

DIAMETER GROWTH AND SURVIVAL OF LOCAL HALF-SIB FAMILIES OF SCOTS PINE (*Pinus sylvestris* L.) IN YUNDOLA, BULGARIA

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The paper presents results of a study on a progeny test in Yundola established in 1966 and comprising 26 local plus trees of Scots Pine. Diameters at breast height (DBH) were measured in 1996 and 2012. There were substantial differences among the performance of half-sib families regarding the diameter growth. The best performing families 30 and 46 years after planting were the same, but there were statistically significant changes in the rank of the families as a whole. Mean survival rate was 91% at the age of 30 and 79% at the age of 46, which is relatively high. Individual heritability increased from 0.2 to 0.5-0.6 from the age 30 to 46 and additive genetic coefficient of variation was 5.2% at the age of 30 and 10.1% at the age of 46. Heritabilities and estimated response to selection were within the range of the results reported in other studies on Scots Pine. The main inferences of the study are that individual selection could be a promising tool for genetic improvement of Scots Pine in the region, and genetic parameters could change over time even after 30 years of age.

Key words: heritability, genetic gain, progeny test, tree breeding

INTRODUCTION

Scots Pine (*Pinus sylvestris* L.) is among the most studied forest tree species in Europe. The attention to this species is due to its economic importance and wide distribution, and investigations comprise broad range of topics and problems, including its genetics and breeding (ERIKSSON, 1998, 2008). It was also among the first species included in IUFRO series of provenance research at the beginning of 20th century (GIERTYCH and OLEKSYN, 1992). Experiments aiming at improvement of the species and including selected genetic entries have gained increasing attention in all parts of its natural area of distribution, and particularly where it is considered one of the most important species from economic point of view. For example, in Finland almost six million trees were planted in progeny trials (ERIKSSON, 2008; HAAPANEN, 2002). Currently the extensive studies on genetics of Scots Pine encompass a broad set of

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modern methods, ranging from classical breeding methods to molecular breeding, including identification of candidate genes and QTLs (ABRAHAMSSON, 2011; ERIKSSON, 2008).

Progeny tests could be classified into classical breeding methods. Usually they are aimed at analyzing the family performance and inheritance of the traits of interest. However, the significant environmental effects require establishment of numerous progeny tests for selection of genetic entries appropriate for different breeding zones, representing a complex of ecological conditions. Genotype x environment (GxE) interactions could make this task much more laborious (HAAPANEN, 1996; ZHELEV *et al.*, 2003). There is a lot of information about the genetics and breeding of Scots Pine at northern latitudes, while such information is relatively scarce about other parts of its area of distribution, like Balkans, and Bulgaria, in particular. There are some studies reporting different aspects of Scots Pine genetics on Balkans (e.g., BALLIAN *et al.*, 2009; LUČIĆ *et al.*, 2011a,b). In Bulgaria it is also one of the most studied coniferous species. Numerous experiments have been established starting from 1950s and the trials that survived to date possess a substantial interest for evaluation of long-term development of the genetic entries. However, many of the established trials lack replications, thus making proper statistical analysis difficult. One experiment that meets the modern requirement of experimental design was established in the University forest in Yundola in 1966 (DOBRINOV and KALINKOV, 1977). Unfortunately, nowadays height was technically almost impossible to measure precisely. Therefore, diameter growth was selected as a target trait and the objective of present study was to assess diameter growth and survival rate of half-sib families of local origin in a progeny test in Yundola, Western Rhodopes, Bulgaria. Particularly, for achieving this objective, several tasks were set to: 1) evaluate the performance of half-sib families regarding their diameter growth, 2) calculate heritabilities and to draw some implications for tree breeding and 3) evaluate survival rate.

MATERIALS AND METHODS

The experiment

Progeny trial was established by Professor Ivan K. Dobrinov (1922-2010) in the region of Yundola. In total 26 plus trees were selected, and seedlings produced were planted in 1966 in three experiment trials: at 1200, 1400 and 1600 m a.s.l. The initial objective was to test genotype x environment interactions and it was partially done with the height growth during the early stage of development of seedlings (DOBRINOV *et al.*, 1976). Unfortunately, at the moment of the last measurement (2012) only the trial at 1400 m was in good conditions allowing proper measurement and reliable inferences. The trials at 1200 and 1600 m a.s.l. were destroyed by different reasons – pathogens, abiotic factors, as well as insufficient and improper management. Therefore, only the trial at 1400 m a.s.l. was included in the study.

