p_2730	5.84	2.32±0.744	2.26±0.766	-0.728±0.744	***	0.032	0.026
	No. sylleptic branches	VIII	p_2607–o_381a	8.38	-0.921±0.649	-2.52±0.696	-3.16±0.7	**	0.007	0.031
	No. sylleptic branches	X	p_2571–p_2887	6.04	-1.13±0.648	-3.29±0.744	-0.815±0.774	**	0.009	0.052
	No. sylleptic branches	XIV	CACAC-I–p_571	18.8	-4.96±0.748	-3.66±0.811	-3.5±0.786	0.137	0.063	0.061
No. sylleptic branches	XVI	o_064–p_2804	12	4.81±1.39	3.67±1.45	4.17±1.51	0.205	0.117	0.114	
Year 2	Height	II	p_422–p_684	8.52	-28.9±7.43	-32.7±8.88	-35.6±14.5	0.060	0.059	0.039
	Height	III	o_203–CACAC-26	6.3	29.2±9.85	33.8±12.3	17.7±22.3	0.064	0.065	0.021
	Height	VII	p_2140–w_17a	9.19	-25.7±14.3	-32.9±19.8	-21.2±32.5	0.095	0.128	0.043
	Height	VII	CACTG-19–CCCCT-34	9.19	29.6±12.3	43.7±17.6	4.66±33.2	*	0.095	0.128
	Height	X	p_2571–p_2887	4.03	-19.2±8.51	-22.7±9.32	-5±16.5	0.030	0.031	0.008
	Height	Xb	CTCAG-34–o_049	8.91	-20.6±7.94	-23.1±8.75	-46.7±14.2	0.044	0.037	0.122
	Height	Xb	o_049–CTCAG-23	–	11.1±10.8	-0.454±11.7	49.4±22.9	***	–	–
	Height	XVII	p_2889a–p_2030ab	4.85	-29.1±18.5	-29.7±20.8	-9.53±23	0.072	0.066	0.015
	Height	XVIII	p_2525–p_2880	11.5	39.8±11.3	45.8±12.9	75.3±21.9	0.118	0.108	0.139
	Height	XVIII	p_2880–AGCGA-26	–	-34.1±11.9	-31.7±15.2	-39.6±26.2	–	–	–
	Stem circumference	II	p_422–p_684	6.14	-6.21±4.12	-9.67±3.73	-7.51±5.58	0.034	0.06	0.028
	Stem circumference	III	o_203–CACAC-26	7.53	8.35±3.4	13.3±3.32	9.62±5.96	0.048	0.104	0.04
	Stem circumference	X	p_2571–p_2887	4	-6.29±2.8	-6.93±3.11	-2.25±5.39	0.029	0.032	0.011
	Stem circumference	Xb	CTCAG-34–o_049	7.93	-8.39±2.77	-7.39±2.7	-10.3±4.36	0.066	0.045	0.084
	Stem circumference	Xb	o_049–CTCAG-23	–	5.61±4.69	2.99±4.41	11.9±7.22	–	–	–
	Stem circumference	XVII	p_2889a–p_2030ab	5.91	-10.9±5.34	-9.86±5.59	-3.11±5.71	0.083	0.07	0.013
	Stem circumference	XVIII	p_2525–p_2880	9.83	13.8±5.39	12.3±4.02	25.1±11.2	0.117	0.098	0.185
	Stem circumference	XVIII	p_2880–AGCGA-26	–	-11.8±6.39	-11.4±4.87	-19.4±13.2	–	–	–
	Stem Volume	II	p_667–p_422	10.1	-210±84.5	-399±99	-491±140	0.029	0.068	0.039
	Stem Volume	III	o_203–CACAC-26	9.31	282±79.7	444±94.4	398±130	0.048	0.082	0.026
	Stem Volume	Xb	CTCAG-34–o_049	11.3	-311±72.5	-329±94.1	-353±135	0.068	0.058	0.049
	Stem Volume	Xb	o_049–CTCAG-23	–	122±107	136±138	428±163	–	–	–
	Stem Volume	XVII	p_2889a–p_2030ab	5.52	-270±116	-327±187	-8.16±169	0.048	0.056	0.004
	Stem Volume	XVIII	AGCGA-26–o_028a	9.66	667±338	611±287	1280±985	0.221	0.15	0.23
	Stem Volume	XVIII	AGCGA-26–o_028a	–	-637±380	-620±300	-1120±1050	–	–	–
	Biomass	II	p_422–p_684	5.47	-0.0772±0.0741	-0.245±0.098	-0.178±0.134	0.023	0.07	0.023
	Biomass	III	o_203–CACAC-26	7.13	0.139±0.0567	0.301±0.0873	0.27±0.129	0.045	0.098	0.042
	Biomass	Xb	CTCAG-34–o_049	6.73	-0.163±0.0573	-0.2±0.0733	-0.115±0.127	0.084	0.065	0.046
	Biomass	Xb	o_049–CTCAG-23	–	0.118±0.0847	0.121±0.125	0.236±0.166	–	–	–
	Biomass	XVII	p_2889a–p_2030ab	4.42	-0.15±0.0784	-0.211±0.12	0.0119±0.14	0.055	0.059	0.009

