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Full Length Research Paper

Genetic variation in *Pinus brutia* Ten. seed stands and seed orchards for growth, stem form and crown characteristics

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In this study, some adaptive traits (growth, stem, branching and crown characteristics) in a seven-year old plantation of Turkish red pine (*Pinus brutia* Ten.) were studied in 2008. The experiment was established by using 1+0 bare-root seedlings from 5 seed stands and 5 seed orchards from the same origins in 2001. Randomized block design with three replications was used in the field. There were a total of 100 families, 10 from each of these 10 populations were used in the experiment. Each family was represented with 10 seedlings in each replication. Populations and families within each population were significantly different for all traits both in the seed stands and in the seed orchards. The percent of genetic variation caused by population was considerable except for branch length ranging from 0.19 to 18.28% especially in the seed stands. Variance components due to families in the seed orchards were in general higher than those in the seed stands. Individual heritabilities varied in 0.45 – 0.90 range. Family heritabilities ranged from 0.76 to 0.88. These results indicated that combined population, family and within family selection for studied traits would result in considerable gain in this species.

Key words: Genetic variation, heritability, *Pinus brutia* Ten., seed stand, seed orchard.

INTRODUCTION

Turkish red pine (*Pinus brutia* Ten.) constitutes nearly 20% of forests and takes the first place among the species preferred to be used for forestation activities in Turkey (Anonymous, 2001). The species is an important commercial tree species and used widely in forestation programs in southern and western Turkey. Turkish Red Pine naturally grows from sea level up to 1200 m, occasionally to 1400 m elevation in the Taurus Mountains along the Mediterranean Coast. Within its altitudinal and horizontal distribution ranges, Turkish red pine exhibits significant amount of variation in various form and growth characteristics (Arbez, 1974; Işık, 1986; Işık et al., 1987; Atalay et al., 1998; Kandemir, 2002). It grows on a variety of sites with very different annual precipitation and climatic conditions (Arbez, 1974; Panetsos, 1981). It is also considered as a fast growing conifer when compared to other native forest tree species in Turkey (Işık et al., 1987). The species has been introduced to several countries in the Mediterranean region and to overseas countries such as Australia and Mexico (Palmberg, 1976; Fisher et al., 1986; Weinstein, 1989a; Weinstein, 1989b). It is included in the National Tree Breeding and Seed Production Program of Turkey (Koski and Antola, 1993) and National Plan for in-situ Protection of Plant Genetic Diversity (Kaya et al., 1998). Tree breeding zone designnations and plus tree selections for each breeding zone have been completed and progeny tests have been established on multiple sites to evaluate the genetic merits of the selected trees. There are also well distributed clonal seed orchards established to represent the breeding zones of the species. Some of these seed orchards, however, are still too young to provide sufficient seed to meet forestation needs. The main seed sources for all kinds of plantation activities are seed stands, which are reserved natural stands of this species.

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Table 1. Description of the seed stands.

Seed stands	Code	Region	Altitude	Latitude	Longitude
Çetibeli	34	Muğla	60	37º02´30´´	28º16´20´´
Yaraş	35	Muğla	750	37º06´30´´	28º32´45´´
Lengüme	9	Antalya	1050	37º24´30´´	28º30´00´´
Kumluca	39	Antalya	250	37º26´30´´	30º15´45´´
Karaköy	18	Çanakkale	450	37º50´00´´	26º55´30´´

Table 2. Description of the seed orchards.

Seed orchards	Code	Provenance	Region	Latitude	Longitude	Number of clones
Çetibeli	31	Çetibeli SS	Muğla	37º01´20´´	28º30´30´´	34
Yaraş	14	Yaraş SS	Muğla	37º08´30´´	28º23´20´´	26
Lengüme	13	Lengüme SS	Muğla	36º56´30´´	29º18´30´´	30
Kumluca	11	Kumluca SS	Antalya	37º52´20´´	30º37´00´´	25
Karaköy	32	Karaköy SS	Balıkesir	40º15´40´´	27º35´00´´	30

SS; Seed Stands.