The design of experiment was complete randomized block design, with three blocks (replications) and 25 individuals per half-sib family per block.

Measurement and data analysis

Diameters (DBH) of all trees were measured to the nearest cm in 1996 and 2012, at the age of 30 and 46 years after planting, respectively. The real age of seedlings is probably two years more, but hereafter the age after planting will be used, i.e. 30 and 46 years. Survival rate was calculated as percent of survived trees of the total number of planted seedlings.

Diameter data were log-transformed before the statistical analysis (KUNG, 1988) and ANOVA was run on transformed and non-transformed (original) data for comparison. When

presenting the ranking of families, original (non-transformed) data were used. The following model was applied:

$$Y_{ijk} = \mu + F_i + B_j + FB_{ij} + e_{ijk},$$

where:

Y_{ijk} is the value of k -th individual of i -th half-sib family in j -th block;

μ is the overall mean;

F_i is the random effect of i -th half-sib family ($i = 1, \dots, 26$);

B_j is the fixed effect of j -th block ($j = 1 \dots 3$)

FB_{ij} is the effect of interaction between i -th half-sib family and j -th block;

e_{ijk} is the residual error;

Survival data were *arcsin*-transformed before the ANOVA, but the analysis was based on family values for block.

Significance of rank change of half-sib families from the age of 30 to 46 was tested by two approaches. The first one included fitting of a linear model:

$$Y = Time + Fam + Time \times Fam,$$

where the effect of half-sib families (*Fam*) was considered random. The rank change was considered significant when the effect of interaction term *Time x Fam* was significant.

The second approach consisted of testing the significance of rank correlation coefficients of Spearman (ρ) and Kendall (τ). Lack of statistical significance indicated significant change of ranks from the age 30 to 46.

Hertiability of diameter growth (single-tree basis) was calculated using the formula of COTTERILL (1987), and additive (family) variance component was multiplied by 3, which is considered as more realistic approach, not overestimating heritability (WILLIAMS *et al.*, 2002). The additive genetic coefficient of variation (CV_A) was calculated as percentage of additive genetic standard deviation (σ_A) of the phenotypic mean (\bar{x}):

$$CV_A = 100 \left(\frac{\sigma_A}{\bar{x}} \right)$$

Predicted response to selection (genetic gain) was calculated for 10% selection intensity (FALCONER and MACKAY, 1996):

$$\Delta G = h^2 \cdot S = h^2 \cdot i \cdot \sigma_p,$$

Where:

ΔG is the genetic gain,

h^2 is heritability,

S is selection differential,

i is selection intensity, and

σ_p is phenotypic standard deviation.

RESULTS AND DISCUSSION

Progeny performance – diameter growth and survival

ANOVA test showed that the two factors – family and block and their interaction had significant effect on the variation of diameter (Table 1). There were no principal differences in

the results when original and log-transformed data were used. The significance of factors' effect increased with increasing of the age of trees (Table 1).

Table 1. Analysis of variance

Source of variation	DF ¹⁾	Mean Square		F-ratio		p-value	
		NT	LT	NT	LT	NT	LT
1996 measurement							
REP	2	15450.467	0.665	5.033	5.258	0.010	0.008
FAM	25	7042.103	0.319	2.290	2.520	0.006	0.003
REP * FAM	50	3082.383	0.127	4.173	3.271	<0.001	<0.001
ERROR	1749	738.716	0.039				
2012 measurement							
REP	2	44803.137	1.232	18.678	19.072	<0.001	<0.001
FAM	25	25013.569	0.603	10.392	9.312	<0.001	<0.001
REP * FAM	50	2420.406	0.0651	1.814	1.678	0.001	0.002
ERROR	1487	1334.009	0.0388				

¹⁾Legend: NT – non-transformed (original) data; LT – log-transformed data; DF – degrees of freedom; REP – replication; FAM – half-sib family.

