



Genetic relationships between terminal shoot length, number of flushes and height in a 4-year-old progeny test of *Pinus brutia* Ten.

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Abstract

• **Key message** In this study, genetic variation in polycyclic growth was investigated in a young *Pinus brutia* Ten. study in Turkey. The number of flushes was partially under additive genetic control and was moderately correlated with the tree height at age 4.

• **Context** *Pinus brutia* is the most economically important tree species in Turkey. Previous limited studies suggested that its cyclic shoot elongation pattern can be useful for selecting seed sources for breeding and conservation of genetic resources.

• **Aims** Understand the degree of genetic control of terminal shoot growth, number of flushes, and total tree height at early ages and assess the genetic relationships between the traits to guide decisions for breeding and gene conservation.

• **Methods** Open-pollinated progenies of 188 trees from eight different seed sources were tested in three locations in the Aegean region of Turkey. Variance components, heritability and additive genetic correlations were estimated for tree height, terminal shoot length, and number of flushes.

• **Results** Traits were moderately under genetic control at the family. Terminal shoot length explained 76% of the variation in tree height. A strong genetic correlation (0.96) was found between tree height and terminal shoot length, while the number of flushes had a moderate genetic correlation with height (0.59). Northern seed sources tended to display less height growth, partially due to fewer flushes and lower shoot length.

• **Conclusion** Shoot elongation in the species is partially under additive genetic control and could be useful to select for early height in breeding programs.

Keywords Heritability · Genetic correlations · Polycyclic growth · Shoot elongation · Breeding

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Contribution of the co-authors Alan conceived the study, helped to establish and manage the field trials, collected data, helped with data analysis, and wrote the first draft of the manuscript. Isik wrote codes for linear mixed models and contributed to writing and editing the manuscript.

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1 Introduction

Shoot elongation patterns in *Pinus* sp. can be classified into three general modes: free growth, predetermined growth, and a combination of the two, which can be further divided into more patterns for pines growing in cold and temperate regions (Lanner 1976). Free growth mode refers to polycyclic growth (multiple shoots a year), while the predetermined mode refers to monocyclic growth or one shoot growth in a year. Girard et al. (2011) defined the polycyclic growth as the ability of a tree species to produce multiple flushes in the same growing season, and it is sometimes called the “*elliottii* pattern” (Pollard and Logan 1974; Joyce 1987). Some species have been observed to display multiple shoot elongation patterns, such as *Pinus radiata* D. Don (Bannister 1962; Codesido and Fernández-López 2009), *Pseudotsuga menziesii* (Mirb.) Franco (Schermann et al. 1997; Kaya et al. 1994),

Pinus pinaster Aiton (Alia et al. 1997), *Pinus banksiana* Lamb. (Kremer and Larson 1983), *Pinus halepensis* Mill. (Pardos et al. 2003; Girard et al. 2011), and *Pinus brutia* Ten. (Calamassi et al. 1988; Isik et al. 1999). In some species, the number of annual flushes decreases with age, such as *Pseudotsuga menziesii* (Greenwood 1984; Greenwood et al. 1989; Ritchie and Keeley 1994), while in other species, there does not appear to be a dependence on age, such as *Pinus radiata* (Sweet 1973). Greenwood (1995) defined phases of maturation and described decreasing growth rates in woody plants after the first phase.

Genetic variation in shoot growth patterns within a species can be used to select seed sources for breeding programs. Information on the level of genetic control between and within seed sources and the genetic relationships between traits can provide guidance for the planning and implementation of genetic conservation strategies, as traits such as late frost and drought tolerance are related to adaptation (Isik et al. 2002; Girard et al. 2011). In a *Pinus radiata* common garden experiment, the variation in shoot elongation patterns between open-pollinated families was partly explained by genetic differences (Codesido and Fernández-López 2009). The occurrence of second flushing in a population of Douglas fir was under genetic control at the family level (Schermann et al. 1997).

Polycyclic growth could have some disadvantages for adaptation in a breeding program. For example, extension of the growth period could delay terminal shoot or bud hardening, which may result in drought and early frost damage (Cannell and Johnstone 1978; Kaya et al. 1989; Codesido and Fernández-López 2009). Frost damage may cause stem defects, such as forking and multiple tops, and even result in tree mortality (Adams and Bastien 1994; Codesido and Fernández-López 2009). On the other hand, polycyclic growth is often an advantage: multiple flushing increases total photosynthetic area and plays an important role in total annual growth (Girard et al. 2011). In a common garden experiment of *P. brutia* established in the Mediterranean region of Turkey, seed sources from the middle elevation distribution of the species were superior for growth and also produced a higher number of flushes than the seed sources from the peripheral distribution (Isik et al. 1999). Jayawickrama et al. (1998) reported seed source variation in the date of cessation of annual height growth and a moderately strong correlation with total height growth, such that coastal seed sources tended to grow longer in the year and produce taller trees in *Pinus taeda* L. Shoot elongation patterns of pines could be adaptive, controlled by multiple environmental and genetic factors (Girard et al. 2012; Hover et al. 2017; Matisons et al. 2019).