Two recent studies involving Turkish red pine indicate that impact of humans on genetic resources of the species are significant (Kandemir et al., 2004; İçgen et al., 2006). They reported that there is a large amount of genetic diversity within and among Turkish red pine seed stands, but no distinct pattern of genetic diversity according to the geography, elevation or breeding zones, implying the occurrence of past human disturbances such as forest fires, in turn and artificial regeneration. Furthermore, Içgen et al. (2006) studied the potential impact of forest management and breeding practices on established Turkish red pine plantations. They reported that the genetic relationships between seed sources (seed stands, seed orchards and plantations originating from the same locality) varied with respect to seed source locations. In general, seed stands were genetically distant to seed orchards and plantations, demonstrating that some genetic changes have taken place during the course of seed orchard (plus tree selection) and plantation (seed and/or seedling production) establishment.

The overall goal of this study was to investigate the degree and extent of genetic variation on some growing and branching traits between and within five seed stands and five seed orchards which below the same origin of *P. brutia* planted in a common garden experiment. The specific objectives were (i) to estimate proportions of variation contributed by populations and families within the seed stands and seed orchards respectively, (ii) to estimate individual and family heritabilities and (iii) to investigate the degree of relationships among the traits studied and relationships between them.

MATERIALS AND METHODS

Genetic material, experimental design and measurements

Ten populations, five from seed stands and five from seed orchards were sampled from the western and southern Turkey (Tables 1 and 2). Each population was represented by ten randomly-selected open pollinated families. There were a total of 100 families, 10 from each of these 10 populations were used in the experiment. Each family was represented with 10 seedlings in each replication. Seedlings were raised in 2000 in Eğirdir state nursery, near Isparta. The experiment was set up in a single site at Aydoğmuş using 1+0 bare root seedlings in the winter of 2001. Two meters x three meters spacing was used and borders consisted of two rows. The site's latitude, longitude and altitude are, respectively, 37º 58' N and 30º 14' E and 1035 m. The mean annual rainfall was approximately 650 mm being the rain season mostly during winter months. The mean annual temperature is approximately 15ºC. Randomized block design with three replications was used in the field. The trial was observed and measured in 2008 (seven years after planting). Vegetation periods (VP, days) of each sapling were observed from September, 2007 to May, 2008 once a week. Sapling height (SH, cm), number of growth cycles (NGC), number of branches (NB), branch diameter (BD, mm), branch length (BL, cm) and branch angle (BA, degree) were measured in March, 2008. Additionally, stem form (SF), one of the most important indicators of wood quality in the future, was estimated according to the scale that was described by Işık et al. (2002) (Figure 1).

Data analyses

Before performing the analysis, data were examined for conformity to normal distribution and homogeneity of the variance assumptions. Prior to analyses, the outliers were removed from the data. The number of branch, branch angles and number of growth cycles

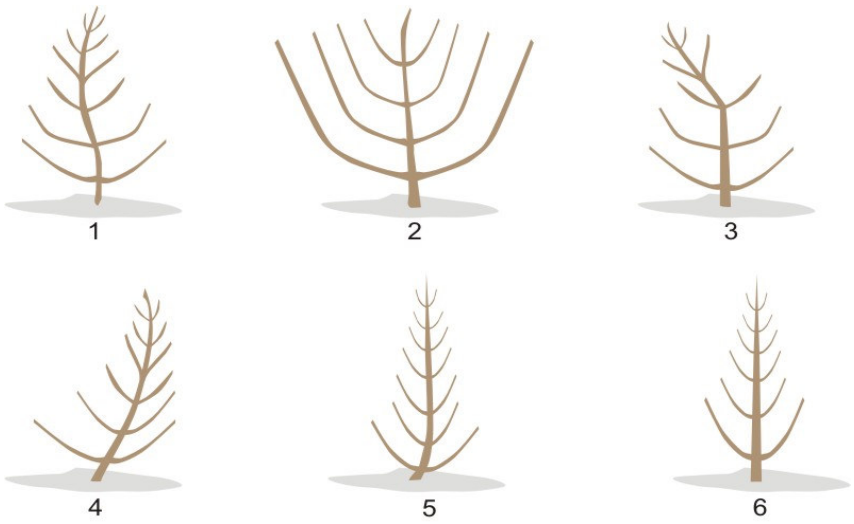

Figure 1. The scale used to determine stem form (Işık et.al., 2002).

significantly deviated from these assumptions. Therefore, the data of the number of branch and growth cycles were square root-transformed before analysis. The following ANOVA model was used for the analyses of variance in the seed stands and seed orchards respectively.