At the age of 30 (1996 measurement) the best half-sib families were № 25 and № 1 (mean diameter 16.8 cm each), followed by № 7 (16.7 cm), and № 14 (16.4 cm). The worst performing were half-sib families № 21 and № 26 (mean diameter 12.9 and 12.8 cm, respectively) (Fig. 1 and Appendix 1). The difference between the best and worst performing families was 4.0 cm. Coefficient of variation, representing the standard deviation as a percentage of mean, ranged from 15.1 to 25.4 % (see Appendix 1), which can be evaluated as moderate to high variation. It indicates relatively high inter-individual variation within the half-sib families. The lowest minimum values were within the range 5-7 cm, but these could be some damaged trees, i.e. the reasons for these low extremes can be due to external reasons. However, all they were included in the analysis. The highest maximum values were within the range 26-32 cm (Appendix 1).

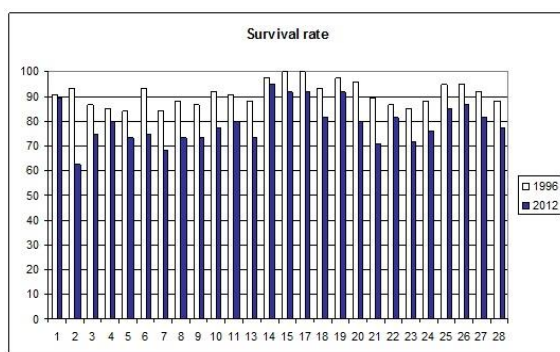


Figure 1. Mean diameter (DBH) of the progenies measured 1996 and 2012. The means connected with a solid line are not significantly different at $p \leq 0.05$, tested by One-way ANOVA and post-hoc Tukey test.

The results showed that three groups could be distinguished, even though with some approximation: best performing, moderately performing and worst performing families. There were more or less pronounced differences among these groups, but there was also continuous, cline-like change of the mean values and therefore, overlapping of the borders of the three groups mentioned above. This fact shows that the selection of plus trees still cannot be completely verified at the age of 30, based solely on diameter growth.

At the age of 46, best performing family was № 1 (mean diameter 26.8 cm), followed by № 25 (20.3 cm), № 7 (19.4 cm) and № 9 (19.4 cm). Again, the worst performing families were № 21 and № 26 (mean diameter 16.0 and 15.8 cm, respectively) (Fig. 1 and Appendix 1). Here the best half-sib family was significantly different from the others, but the significance of differences among the other half-sib families was not clearly expressed (Fig. 1). Even the mean diameter of the worst performing half-sib family (№ 26) was not significantly different from the means of the next eleven families in ascending order (Fig. 1). The coefficient of variation ranged from 14 to 28 %, which is practically of the same magnitude as at the age of 30. The lowest minimum values were within the range 8-10 cm, and the highest maximum values – within the range 33-36 cm (Appendix 1).

In spite of the fact that the best and worst families were generally the same, there was significant change in the ranking of families from the age of 30 until the age of 46. The interaction *Time x Fam*, tested by ANOVA, was highly significant ($F = 5.75$, $DF = 25$, $p < 0.001$), while the rank correlation coefficients were not: Spearman's rho ($\rho = 0.027$; $p = 0.89$) and Kendall's tau ($\tau = 0.046$; $p = 0.76$). The significant changes in ranks indicate that in some experiments 30 years could not be enough for final evaluation of diameter growth in Scots pine half-sib families. However, it should be noted that in many cases the differences among family means were not statistically significant (see Fig. 1). In other words, the change of ranks was generally significant but of small magnitude.

Data transformation had practically no effect on the results at the age of 30 and the age of 46 (Table 1), which confirms that the distribution of the original (non-transformed data) is close to the normal one. Probably the lack of substantial deviations of the actual data distribution from the normal one is due to the large number of individuals per progeny per replication. Log-transformation usually improves not only normality, but also additivity (KUNG, 1988). It could be useful tool when the data lack normality or when ratios are used, because ratios introduce non-linearity in a linear model, but log-transformation converts ratios in a difference between the logarithms of nominator and denominator, which is also a linear relationship (NEWMAN and JANCEY, 1983). The results of the present study indicate that the distribution of raw, non-transformed data of the present study was close to the normal one.

