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MALE FERTILITY IN CLONAL SEED ORCHARD OF SPHEROID SCOTS PINE

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Abstract: *Spheroid scots pine (Pinus sylvestris L. var. compacta (Tosun) Ü. Akkemik) clonal seed orchard was established in April 2004 including 823 ramets of 26 clones at 5m x 5m spacing in Eflani of Karabuk, Turkey. Five ramets per clone were chosen randomly and all of the male strobili over the entire crown on all grafts were counted in 2016 and 2017. Average numbers of male strobili per clone were ranged from 130.0 to 83710.7 for two consecutive years. Pearson correlation for two consecutive years on male strobili production was 0.91 showing stability 2016 and 2017. The top 25% of clones produced 48% of total male strobili in two years. Male fertility variation, effective status number and relative status number were 1.56, 16.61 and 0.64, respectively. Estimation of broad sense heritability and clonal coefficient of genetic variation for male production was 0.49 and 12% respectively. The parameters were discussed for management of Spheroid scots pine clonal seed orchard aiming ex situ gene conservation and landscape activities.*

Key words: *Pinus sylvestris, heritability, male fertility variation, status number.*

1. Introduction

Breeding activities were started in 1964, but first National Tree Breeding Program (NTBP) of Turkey was implemented in 1994 [11]. *Pinus sylvestris* L. (Scots pine) was one of the target species in NTBP. In this context, 21 seed orchards occupying 116.4 ha in Scots pine have been established so far [18]. Two out of 21 seed orchards were established for ex situ conservation in *Pinus sylvestris* L. var. *compacta* (Tosun) Ü. Akkemik (Spheroid Scots pine). These orchards were clonal seed orchards, established by using grafted clones like other Scots pine seed orchards in Turkey. Spheroid Scots pine is a special variety (spheroidal, short, and bushy) and its distribution is scattered and limited in only Bolu province of Turkey [17]. Due to the special form, scions were collected from each parent tree in Bolu province and the scions were grafted to seedlings, then a clonal seed orchard was established by the grafted seedlings for *ex situ* conservation.

Flowering processes in an orchard are of great importance since they affect gene exchange among clones and genetic configuration of seeds obtained from seed

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orchard. Thus, genetic composition of orchards seeds is mainly determined by the numbers of female and male strobili produced by each clone [6, 9, 15]. The high male/female ratio and tremendous pollen production capacity indicate high male competition among trees within populations for successful out-crossing and maximum seed set through sufficient pollen grains reaching each megasporophylls, which addresses ultimately the evolutionary cause of this pattern [16]. On the other hand, male fertility is markedly affected not only by the quantity of fruit and seed production (siring success) but also by progeny quality i.e. seed germination rate and seedling survivorship [19].

Some researches on the flowering system were carried out for Scots pine clonal seed orchards in Turkey [2-3, 5]. There is also a research on the female fertility for Spheroid clonal seed orchard [1], but there is no research on the male flowering system. These orchards were established for ex situ conservation, but they can be also used for landscape service due to its special form [17]. Data on the reproductive mechanism of population of tree species are indispensable for effective genetic conservation and as working tools to our understanding of evolutionary mechanisms [16]. In this context, findings of a research on male flower production can be used for seed orchard management aiming *ex situ* conservation and landscape service. This study aimed to gather information about male production on two consecutive years and to obtain some parameters related to male production and to use it for seed orchard management.

2. Material and Methods

Spheroid scots pine seed orchard was established in April 2004 including 823 ramets of 26 clones at 5m x 5m spacing in Eflani of Karabuk, Turkey (latitude 41º 23´ 45 ˝N, longitude 32º 49´ 07˝E and altitude 890 m). A number of ramets per clone varied from 3 to 37. Usually, Scots pine seed orchard in Turkey initiates seed production at age ten [12]. The seed orchard was at 12 ages when data were collected in 2016. So, the seed orchard can be considered as a young seed orchard. Clone #339 has 4 and clone #341 has 3 ramets in the seed orchard. Except these two clones, five ramets per clone were chosen randomly and male strobili in 127 ramets were counted in May 2016 and 2017. All of the male strobili over the entire crown were counted on all chosen ramets. Modified Keskin (1999) method was used for counting of male flowers. Ten clusters were determined in the seed orchard including from 5 to 40 number flowers. The cluster number in each ramet was multiplied male flower number. Then multiplied values were summed up for each ramet. Logarithmic transformation was applied prior to analyses of variance for flower numbers. Height and crown diameter were measured and calculated volume index of sphere or cone shape of grafts. Volume index of each graft was used as a covariate in the statistical model. Following the statistical model used was:

$$y_{ijk} = \mu + AX_{ijk} + c_i + t_j + ct_{ij} + e_{ijk} \quad (1)$$

where:

y_{ijk} is the observation of the j^{th} ramet in the i^{th} clone and the k^{th} year;

μ - the overall mean;

A - the regression coefficient;
X_{ijk} - the volume index of clones in the t^{th} year (ijk=1,..254);
c_i - the random effect of the i^{th} clone (i=1,...26);
t_j - the random effect of the j^{th} year (j=1,2);
ct_{ij} - the clone x year interaction;
e_{ijk} - the experimental error.

