

Early evaluation of growth traits of *Larix kaempferi* clones

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Received: 6 December 2016 / Accepted: 22 December 2016 / Published online: 23 October 2017
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Abstract Early selection is an important method to shorten the breeding cycle for tree species, which may differ in the time for early selection. To evaluate the early selected time for *Larix kaempferi*, tree height and diameter at breast height of 57 *L. kaempferi* clones were measured over many different growth years. The results indicated that, except for age × clone interaction for diameter at breast height ($P = 0.741$), there were significant differences among all variation sources ($P < 0.01$). The coefficient of phenotypic variation ranged from 14.89 to 35.65%

for height and from 19.17 to 23.86% for diameter at breast height in different growth years. The repeatability of height and of diameter at breast height among clones was high, ranging from 0.6181 to 0.8531 (height) and from 0.8443 to 0.8497 (diameter at breast height), in different growth years. There were significant positive correlations between all pairs of growth traits except between height in the 2nd growth year and height in the 30th growth year; and between height in the 2nd growth year × diameter at breast height in the 30th growth year. With a comprehensive evaluation method and a selection ratio of 10%, L65, L1, L62, L9, L15, and L78 were selected as excellent clones in the 30th growth year. Their average values of height and diameter at breast height were 9.81 and 16.57% higher than the overall average, representing genetic gains of 6.46 and 13.99%, respectively. This study provides a theoretical foundation for the genetic improvement of *L. kaempferi*.

Project funding: The work was supported by the Innovation Project of State Key Laboratory of Tree Genetics and Breeding (Northeast Forestry University) (No. 2016C02) and China Postdoctoral Science Foundation (2014M561315).

The online version is available at <http://www.springerlink.com>

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Keywords *Larix kaempferi* · Genetic · Variation · Comprehensive evaluation

Introduction

Larix kaempferi (*Larix* Mill) has become one of the most important timber and afforestation species in China since it was introduced from Japan 100 years ago (Toda and Mikami 1976). Because of its strong adaptability, wide distribution, and rapid growth rate (Kurinobu 2005), *L. kaempferi* is used for many purposes, such as building, furniture making, paper making, and biofuel (Buczyc et al. 1997; Fukatsu et al. 2013). Previous studies on *L. kaempferi* have focused on the selection of provenances (Teruyoshi et al. 2014; Nagasaka et al. 2011), families (Hou

et al. 2012), and clones (Liu et al. 2009), as well as its wood properties (Pâques et al. 2010; Apiolaza 2009), photosynthetic characters (Fukatsu et al. 2008; Crane et al. 1983), genetic diversity (Achere 2004; Arcade et al. 2000), and molecular markers (San et al. 2009; Moriguchi et al. 2008).

Among the several studies that focused on genetic variation in growth traits of *L. kaempferi*, most were based on trait measurements in a single year (Xu et al. 2014; Zhang et al. 2013; Takata et al. 1992). However, for selecting superior materials, genetic variations in the chosen variables need to be monitored over several growth years (Zhao et al. 2013; Xia et al. 2016). Early selection can be conducted if growth parameters are correlated among different growth years (Weng et al. 2007). In previous studies, Jiao et al. (2005) determined that the early selection time for *L. kaempferi* clones should be the 6th growth year, in contrast to the 10th year reported by Ma (2006). Different materials and growth environments may lead to different phenotypes, resulting in a range of estimates for the best early selection time. In the present study, for determining the most appropriate time for early selection of *L. kaempferi* seedlings, we measured tree height and diameter at breast height of 57 *L. kaempferi* clones over many years. Different clones were selected as excellent materials in different years under various selection ratios, and a suitable early selected time was proposed. This research provides a theoretical basis for breeding improvement of *L. kaempferi*.

Materials and methods

Materials and experimental design

The experimental field was located at Wudaogou Forestry Farm, Liuhe County (N 41°54', E 125°17'), Jilin Province, China. This area has a continental monsoon climate. The annual average temperature, precipitation, frost-free period, and duration of sunshine of this area are 5.5 °C, 736 mm, 145 days, and 2479 h, respectively.