ANOVA test of survival data (complete results not shown) revealed that both family ($p = 0.025$) and replication ($p < 0.001$) effects were significant at both ages of measurement. The mean survival rate was relatively high – 91% at the age of 30 (1996) and 79% at the age of 46 (2012). Minimum survival rate in 1996 was 84% (family № 7), and the maximum one was 100% (families № 15 and № 17; Fig. 2). Minimum survival rate in 2012 was 63% (family № 2) and maximum – 95% (family № 14). Survival rate of 79% at 46 years could be evaluated as relatively high.

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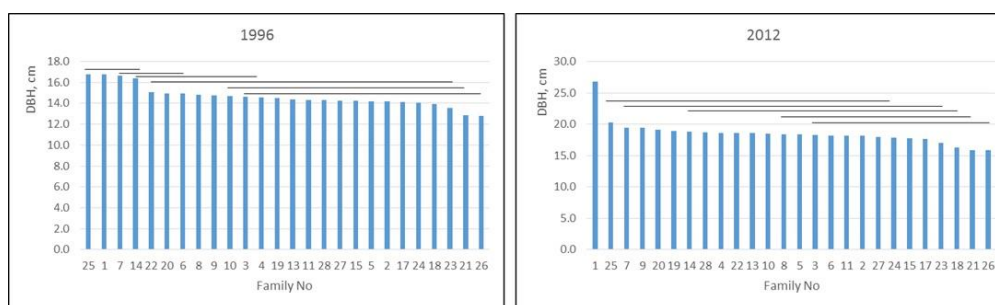


Figure 2. Survival rate (in %) of the half-sib families estimated in 1996 and 2012. Straight lines relate means, which are not significantly different at $p \leq 0.05$.

Usually survival is considered very important evolutionary trait and is used for analysis of provenance transfer (EICHE, 1966; ERIKSSON *et al.*, 1980; PERSSON, 1994). The empirical results have shown that the growth traits are less affected by provenance transfer (BASTIEN and ALIA, 2000). HERTEL and SCHNECK (1999) reported survival 38-57% of 72 provenances. Surprisingly, in the literature there were some higher survival rates in trials out of the natural area of distribution of *P. sylvestris* than within it. For example, CUNNINGHAM and VAN HAVERBEKE (1991) reported mean survival rate of 93% at age of 22 in a provenance trial in North Dakota (USA), while PERSSON (2006), and PERSSON and ANDERSSON (2003) reported 66% average survival across trials at 9-13 years of age. This could be explained by the harsher conditions at higher latitudes, causing repeated injuries on the plants over several years (see PERSSON, 2006 and the references therein).

Heritability and genetic gain

Variance components changed with age. Additive variance component from the age of 30 to 46 increased from 6.62% to 20.10% (non-transformed data). The component due to the interaction family x replication and the error term slightly decreased. Log-transformed data

showed similar values and trend, thus confirming again that transformation was practically not necessary in the particular data set used in the present study (Table 2).

Table 2. Variance components (REML method) and single-tree heritability (h_i^2) estimates

Component	1996				2012			
	Non-transformed data		Log-transformed data		Non-transformed data		Log-transformed data	
	Value	%	Value	%	Value	%	Value	%
FAM	59.072	6.55	0.0029	6.33	349.32	20.10	0.00843	17.36
FAM*REP	103.806	11.51	0.0039	8.52	54.317	3.13	0.00132	2.72
ERROR	738.639	81.93	0.0390	85.15	1334.49	76.78	0.0388	79.92
Single tree heritability								
$h_i^2 = 0.20$		$h_i^2 = 0.19$		$h_i^2 = 0.60$		$h_i^2 = 0.52$		

Individual heritability (h_i) increased from 0.2 to 0.5-0.6 from the age of 30 to 46. Data transformation had only slight effect on the heritability (0.52 vs 0.60), and only at the age of 46. These values are relatively high for Scots Pine, where most estimates of heritability of growth traits are seldom higher than 0.3. CORNELIUS (1994) summarized 67 studies on forest trees and revealed that heritabilities of most traits were within the median range 0.18-0.26, and a notable exception was wood specific gravity (median 0.48). The studies published after CORNELIUS (1994) review confirmed this trend, at least for Scot Pine (see ERIKSSON, 2008, for review). However, it should be noted that the heritability always tends to be higher if estimated in one test site only. Calculation of heritability across several sites (more than one) adds one more term in the denominator – the one of genotype x environment interaction – and therefore, heritability estimates could decrease to an extent depending on the value of this term. For example, for *Pinus sylvestris* JANSSON (2008a,b) estimated individual heritability of diameter growth 0.11 across sites and 0.14 to 0.24 in single sites, and ZHELEV *et al.* (2003) reported 0.07 across sites and values from 0.07 to 0.19 in individual sites.

