Phenotypic plasticity in *Populus trichocarpa* clones across environments in the Nordic–Baltic region

Ann Christin Rönnberg-Wästljung, Anneli Adler, Almir Karacic, Kaspars Liepins, Thomas J. Richards, Pär K. Ingvarsson & Martin Weih

Taylor & Francis
Taylor & Francis Group

BRIEF REPORT

🔓 OPEN ACCESS | Check for updates

Phenotypic plasticity in *Populus trichocarpa* clones across environments in the Nordic–Baltic region

Ann Christin Rönnberg-Wästljung 🅘[a], Anneli Adler[b], Almir Karacic[b], Kaspars Liepins[c], Thomas J. Richards[a,d], Pär K. Ingvarsson 🅘[a] and Martin Weih 🅘[b]

[a]Department of Plant Biology, Uppsala BioCentrum, Linnean Centre for Plant Biology, Swedish University of Agricultural Sciences, Uppsala, Sweden; [b]Department of Crop Production Ecology, Linnean Centre for Plant Biology, Swedish University of Agricultural Sciences, Uppsala, Sweden; [c]Department of Forest Regeneration and Establishment, Latvian State Forest Research Institute (SILAVA), Salaspils, Latvia; [d]School of Biological Sciences, The University of Queensland, St. Lucia, QLD, Australia

ABSTRACT

Transition towards a bio-based society requires large amounts of woody biomass to be converted into biofuels and biomaterials. *Populus* species are good candidates for growth in short rotations, but there is a lack of climate-adapted plant material suitable for growth at the high latitudes of the Nordic–Baltic region. Here we studied the growth and phenology traits in 63 *Populus trichocarpa* clones earlier preliminary selected for growth at northern latitudes, in three different field sites; i.e. in central Sweden, eastern and western Latvia. The material showed moderate broad sense heritabilities, with high values for phenology traits, indicating opportunities for selection. Genotype × environment (g × e) interaction was identified for all traits, but the phenotypic correlation between pairs of sites provided more detailed information indicating the strength of the g × e interaction. The between-clone variation in plasticity was high, and we identified some clones showing a high and stable performance across the three sites. These clones are of particular interest for the commercial deployment and future breeding of *Populus* material for the Nordic–Baltic region.

Introduction

Fast-growing *Populus* species represent an interesting alternative for meeting the increasing demands of woody material for the production of biofuels and biomaterials. A rising interest for growing poplars in northern Europe and specifically in the Nordic–Baltic region actualised the issues of climate and/or photoperiod adaptation since most commercially available poplar clones in Europe are adapted to and used in more southern temperate regions. Such a southern poplar material has been previously tested in Sweden and Lithuania with the aim to select clones usable at northern latitudes (Ilstedt 1996; Pliura et al. 2014). Previous studies indicated that transferring material from the photoperiod conditions at southern latitudes often leads to a delayed (or late) growth cessation in the fall and an increased risk of early frost damage when they are grown under the photoperiod conditions at northern latitudes (Weih 2004). Consequently, new poplar plant material is needed that is better adapted to a shorter growing season and the specific photoperiod conditions at northern latitudes. Breeding efforts directed towards an earlier growth cessation of the material were made during the 1990s and resulted in a population of better adapted *P. trichocarpa* clones (Istedt 2005), which are the basis for the investigations in this study.

One issue of particular importance in the study of the new poplar material is related to genotype × environment (g × e) interaction, meaning that the plant material (genotypes, clones) shows differential trait responses when moved from one environment to another, resulting in a genotype or clone rank shift when the trait values measured in the different environments are compared. A substantial g × e interaction must be taken into consideration by either dividing the environments into different breeding zones or by selecting plant material with stable performances across environments. In a study of poplar material across various sites in Sweden and the Baltic countries, Adler et al. (2021) suggested that the g × e interactions should lead to a division of the whole region into three different breeding zones. Still another way of characterising the plant material is a classification according to its plasticity across environments, with plasticity defined as the variation in the phenotypic expression observed under different environmental conditions (Nicotra et al. 2010).

In this study, we used a set of 63 *Populus trichocarpa* clones that previously had been selected for more northern conditions (Istedt 2005). The aim was to evaluate clonal variation with respect to the growth and phenology traits observed across three different sites in the Nordic–Baltic region, including the g × e interaction and the plastic

---

responses of these clones when grown in three different environments.