Two Mediterranean pines, *P. halepensis* and *P. brutia*, are known for polycyclic growth patterns. *P. brutia*, naturally found in the eastern Mediterranean basin up to 1000 m above sea level (Arbez 1974), can produce up to six flushes per growing season. In a common-garden experiment in the Mediterranean region of Turkey, the fast growth of middle elevation populations was attributed to the higher number of summer flushes and their length (Isik et al. 2002). In a nursery study of *P. brutia*, families from the low- and middle-elevation populations had more flushes than the high-elevation families (Kaya and Isik 1997). *P. halepensis*, a pre-dominantly western Mediterranean species, covers extensive areas in the Mediterranean basin (Fady et al. 2003) and exhibits similar shoot elongation patterns as *P. brutia*. The species can produce up to four annual growth flushes in a growing season (Serre 1976), and the polycyclic growth pattern explained up to 60% of the variation in total annual growth (Girard et al. 2011).

P. brutia is the most economically important tree species in Turkey, covering 5.4 million hectares (25% of total forest area) and producing about half of raw wood material in the country (Boydak 2004; Kurt et al. 2012). While individual trees of *P. brutia* were first selected to establish seed orchards beginning in the 1960s, the modern National Tree Breeding Program of *P. brutia* started in earnest in 1994 (Alan 2006). The program defined nine zones, two of which were designated as gene conservation zones and the other seven for progeny testing. Progeny tests have since been established in five zones, including open-pollinated progeny tests established in the lower elevations of the Aegean region. In order to understand the variation in polycyclic growth and its relationship with tree height, this study investigated shoot growth patterns. Previous studies of shoot growth patterns in *P. brutia* were based on small sample sizes (Isik et al. 1999) or were based on young seedling characteristics (Isik 1986; Kaya and Isik 1997). Some internal results from progeny tests assessments were produced by the Turkish Tree Breeding Research Institute (personal communications with Dr. Hikmet Ozturk), but were never published.

This study is based on a comprehensive common-garden experiment established in the coastal Aegean region of Turkey. In the study, 188 open-pollinated families sampled from eight seed stands were tested in three locations for selection. The objectives of the study were (1) estimate genetic parameters of tree height, number of flushes, and terminal shoot length; (2) understand the phenotypic and genetic relationships between traits; and (3) assess the potential implications for breeding of the species.

Table 1 Geographic information of natural seed stands, number of plus trees selected from each seed stand included in the study (188 in total). All the seed stands are located within the Aegean breeding zone of *Pinus brutia* in Turkey

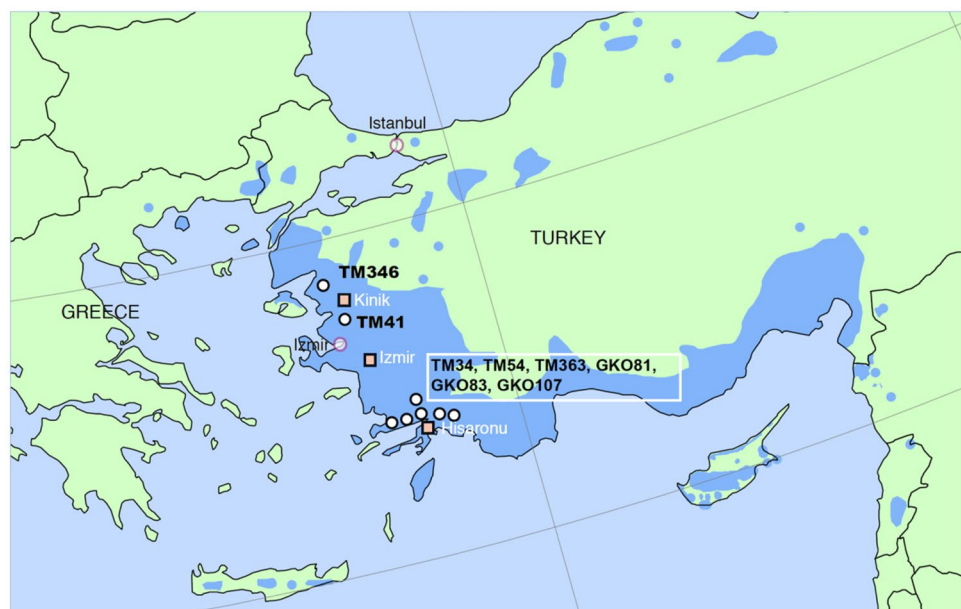
Seed stand	Latitude (N)	Longitude (E)	Altitude (m)	Number of plus trees selected
TM34	37°02'30"	28°16'20"	60	53
TM41	38°50'11"	28°04'32"	350	10
TM54	37°00'39"	28°24'30"	270	29
TM346	39°12'55"	26°57'25"	460	46
TM363	37°07'00"	27°50'11"	350	12
GKO81	37°04'16"	27°44'50"	410	8
GKO83	37°12'17"	27°54'46"	395	23
GKO107	37°07'30"	27°50'35"	350	7

2 Materials and methods

2.1 Genetic material

A total of 188 plus trees were selected from eight natural seed stands of *P. brutia*, with 7 to 53 trees per stand in the Aegean region of Turkey (Table 1; Fig. 1). Open-pollinated seeds were collected from plus trees in 1998 and in 1999. In addition, six checklots consisting of bulk seeds from natural stands were included in the study to estimate genetic gain and link the progeny tests across different breeding zones. All 188 plus trees used in this study were selected from and tested in the Aegean region (Fig. 1).