$$Y_{ijkm} = \mu + R_i + P_j + F(P)_{k(j)} + RP_{ij} + RF(P)_{ik(j)} + e_{m(ijk)}$$

Where Y_{ijkm} is the measurement on the m^{th} sapling of the k^{th} family from the j^{th} population in the i^{th} replication; μ is the overall mean; R_i is the effect of i^{th} replication (I = 1, 2, 3); P_j is the effect of j^{th} population (j = 1, 2, 3, 4, 5); $F(P)_{k(j}$ is the effect of k^{th} family in j^{th} population (k = 1, 2, 3, …, 10); RP_{ij} is the interaction effect between i^{th} replications and j^{th} population; $RF(P)_{ik(j)}$ is the interaction between i^{th} replication and k^{th} family within j^{th} population; and $e_{m(ijk)}$ is the residual.

Except μ, all effects on the right side of the model were considered random with zero expectation and respective variances. The restricted maximum likelihood estimates of variance components were calculated using PROC MIXED (Sas Inst. Inc., 1989). Individual heritability was estimated after Shelbourne (1969, 1992) and Falconer and Mackay (1996):

$$h_i^2 = \frac{\sigma_A^2}{\sigma_u^2} = \frac{4\sigma_{F(P)}^2}{\sigma_u^2}$$

Where h_i^2 = individual heritability, σ_A^2 = additive genetic variance, $\sigma_{F(P)}^2$ = between-family-within-population variance component and σ_u^2 = phenotypic variance calculated as:

$$\sigma^2_u = \sigma^2_{F(P)} + \sigma^2_{RF(P)} + \sigma^2_e$$

where $\sigma_{RF(P)}^2$ = variance due to interaction between replication and family-within-population (plot to plot error) and σ_e^2 = variance among individuals within family (residual). Standard errors of individual heritability were determined according to Falconer (1981):

$$\text{S.E. } (h^2_i) = \sqrt{16x\frac{Var(\sigma^2_{F(P)})}{(\sigma^2_u)^2}}$$

Family mean heritability (h_f^2) estimated Shelbourne (1992):

$$h_f^2 = \frac{\sigma_{F(P)}^2}{\sigma_{F(P)}^2 + k_2/k_3\,\sigma_{RF(P)}^2 + \sigma_e^2/k_3}$$

Where h_f^2 = family mean heritability, k_2 and k_3 are coefficients for and $\sigma_{RF(P)}^2$ in the expected mean squares. To compare the genetic variation of different traits at the standard level, the coefficients of genetic variation estimated for the traits studied (Cornelius, 1994):

$$\%CV_g = \left(\frac{\sqrt{3\sigma_{F(P)}^2}}{\bar{x}}\right)x100$$

Where %CVg = Coefficients of genetic variation, $\sigma_{F(P)}^2$ = between-family-within-population variance component, $\bar{x}$ = the mean of trait. Genetic correlations were estimated from the component of variance and covariances (Falconer, 1981) substituted into the standard equation for the product moment correlation coefficient:

Table 3. Components of total variance (%) and some genetic parameters for sapling traits in the seed stands.

Trait	$\bar{x}$ ± SE	σ^2_P	$\sigma^2_{F(P)}$	σ^2e	CV_g *(%)*	h^2_i ± S.E	h^2_f ± S.E
SH	149.75 ± 1.16	11.73***	6.55***	81.72	12.41	0.30 ± 0.09	0.66 ± 0.21
BL	80.14 ± 0.59	0.19**	11.09***	88.73	15.04	0.44 ± 0.13	0.76 ± 0.21
NB	13.08 ± 0.10	2.68***	21.03***	76.28	22.83	0.86 ± 0.21	0.87 ± 0.21
BA	64.93 ± 0.23	18.28***	9.47***	72.23	7.11	0.46 ± 0.13	0.76 ± 0.21
BD	25.03 ± 0.22	6.26***	19.79***	73.96	2.87	0.84 ± 0.20	0.87 ± 0.20
NGC	10.76 ± 0.06	14.38***	12.93***	72.69	2.05	0.60 ± 0.16	0.81 ± 0.21
SF	4.20 ± 0.05	10.08***	6.40***	83.53	9.72	0.39 ± 0.12	0.65 ± 0.21
VP	179.66 ± 0.68	2.91***	10.93	85.97	7.66	0.45 ± 0.13	0.76 ± 0.22

SH = Sapling height (cm), BL = branch length (cm), NB = number of branches, BA = branch angle (°), BD = branch diameter (mm), NGC = number of growth cycles, SF = stem form, and VP = vegetation periods (days).