Broad sense heritability and clonal genetic coefficient of variation for male fertility were calculated following formulas:

$$H_c^2 = \frac{\sigma_c^2}{\sigma_c^2 + \sigma_{ct}^2 + \sigma_e^2} \quad (2)$$

$$Cv_g = \frac{\sqrt{\sigma_c^2}}{\bar{X}} \cdot 100 \quad (3)$$

Where:
H_c^2 is the broad sense heritability;
σ_c^2 - the clonal variance;
σ_{ct}^2 - the clone year interaction variance;
σ_e^2 - the error variance;
Cv_g - the clonal genetic coefficient of variation;
$\bar{X}$ - the general mean.

Male fertility variation (Ψ_m) can be described by coefficient of variation (*CV*) in strobilus production, a measure suggested by Kang & Lindgren (1999); Kang & Mullin (2007) as:

$$\Psi_m = CV_m^2 + 1 \quad (4)$$

where CV_m^2 is the coefficient of variation in male strobilus production among clones. Effective number was calculated by the concepts of status number [13] and effective parent number [8]. Status number calculations were based on the fertility variation at male level [7, 9] as:

$$N_{s(m)} = N/\Psi_m \quad (5)$$

where: $N_{s(m)}$ is the status effective number of male strobilus, and *N* is the census number of clone.

Relative status number (N_r) was calculated as the ratio of the status number ($N_{s(m)}$) over census number (*N*) [9].

3. Results

Average, minimum, maximum male strobilus production, the coefficient of variation and standard deviation are presented in Table 1. The average of male strobilus production was 2420.74, 4018.53 and 3219.64 per clone in 2016, 2017 and total, respectively. The male strobilus production was ranged from 0.00 to 104482.00 per clone in two years.

Table 1

Average, minimum, maximum male strobilus per clone, coefficient of variation (*CV*), standard deviation (Sd)

Year	Average	Minimum	Maximum	*CV*	Sd
2016	2420.74	0.00	62939.40	0.85	2075.53
2017	4018.53	260.00	104482.00	0.72	2873.12
Pooled	3219.64	130.00	83710.70	0.75	2419.43

The analysis of variance was revealed statistically significant differences among clones and years for male strobilus production. But, differences among clones regarding volume index and clone-year interaction were not statistically significant (Table 2).

Broad sense heritability (H_c^2) was 0.49±0.14, clonal genetic coefficient of variation (CV_g) was 12% for two years. Pearson correlation between 2016 and 2017 on male fertility was 0.91.

Male fertility variation (Ψ_m), status number ($N_{s(m)}$), and relative status number (N_r) were 1.74, 15.0.and 0.58 for 2016, 1.51, 17.2 and 0.66 for 2017 and 1.56, 16.6 and 0.64 for pooled data respectively (Table 3).

Table 2

The analyses of variance for male fertility

Source of variation	Degree of freedom	Mean square	F Value	P
Volume index	1	2.340	0.02	0.903
Clones	25	2.694	2.47	0.012
Years	1	64.458	57.23	<0.0001
Clone*year	20	1.041	0.68	0.829
Error	75	1.523		

Table 3

Male fertility variation (Ψ_m), status number ($N_{s(m)}$), relative status number (N_r) for male strobilus production

Year	Ψ_m	$N_{s(m)}$	N_r
2016	1.74	15.0	0.58
2017	1.51	17.2	0.66
Pooled	1.56	16.6	0.64

The most fertile clone was #339, the less fertile one was #361. The top 25% of clones produced 48% of total male strobili, and the lowest 25% of clones produced 5 % of total male strobili (Figure 1).

4. Discussion

The production of male strobili was found to vary among clones in the clonal seed orchard. Average male strobilus production was 3219.64 per clone in pooled data. On the other hand, the highest production was 8697.5 for clone #339, the lowest production was 130.0 for clone #361 indicating unequal male strobilus contribution at clonal level in the seed orchard based on the 2-year average. The analysis of variance was revealed statistically significant differences among clones (P=0.012) and years (P<0.0001) on male strobilus production but was not revealed statistically significant differences in clone-year interaction (P=0.829). Volume index also was not important on strobilus production as a covariate, in other words, male strobilus production was not related to the crown size of grafts. The difference between years was higher than the difference between clones on male strobilus production, but Pearson correlation between years was 0.91 showing stability of male strobilus production of clones.