Fig. 1 The natural distribution of *Pinus brutia* Ten. (dark blue shaded area), seed stands (white circles) where parent trees were selected and three test site locations (orange squares) in the Aegean region of Turkey. Map source: EUFORGEN 2009 (www.euforgen.org)



2.2 Experimental design and data collection

Open-pollinated progeny tests were established at three locations in the Aegean region of Turkey (Hisaronu, Izmir, and Kinik) in March 2000. Seedlings were reared in tray-pots for 1 year prior to planting. A randomized complete block design with four-tree row plots was used at each site. The Hisaronu site had four blocks, while the other two sites had seven blocks each, making a total of about 72 half-sib progeny for each plus tree (parent) across the three test sites. Each block was split into four sets to accommodate the large block size, with checklots included in every set. In total, about 166 half-sib progenies and checklots were planted in each block. The spacing among seedlings was 2 × 3 m at each site.

Survival was assessed after the first growing season after planting and was determined to be adequate at the İzmir and Kinik sites (94% and 91%, respectively). The Hisaronu site had unacceptable initial survival (52%) and was replanted with about 1290 2-year-old seedlings of the same families, which were grown in a nursery near Marmaris in the Aegean region. At age 4 years after planting (2004), tree height (cm), terminal shoot length (cm), and the number of flushes were measured on every living tree. In total, approximately 12,100 trees were assessed across the three locations (Alan and Isik 2021).

2.3 Statistical analysis

The following linear mixed model was used to partition the observed phenotypic variance into genetic and environmental components for tree height, terminal shoot length, and number of flushes:

Table 2 Summary statistics for each test site (mean ± standard error). Trees grew faster at the Izmir test site compared to other two test sites

Test site	Number of trees	Height (cm)	Terminal shoot length (cm)	Number of flushes
Hisaronu	2229	90 ± 0.57	34 ± 0.26	2.8 ± 0.017
Izmir	4844	111 ± 0.40	39 ± 0.18	3.0 ± 0.010
Kinik	5033	96 ± 0.35	31 ± 0.15	2.5 ± 0.010

$$y_{ijklmnp} = \mu + S_i + B_{j(i)} + C_{k(i)} + R_{l(i)} + P_m + SP_{im} + SF_{i(n)} + BF_{jn(i)} + e_{ijklmnp} \tag{1}$$

where $y_{ijklmnp}$ is the p^{th} observation in the n^{th} family of the m^{th} seed stand in the l^{th} row, in the k^{th} column, and the j^{th} block at i^{th} test site; μ is the overall mean, S_i is the fixed effect of the test site ($i = 1, 2, 3$), $B_{j(i)}$ is the random effect of the j^{th} block within the i^{th} test site $\sim NID(0, \sigma_{b(s)}^2)$, $C_{k(i)}$ is random effect of the k^{th} column within i^{th} site $\sim NID(0, \sigma_{c(s)}^2)$, $R_{l(i)}$ is random effect l^{th} row within i^{th} site $\sim NID(0, \sigma_{r(s)}^2)$, P_m is the fixed effect of the m^{th} seed stand ($m = 1, 2, \dots, 8$), SP_{im} is the fixed interaction effect of site i and seed stand m , $SF_{in(m)}$ is the random n^{th} family effect nested within i^{th} site $\sim NID(0, \sigma_{s(f)}^2)$, $BF_{jn(i)}$ is the random plot effect (interaction effect of the j^{th} block and n^{th} family within i^{th} site) $\sim NID(0, \sigma_{plot}^2)$, and $e_{ijklmnp}$ is the random residual with $\sim NID(0, \sigma_e^2)$. The seed stand by block interaction was statistically insignificant and was dropped from the final model.

A uniform correlation and heterogeneous genetic variance structures were fit to the compound term family nested within site SF_{in} . This structure assumes that each site has a different family variance but the additive genetic correlation between pairs of sites is the same. We also fit diagonal covariance structures to the residual effect and the plot term nested within blocks to improve the model fit. The diagonal structure for the residuals takes the form as $R = \oplus_{j=1}^s R_j$ (Isik et al. 2017), where \oplus is the direct sum operator, and R_j is the site-specific residual variance. This structure assumes that each site had its own residual plot variance.