$\bar{x}$ ± SE: means and standard errors, σ^2_P: Estimated variance components of populations, $\sigma^2_{F(P)}$: Estimated variance components of families within populations, σ^2_e: Estimated variance components of individuals within families, CVfm: Coefficients of phenotypic variation at family levelCVg: Coefficients of genetic variation, h2i: Individual heritability, h2f: family heritability, ns: none significant; *: significant at P < 0.05; **: significant at P < 0.01; ***: significant at P < 0.001.

$$r_g = \frac{COV_{f(x,y)}}{\sqrt{\sigma^2{}_{f(x)}}\sqrt{\sigma^2{}_{f(y)}}}$$

Where r_g = estimated genetic correlation between traits *x* and *y*, $\sigma^2{}_{f(x)}$ = estimated components of variance of families within populations for trait *x*, $\sigma^2{}_{f(y)}$ = estimated components of variance of families within populations for trait *y* and $COV_{f(xy)}$ = estimated component of covariance of families within populations between traits *x* and *y*. The phenotypic correlation between traits *x* and *y* were calculated from family mean squares and mean cross products for the traits according to Kaya et al. (1989).

RESULTS AND DISCUSSION

There was significant variation among populations both in the seed stands and in the seed orchards for all sapling traits (Tables 3 and 4). The components of the total variance attributed to variation among populations ranged from 0.19% in branch length to 18.28% in branch angle in the seed stands. On the other hand it ranged from 0.15% in branch diameter to 19.50% in stem form in the seed orchards. Families within populations of both in the seed stands and seed orchards showed significant variation for all traits. The components of the total variance attributed to variation among families within populations ranged from 6.40% in stem form to 21.03% in number of branch in the seed stands (Table 3), from 11.32% in branch angle to 20.02% in branch diameter in the seed orchards (Table 4). On the other hand, there was no significant variation among replications, replication x population and replication x family interactions for all traits. As the components of the total variance attributed to these factors were 0.0%, they were not presented in the Tables 3 and 4.

Co-efficients of genetic variation varied from 2.05% (NGC) to 22.83% (NB) in the seed stands, whilst from 2.56% (NGC) to 21.43% (NB) in the seed orchards. These results showed that genetic variation in the seed orchards was close or a bit higher than that in the seed stands in terms of all characteristics except for NB. As selection was made in terms of desired phenotypic properties (families with thin and a bit branch, straight and long stem form etc.) in tree breeding programme, the genetic base was expected to be narrower in seed orchards. However, in this study it was identified that genetic diversity was preserved in the seed orchards. Similarly, in the study conducted in order to compare the genetic diversities of these seed sources, examined in the present study, using molecular markers, it was stated that genetic diversity was preserved in the seed orchards (Velioğlu et al., 2003). Furthermore, there are several studies proving that the genetic diversity in seed orchards are equal to or more than that in natural populations (Knowles, 1985; Chaisurisri and El-Kassaby, 1994; El-Kassaby and Ritland, 1996; Schmidtling and Hipkins, 2004; Zheng and Ennos, 1999). The high genetic diversity calculated in terms of observed characteristics may be due to the sampled populations being selected from different regions and their geographical distances being far. Then it is stated that as the distance between the

Table 4. Components of total variance (%) and some genetic parameters for sapling traits in the seed orchards.