The 57 clones of *L. kaempferi* (L1–L26, L60–L90) used for this study were propagated by grafting, and experimental plantations were established with 1-year-old plants in the spring of 1988 in a completely randomized block design (Marron and Ceulemans 2006) with 4 replication blocks and 10-tree row plot and 3.0 × 4.0 m spacing.

Data collection

The tree height (H) of all living and unbroken trees was measured from 1988 (2nd growth year) to 1994 (8th growth year), and in 1996 (10th growth year), 2003 (17th growth

year) and 2016 (30th growth year), respectively. The diameter at breast height (DBH) of each tree was measured in 2003 (17th growth year) and in 2016 (30th growth year), respectively.

Statistical analyses

All data were analyzed using SPSS software, version 19.0 (IBM, Armonk, NY, USA) (Shi 2012). The significance of fixed effects was tested by analysis of variance (One-way ANOVA) *F* tests. Variations among families in different years were determined by ANOVA according to Hansen and Roulund (1997), as follows:

$$y_{ij} = \mu + \alpha_i + b_j + \alpha b_{ij} + \varepsilon_{ij}, \quad (1)$$

where y_{ij} is the performance of an individual of clone i in year j , μ is the overall mean, α_i is the clone effect, β_j is the year effect, $\alpha\beta_{ij}$ is the random effect of clone i in year j , and ε_{ij} is the random error.

Variations among clones in the same year were determined by ANOVA according to Xu (2006) using the following equation:

$$y_{ij} = \mu + \alpha_i + \varepsilon_{ij}, \quad (2)$$

where y_{ij} is the performance of individual clone i , μ is the overall mean, α_i is the clone effect ($i = 1, \dots, 50$), and ε_{ij} is the random error.

The coefficient of phenotypic variation (PCV) was calculated using the formula (Hai et al. 2008):

$$\text{PCV} = \text{SD}/\bar{X} \quad (3)$$

where SD is the standard deviation and \bar{X} is the phenotypic mean of the trait, respectively.

Repeatability (R) was calculated according to Hansen and Roulund (1997) as:

$$R = \frac{\sigma_A^2}{\sigma_A^2 + \sigma_b^2 + \sigma_e^2}, \quad (4)$$

where σ_A^2 is the additive genetic variance component among clones, σ_b^2 is the block variance, and σ_e^2 is the error variance component.

The phenotypic correlation, $r_A(xy)$, of traits x and y was calculated according to Bi et al. (2000), as:

$$r_A(xy) = \frac{\text{cov}_p(x, y)}{\sigma_p(x)\sigma_p(y)}, \quad (5)$$

where $\text{cov}_p(x, y)$ is the covariance between trait x and y , $\sigma_p(x)$ is the variance component for trait x , and $\sigma_p(y)$ is the variance component for trait y .

Comprehensive evaluations of different clones were calculated as described by Zhao et al. (2016), as:

$$Q_i = \sqrt{\sum_{j=1}^n a_i} \tag{6}$$

where $a_i = X_{ij}/X_{jmax}$, Q_i is a comprehensive evaluation value for each clone, X_{ij} is the average value of one trait, X_{jmax} is the maximum value of the trait; and n is the number of traits.

Genetic gain was estimated using the formula of Francisco et al. (2008):

$$\Delta G = h^2 W / \bar{X}, \tag{7}$$

where W is the selection difference, and \bar{X} and h^2 are the mean value and heritability of a given trait, respectively.

Results

Analyses of variance for H and DBH data

The H and DBH of 57 *L. kaempferi* clones were compared among different growth years by ANOVA (Table 1). All of the variance sources were significant ($P < 0.01$) except for the age \times clone interaction ($P = 0.741$) for DBH.

The ANOVA results for H and DBH among clones in the same growth year are shown in Table 2. For both H and DBH, there were highly significant differences among different clones in the same growth year ($P < 0.01$).