2.4 Linear combinations of variance components

Linear combinations of variance components were used to calculate phenotypic variances, individual tree narrow-sense heritability (h_i^2), and family mean heritability (h_{hs}^2):

$$h_i^2 = \frac{4\bar{r}_A \tilde{\sigma}_f^2}{\tilde{\sigma}_f^2 + \bar{r}_A \tilde{\sigma}_f^2 + \tilde{\sigma}_{plot}^2 + \tilde{\sigma}_e^2} \tag{2}$$

$$h_{hs}^2 = \frac{4\bar{r}_A \tilde{\sigma}_f^2}{\left(\frac{\tilde{\sigma}_f^2}{s} + \frac{(s-1)\bar{r}_A \tilde{\sigma}_f^2}{s} + \frac{\tilde{\sigma}_{plot}^2}{bs} + \frac{1}{s^2} \sum \frac{\sigma_e^2}{n_h}\right)} \tag{3}$$

where $\tilde{\sigma}_f^2$ is the average family variance, $4\bar{r}_A \tilde{\sigma}_f^2$ is the average additive genetic variance scaled by the average additive genetic correlation (\bar{r}_A), $\tilde{\sigma}_{plot}^2$ is the average plot variance (block by family interaction), σ_e^2 is the residual variance, $\tilde{\sigma}_e^2$ is the average residual variance, s is the number of sites, b is the number of blocks per site, and n_h is the harmonic mean number of trees per plot.

2.5 Phenotypic and genetic correlations between traits

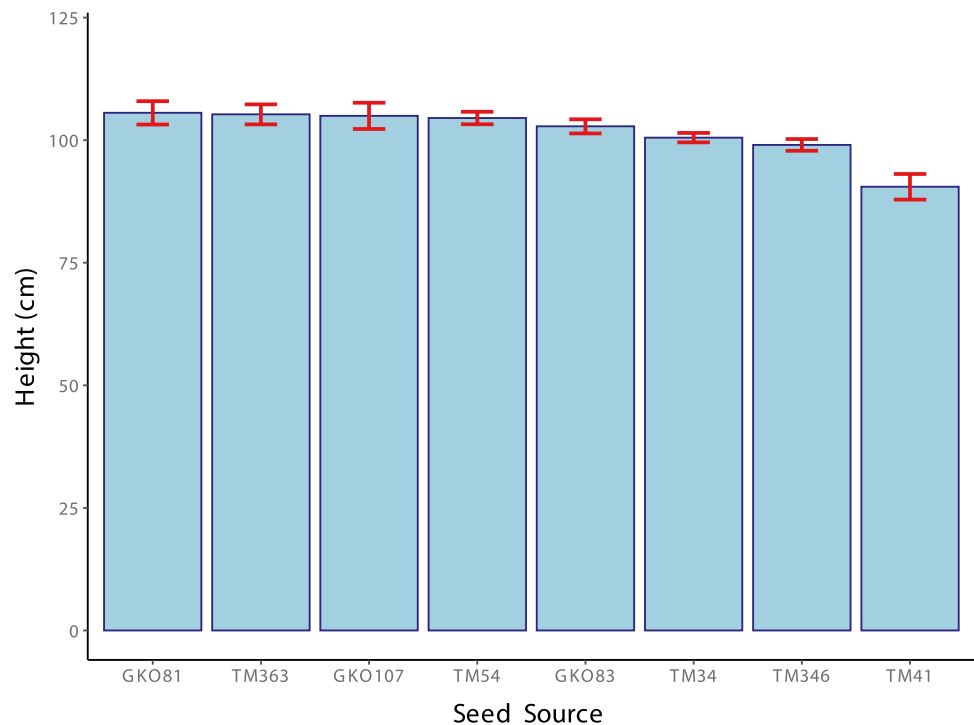
A multivariate model was fit to estimate additive genetic correlations between pairs of traits. The model in matrix form is (Isik et al. 2017):

$$y_{nxd} = X_{nx(p+1)} b_{(p+1)xd} + Z_{nxs} u_{rsd} + e_{nxd} \tag{4}$$

Table 3 Wald incremental F-tests of the fixed effects. Test site and seed stand effects were significant different at Pr=0.05 level. The difference between replanted seedlings after 1-year survival assessment and originally planted trees was highly significant at the Hisaronu site

Source of variation	Height		Terminal shoot length		Number of flushes	
	F-inc	Pr	F-inc	Pr	F-inc	Pr
Site	45.49	<0.001	29.51	<0.001	31.32	<0.001
Seed stand	4.15	<0.001	2.30	0.0111	5.44	<0.001
Replanted (Hisaronu)	230.88	<0.001	154.44	<0.001	198.94	<0.001

Fig. 2 Seed stand height means (cm) and 95% confidence intervals (red bars at the top). Seed sources were significantly different for tree height at age 4 (F test $Pr < .001$). The significance mainly originated from the slower growth of northern seed source TM41



where $y_{n \times d}$ is the matrix of response variables, n is the number of rows, d is the number of response variables, \mathbf{X} is the design matrix for the fixed effects, p is the number of predictors for one trait including one additional column for the intercept, \mathbf{b} is the matrix of fixed effects, \mathbf{Z} is the design matrix for the random effects, r is the number of random predictors per trait, \mathbf{u} is the matrix of random effects, and \mathbf{e} is the matrix of residuals with $n \times d$ dimension (number of rows and traits). The variance-covariance matrix of the random effects was scaled by the additive genetic relationship matrix derived from the pedigree.

