

## Influence of growth on reproductive traits and its effect on fertility and gene diversity in a clonal seed orchard of scots pine, *Pinus Sylvestris* L.

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**Abstract:** This study was carried out in a clonal seed orchard of scots pine (*Pinus sylvestris* L.), to determine the difference and interaction for reproductive and growth characters among clones and its impact on fertility variation and gene diversity. Numbers of female and male strobili, and height and diameter at breast height were studied on six grafts chosen randomly in each of the 27 clones for the purpose. One-way analysis of variance revealed large differences in both reproductive and growth characters among clones. The differences were higher in growth characters than in reproductive traits. There was significant phenotypic correlation among growth and reproductive characters. So, growth characters had a greater effect on male and female fertility. Estimates of total fertility variation (Sibling coefficient= 1.012), status number (26.8) and relative gene diversity (0.981) were computed. Fertility variation among clones was low, which caused a high relative population size (99% of census number). The positive phenotypic correlation between growth and reproductive characters showed that enhanced growth rate could be effective in improving fertility and gene diversity of seed orchard crop. The results of the study have implications in breeding and selection of plus tree and populations, establishment and thinning of seed orchards of the species.

**Key words:** *Pinus sylvestris*, Sibling coefficient, Status number, Strobili  
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### Introduction

Seed orchards are expected to produce adequate quantity of improved seeds for afforestation or reforestation. They are established with vegetative or generative materials collected from plus trees selected phenotypically from natural stands or plantations. Adequate gene diversity in seed orchard crop is important for sustainable forestry and tree breeding. Gene diversity is influenced by variation in strobili production of clones in an orchard. It can be also related to growth characters such as height, diameter, number of branches, and crown diameter of clones.

A positive relation between reproductive and growth characters is reported in scots pine (*Pinus sylvestris* L.) in some studies (Jonsson *et al.*, 1976; Bhumibhamon, 1978; Burczyk and Chalupka, 1997), while certain other studies reported negative correlations among them (Nikkanen and Velling, 1987).

Gene diversity estimation based on fertility variation has been made recently in different forest tree species (Kang, 2001; Kang and Lindgren, 1998; Bilir *et al.*, 2002; Bilir *et al.*, 2004; Varghese *et al.*, 2006; Ayan *et al.*, 2006; Pandeya *et al.*, 2007). Estimation of fertility variation is used for many purposes such as genetic management of populations, breeding (El-Kassaby, 1995; Griffin, 1982), and gene conservation programs (Kang *et al.*, 2003).

There is a total of 114 ha of scots pine seed orchards at 21 places in Turkey (Anonymous, 2001) and 9.2% of annual seed

demand for plantations in the species was covered from the orchards. The remaining requirement is covered from seed stands selected phenotypically (Cengiz, 2003).

This study was conducted to examine the correlation between reproductive and growth characters in a Scots pine seed orchard and to determine its effect on fertility and gene diversity in seed orchard crop. The results of the study are discussed and based on breeding strategies of the species and establishment and management of seed orchards.

### Materials and Methods

**Seed orchard and data collection:** The seed orchard of *Pinus sylvestris* was established at Sogutlu-Adapazari (latitude 40°52'N, longitude 30°42'E, altitude 120 m) in the northern part of Turkey in 1986. Grafts of 29 clones (total of 1265 grafts) (Anonymous, 2001), which were originated from plus trees selected in a seed stand at Dirgine-Zonguldak, were planted at a spacing of 7 m x 7 m. Data on the reproductive (female strobili,  $N_f$ , and male strobili,  $N_m$ ) and growth (height, H, and diameter,  $d_{1.30}$  at breast height of graft/clones) characters were recorded from six randomly chosen ramets each of the 27 clones at the beginning of May 2005.

**Fertility variation, status number and gene diversity:** In this study, fertility variation is estimated based on the variation in strobili production among clones in the seed orchard. The female ( $\psi_f$ ) and

male ( $\psi_m$ ) fertility variation was estimated by Kang and Lindgren (1999) as:

$$\psi_f = N \sum_{i=1}^N \left( \frac{f_i}{\sum f_i} \right)^2, \psi_m = N \sum_{i=1}^N \left( \frac{m_i}{\sum m_i} \right)^2$$

(1a and b)

where  $N$  is the census number,  $f_i$  is the number of female strobili of the  $i^{\text{th}}$  individual,  $m_i$  is the number of male strobili of the  $i^{\text{th}}$  individual.