Linkage groups (LG) and marker intervals to which QTL mapped are shown with LOD scores. The estimated additive genetic effect (*a*) of each QTL at each site is shown and where significant differences between a across sites is indicated. The percentage genetic variation explained by the mapped QTL at each site is shown.

**Table 7** QTL identified from the paternal, 53–242, analysis

Year	Trait	LG	Marker position	LOD	Additive genetic effect			<i>p</i> Value	PEV			
					UK	France	Italy		UK	France	Italy	
Year 1	Stem height	I	CCCCT-39-p_634	15.3	-32.3±8.85	-31.5±7.57	-10.5±14.4		0.219	0.219	0.113	
	Stem height	I	p_2789-o_30a	–	33.1±8.88	34±7.76	28.1±16.4		–	–	–	
	Stem height	IV	p_2277a-p_204.02	17.7	7.85±17.1	513±19.5	19.2±28.6		0.178	0.177	0.079	
	Stem height	IV	o_030b-o_203	–	-28.9±17.1	5-20±16.9	-23±30.8		–	–	–	
	Stem height	V	p_2156-p_2839	4.89	-6.43±5.64	-4.37±5.27	-21.4±6.19	*	0.017	0.01	0.073	
	Stem height	VIII	o_381a-p_2610	4.72	-2.74±4.91	-6.89±4.27	-20.1±5.57	*	0.007	0.014	0.064	
	Stem height	IX	p_2522-o_023	3.71	-1.53±3.58	-0.586±3.99	-17.9±4.85	**	0.003	0.004	0.051	
	Stem height	X	CACTG-38-p_2571	5.53	13±5.05	16.5±4.97	5.72±6.33		0.044	0.065	0.011	
	Stem circumference	I	p_634-p_2789	4.47	1.51±3.43	4.81±4.14	4.41±3.12		0.029	0.068	0.03	
	Stem circumference	IV	p_2501a-o_030b	12.1	5.86±10.2	1.39±10.1	16.1±15.3		0.210	0.142	0.201	
	Stem circumference	IV	o_030b-o_203	–	-11.2±10.1	5-4.62±9.76	-18.9±15.8		–	–	–	
	Stem circumference	IX	p_2522-o_023	4.98	-2.25±1.3	-2.11±1.55	-7.19±1.9	*	0.014	0.012	0.058	
	Stem circumference	X	p_2887-CACTG-38	10.9	1.86±2.61	3.07±2.32	-0.536±4.38		0.085	0.100	0.052	
	Stem circumference	X	CACTG-38-p_2571	–	4.87±1.91	5.74±2.08	6.1±3.74		–	–	–	
	Stem Volume	I	p_2789-p_2852	5.07	13±24	41.8±41.5	50.2±27.5		0.029	0.069	0.03	
	Stem Volume	IV	o_030b-o_203	6.82	-42.2±11.3	-51±19.1	-12.9±32.3		0.075	0.060	0.012	
	Stem Volume	X	CACTG-38-p_2571	9.39	47.2±12.8	72.6±17.4	58.2±19.4		0.094	0.112	0.037	
	Stem Volume	XIV	CACTG-25-CCCTC-4	7.39	14.4±54	1-30.6±85.1	164±135	***	0.04	0.055	0.091	
	Stem Volume	XIV	CCCTC-4-p_2515	–	-10.3±56.2	30.3±88.3	-127±134	***	–	–	–	
	No. sylleptic branches	IV	o_030b-o_203	5.53	-2.35±1.18	-3.26±1.4	-3.53±1.65		0.03	0.045	0.056	
	No. sylleptic branches	V	p_2156-p_28402	7.62	5.08±1.23	4.37±1.41	1.06±1.8	**	0.119	0.075	0.016	
	No. sylleptic branches	VIII	o_381a-p_2610	7.09	4.13±1.05	3.75±1.08	-1.17±1.29	***	0.079	0.055	0.011	
	No. sylleptic branches	X	CACTG-38-p_2571	9.27	4.24±1.17	5.88±1.32	2.47±1.69		0.085	0.13	0.034	
	No. sylleptic branches	XII	p_495-p_2885	3.42	-1.96±0.901	-0.236±1.02	-3.19±1.24	*	0.021	0.004	0.043	