Co-heritability of pairs of traits was calculated as $r_{xy}h_xh_y$ (Falconer and Mackay 1996), where r_{xy} is the additive genetic correlation, and h_x and h_y are the square roots of heritabilities of two traits. Standard errors of all linear combinations of variance components were approximated by the Delta method (Lynch and Walsh 1998). All statistical analyses were conducted by using the ASReml software version 4.1 (Gilmour et al. 2015). Tree height was regressed on terminal shoot length and number of flushes to understand the effect of shoot growth patterns on the tree height using the R software (Core R Team 2018) (Alan and Isik 2021).

3 Results

Trees displayed an average height of 111 cm at the Izmir site, and 90 cm at the Hisaronu site at age 4 (Table 2). Seed stands, where plus trees were selected from, had significant

differences for height, number of flushes, and terminal shoot length (Table 3). Northern seed stands (TM41 and TM346) had lower height growth than the rest of the seed stands (Fig. 2). Test sites were significantly different for all traits at $Pr < 0.001$ level. On average, trees displayed longer terminal shoots, and a greater number of flushes at the Izmir site than at other two sites (Table 2). At Hisaronu site, the difference between replanted seedlings (1290) after 1-year survival assessment and originally planted trees (1398) was highly significant for all three traits ($Pr < 0.001$). Original trees had more height growth, longer terminal shoot, and a greater number of flushes than replanted trees (Table 3).

3.1 Variance components and heritability estimates

A uniform genetic correlation with heterogeneous genetic variance structure for sites improved model fit statistics. Plot term variance was large, explaining 20.3% and 20.6% of the total variance for height and terminal shoot length, and 12.4% of the total variance for number of flushes (Table 4). On the other hand, the general combining ability variance (variation due to differences between parents) explained about 4.3%, 3.4%, and 4.1% of the total variance for the same traits pooled across three sites. Variance explained by family and site interaction was not significant for any of the traits, and this term was dropped from the final analyses.

Variance components for individual sites are given in Table 5. Variance explained by the random block effect

Table 4 Pooled estimates of variance components, percent variance explained by each term (%), narrow-sense individual tree (h^2_i), and family mean (h^2_{hs}) heritability for height, terminal shoot length, and the number of flushes. A uniform correlation with heterogeneous variance for family effect was implemented (each site had a unique

female variance, and the correlation between pairs of sites was uniform). Standard errors (\pm SE) are provided following the estimate. The plot effect (family by block interaction) explained a large proportion of total variance for all the traits, suggesting a suboptimal field design (row plots) and reducing heritability estimates

Estimates	Height \pm SE	%	Terminal shoot length \pm SE	%	Number of flushes \pm SE	%
Block	12.78 \pm 10.21	1.8	5.14 \pm 3.24	3.5	1.25 \pm 0.89	2.3
Stand \times block	40.14 \pm 14.28	5.6	7.49 \pm 2.60	5.1	0.78 \pm 0.44	1.4
Row	14.47 \pm 6.50	2.0	4.97 \pm 1.70	3.4	2.08 \pm 0.67	3.8
Column	8.56 \pm 4.28	1.2	1.37 \pm 0.79	0.9	0.58 \pm 0.33	1.1
Family	31.02 \pm 5.48	4.3	4.99 \pm 1.02	3.4	2.23 \pm 0.45	4.1
Plot	145.26 \pm 9.11	20.3	30.03 \pm 1.85	20.6	6.77 \pm 0.66	12.4
Residual	461.81 \pm 8.06	64.7	91.98 \pm 1.66	63.0	40.85 \pm 0.76	74.9
GxE (correlation)	0.89 \pm 0.13		0.87 \pm 0.19		0.67 \pm 0.161	
h^2_i	0.17 \pm 0.03		0.14 \pm 0.03		0.12 \pm 0.04	
h^2_{hs}	0.60 \pm 0.04		0.41 \pm 0.05		0.46 \pm 0.04	

and block by seed stand interaction was essentially 0 at Hisaronu site. The plot variance was large for all traits at all three sites. For example, at the Izmir site, the plot variance explained 29% of the total variance for height.

The family by site interaction for height and terminal shoot length were negligible as suggested by high (0.89 and 0.87) additive genetic correlation between all pairs of sites (Table 4), whereas the family by site interaction was noticeable (0.67) for the number of flushes. Narrow-sense individual tree heritability estimates were modest for all the traits, being 0.20 for height, 0.14 for terminal shoot length, and 0.12 for number of flushes (Table 4). The estimates were precise and associated with small standard errors. Family mean heritability estimates were moderate, with a range of 0.41 and 0.60.

3.2 Relationships between traits

Pearson product-moment correlation at age 4 between height and terminal shoot length was strong (0.87). Tree height had a moderate phenotypic correlation (0.59) with number of flushes (Fig. 3). Genetic correlations between traits were similar to product-moment correlations (Table 6). Height and terminal shoot length had a strong positive genetic correlation (0.96) whereas the correlation of height with number of flushes was moderate (0.59). Similarly, the genetic correlation between terminal shoot length and number of flushes was moderate (0.64). Regressing tree height on terminal shoot length explained 76% of the variation in height (Fig. 4). Number of flushes did not explain any additional variation (partial $R^2 = 0.0014$) in height.