Trait	$\bar{x}$ ±SE	σ^2_P	$\sigma^2_{F(P)}$	σ^2e	CV_g *(%)*	h^2_i±S.E	h^2_f±S.E
SH	157.82 ± 0.21	5.00***	13.71***	81.29	18.52	0.58 ± 0.15	0.81 ± 0.21
BL	81.03 ± 0.34	0.22***	14.72***	85.06	17.76	0.59 ± 0.15	0.81 ± 0.20
NB	13.05 ± 0.23	2.87***	18.21***	78.57	21.43	0.75 ± 0.19	0.85 ± 0.21
BA	62.76 ± 0.59	0.00***	11.32***	88.68	8.05	0.45 ± 0.12	0.76 ± 0.20
BD	23.77 ± 1.24	0.15*	20.02***	79.83	3.25	0.80 ± 0.17	0.86 ± 0.19
NGC	11.29 ± 0.20	12.92***	19.60***	67.49	2.56	0.90 ± 0.22	0.88 ± 0.21
SF	4.57 ± 0.02	19.50***	14.74***	75.77	13.02	0.65 ± 0.17	0.83 ± 0.21
VP	182.66 ± 0.01	0.56***	12.12***	86.37	8.53	0.49 ± 0.13	0.78 ± 0.21

SH = Sapling height (cm), BL = branch length (cm), NB = number of branches, BA = branch angle (°), BD = branch diameter (mm), NGC = number of growth cycles, SF = stem form, and VP = vegetation periods (days). $\bar{x}$ ± SE: means and standard errors, σ^2_P: Estimated variance components of populations, $\sigma^2_{F(P)}$: Estimated variance components of families within populations, σ^2_e: Estimated variance components of individuals within families, CVfm: Coefficients of phenotypic variation at family levelCVg: Coefficients of genetic variation, h2i: Individual heritability, h2f: family heritability, ns: none significant; *: significant at P < 0.05; **: significant at P < 0.01; ***: significant at P < 0.001.

populations increases, the gene flow decreases (Parker et al., 1997).

The heritability estimated on both individual and family level were found to be higher in the seed orchards compared to the seed stands for all characteristics apart from number of branch and branch angle. Whilst in branch diameter, they were observed to be almost equal. In the seed stands, individual heritabilies ranged from 0.30 (sapling height) to 0.86 (number of branches) and family heritabilities ranged from 0.65 (stem form) to 0.87 (number of branches and branch diameter). In the seed orchards, the heritability degrees ranged between 0.45 (branch diameter) and 0.90 (number of growth cycle) on the individual level and between 0.76 (branch diameter) and 0.88 (number of growth cycle) on the family level. In previous studies conducted into Turkish red pine, high heritability degrees were estimated for various sapling characteristics on the individual and on the family level. In some of these, it is reported that the estimated heritability degrees for height ranged between 0.09 and 0.74 (Işık, 1986; Işik and Kaya, 1995; Kaya and Işık, 1997; Işık, 1998; Işık et al., 1999; Işık and Işık, 1999). In previous studies, high heritabilities were calculated for both Turkish red pine and other tree species (Yıldırım, 1992; Işık et al., 2001; Vargas-Hernandez et al., 2003; Fedorkov et al., 2005; Xie et al., 2007; Codesido and Fernandez-Lopez, 2008; Gülcü and Üçler, 2008).

In most observed characteristics, family heritabilities were found to be higher than individual heritabilities both in the sees stands and seed orchards. This result indicates that it can be achieved higher genetic gain with family selection in Turkish red pine populations. In fact, results obtained from previous studies conducted into Turkish red pine also support this view (Işık, 1998; Öztürk et al., 2004).

It was observed that the genetic and phenotypic correlations among the observed characteristics were in general high and positive (Table 5). The genetic correlations calculated between some characteristics were higher than the phenotypic correlations. Genetic correlations being higher than phenotypic correlations are explained with the negative effects of environmental conditions creating a negative relationship between the two characteristics (Işık and Kaya, 1995).

High, positive genetic and phenotypic correlations were identified between sapling height and number of growth cycle, number of branches and branch length. In fact, in studies conducted into Turkish red pine and some other species, it is stated that fast growing genotypes also had a tendency to form more and longer branches and produce more growth cycle (Işık et al., 1987; Işık, 1998; Vargas-Hernandez et al., 2003; Tucic et al., 2006; Sebbenn et al., 2007; Coseido and Lopez, 2008).

The highest correlation with stem form was observed in number of growth cycle, followed by sapling height. It was identified that the genetic correlation between sapling height and stem form ranged between 0.05 and 0.90 in studies about Turkish red pine and some other tree species (St. Clair, 1994; Hodge and White, 1992; Işık, 1998; Vargas-Hernandez et al., 2003; Tucic et al., 2006; Sebbenn et al., 2007; Coseido and Lopez, 2008). Whilst in some conifer species, a negative genetic correlation was recorded between height and stem form (Woolaston et al., 1990; Dean et al., 1986; Dean and Stonecypher,

Table 5. Genetic (below diagonal) and phenotypic (above diagonal) correlations between traits.