	No. sylleptic branches	XIII	AGCGA-N5-p_2658	6.32	-2.74±0.848	-3.51±1.08	-1.27±1.33		0.036	0.049	0.013	
	Year 2	Stem height	I	CCCCT-39-p_634	17.4	-38.8±10.3	-63.2±10.7	-12±16.3	***	0.124	0.206	0.057
		Stem height	I	p_2789-o_30a	17.4	41.9±10.4	56.6±9.79	39.3±20.5		–	–	0.057
		Stem height	III	p_2277a-p_204.02	11.9	27.9±6.5	26.5±6.61	46±10.6		0.063	0.045	0.081
		Stem height	IV	p_2277a-p_204.02	23.4	7.74±9.05	32.4±9.13	23.7±12.3	*	0.141	0.194	0.037
		Stem height	IV	o_030b-o_203	–	-41.9±8.8	-48.9±8.12	-15.2±13.2	*	–	–	–
		Stem height	VII	p_2140-w_17a	13.4	-25.1±12.4	-41.9±9.34	-52.7±21.5		0.107	0.106	0.132
		Stem height	VII	o_312a-CCCCT-N16	–	37.4±12.8	31.5±11.3	53.9±20.4		–	–	–
		Stem height	IX	p_2522-o_023	6.86	-0.883±5.85	11.9±5.91	-46.3±9.23	***	0.003	0.011	0.081
Stem height		X	CACTG-38-p_2571	11	29.7±6.83	29±7.53	35.1±12.3		0.071	0.054	0.049	
Stem height		XV	CCCAT-21-o_430	12.6	-25.5±10.7	-27.5±9.15	-55.5±18.3		0.128	0.137	0.125	
Stem height		XV	p_520-p_2585	–	34.7±8.55	42.5±8.49	19.9±16.1		–	–	–	
Stem circumference		III	p_2277a-p_204.02	5.35	8.2±5.01	3.52±4.63	9.55±5.18		0.056	0.019	0.038	
Stem circumference		IV	o_030b-o_203	5.71	-9.21±3.88	-8.79±5.31	-6.05±4.68		0.06	0.059	0.019	
Stem circumference		IX	p_2522-o_023	5.67	-4.43±2.52	-4.85±2.65	-14.4±3.57	*	0.016	0.017	0.072	
Stem circumference		X	CACTG-38-p_2571	11	11.8±3.16	13.7±3.8	18.5±4.58		0.089	0.112	0.117	
Stem circumference		XV	CCCAT-21-o_430	6.57	-6.86±4.83	-4.14±4.06	-14.1±5.45		0.092	0.051	0.076	
Stem circumference		XV	CACAC-48-p_520	–	10.1±3.72	47.65±3.89	1.7±6.31		–	–	–	
Stem circumference		XIX	CACAC-17-AGCTG-25	5.59	6.17±7.09	4.33±9.91	2.31±12.2		0.052	0.062	0.045	
Stem Volume		III	p_2277a-p_204.02	4.53	197±263	84.8±237	295±268		0.058	0.023	0.017	
Stem Volume		IV	o_030b-o_203	5.28	-298±133	-330±192	-344±226		0.058	0.054	0.018	
Stem Volume		V	o_312b-p_2156	9.3	46.4±301	-95.9±456	998±1170	***	0.045	0.083	0.241	
Stem Volume		V	p_2156-p_28402	–	25±296	288±459	-1430±1100	***	–	–	–	
Stem Volume		IX	p_2522-o_023	3.93	-92.1±86.6	-184±109	-535±170	*	0.009	0.017	0.034	
Stem Volume		X	CACTG-38-p_2571	10.3	401±109	532±145	795±217		0.094	0.112	0.078	
Biomass		III	p_2277a-p_204.02	4.22	0.0923±0.133	0.0369±0.145	0.129±0.142		0.055	0.023	0.013	
Biomass		IV	o_030b-o_203	5.96	-0.173±0.0568	-0.214±0.104	-0.213±0.119		0.07	0.059	0.021	
Biomass		V	o_312b-p_2156	11	0.00881±0.154	-0.0624±0.256	0.64±0.642	***	0.046	0.077	0.239	
Biomass		V	p_2156-p_28402	–	0.0411±0.148	50.175±0.266	-0.809±0.609	***	–	–	–	
Biomass		IX	p_2522-o_023	3.08	-0.0546±0.0416	-0.125±0.0641	-0.226±0.103		0.01	0.021	0.022	
Biomass		X	CACTG-38-p_2571	10.2	0.212±0.0523	0.33±0.0821	0.396±0.123		0.1	0.121	0.061	