Genetic and phenotypic variations in different traits

The genetic and phenotypic variations in H and DBH among different clones are shown in Table 3. The average H of the 57 clones ranged from 0.53 to 19.09 m (from the 2nd to the 30th growth year). The average DBH of all clones was 17.56 cm in the 17th growth year and 29.03 cm in the 30th growth year. The PCVs of H and DBH in different growth years ranged from 14.89 to 35.65% and from 19.17 to 23.86%, respectively. The PCVs of H decreased as the trees grew. The PCVs of DBH were higher than those of H in the 17th and the 30th growth year. The R of H increased as the trees grew, and the R values were all higher than 0.6000. In the 17th and the 30th growth

year, the R of DBH was 0.8497 and 0.8443, respectively, indicating high repeatability.

Correlation analysis

Correlations among H and DBH in different growth years are shown in Table 4. There were significant positive correlations between all pairs of growth traits except for H (in the 2nd growth year) \times H (in the 30th growth year); and H (in the 2nd growth year) \times DBH (in the 30th growth year). The correlation coefficients between H at different growth years ranged from 0.018 (between 2nd and 30th growth year) to 0.974 (between 7th and 8th growth year) and the values decreased as the age interval increased. The correlation coefficients between H in consecutive years increased from the 2nd growth year (0.749) to the 8th growth year (0.974). The correlation coefficient between H and DBH in the same growth year was extremely significant (0.483 in the 17th growth year; 0.644 in the 30th growth year).

Comprehensive evaluation

In this study, excellent clones were selected based on H (in the 2nd, 4th, 7th, and 10th growth year) and Q_i values (in the 17th and the 30th growth year) with different selection ratios in different growth years. The results are shown in Tables 5 and 6. Thirty-five excellent clones were selected in the 2nd growth year under a 60% selection ratio. The average H of the selected clones was 0.56 m, which was 6.59% higher than the overall average value, and the genetic gain was 4.08%. Twenty-nine excellent clones were selected in the 4th growth year under a 50% selection ratio. The average H of selected clones was 2.06 m, which was 7.20% higher than the overall average value, and the genetic gain was 5.45%. In the 7th growth year, 23 excellent clones were selected under a 40% selection ratio. The average H of selected clones was 4.29 m, which was 7.56% higher than overall average value, and the genetic gain was 5.54%. In the 10th growth year, 18 excellent clones were selected under a 30% selection ratio. The average H of selected clones was 5.92 m, which was 7.74%

Table 1 ANOVA of H and DBH of 57 *L. kaempferi* clones

Traits	Variance source	SS	df	MS	F	Sig.
H	Age	409,896.588	9	45,544.065	22,104.679	0.000
	Clone	2039.296	56	36.416	17.674	0.000
	Age \times clone	3803.327	504	7.546	3.663	0.000
DBH	Age	98,253.091	1	98,253.091	4797.225	0.000
	Clone	14,012.702	56	250.227	12.217	0.000
	Age \times clone	998.921	56	17.838	0.871	0.741

Table 2 ANOVA of different traits in the same year of 57 *L. kaempferi* clones

Traits	Age	Variance source	SS	df	MS	F	Sig
H	2	Clone	4.931	56	0.088	2.619	0.000
	3	Clone	17.001	56	0.304	3.738	0.000
	4	Clone	50.520	56	0.902	4.126	0.000
	5	Clone	85.258	56	1.522	3.795	0.000
	6	Clone	121.702	56	2.173	3.742	0.000
	7	Clone	144.280	56	2.576	3.753	0.000
	8	Clone	178.608	56	3.189	3.876	0.000
	10	Clone	221.231	56	3.951	3.771	0.000
	17	Clone	738.470	56	13.187	6.810	0.000
DBH	30	Clone	1240.297	56	22.148	2.930	0.000
	17	Clone	5437.702	56	97.102	6.652	0.000
	30	Clone	9315.103	56	166.341	6.421	0.000