	SH	NB	NGC	BL	BA	BD	SF	VP
SH	-	0.70***	0.76***	0.78***	0.09***	0.49***	0.34***	0.26***
NB	0.71 ± 0.09	-	0.70***	0.57***	0.19***	0.45***	0.26***	0.20***
NGC	0.69 ± 0.10	0.82 ± 0.06	-	0.53***	0.12***	0.31***	0.38***	0.20***
BL	0.89 ± 0.04	0.66 ± 0.10	0.59 ± 0.12	-	0.32*	0.64***	0.15***	0.21***
BA	0.10 ± 0.19	0.36 ± 0.16	0.17 ± 0.18	0.06 ± 0.19	-	0.06*	0.02**	0.05**
BD	0.71 ± 0.09	0.67 ± 0.10	0.47 ± 0.14	0.83± 0.06	0.13 ± 0.18	-	0.07***	0.19***
SF	0.38 ± 0.17	0.35 ± 0.16	0.53 ± 0.13	0.25± 0.18	0.11 ± 0.19	0.08 ± 0.18	-	0.10***
VP	0.26 ± 0.18	0.10 ± 0.18	0.07 ± 0.18	0.31± 0.17	0.00 ± 0.19	0.37 ± 0.15	-0.03 ± 0.19	-

SH = Sapling height (cm), BL = branch length (cm), NB = number of branches, BA = branch angle (°), BD = branch diameter (mm), NGC = number of growth cycles, SF = stem form, and VP = vegetation periods (days).
*: Significant at P < 0.05; **: significant at P < 0.01; significant at P < 0.001.

2006).

The genetic and phenotypic correlations between branch angle and other observed characteristics were found to be quite low. Branch angle had the highest genetic correlation with number of branches and the highest phenotypic correlation with branch length. There are some studies reporting low genetic and phenotypic correlations between the growth characteristics and branch angle in Turkish red pine and some other species (Işık, 1998; Vargas-Hernandez et al., 2003; Sebbenn et al., 2007).

Conclusion

Statistically significant differences were observed among populations and families within the populations both in the seed stands and seed orchards for all characteristics. It was determined that the seed orchards were better than the seed stands in terms of sapling height, stem form, number of growth cycle, branch length and branch diameter. At the same time, the seed orchards were observed to have a little more genetic diversity compared to the seed stands. In other words, the high genetic diversity in the seed stands could be successfully transferred to the seed orchards. It could be explained that the seed stands might have been manipulated by tending, pruning and other treatments.

Whilst in the seed stands, variance observed in characteristics apart from number of branches and vegetation period was more due to the differences between populations; in the seed orchards, it was more due to the differences between intra-population families in all characteristics apart from stem form. In the seed stands, some characteristics were kept under genetic control on the population level and some on the family level. Whereas in the seed orchards, all characteristics apart from stem form were genetically controlled more at the family level. Therefore, the selection to be conducted in terms of the characteristics should be done on both the population and the family level in seed stands; whilst in seed orchards it should be performed more on the family level. A considerable part of the total variance calculated for all characteristics stems from genetic differences between half-sib individuals within the families. This situation also means that the intra-family genetic diversity is high, which is a significant potential in terms of increasing the genetic gain. This potential should be taken into consideration in breeding programme to be conducted in Turkish red pine.

Medium and high levels of individual and family heritabilities were estimated in terms of all characteristics. Heritabilities were higher in the seed orchards compared to the seed stands. Both in the seed stands and seed orchards, family heritabilities were found to be relatively higher in proportion to the individual heritabilities for all traits. This result indicates that more genetic gain can be achieved with selection to be conducted on the family level in this species.

Strong positive correlations were identified between branching characteristics except for sapling height and branch angle. That is to say, when selection is being conducted for height in Turkish red pine breeding programme, individuals which branch more and coarsely will also be selected. However, as branching situation will change with further age due to natural pruning, correlations between branching characteristics and sapling height should be re-examined in future years of the trial.

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