Linkage groups (LG) and marker intervals to which QTL mapped are shown with LOD scores. The estimated additive genetic effect (*a*) of each QTL at each site is shown and where significant differences between a across sites is indicated. The percentage genetic variation explained by the mapped QTL at each site is shown.



**Fig. 2** QTL for stem, biomass and sylleptic branch traits, which showed differential additive genetic effects across the UK, French and Italian sites and stability QTL in the  $F_2$  pedigree (POP1). QTL denoted in red were identified in the maternal analysis and those

shown in blue identified in the paternal analysis. Solid intervals denote trait QTL, which showed significant differences in additive effect across sites, and hatched intervals indicate stability QTL. QTL are shown displayed on the marker interval to which they mapped

interval on LGI on the paternal map had a decreasing effect on height and so can be assumed to be the same QTL. This reduces the number of unique QTL for stem traits to 23 mapped to the maternal map and 24 for the paternal map.

Plasticity parameters

The AMMI model resulted in two stability parameters for each trait; however, the second parameter for year 2 stem volume and biomass were not significant, so were attributed as noise and were not included in the QTL analysis. A total of 14 and 21 QTL were mapped for stability on the maternal and paternal maps, respectively. The total PEV per stability QTL ranged from 3.8 to 57.4%, with stability QTL for the number of sylleptic branches showing the highest PEV in both parental maps.