Table 3 Variation parameters of different clones in different years

Growth year	Traits	Variation range	X ± SD	PCV	R
2	H	0.38–0.63	0.53 ± 0.19	35.65	0.6181
3	H	0.89–1.33	1.12 ± 0.29	26.58	0.7325
4	H	1.59–2.32	1.92 ± 0.49	25.68	0.7576
5	H	2.17–3.09	2.63 ± 0.67	25.29	0.7365
6	H	2.74–3.86	3.31 ± 0.80	24.14	0.7327
7	H	3.42–4.57	3.99 ± 0.86	21.77	0.7336
8	H	4.03–5.39	4.68 ± 0.95	20.35	0.7420
10	H	4.72–6.35	5.49 ± 1.07	19.54	0.7348
17	H	7.69–11.12	9.03 ± 1.53	16.94	0.8531
	DBH	14–21.69	17.56 ± 4.19	23.86	0.8497
30	H	17.09–23.18	19.09 ± 2.84	14.89	0.6588
	DBH	24.55–34.51	29.03 ± 5.56	19.17	0.8443

The unit of H was m, DBH was cm, PCV was %

Table 4 Correlation analysis of different traits in different growth years in *Larix* clones

	3H	4H	5H	6H	7H	8H	10H	17DBH	17H	30DBH	30H
2H	0.749**	0.581**	0.496**	0.439**	0.393**	0.390**	0.373**	0.149**	0.130**	0.018	0.032
3H		0.844**	0.750**	0.678**	0.633**	0.610**	0.579**	0.286**	0.215**	0.122**	0.092**
4H			0.923**	0.856**	0.811**	0.784**	0.752**	0.441**	0.338**	0.256**	0.143**
5H				0.957**	0.921**	0.892**	0.860**	0.552**	0.406**	0.354**	0.193**
6H					0.966**	0.946**	0.916**	0.605**	0.457**	0.399**	0.198**
7H						0.974**	0.943**	0.650**	0.453**	0.470**	0.273**
8H							0.967**	0.651**	0.494**	0.460**	0.258**
10H								0.661**	0.507**	0.467**	0.265**
17DBH									0.644**	0.764**	0.380**
17H										0.473**	0.219**
30DBH											0.483**

**Correlation is significant at 0.01 level (2-tailed)

higher than the overall average value, and the genetic gain was 5.69%.

In the 17th growth year, 12 excellent clones were selected under a 20% selection ratio with the

comprehensive evaluation method. The average H and DBH of selected clones was 10.19 m and 20.67 cm, which were 12.78 and 17.71% higher than their respective overall average values, and the genetic gain was 10.91 and

Table 5 Excellent clones selected under different selected rate in different growth years

The 2nd growth year		The 4th growth year		The 7th growth year		The 10th growth year		The 17th growth year		The 30th growth year	
Clones	H	Clones	H	Clones	H	Clones	Qi	Clones	Qi	Clones	Qi
L78	0.63	L9	2.32	L9	4.57	L9	6.35	L15	1.41	L65	1.41
L12	0.62	L62	2.23	L62	4.55	L65	6.15	L1	1.41	L1	1.39
L15	0.62	L78	2.22	L65	4.53	L62	6.11	L10	1.39	L62	1.39
L25	0.61	L1	2.19	L15	4.50	L1	6.07	L9	1.38	L9	1.39
L63	0.61	L65	2.19	L1	4.48	L15	6.07	L8	1.38	L15	1.38
L14	0.60	L72	2.16	L72	4.41	L8	6.02	L62	1.37	L78	1.38
L9	0.60	L3	2.13	L78	4.34	L25	5.97	L65	1.37		
L86	0.60	L10	2.13	L10	4.31	L78	5.95	L78	1.37		
L60	0.60	L25	2.12	L86	4.31	L10	5.93	L86	1.35		
L81	0.60	L20	2.12	L20	4.31	L72	5.91	L20	1.31		
L71	0.60	L15	2.11	L25	4.28	L86	5.86	L6	1.31		
L8	0.59	L14	2.11	L8	4.27	L14	5.78	L7	1.29		
L90	0.58	L86	2.10	L90	4.26	L87	5.77				
L10	0.57	L81	2.10	L60	4.23	L20	5.77				
L65	0.57	L90	2.08	L67	4.21	L85	5.74				
L62	0.57	L24	2.07	L3	4.20	L60	5.70				
L1	0.56	L13	2.02	L24	4.19	L67	5.70				
L72	0.56	L80	2.01	L87	4.16	L4	5.69				
L80	0.56	L63	2.01	L66	4.16						
L76	0.56	L8	2.00	L14	4.13						
L2	0.55	L66	2.00	L85	4.12						
L6	0.55	L71	1.99	L17	4.12						
L26	0.55	L87	1.98	L82	4.06						
L77	0.54	L67	1.95								
L61	0.54	L17	1.95								
L66	0.53	L61	1.95								
L16	0.53	L16	1.94								
L24	0.53	L12	1.93								
L75	0.53	L7	1.92								
L4	0.53										
L23	0.52										
L74	0.52										
L11	0.51										
L13	0.50										
L17	0.50										