4 Discussion

4.1 Seed source variation

There were significant differences between seed stands for height, terminal shoot length, and the number of flushes. Trees from the southern seed stands displayed higher height growth, longer terminal shoots, and higher number of flushes than the northern seed stands. Large provenance variation in *P. brutia* growth traits have been previously reported (Isik et al. 1999, 2000). Granhus et al. (2019) also reported a relationship between shoot formation and the latitude of the origins in *Picea abies*, with the frequencies of trees with multiple shoots decreasing with increasing elevation. Similarly, Rweyongeza et al. (2010) observed negative association between height and latitude, longitude, and elevation of seed sources in *Picea glauca* in a seedling study. Significant provenance effects on growth and the number of flushes were observed in *Pinus taeda*, with the southern populations having more growth and more frequent flushing (Quesada et al. 2017). These trends in height growth patterns among seed source are important when considering breeding strategies for *P. brutia*.

4.2 Shoot elongation and relationship with height

Shoot elongation in *P. brutia* occurs as a result of a spring shoot and simultaneous initiation and elongation of multiple summer flushes in a growing season (Isik et al. 2002). The number of summer shoots can vary from year to year,

Table 5 Individual test site variance components, percent variance explained by each term (%), narrow-sense individual tree (h^2_i), and family mean (h^2_{hs}) heritability for height, terminal shoot length, and the number of flushes using the heterogeneous variance with uniform correlation structure for the family effect. Standard errors (\pm SE) of the estimates are provided. Terms with NA did not explain a significant variance based on the likelihood ratio tests and were dropped from the final model

Estimates	Height \pm SE	Terminal shoot length \pm SE	Number of flushes \pm SE
(a) Hisaronu site			
Block	NA	NA	NA
Block \times stand	NA	NA	NA
Column	11.79 \pm 5.69	1.47 \pm 0.99	0.86 \pm 0.49
Row	24.90 \pm 10.78	7.73 \pm 2.67	4.50 \pm 1.31
Family	21.24 \pm 8.88	5.35 \pm 2.13	3.25 \pm 1.10
Plot variance	154.96 \pm 19.79	24.74 \pm 3.84	4.77 \pm 1.53
Residual	430.33 \pm 17.04	95.17 \pm 3.75	45.77 \pm 1.80
h^2_i	0.14 \pm 0.06	0.17 \pm 0.06	0.24 \pm 0.08
h^2_{hs}	0.22 \pm 0.07	0.27 \pm 0.08	0.39 \pm 0.09
(b) Izmir site			
Block	13.93 \pm 12.78	NA	0.61 \pm 0.61
Block \times stand	NA	7.49 \pm 2.60	0.79 \pm 0.44
Column	5.34 \pm 2.87	1.28 \pm 0.60	0.56 \pm 0.24
Row	NA	NA	1.12 \pm 0.46
Family	43.78 \pm 10.82	5.96 \pm 1.94	2.52 \pm 0.64
Plot variance	227.51 \pm 16.67	51.04 \pm 3.57	10.99 \pm 1.02
Residual	445.89 \pm 11.53	87.46 \pm 2.27	35.53 \pm 0.92
h^2_i	0.24 \pm 0.06	0.16 \pm 0.05	0.21 \pm 0.05
h^2_{hs}	0.73 \pm 0.20	0.37 \pm 0.08	0.46 \pm 0.07
(c) Kinik site			
Block	11.63 \pm 7.65	5.14 \pm 3.23	1.89 \pm 1.18
Block \times stand	NA	NA	NA
Column	NA	NA	0.31 \pm 0.27
Row	4.03 \pm 2.21	2.21 \pm 0.74	0.61 \pm 0.24
Family	28.03 \pm 6.02	3.66 \pm 1.01	0.92 \pm 0.34
Plot variance	53.35 \pm 9.01	14.32 \pm 1.84	4.55 \pm 0.77
Residual	509.34 \pm 12.71	93.34 \pm 2.34	41.26 \pm 1.03
h^2_i	0.19 \pm 0.04	0.13 \pm 0.03	0.08 \pm 0.03
h^2_{hs}	0.47 \pm 0.12	0.39 \pm 0.09	0.29 \pm 0.08

depending on the availability of soil moisture, but these studies and others confirm that the trait is under strong genetic control at the provenance level (Kaya and Isik 1997; Isik and Isik 1999). We observed a maximum of six flushes in this study. Similarly, up to six flushes were observed for seed sources sampled from the middle elevation range of the

species in the Mediterranean region (Isik and Isik 1999). In the same study, growth differences between seed sources were attributed mainly to the number of summer flushes. Summer shoot growth was the major factor differentiating seed sources for height growth. In this study, a moderately strong genetic correlation (0.59) between the number of flushes and total height growth suggested importance of summer flushes in total height growth. Regressing height on number of flushes explained 36% of the variance in height (data not reported).