Figure 2 shows the positions of trait QTL for which the additive genetic effects differed significantly across sites shown as solid blocks, and QTL for stability parameters shown as hatched blocks. From this, it can be seen that few stability QTL collocated or overlapped with those for traits. For the maternal map analysis, LGII shows a QTL for stem

volume in the second year of growth that collocates to a stability QTL for stem volume in the first year, and on LG XIII, a QTL for height in the first year overlaps to a stability QTL for height in year 2. The analysis of the paternal map showed a stability QTL for first-year stem circumference to collocate to QTL for biomass and second-year stem volume on LGV. On the same LG, a stability QTL for sylleptic branching collocated to QTL for biomass, stem volume, height and number of sylleptic branches. LGVIII had a region to which stability QTL for stem height, sylleptic branching, stem volume and circumference over 2 years collocated with trait QTL for height and sylleptic branching. A further stability QTL for height collocated to a QTL for height, circumference and stem volume on LGIX.

Discussion

This study has not only identified QTL for important yield components but also the influence of environment on their determination. The  $F_2$  mean biomass estimation was rela-

tively low at all three sites, but high transgressive segregation was observed. The large phenotypic range both within and across sites (0.18–10.31 ODT ha<sup>-1</sup> year<sup>-1</sup> in the UK, 0.06–14.93 ODT ha<sup>-1</sup> year<sup>-1</sup> in France and 0.77–18.06 ODT ha<sup>-1</sup> year<sup>-1</sup> in Italy) suggests that this population has much potential for selective breeding. The primary aim of this study was to understand the genetic control of biomass and the interaction with the environment. For the maternal map, 2 of the 16 stability QTL collocated to trait QTL that showed differential expression at the different sites, while 8 of the 24 stability QTL collocated to trait QTL in the paternal parent analysis. This suggests that while there is some evidence of allele sensitivity to the environment, the majority of stability traits did not collocate to trait QTL, which showed genetic variation across sites; therefore, there is strong evidence for the gene regulatory model of G×E control.

Overall the southerly Italian site produced superior biomass yields at the end of 2 years growth. This may be of great relevance for future breeding programs in northern Europe, as future climate change is predicted to increase average summer temperatures by 2–5°C, and summer precipitation may decrease (Broadmeadow et al. 2005), creating a southern UK climate similar to that of the present-day Italian climate, although the solar radiation and soil type also needs to be taken into account. This is of significant importance, implying that future breeding programs need to consider the climate and environment in which the trees are to be grown, as well as predictions about future climate change.

The high mortality rate at the Italian site (11.6% of genotypes lost) is thought to be due to rooting difficulties. It may be suggested that the superior mean biomass production at this site was due to the loss of the weakest genotypes, but the biomass range produced at this site suggests many genotypes performed better at this site than at the more northerly sites. A significant genotype effect was seen for mortality ( $p < 0.001$ ), but no significant effect of genotype survival was seen for biomass ( $p = 0.382$ ). This suggests that although survival was not random to genotypes, there was no relation between poor biomass yields and survival rate. Previous studies of this population at the same UK field site have reported biomass yields ranging from 0.04 to 23.68 ODT ha<sup>-1</sup> year<sup>-1</sup> (Rae et al. 2004), resulting from short rotation coppiced (SRC) trees grown at a higher density. There are mixed reports whether superior yields are achieved from coppiced or single-stemmed poplar plantations (Hervé and Ceulemans 1996; Sims et al. 2001). It is generally accepted that wider spacing produces larger trees, but productivity per hectare is reduced (Proe et al. 1999). A comparison of the Rae et al. (2004) high-density SRC trial with this single-stemmed trial in the UK confirms this, suggesting that even higher yields may be possible at the Italian site with denser planting.