Material and methods

The plant material used for this study consisted of F_1 genotypes resulting from 25 intraspecific crosses between selected *Populus trichocarpa* ecotypes from North America (see Apuli et al. 2021; Istedt 2005; Richards et al. 2020). One to twelve genotypes from the different families, and in total, 63 genotypes were used. Originally, vegetatively propagated F_1 genotypes from a larger set of crossings were planted 2003 in a field experiment at the site Krusenberg located nearby Uppsala, Sweden (59°44′44.2″N, 17°40′31.5″E) (Supplementary file 1), using an experimental set-up with four completely randomised blocks (Richards et al. 2020). All 63 clones used in this study originated from this field experiment, and each clone had from one to seven plants per block, in total 544 plants. Twig material from nine-year-old trees in Krusenberg was taken for vegetative propagation of the selected set of clones for planting in two Latvian field experiments, Talsi (57°23′ 20.57″N, 22°27′ 39.20″E) and Ludza (56°43′ 03.39″N, 27°40′59.61″E), where Talsi has a more maritime climate compared to the more continental climate in Ludza (Supplementary file 1). The Latvian experiments were planted in 2013 in four blocks with five randomly positioned plants per clone within each block. A total of 1181 and 995 plants in Ludza and Talsi, respectively, were selected for analyses of those 63 clones which were in common between the three sites. At all field experiments, measurements were taken during 2016 (height) and 2017 (phenology and growth traits) at a plant age of 13 or 14 years in Krusenberg and at a plant age of 4 or 5 in the Latvian experiments. During spring 2017, bud burst was scored repeatedly during the season according to a scale from 0 to 5, where 0 indicates that bud burst was not initiated and five means that buds were fully leafed out (Richards et al. 2020). In the fall of 2017, colouring of the leaves was scored (col17) using a scale of 1–8 where colouring grade 1 represented 100% green canopy and colouring grade 8 a 100% yellow canopy. Leaf senescence (ls17) was scored in 2017 using a scale from 1 to 3, where 1 = full foliage, 2 = half the foliage shed and 3 = fully defoliated (Richards et al. 2020). Diameter at breast height (dbh17) as well as height (h17) of the tree was measured during fall 2017. When repeated measurements were taken for phenology traits, the time point with the largest standard deviation across clones was chosen for the analyses.

All statistical analyses were made with the programme JMP (SAS Institute Inc. 2019. JMP® 15 Documentation Library. Cary, NC: SAS Institute Inc.). Analysis of variance (ANOVA) was conducted using the complete dataset with the model:

$$Y_{ijk} = m + s_i + b_{j(i)} + c_k + sc_{ik} + e_{ijk}. \qquad (1)$$

Where Y_{ijk} represents the observed value for a trait of an individual, m = the overall mean, s_i the fixed site effect (i), $b_{j(i)}$ the fixed block effect (j) within site (i), c_k the fixed clone effect (k), scik the interaction effect between site and clone,

and e_{ijk} the experimental error. For each experimental site separately, the analysis was made with the model:

$$Y_{ij} = m + b_i + c_j + e_{ij}. \qquad (2)$$

The effects have the same explanations as shown in model (1) except for the clone effect that here was considered random to be able to estimate variance components. Broad sense heritabilities (H^2) were calculated from variance components taken from (2), according to the formula:

$$H^2 = \sigma_c^2/(\sigma_c^2 + \sigma_e^2). \qquad (3)$$

Phenotypic plasticity was estimated as the standardised (var = 1, mean = 0) trait difference on the BLUP-values from model (2) between pairs of experiments. Phenotypic correlations between experimental sites and between traits at the same site were estimated with Pearson product-moment correlations based on BLUP values taken from the analysis with model (2).

Results

Broad sense heritabilities varied between 0.13 and 0.50 for the growth traits and between 0.35 and 0.61 for the phenology traits (Table 1), the lowest values always being recorded in Talsi. Still, the estimated heritabilities indicate a considerable clonal variation for most of the traits (Table 1).

The ANOVA, using the overall model, showed highly significant ($p < .001$) differences between sites, block(site) and clones for all traits and the interaction between clone and site was also highly significant ($p < .001$) for all traits. The phenotypic correlations between sites provided more specific information on which pairs of sites caused the g × e interactions, and correlation values close to 0 indicated strong g × e interaction and values closer to 1 indicated weak or no g × e interaction. We found significant correlations between the different site pairs for all traits, but the highest correlations were found among phenology traits, thus implying a low g × e interaction. This was especially true for bud burst between Talsi and Ludza (Table 1). The autumn phenology traits for both leaf colouring and senescence in 2017 (col17 and ls17) showed lower correlations between Krusenberg and Ludza, indicating strong g × e interaction (Table 1). The lowest correlation for the growth traits was for diameter at breast height (dbh17) between Krusenberg and Talsi, indicating strong g × e interaction; the remaining site correlations for growth traits were around 0.5, demonstrating moderate g × e interaction (Table 1).