15.04%, respectively. In the 30th growth year, six excellent clones were selected under a 10% selection ratio with the comprehensive evaluation method. The H and DBH of selected clones was 20.96 m and 33.84 cm, respectively, 9.81 and 16.57% higher than their overall average values. The genetic gain in H and DBH in the 30th growth year was 6.46 and 13.99%, respectively.

Discussion

Analysis of variance

Among the various statistical analyses, ANOVA is the most important method to estimate the extent and significance of variability (Zhao et al. 2014). In this study, the ANOVA results showed that all sources of variance were highly significant ($P < 0.01$), except for the age \times clone interaction for DBH ($P = 0.741$). These results were consistent with those reported for *Pinus taeda* (Isik et al.

Table 6 Average and variation parameters of selected clones in different year

Growth year	Traits	Average	Higher than overall average value (%)	Genetic gain (%)
The 2th	H	0.56	6.59	4.08
The 4th	H	2.06	7.20	5.45
The 7th	H	4.29	7.56	5.54
The 10th	H	5.92	7.74	5.69
The 17th	H	10.19	12.78	10.91
	DBH	20.67	17.71	15.04
The 30th	H	20.96	9.81	6.46
	DBH	33.84	16.57	13.99

The unit of H was m, DBH was cm

2003) and *L. kaempferi* (Nakada et al. 2005), indicating that the selection of excellent clones is practical and feasible.

Genetic variation analysis

Genetic and phenotypic variations are the main factors in tree genetic and breeding (Mwase et al. 2008), and both are very important for the selection and evaluation of excellent clones (Safavi et al. 2010). In the process of tree growth, genetic and phenotypic variations in growth traits undergo dynamic changes and show certain patterns (Karacic and Weih 2006). Understanding the dynamic changes in genetic variables is important for early selection (Pliura et al. 2007) and the genetic improvement of forest trees (Sixto et al. 2011; Neale 2007). In this research, the PCVs of H ranged from 35.65 to 14.89%, and the PCVs decreased as the trees grew. A similar phenomenon was reported in other studies (Shigematsu 1991; Oshima 1998); that is, the differences in H among clones decreased as the trees grew. In the 30th growth year, the PCV value was 14.89%, suggesting that tree height was close to the critical value.

The size of the *R* value indicates the reliability with which the genotype will be recognized by its phenotypic expression (Zhao et al. 2013). The *R* values of H and DBH ranged from 0.6181 to 0.8531 and from 0.8443 to 0.8497, respectively, in different growth years. These high values indicated that the variation in H and DBH was strongly controlled by genetic factors, consistent with the results of a previous study (Du et al. 2015). For H, the *R* values increased as the trees grew. Similar findings were also reported for *Picea abies* (Isik et al. 2010) and *Pinus sylvestris* (Haapanen 2001), and indicated that the effect of the environment decreased as the trees grew. Overall, high *R* values indicate that the selection for excellent traits would be effective (Maniee et al. 2009). The high variation and *R* values of growth traits in this study were useful for the selection and evaluation of excellent clones.