In *P. halepensis*, another Mediterranean pine species, polycyclic shoots contributed between 10 and 45% of total annual growth in a study established in southern France (Girard et al. 2011). This species produces up to four annual growth flushes in a growing season (Serre 1976; Girard et al. 2011). In an irrigated seed orchard, grafted *P. halepensis* clones have reached up to seven successive annual flushes (Pardos et al. 2003). Girard et al. (2011, 2012) attributed the polycyclic growth to summer temperatures of the current and preceding year and rainfall of the first half of preceding year through winter. In *P. taeda*, significant differences among six families were reported for number of summer shoots as well as annual height growth (Bridgwater et al. 1985).

The relationship between polycyclic growth and height is not consistent among conifers. In *Pinus elliotii* Engelm., *Pseudotsuga menziesii*, and *Pinus radiata*, genetic correlations among height and shoot number and shoot length have been found to be low and even negative (Smith et al. 1993; Schermann et al. 1997; Codesido and Fernández-López 2009). Adams and Bastien (1994) found that second and subsequent flushing in *Pseudotsuga menziesii* seedlings contributed negatively to stem form, such as forking, coarse branches, and multiple tops. In *Picea abies* L., Skrøppa and Steffenrem (2016) reported that the occurrence of lammas shoots increased the probability of multiple tops (forking), causing a negative effect in timber quality. However, studies with *P. brutia* have indicated that populations with higher number of flushes at the ages of 13 and 17 had more desirable stem straightness, taper, and lower forking incidence compared with populations with fewer of flushes (Isik and Isik 1999; Isik et al. 1999). The early assessment (age 4) in this study prevented quantification of stem form and its relationship with polycyclic growth.

4.3 Heritability estimates

Heritability estimates in this study were somewhat lower than the estimates reported for the same species in earlier studies (Isik et al. 1999). Low heritability estimates in this study were likely due to the sub-optimal experimental design, as suggested by large plot-to-plot variances. The