Increase of heritability with age is not a common phenomenon in Scots Pine. For example, HAAPANEN (2001) did not find any consistent pattern in the development of height-growth heritability over time, while JANSSON *et al.* (2003) detected a slight tendency for increase of height-growth heritability with age. ZHELEV *et al.* (2003) reported also a slight increase of heritability of all traits studied in the period from 12 to 16 years.

The recent studies in conifers reported for heritability of diameter growth values of similar magnitude. The family heritability of diameter growth in Corsican Pine (*Pinus nigra* subsp. *laricio* (Poir.) Maire) was 0.6 in the study of LEE and CONNOLLY (2004), but GÜLCÜ *et al.* (2013) estimated for diameter growth of Anatolian Black Pine (*Pinus nigra* Arn.) values of individual heritability 0.44 and family heritability 0.71. MIHAI and MIRANCEA (2016) in *Abies alba* reported individual heritabilities 0.21 and 0.23 for the diameter growth at the age of 25 and 34, respectively. The family heritabilities for the same traits were 0.37 and 0.41, respectively. MIHAI and TEODOSIU (2009) reported broad sense heritability for diameter growth 0.77 in the European Larch (*Larix decidua* Mill.). In Norway Spruce, HANNRUP *et al.* (2004) estimated low heritability for diameter growth – 0.19. When summarizing the results of studies in heritability of

coniferous forest tree species during the last two decades, we could see that they fit into the general trends estimated by CORNELIUS (1994).

HOULE (1992) recommended using additive genetic coefficient of variation (CV_A) as a measure combining variation and evolvability of populations. In the present trial CV_A was 5.2% at the age of 30 and 10.1% at the age of 46. These values are well within the range summarized by CORNELIUS (1994) for diameter growth – median 8.6%. JANSONS (2008b) reported similar values – 7.5% across sites and 8 to 11% on individual sites, and ZHELEV *et al.* (2003) – from 6.9 to 12.2 % on individual sites.

Genetic gain, as estimated based on individual heritability and at 10% selection intensity, was 1.18 cm (or about 8%) at the age of 30, and 4.6 cm (about 25%) at age 46. The latter seems rather high but it should be noted that the heritability and respectively, genetic gain, could be overestimated when based on a single site (see above).

Usually genetic gain in diameter is larger than that in height (ZHENG *et al.*, 1994). JANSONS (2008b) reported values of genetic gain 11.3% across sites and mean 8.3% in single site at 10 % selection intensity. He found the highest genetic gain for volume growth ranging from 11.9 to 33% in single site, which concords well with the results of the present study.

CONCLUSIONS

The study showed that successful breeding activities could be performed even within a relatively small region. Differences found among the half-sib families indicate that selecting the best families could be a promising activity in the breeding of local Scots Pine. Individual heritability of diameter growth had moderate value and increased almost twice from the age 30 to 46 years. The study found a reasonable concordance between using original and log-transformed data, which was probably due to a large number of individuals per half-sib family. The local half-sib families demonstrated high survival rates, when grown under optimal conditions. Genetic gain in the breeding of Scots Pine can be reasonable even when selection takes place within one large natural population.