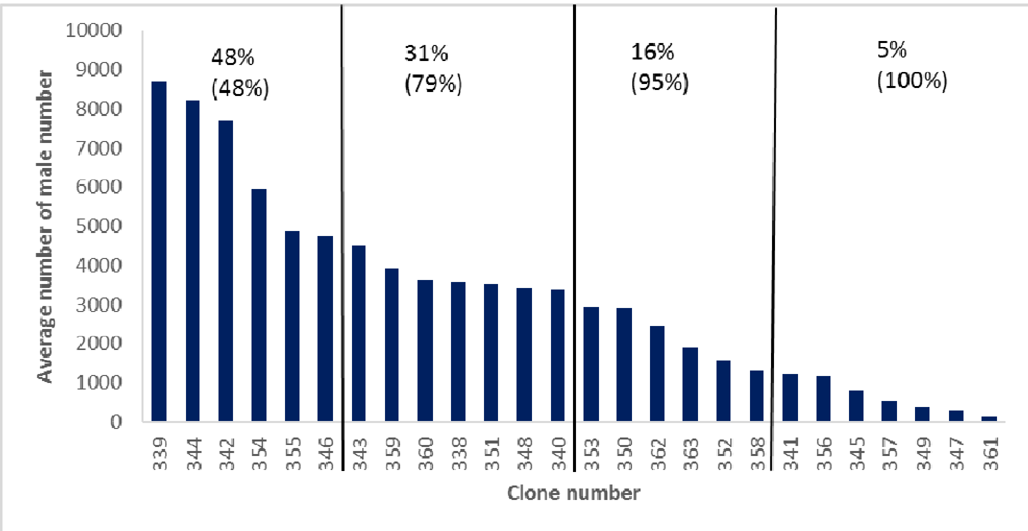

Fig. 1. The contribution of clones in male strobili production based on 2-year average (values in brackets are cumulative percent's)

Broad sense heritability (H_c^2) of male fertility was high in this research (0.49). Bilir et al. (2006) found that broad sense heritability of male fertility was 0.13 for pooled data of three mature scots pine seed orchards. Due to high heritability, selection on male strobilus production could be done in the Spheroid scots pine seed orchard [14]. The clonal genetic coefficient of variation (CV_g) was 12 % for two years showing moderate genetic diversity on clonal male strobilus production.

The sibling coefficients showing fertility variation were 1.74, 1.51 and 1.56 for 2016, 2017 and pooled data in the present research, respectively. If sibling coefficient is 1, then, the coefficient of variation is zero and each clone contributes with equal strobili in a seed orchard [15]. Dutkuner et al. (2008) found that sibling coefficients of male strobili were 1.015 in a mature Scots pine seed orchard, Bilir and Temirağa (2012) found also 1.02 and 1.03 in two mature Scots pine seed orchard. All sibling coefficients founded in mature Scots pine seed orchards were very close to 1 indicating that each clone producing equal strobili. In the present study, the sibling coefficient founded for Spheroid Scots pine was higher than the sibling coefficient of the mature Scots pine seed orchards. This situation might be sourced that Spheroid scots pine seed orchard was young and its grafts were specially formed. In this context, Prescher et al. (2007) claimed that variation in fertility seemed to be higher in young seed orchards and in years when seed production was low.

The status number and the relative status number ranged from 15.0 to 17.2

and from 0.58 to 0.66, respectively in this research. The status number explains how many ideal orchard clones would give rise to the considered crop. It gives a measure to compare different seed orchard designs, seed orchard management actions, consequences of pollen contamination, etc., for the expected relatedness and gene diversity in a seed orchard crop [13]. Due to the relation between the status number and the census number of each seed orchard, using of relative status number is better than using the status number to compare other studies. Dutkuner et al. (2008); Bilir & Temirağa (2012) found that male strobilus relative status numbers were ranged from 0.98 to 0.99 for one year in mature Scots pine seed orchards. The relative status number in Spheroid scots pine was lower than the relative status number in mature scots pine. So, gene diversity in Spheroid scots pine was about half of gene diversity in mature scots pine.

The contribution of male strobilus production was different for each clone in Spheroid scots pine as an averaged 2-year. The top 25% of clones (6 clones) and the lowest 25% of clones (7 clones) produced 48% and %5 of total male strobili, respectively in this research. In the *Pinus koreansis* S. et Z., the top 25% of clones and the lowest %25 of clones produced 90.4% and 0.1% of total female strobili, respectively for five years (Kang & Lindgren, 1999). In the other hand, Burzcyk & Chalupka (1987) found that the top 25% of clone and the lowest 25% of clones produced 45.58 % and 10.42 of total pollen in Scots pine for three years. These explanations were revealed that clonal contributions on any production (flower, pollen etc.) in the seed orchards were not balanced.

5. Conclusions

The study on male fertility was carried out two consecutive years. Some parameters related to male fertility which were obtained could be used for seed orchard management. The clonal contribution on male strobilus production for the two-year was unbalanced like in other seed orchards. Sibling coefficient of male fertility and the relative status number in Spheroid seed orchard were lower than in mature Scots pine seed orchards'. The differences among clones and years on male fertility were high but clonal fertility was stable between years. The spheroid scots pine clonal seed orchard was established for ex situ conservation, but due to a special form, in the same time, the orchard can be also used for landscape service. In this context, the findings of this study can be used for the management of young spheroid Scots pine seed orchard aiming ex situ conservation and landscape services.

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