Plasticity was defined as the difference in standardised BLUP values between sites, high and low values show that the respective clones have differential responses in growth or phenology traits, while values close to zero mean stable trait values across sites. Plasticity varied greatly between clones for all traits and for all site comparisons (Figure 1). The comparison between Ludza and Talsi revealed a lower variation of plasticity values than the comparisons with the Swedish site, indicating a more stable clonal trait expression between the two Latvian sites (Figure 1(C, G)). Furthermore, the clones with the greatest plasticity values in the

Table 1. Broad sense heritabilities in % (bold italics in the diagonals) and phenotypic correlations between sites for 63 poplar clones.

		Krus	Ludza	Talsi		Krus	Ludza	Talsi
			bb17				**h16**	
Krus	col17	*61* / *61*	0.56	0.51	h17	*37* / *37*	0.44	0.51
Ludza		0.41	*53* / *56*	0.89		0.41	*20* / *18*	0.51
Talsi		0.67	0.67	*52* / *48*		0.45	0.48	*18* / *13*
Krus	ls17	*52*			dbh17	*50*		
Ludza		0.33	*58*			0.52	*22*	
Talsi		0.55	0.48	*35*		0.26	0.42	*17*

Note. Red indicate significance at $p < .0001$, blue indicate significance where $.0001 < p < .001$ and black $.001 < p < .05$. Trait abbreviations: Krus = Krusenberg; bb = bud burst; col = colouring of leaves; ls = leaf shed; h = height of the tree; dbh = diameter at breast height; number after abbreviation equals a year of measurement.

comparison between Krusenberg and Ludza had also large values in the comparison between Krusenberg and Talsi, while they were closer to zero in the Ludza–Talsi comparison; indicating that the Latvian sites were more similar in terms of growth and phenology than each of them compared to the Swedish site (Figure 1). Despite the larger differences in clonal responses between Krusenberg and the Latvian experiments, there were some high-performing and early-starting clones with a stable performance across all sites (Figure 1). For bud burst in 2017 (bb17), four out of the five most stable and early-starting clones in each site were the same, and five of the most stable clones were the same in two

comparisons (Figure 1). Similarly, for height in 2017 (h17), clone 722.16 was stable, high producing and early-starting across all sites (Figure 1).

In terms of phenotypic correlations between traits within sites, we found high correlations between h17 and dbh17 at all sites, as well as between the two autumn phenology traits (Supplementary file 2). In Krusenberg, the trait col17 was significantly negatively correlated with all growth traits, indicating that early leaf colouring generally is associated with lower biomass growth. Only in Ludza, the leaf shedding trait (ls17) was inversely correlated with all growth traits (Supplementary file 2). The spring phenology trait bud burst
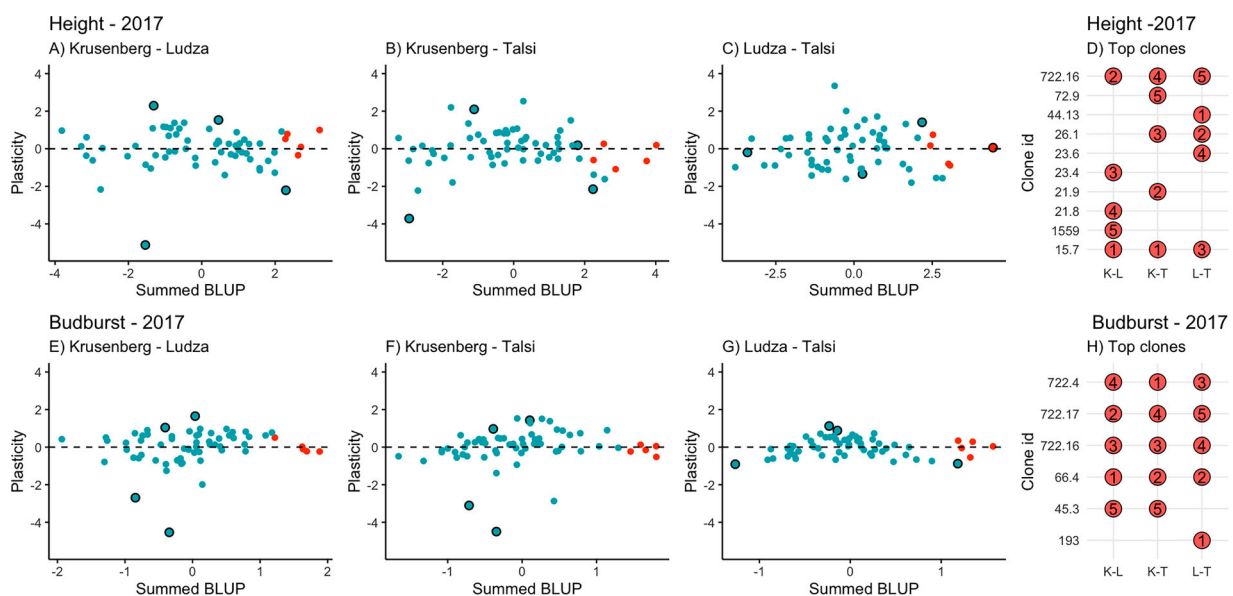
Figure 1. Plots of plasticity and summed standardised BLUP (summed BLUP) values for height of 63 poplar clones measured 2017 (panel A–C) and bud burst measured 2017 (panel E–G) for the three pairs of sites: Krusenberg–Ludza (K–L), Krusenberg–Talsi (K–T) and Ludza–Talsi (L–T). In panel D and H the top clones based on low plasticity and high summed BLUP values are shown with the ranking (1–5) for height 2017 and bud burst 2017 respectively. Clones with the extreme (highest and lowest) plasticity values in the comparison between Krusenberg and Ludza are indicated with a circle (A, E), the same individuals are also marked with circles in the other pairs of comparison (B, C and F, G).