Total fertility variation ( $\Psi$ ) was calculated by Bilir *et al.* (2005) as:

$$\Psi = \left( \frac{CV_f^2 + CV_m^2}{4} \right) + 0.5 \left( N \sum_{n=1}^N \frac{f_n m_n}{\sum f_n \sum m_n} + 1 \right) \quad (1c)$$

where  $N$  is the census number,  $CV_f$  is the coefficient of variation in female fertility, and  $CV_m$  is the coefficient of variation in male fertility,  $f_n$  and  $m_n$  are the number of female and male strobili of the  $n^{\text{th}}$  individual;  $f$  and  $m$  are used as index for the female and male strobili, respectively.

If the clones are unrelated and non-inbred, the status numbers of female ( $N_{s(f)}$ ) and male parents ( $N_{s(m)}$ ) are calculated (Lindgren *et al.*, 1996) as:

$$N_{s(f)} = \frac{1}{\sum_{i=1}^N P_{i(f)}^2}, \quad N_{s(m)} = \frac{1}{\sum_{i=1}^N P_{i(m)}^2} \quad (2a \text{ and } b)$$

where  $p_{i(f)}$  is the female fertility of the clone  $i$ ,  $p_{i(m)}$  is the male fertility of the clone  $i$  and  $N$  is the census number in the seed orchard. Here, fertility is estimated based on the strobili assessment.

Status number on total fertility was calculated as:

$$N_s = \frac{4N}{[\psi_f + \psi_m + 2 + 2r\sqrt{(\psi_f - 1)(\psi_m - 1)}]} \quad (2c)$$

where  $N$  is census numbers of clone,  $\psi_f$  and  $\psi_m$  are the fertility variation of female and male parents and  $r$  is the correlation coefficient between female and male fertility (Kang and El-Kassaby, 2002).

Gene diversity (GD) in seed orchard crop was estimated based on total fertility variation ( $\psi$ ) and census number of clones ( $N$ ) as:

$$GD = 1 - \frac{0.5\Psi}{N} \quad (3)$$

**Statistical analysis:** Differences among clones for both reproductive and growth characters were determined by following model of one way-analysis of variance:

$$Y_{ij} = \mu + C_i + e_{ij}$$

where  $Y_{ij}$  is the observation from the  $j^{\text{th}}$  graft of the  $i^{\text{th}}$  clone,  $\mu$  is overall mean,  $C_i$  is the random effect of the  $i^{\text{th}}$  clone and  $e_{ij}$  is random error. The error includes environmental variation as well as random sources of genetic deviation due to somatic mutations and genetic variation among rootstocks.

The characters were related by Pearson's correlation analysis using SPSS statistical package program. Besides clones were grouped by Duncan's multiple range test for the characters.

## Results and Discussion

**Characters:** As seen from Table 1, there was a large difference for the characters among clones. For instance, the clone with the highest strobili production (355 for  $N_f$  and 1034 for  $N_m$ ) produced about 80 % more strobili than that of the lowest contribution (203 for  $N_f$  and 596 for  $N_m$ ). The differences were also showed by analysis of variance (Table 2).

Averages of female and male strobili production were 201.4 and 531.3 respectively in the orchard five years ago (Bilir *et al.*, 2002). Variable flowering may occur in seed orchards because of many factors such as location and years (Lindgren *et al.*, 1977).

The difference among clones is important for their contribution to gene pool and gene diversity. Results of the analysis of variance showed significant ( $p < 0.01$ ) differences in both growth and reproductive characters among clones (Table 2). The clones were grouped by Duncan's multiple range test after determination of differences for the characters among clones. According to results of Duncan's multiple range test, clones were at three groups for  $N_f$ , five groups for  $N_m$ , seven groups for H and nine groups for  $d_{1,30}$ . It showed that the differences among clones for growth characters were larger than that for reproductive traits. Differences in gamete contribution among clones could be influenced by management of orchards (Zobel and Talbert, 1984).