Site effect was treated as a random factor in this study. This is in keeping with another related study of a different pedigree grown at the same three sites (Marron et al. 2006). However, as there are only three sites investigated here, the estimates of site variance may be unreliable, so it should be recognised that these results are conditional on the observed three sites. All traits showed moderate to high broad-sense heritabilities. For the majority of traits, heritability was highest at the French site, followed by the Italian site. A previous study by Wu and Stettler (1997) on the same pedigree found genetic variances for growth traits, especially basal area and volume index, substantially larger in a warm, high-radiation, well-watered environment than in cooler conditions. It could be argued that this is also the case here because, although the French site had the intermediate temperature, it was more regularly irrigated than the Italian site. As expected, all heritabilities calculated for each site were higher than those for the pooled data across the three sites. Pooled values are suggested to be more accurate estimates because they are not affected by genotype by macro-environment interactions. Heritability values from this study are comparable to those reported by Wu and Stettler (1997).

A number of QTL for correlated traits were seen to collocate. Genetic correlations of the traits can be used to identify the extent to which the traits share the same genetic basis, offering a criterion to carry out simultaneous selection for correlated traits. It can be assumed that the collocation of QTL for traits that showed a high genetic correlation showed pleiotropic effects, so that inferences may be made about the number of unique QTL mapped. In this study, stem height, circumference, volume and biomass were seen to have high genetic correlations over the 2 years, and collocation of QTL for these traits will enable selection for overall stem growth. Interestingly, the number of sylleptic branches did not show high genetic correlation to the other stem traits despite having been reported to show strong phenotypic correlations to biomass in previous studies (Rae et al. 2004). This suggests that although this trait does contribute to final biomass, it is not necessarily under the same genetic control as the other stem traits.

QTL for growth and biomass mapped in a related backcross pedigree sharing the same maternal grandparent have been reported previously (Wullschleger et al. 2005). The use of the same microsatellite primers in the production of the linkage maps for the two pedigrees enabled the identification of QTL, which collocate to similar regions. For example, in the backcross pedigree, a QTL for stem biomass mapped at the bottom of LGIII to a similar region as stem and biomass QTL in the maternal map for the F<sub>2</sub> pedigree, biomass QTL mapped to LGIV in the backcross to a similar region as stem and biomass QTL in the F<sub>2</sub> pedigree, stem biomass QTL mapped to a similar region as

F<sub>2</sub> stem height QTL, stem and branch QTL mapped to a similar region as stem height and sylleptic branch QTL on LGXIII and branch biomass QTL on LG XIV mapped to a similar region as a QTL for sylleptic branching in the F<sub>2</sub> pedigree.

In addition, the microsatellites used in the current study were assessed in MAPMAKER for grouping with the RFLP markers used previously to produce a linkage map in this pedigree (Bradshaw et al. 1994). This enabled a rough comparison of QTL previously mapped in this pedigree. For example, both Bradshaw and Stettler (1995) and Wu et al. (1998) previously mapped QTL for stem traits to LGs D, E, M and O, which correspond to LGXVI, LGXI, LGI, and LGXIX in the present study, and a biomass QTL to LG J, which corresponds to LGX. With the exception of LGXI, QTL for stem and biomass traits also mapped to these LGs in the present study. Although it is not possible to precisely align the maps used in this previous work, it is possible to use this information to back up the presence of stem and biomass QTL across the different studies and even different mapping pedigrees.

The percentage of phenotypic variation assigned to the genotype and heritability values were seen to be higher for traits scored after the second year of growth than after the first one, possibly because of the environmental effects of cutting quality and rooting ability during the first year of growth. This, added to the fact that the plants grew exponentially, means that the phenotypic variation in response to genetic differences became more pronounced in the second year. This may also explain why, in both parental analyses, more stem QTL were mapped in the second year than in the first. The high genetic correlations and collocation of QTL for traits over the 2 years imply that measurements of traits after the first year of growth are representative of longer-term growth characteristics. However, some caution should be taken when using early growth information for future growth predictions because of the environmental effects.