(bb17) was significantly positively correlated with the diameter at breast height (dbh17) only in Krusenberg, indicating that an early bud burst is connected to higher biomass growth (Supplementary file 2).

Discussion

In this study, we compared a set of 63 *Populus trichocarpa* clones across three sites in the Nordic–Baltic region to identify plant material with a high potential to be commercially deployed and further bred towards better climate adaptation and increased poplar cultivation in the region. Our attempt was to study variation, genotype × environment interaction and the plasticity of clones, and to identify plant material with stable and high performance in growth across the three environments investigated.

The generally moderate heritabilities observed in this material show that the existing variation can be utilised for breeding. Lower heritabilities for growth-related traits compared to phenology traits, as we have found in this study, are commonly observed and have been reported frequently in *Populus* and *Salix* species (Adler et al. 2021; Hallingbäck et al. 2019; Richards et al. 2020). This was especially the case in the younger field experiments, which could be an effect of the inter-annual weather variation during establishment and early years of growth. The generated knowledge of g × e interactions in this study is important for the further breeding process based on the clonal population addressed here. Different breeding strategies could be adopted, either to breed for clonal material with high production potential across all sites or to breed for clones that are specifically adapted to the climate and site conditions in a specific environment. In our study, g × e interaction was highly significant for all traits, reflected by rank shifts between clones from one site to another. With a closer look into the g × e using phenotypic correlations between pairs of sites, we found significant correlations indicating weak or no g × e interaction between all the site combinations, although some of these correlations had very low correlation coefficients. For bud burst, the correlation coefficients were high, especially for the Latvian sites. In an earlier study including six sites in the Nordic–Baltic region, Adler et al. (2021) showed that southern and central Sweden and Latvia could be the same breeding zone based on high correlations between sites and relatively few rank shifts from one site to another. In that study (Adler et al. 2021), a smaller number of clones was included compared to the current study, which influences the strength of the pairwise correlations. Also, Vico et al. (2021) reported a few shifts in the clone ranks between the studied sites in central Sweden and Latvia, indicating low g × e interaction. In this study (Vico et al. 2021) only addressed phenology traits and included relatively few clones, but for bud burst, their results were similar as in the current study and both reported low g × e interaction. To further explore the trait performances of specific clones across all sites, we studied clonal plasticity between the three possible pairs of sites. In general, variation in plasticity was considerable. The individuals showing extreme plasticity values in the comparison between Krusenberg and Ludza also represented extremes in the comparison between Krusenberg and Talsi. In the comparison of Latvian sites, the same clones were closer to zero plasticity value, which indicates a lower g × e interaction between these sites. When combining plasticity values with a summed performance measure (summed BLUP) across all paired site combinations, we identified several clones that were stable (plasticity close to zero) and had high-performance values with respect to biomass and phenology traits. In particular, one stable, early-starting and highly productive clone (722.16) was identified, and we consider this clone as a good candidate for the commercial deployment and further breeding for the range of latitudes represented by our experimental sites in the Nordic–Baltic region.