Correlation analysis

In previous studies, age–age correlations of growth traits were important parameters for early selection (Goncalves et al. 2005). Age–age correlations for growth have been reported for *Pinus radiata* (Matheson et al. 1994; King and Burdon 1991), but the relationship between H and DBH was rather complex (Sumida et al. 2013). In this study, there were significant positive correlations between all pairs of growth traits except for H (in the 2nd growth year) \times H (in the 30th growth year); and H (in the 2nd growth year) \times DBH (in the 30th growth year). These results indicated that there was a close relationship between early and late growth traits.

The results indicated that early selection of trees was feasible (Pâques 2004). The correlation coefficients between DBH (in the 17th and 30th growth year) and H (in the 2nd to 10th growth year) were higher than those between H (in the 17th and 30th growth year) and H (in the 2nd to 10th growth year). Therefore, although H would be a good selection variable for clones with fast growth at an early stage, DBH would be a better to select clones with strong growth at a later stage, consistent with the findings of another study (Zas et al. 2004). The high correlation coefficients also suggested that excellent clones could be selected early based on their measured traits.

Selection of excellent clones

Early selection is an important method to shorten the breeding cycle and accelerate the selection process (Kroon et al. 2011; Kumar and Singh 2001). The optimum age for selection depends on the correlation coefficients between various variables and on genetic stability (Gwaze and Bridgwater 2002). The optimum age for early selection of *L. kaempferi* differed depending on the selected breeding targets (Fujimoto et al. 2006; Diao et al. 2016). In terms of growth traits, some studies found that H was the best factor for early selection (Sun et al. 2004; Lai et al. 2014). The optimum age for early selection was reported to be the 6th

to the 8th growth year (Sun 2003; Balocchi et al. 1993). Other studies suggested that DBH was a more appropriate than H for early selection (Vasquez and Dvorak 1996) because it is harder to measure H accurately as trees grow and the DBH measurements were more accurate (Zhou et al. 2008). In this research, H was selected as an early evaluation index, and different clones were selected in different growth years under different selection ratios. Under a selection ratio of 10%, the same excellent clones were selected in the 7th and the 30th growth year, indicating that the early selection of these materials could be conducted from the 6th to the 8th growth year.

Some studies reported that selection based on dual growth traits (H and DBH) was more effective than that based on a single trait (Collet and Chenost 2006; Chen et al. 2003), which has important implications for breeding programs. In this study, the combination of H and DBH was used to select excellent clones in the 30th growth year, and L65, L1, L62, L9, L15, and L78 were selected as the excellent clones. Among these clones, L65 and L62 showed fast growth in the later growth stage, but clones L1, L9, L15, and L78 grew fast all the time. Thus, the clones had different growth patterns, consistent with the findings of another study (Jansson et al. 2003). In the process of selecting clones, the use of appropriate selection ratios and the choice of appropriate selection years are vital to select the best clones (Leksono et al. 2006).

Genetic gain

Genetic gain was one of the most important variables for selecting excellent materials. The level of genetic gain depends on the repeatability, amplitude of genetic variation, and selection rate (Zhou et al. 2014). Most breeding programs aim to increase genetic gain via selection (Montes et al. 2008). In our research, the genetic gains of H increased from the 2nd to the 17th growth year (4.08 to 10.91%), but decreased from the 17th to the 30th growth year (from 10.91 to 6.46%), possibly because of smaller annual increases in height that led to smaller differences among clones, as reflected by the low PCV values.

Conclusions

Early selection is very important to shorten the breeding cycle and improve breeding efficiency. In this research, there were highly significant differences in all the traits among 57 *L. kaempferi* clones, and the high PCV and *R* values indicated that it was feasible to select excellent clones. There were significant positive age–age correlations in growth traits among different growth years. Under the same selection ratio, the clones selected in the 7th

growth year were the same as those selected in the 30th growth year, suggesting that the optimum early selection age is the 6th–8th growth year. The selected clones could serve as materials for the genetic improvement of *L. kaempferi*. This study provides a theoretical foundation for the early selection of *L. kaempferi*.

Author's contributions YP, SL and CW have contributed equally to this work.

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