The percentage of the total genetic variance explained by individual QTL was relatively low (shown in Tables 6 and 7). Previous studies have suggested that stem traits were controlled by several loci with a relatively large effect (Bradshaw and Stettler 1995; Grattapaglia et al. 1996). These studies, however, were carried out with considerably smaller sample sizes and so were less likely to detect QTL of small effect. The sum of the genetic variation explained by QTL for each trait in this study ranged from 3.9 to 75.3% of the total genotypic variation, suggesting that additional, undetected effects may be segregating in this pedigree, in particular for stem volume, as this consistently showed low percentage variation explained by the mapped QTL.

The identification of the genetic regions solely for a trait of interest may miss important regulatory information

particularly in cases where environment sensitivity is present. It is of utmost importance to understand the regulation of G×E whether the breeding aim is for future crops stable across environments or for the selection of individuals best suited to specific environments and cultural practices. An advantage of the QTL mapping technique used here allowed the simultaneous treatment of data from multiple environments providing an increased statistical power of QTL detection and accuracy of the estimates of QTL position and effect (Jansen et al. 1995). Using more traditional methods, the difficulty in detecting QTL of small effect may result in QTL being identified in one environment and not another being falsely assumed to be differential genetic control across environments. The advantage of the method used here is that many environments may be studied at one time, while previous studies have tended to focus on the comparison of just two environments (Wu and Stettler 1997). The simultaneous analysis of the multiple sites resulted in a single logarithmic odds value for the presence of QTL across the sites, i.e. a single test for significance, but PEV and additive genetic effects were calculated as site specific to be able to analyse QTL, which showed site-specific effects.

In this study, advantage has been taken of the MultiQTL software that allows the analysis of different environments simultaneously and the creation of submodels with genetic parameters set as equal or unequal at the different sites. Permutation tests were then used to identify the best model. The combination of MIM with the multiple environment approach increases the accuracy of the estimated QTL position. This makes the MultiQTL software an ideal package for the type of work carried out here. However, a drawback of using this software is that it is designed for pure breeding lines, so it does not allow for more than two alleles segregating at a locus. This meant that, despite using an F<sub>2</sub> pedigree, the data was analysed using a two-way pseudo-testcross approach put forward by Grattapaglia and Sederoff (1994).

The AMMI model combines ANOVA and principal components analysis into a single model with additive and multiplicative parameters. An advantage of the AMMI model over other methods used to obtain stability parameters (relative genotypic ecovalences, for instance; Wricke 1962) is that the resulting parameters display a normal distribution, enabling QTL mapping without the need for transformation of data. Spearman's rank analysis showed evidence of changes in rank order of genotypes at the Italian site when compared to the UK and French sites. The AMMI model is sensitive to crossover interaction (COI), while many other methods give equal weights to COIs and non-COIs. Emebiri and Moody (2006) compared a number of different stability parameters to map as QTL. Their results showed that the parameters obtained using AMMI

were perfectly correlated to those obtained from the much-used regression approach, SLOPE (Eberhart and Russell 1966), and resulted in identical genetic information. Emebiri and Moody (2006) concluded that for the barley pedigree analysed for heading date, the allele sensitivity model, put forward by Via et al. (1995), appears to be prevalent. However, the authors point out that they would be unsure if similar results would be obtained for traits that are more genetically complex than heading date, such as grain yield. Work on *Populus* by Wu et al. (1998) concluded that the gene regulation model was the more likely mechanism controlling phenotypic plasticity for growth traits across heterozygous environments, which is in agreement with the results of this study.

The large biomass yields and high transgressive segregation illuminate the potential of these trees for bioenergy and for future breeding programs. With the release of the poplar genome sequence, the first for a tree, in 2004 (<http://genome.jgi-psf.org/Poptr1/Poptr1.home.html>), combined with the availability of a *Populus* microarray, this should allow the identification of the genes associated with QTL for stem characteristics within the QTL hotspots identified to further understand the interplay of these genes in different environments.

In conclusion, this study found that there was strong evidence of heritable genetic variation for stability parameters and that the genetic regulation model best explains plasticity across environments for stem and biomass traits in this poplar pedigree. This highlights the necessity to identify the regions regulating G×E interactions for future breeding.

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