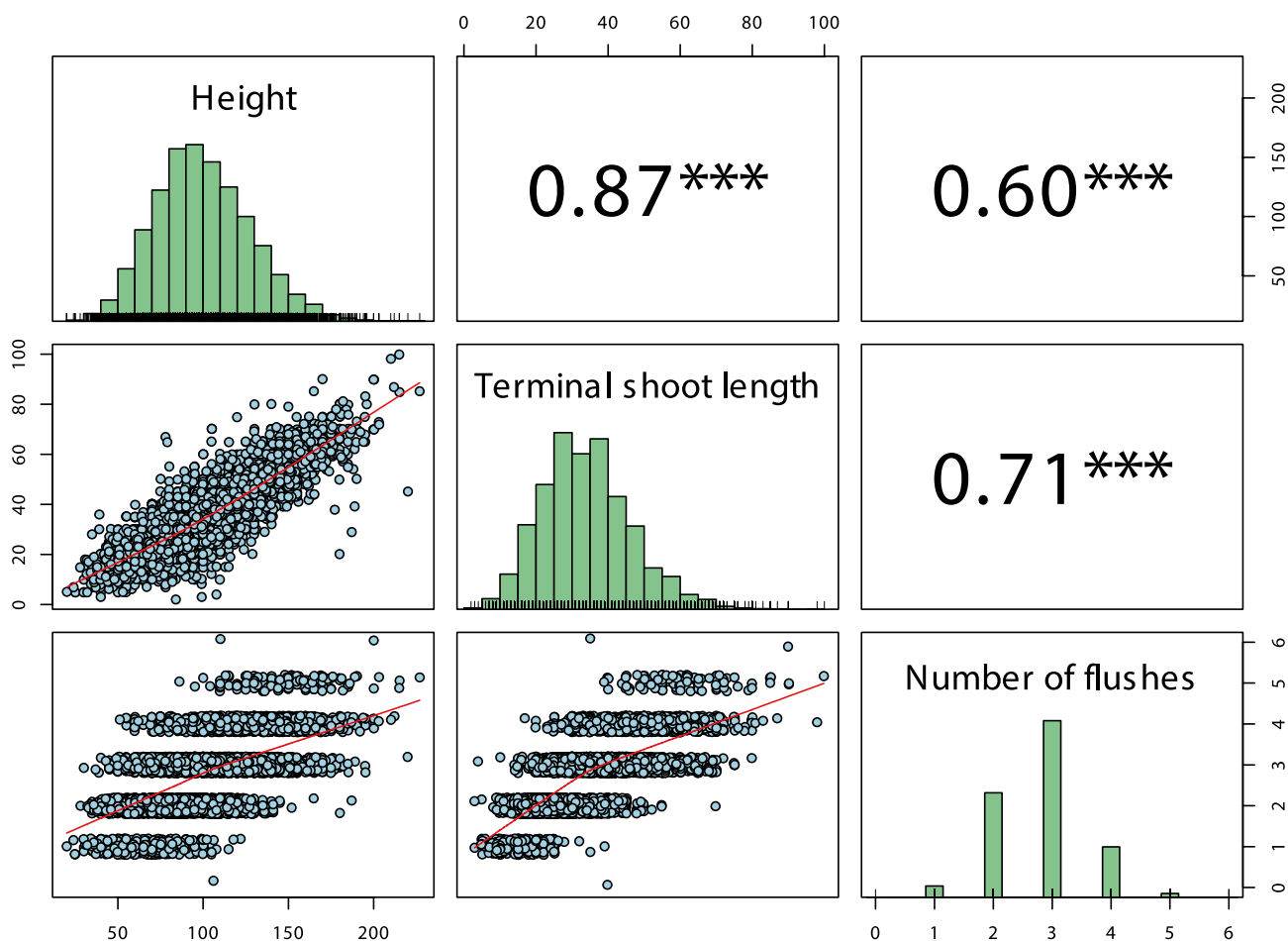


Fig. 3 Pearson product-moment correlations (upper triangle) with significance level, univariate histograms (diagonal), and scatter plots with a linear fit (lower triangle) for tree height, terminal shoot length,

and number of flushes measured at age 4 in pooled data across all sites. All three traits were significantly correlated at $Pr=0.05$ level

randomized complete block design with four-tree row plots used in this study resulted in large block sizes (~0.45 hectare). Incomplete block designs, such as alpha-cyclic row-column designs, have been demonstrated to be more efficient in blocking site heterogeneity for forest trees progeny tests (John and Williams 1995; Isik and McKeand 2019).

Table 6 Multivariate analysis of height, terminal shoot length, and the number of flushes. Additive genetic correlations between traits are in the upper diagonal, family variances are in the diagonal, and co-heritability ($r_{Ah_xh_y}$) estimates are in the lower diagonal of the table. Tree height had a strong additive genetic correlation with terminal shoot length while the correlation with the number of flushes was moderate

Traits	Height	Terminal shoot length	Number of flushes
Height	29.01	0.96 ± 0.016	0.59 ± 0.086
Shoot length	0.14	4.16	0.64 ± 0.082
Number of flushes	0.09	0.08	1.38

Nonetheless, the heritabilities confirm the opportunity for artificial selection for height, shoot length, and number of flushes in this population. Higher heritabilities for the number of flushes and cyclic growth have been reported in other species of *Pinus*, such as individual narrow-sense heritabilities ranging from 0.34 to 0.44 and half-sib family mean heritability ranging from 0.59 to 0.73 in slash pine (*Pinus elliottii* var. *elliottii*) open-pollinated progeny tests at 24 weeks (Smith et al. 1993). The effectiveness of parental (backwards) selection for height growth in *P. brutia* could be improved using open-pollinated trials with advanced progeny test experimental designs.

5 Conclusions

This study revealed considerable genetic variation within *P. brutia* for height growth, terminal shoot length, and the number of flushes at age 4, highlighting the opportunity

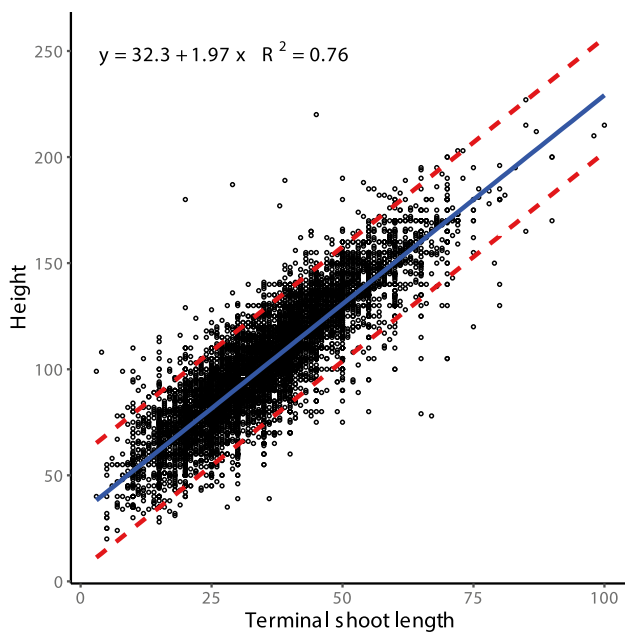


Fig. 4 Linear regression of tree height on terminal shoot length at age 4. Terminal shoot length explained 76% of the variance in height. The 95% prediction intervals of the fit are presented (red dashed lines). Data from all three sites were used in the regression model

for selection on these traits for tree improvement. Southern seed stands had more height growth, longer terminal shoots, and higher number of flushes. A positive genetic relationship between the number of flushes and height growth in *P. brutia* suggests that simultaneous selection for improvement would be effective.

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Availability of data and codes The datasets analyzed in the current study and ASReml software linear models' codes are available in the Zenodo repository; <http://doi.org/10.5281/zenodo.4268819>. Publication date: January 4, 2021.

Declarations

Consent for publication All the authors gave their informed consent to this publication and its content.

Conflict of interest The authors declare that they have no conflict of interest.

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