There were significant ( $p \leq 0.01$ ) correlations among growth and reproductive characters (Table 3).

The positive correlations were also reported by Jonsson *et al.* (1976), Bhumibhamon (1978) and Burczyk and Chalupka (1997) in the species and by Nikkanen and Ruotsalainen (2000) in *Picea abies*, and by Varghese *et al.* (2000) in *Tamarindus indica*, while negative correlations are reported by Nikkanen and Velling (1987) in *Pinus sylvestris* and by Schmidtling (1981) in *Pinus taeda*.

**Table 1:** Average, coefficient of variation and range of the characters

	$N_f$	$N_m$	H	$d_{1,30}$
Average	254.3	742.3	700.2	21.1
CV %	22.1	21.3	16.8	13.7
Range	202.7–355.3	595.7–1034.2	551.7–865.0	17.9–26.5

**Table - 2:** Results of analysis of variance

Characters	Source of variance	Sum of square	D.F.	F value	p
$N_f$	Among clones	139537.2	26	1.960	$p < 0.01$
	Within clone	369690.2	135		
	Total	509227.4	161		
$N_m$	Among clones	1237094.5	26	2.306	$p < 0.01$
	Within clone	2785507.2	135		
	Total	4022601.7	161		
H	Among clones	1193377.8	26	6.028	$p < 0.01$
	Within clone	10227916.7	135		
	Total	2221294.4	161		
$d_{1,30}$	Among clones	681.1	26	5.207	$p < 0.01$
	Within clone	679.2	135		
	Total	1360.3	161		

**Table - 3:** Phenotypic relations among the characters

	$N_f$	$N_m$	H
$N_m$	0.775*	-	-
H	0.276*	0.256*	-
$d_{1,30}$	0.373*	0.356*	-
0.616*			

\* = statistically significant at 0.01 probability level

**Table - 4:** Fertility variation ( $\psi$ ), status number ( $N_s$ ), relative status number ( $N_r$ ) and gene diversity (GD) for female (f) and male (m) parents, and in total gametic gene pool (f+m)

	f	m	f+m
$\psi$	1.012	1.015	1.012
$N_s$	26.7	26.6	26.7
$N_r$	0.99	0.99	0.99
GD	-	-	0.981

There is also a significant positive ( $p \leq 0.01$ ) correlation between number of female and male strobili. Similar trends are also found by Bilir *et al.* (2002), Jonsson *et al.* (1976), Bhumibhamon (1978) and Burczyk and Chalupka (1997).

Determination of relation among reproductive and growth characters is important for breeding activities such as selection of plus trees, establishment and management of seed orchards. Also, seed orchard manager could be effectively managed strobili production and gene diversity (tending, fertilization and pruning).

**Fertility variation, status number and gene diversity:** Sibling coefficient values (fertility variation) were 1.012 for female and 1.015 for male parents and 1.012 for total gametic gene pool (Table 4).

Status number was 26.7, 26.6 and 26.7 for female, male parents and total gametic gene pool, respectively. The relative status number (99% of census number) was quite high. The estimated gene diversity in seed orchard crop based on total fertility was

0.981. Similar results were also reported by Bilir *et al.* (2002) for 2001 of the same orchard data. Gene diversity (GD) is also estimated based on status number ( $N_s$ ) as:  $GD = 1 - 1/(2 \cdot N_s)$  (Kang and Lindgren (1999). Variations of strobili among clones or grafts are effective on contribution in gene pool of the orchard. For instance, female ( $\psi_f$ ) and male ( $\psi_m$ ) fertility variation were related based on coefficients of variation in female ( $CV_f$ ) and male ( $CV_m$ ) strobili production among individuals by Kang and Lindgren (1999) as:  $\psi_f = CV_f^2 + 1$  and  $\psi_m = CV_m^2 + 1$ . So, equal gamete contribution from clones/grafts should be obtained by management activities such as pruning, fertilization in orchards to minimize variations among clones and to maximize gene diversity in seed orchard crop.

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