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RAST PREČNIKA I PREŽIVLJAVANJE LOKALNIH POLUSRODNIH FAMILIJA ŠKOTSKOG BORA (*Pinus sylvestris* L.) U JUNDOLI, BUGARSKA

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Izvod

U radu su predstavljeni rezultati testiranja potomstva 26 drveta Škotskog bora u Jundoli, zasađenih 1966. Prečnici stabla (DBH) mereni su 1996 i 2012.godine. Utvrđene su znatne razlike u rastu prečnika kod polusrodnih familija. Familije sa najboljim performansama bile su iste i posle 30 i posle 46 godina od zasađivanja, ali su bile statistički značajne razlike u rangiranju svih familija. Srednji stepen preživljavanja bio je 90% kod borova starosti 30 i 79% kod drveća starog 46 godina. Pojedinačna heritabilnost se povećala sa 0.2 (drveće staro 30 godina) do 0.5-0.6 (drveće staro 46 godina), a aditivni genetički koeficijent varijacije bio je 5.2%, odnosno 10.1%. Heritabilnost i procenjen odgovor na selekciju bili su u nivou do sad objavljenih rezultata istraživanja na Škotskom boru. Glavni zaključci istraživanja su da individualna selekcija može biti korisna za genetičko poboljšanje Škotskog bora u region, i da se genetički parametri mogu promeniti tokom vremena, čak i posle 30 godina.

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*Appendix I. Descriptive statistics parameters of diameter growth of half-sib families**A) measured in 1996 (at the age of 30)*

	Half-sib family №									
	1	2	3	4	5	6	7	8	9	
Mean ¹⁾	16.75	14.18	14.61	14.56	14.20	14.93	16.24	14.82	14.77	
S. D.	3.34	2.83	2.63	2.82	3.19	3.05	3.19	2.74	2.72	
C.V.	19.92	19.96	18.04	19.36	22.48	20.43	19.63	18.47	18.41	
Min	7.0	5.5	9.0	5.0	1.9	10.5	9.5	9.0	9.0	
Max	24.0	24.0	22.0	20.0	23.0	32.5	26.0	21.0	22.0	
	10	11	13	14	15	17	18	19	20	
Mean	14.68	14.34	14.39	16.37	14.22	14.13	13.95	14.51	14.93	
S. D.	3.12	2.85	3.66	3.46	2.78	2.66	2.49	3.11	2.63	
C.V.	21.25	19.88	25.40	21.13	19.57	18.82	17.84	21.46	17.63	
Min	7.5	9.0	6.5	9.5	8.5	8.5	8.0	6.5	8.5	
Max	25.0	22.0	24.5	24.0	27.0	23.5	21.5	22.0	25.0	
	21	22	23	24	25	26	27	28		
Mean	12.87	15.07	13.53	14.03	16.77	12.80	14.22	14.17		
S. D.	2.30	2.27	2.73	2.68	2.40	2.08	2.67	2.72		
C.V.	17.89	15.08	20.16	19.08	14.29	16.22	18.76	19.18		
Min	7.5	10.5	8.0	8.0	12.5	7.0	8.0	7.5		
Max	20.5	22.0	19.5	23.0	23.0	17.5	21.0	22.0		

B) Measured in 2012 (at the age of 46)

	Half-sib family №									
	1	2	3	4	5	6	7	8	9	
Mean	26.76	18.15	18.32	18.60	18.38	18.16	19.43	18.44	19.42	
S. D.	3.78	3.76	3.53	3.61	4.07	3.92	4.02	4.20	3.28	
C.V.	14.12	20.74	19.28	19.40	22.15	21.59	20.69	22.77	16.90	
Min	18	12	11	13	12	11	10	12	12	
Max	36	33	28	27	31	31	32	28	29	
	10	11	13	14	15	17	18	19	20	
Mean	18.53	18.15	18.56	18.79	17.72	17.68	16.24	18.86	19.14	
S. D.	3.76	3.78	5.32	4.14	3.60	4.12	2.91	3.77	3.65	
C.V.	20.29	20.81	28.63	22.02	20.33	23.28	17.91	19.97	19.06	
Min	11	11	10	12	11	11	9	12	13	
Max	29	29	34	33	28	33	23	30	30	
	21	22	23	24	25	26	27	28		
Mean	15.91	18.59	17.06	17.82	20.33	15.83	17.95	18.74		
S. D.	3.11	3.27	3.86	3.92	3.63	2.78	3.97	3.99		
C.V.	19.54	17.61	22.62	22.01	17.86	17.53	22.13	21.29		
Min	11	12	8	12	14	9	11	10		
Max	25	31	27	31	30	22	29	31		

¹⁾S.D. = standard deviation, C.V. = coefficient of variation. All results are in centimeters, except the coefficient of variation, which is in %.