Earlier studies have shown that especially fall phenology traits often are (negatively) correlated with growth performance traits (e.g. Pliura et al. 2014). Also, in our study, fall phenology (mainly ls17) was inversely correlated with both height and diameter growth at the Ludza site, and, as a tendency for col17 vs. growth traits, also in Krusenberg. In contrast, no significant correlations between fall phenology and growth traits were found in Talsi. A negative correlation between fall phenology and growth traits indicates that, e.g. a late colouring of leaves and late leaf fall will result in a higher potential growth through the extension of the growth period into the late summer/autumn. In general, late autumn phenology implies an increased damage risk due to early autumn frost. However, the clones used in our study, and earlier selected for the climate conditions in central Sweden (Ilstedt 1996), appear to be quite well-adapted even to the conditions of the Latvian climate.

In conclusion, the tested clonal material showed ranking shifts between the environments tested, and thus considerable g × e interaction that could be problematic for further breeding. Still, the material also accommodated some clones that were stable and high performing across all environments investigated. These clones could be used in the further breeding efforts towards well-adapted material in the Nordic–Baltic region.

Data availability

The data used for analyses in this manuscript are available from the corresponding author on request.

Disclosure statement

No potential conflict of interest was reported by the author(s).

ORCID

Ann Christin Rönnberg-Wästljung http://orcid.org/0000-0002-5241-7161
Pär K. Ingvarsson http://orcid.org/0000-0001-9225-7521
Martin Weih http://orcid.org/0000-0003-3823-9183

References

Adler A, Karacic A, Wästljung A-CR, Johansson U, Liepins K, Gradeckas A, Christersson L. 2021. Variation of growth and phenology traits in poplars planted in clonal trials in Northern Europe—implications for breeding. Bioenergy Res. 14:426–444.

Apuli R-P, Richards T, Rendón-Anaya M, Karacic A, Rönnberg-Wästljung A-C, Ingvarsson PK. 2021. The genetic basis of adaptation in phenology in an introduced population of Black Cottonwood (Populus trichocarpa. Torr. & Gray). BMC Plant Biol. 21(1):317.

Hallingbäck HR, Berlin S, Nordh NE, Weih M, Rönnberg-Wästljung AC. 2019. Genome wide associations of growth, phenology, and plasticity traits in willow (*Salix viminalis* (L.)). Front Plant Sci. 10:753.

Ilstedt B. 1996. Genetics and performance of Belgian poplar clones tested in Sweden. Forest Genetics. 3(4):183–195.

Istedt B. 2005. Anpassning av Populus trichocarpa, jättepoppel, till svenskt klimat. In: Christersson L, Verwijst T, editor. Proceedings from a poplar seminar at the department of short rotation forestry. Uppsala: Swedish University of Agricultural Sciences; p. 47–50.

Nicotra AB, Atkin OK, Bonser SP, Davidson AM, Finnegan EJ, Mathesius U, Poot P, Purugganan MD, Richards CL, Valladares F, van Kleunen M. 2010. Plant phenotypic plasticity in a changing climate. Trends Plant Sci. 15(12):684–692.

Pliura A, Suchockas V, Sarsekova D, Gudynaitė V. 2014. Genotypic variation and heritability of growth and adaptive traits, and adaptation of young poplar hybrids at northern margins of natural distribution of Populus nigra in Europe. Biomass Bioenergy. 70:513–529.

Richards TJ, Karacic A, Apuli R-P, Weih M, Ingvarsson PK, Rönnberg-Wästljung AC. 2020. Quantitative genetic architecture of adaptive phenology traits in the deciduous tree. Populus trichocarpa (Torr. and Gray). Heredity. 125:449–458.

Vico G, Karacic A, Adler A, Richards T, Weih M. 2021. Consistent poplar clone ranking based on leaf phenology and temperature along a latitudinal and climatic gradient in Northern Europe. Bioenerg Res. 14:445–459.

Weih M. 2004. Intensive short rotation forestry in boreal climates: present and future perspectives. Can J For Res. 34:1369–1378.

Appendix

Supplementary file 1. Location and climatic data for the three different field experimental sites; Krusenberg, Ludza and Talsi.

Supplementary file 2. Phenotypic correlations between different traits on BLUP values for each poplar clone grown at the sites Krusenberg, Ludza and Talsi. Red numbers indicate phenotypic correlations with significance at $p < .0001$, blue values indicate significance where $.0001 < p < .05$.