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Research Article

Effective Clone Number and Contribution to Gene Pool in a Clonal Seed Orchard of Anatolian Black Pine (*Pinus nigra* Arnold subsp. *pallasiana* (Lamb.) Holmboe) in Kastamonu-Turkey

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Abstract

Aim of study: Clonal seed orchards are really important tools in seed material production. In this study; the effective clone number and clone contribution to gene pool were examined.

Area of study: The studied clonal seed orchard was established on 13 ha of area, located at Hanönü (Kastamonu) in Turkey.

Material and Methods: In 2008, the existed ramets were firstly counted for determining the ramet number for the clones. After that, all cones on the ramets were counted and CGP (clone contribution to gene pool) was analysed. The effective number of clones (Nc) was determined by the variation coefficient (CV).

Main results: There is considerable variation on living ramet numbers among the clones. In the establishment phase, there was also 7 times difference between clone 22 (12 ramets) and clone 2 (85 ramets). This difference both establishment phase and 17 years-old, could stem from epibiot-hipobiot incompatibility and environmental factors.

Highlights: The effective number of clones, describes the gene pool of the orchard. However, incorporation of fertility variation among clones with variation in the number of ramets will give a better prediction of it.

Keywords: *Pinus nigra,* ramet variation, effective clone number, Kastamonu.

Anadolu Karaçamı (*Pinus nigra* Arnold subsp. *pallasiana* (Lamb.) Holmboe) (Kastamonu-Turkey) Klonal Tohum Bahçesinde Etkili Klon Sayısı ve Gen Havuzuna Klon Katkısı

Öz

Çalışmanın amacı: Klonal tohum bahçeleri nitelikli tohum üretiminde önemli bir yer tutmaktadır. Bu çalışmada etkili klon sayısı ve klonların gen havuzuna katkıları incelenmiştir.

Çalışma alanı: İncelenen tohum bahçesi 13 ha alanda, Hanönü (Kastamonu), Türkiye'de tesis edilmiştir.

Materyal ve Yöntem: 2008 yılında öncelikle klonların mevcut ramet sayılarını belirlemek için bütün rametler sayılmıştır. Daha sonra toplam kozalak sayısı belirlenerek CGP (klonların gen havuzuna katkıları) analizi edilmiştir. Etkili klon sayıları (Nc), rametlerin varyasyon katsayısına (CV %) göre belirlenmiştir.

Sonuçlar: Yaşayan klonların ramet sayıları arasında büyük fark bulunmaktadır. Tohum bahçesinin kuruluş aşamasında da klonlara ait ramet sayıları arasında 7 kat fark, klon 22 (12 ramet) ve klon 2 (85 ramet) bulunmaktadır. Kuruluş aşamasında ve 17 yaşında ramet sayıları arasında görülen bu büyük fark aşı uyuşmazlığından ve çevresel faktörlerden kaynaklanmaktadır.

Önemli Vurgular: Etkili klon sayısı tohum bahçesinin gen havuzunu tanımlamaktadır. Bunun yanında, tohum ürününün genetik çeşitliliği ile ilgilendiğimiz zaman, ramet sayılarının farklılığından kaynaklanan döllenme çeşitliliği daha iyi bir öngörü sağlamaktadır.

Anahtar Kelimeler: *Pinus nigra*, ramet varyasyonu, etkili klon sayısı, Kastamonu.

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Introduction

Anatolian black pine is one of the commercially important tree species in Turkey. Also, the area of this species spreads on 4.2 million ha, of which almost 1.8 million ha is degraded forests (Anonymous, 2006). Because of high rate of unproductive forest lands, big amount of seed material is needed for restoration of unproductive lands. Moreover, this pine species seems to be one of the most important species which can be widely used in arid/semi-arid regions in Anatolia. Recently, the semi-arid steppe lands considered as potential afforestation areas by Turkish Foresters. Thus, the importance of Anatolian black pine is increasing continuously. In the present case, the seed material is supplied from 55 (464 ha) of seed orchards and 71 (9087.5 ha) of seed stands in this species (Anonymous, 2019). Mostly, these seed orchards are rather young, because they were set after the year of 1990 (Sıvacıoğlu and Ayan, 2010).

The importance of seed orchard are appreciated as production populations where genetic gain from tree breeding is transferred into practices and to commercial forest crops. The actual seed orchards are set up as to phenotypic selection of plus individuals from seed stands in Turkey. In the time of establishment of clonal seed orchards, selected genotypes (plus trees) are replicated with grafting. After definite period (1 or 2 years) these grafts (ramets), are planted as to a certain design for avoiding self-fertilization and consequent seed production (Kang et al., 2001; Tunçtaner, 2007). Clones in new established clonal seed orchards are in equal proportions (Koski, 1980). The clone number (census number), in an seed orchard, is thus currently used for regulatory purposes and is regarded as essential for decisions on whether forest genetic material is suitable to use. But, the census number has no meaning in terms of gene diversity in case of large variation in ramet numbers per clone and in fertility among genotypes (Tunçtaner, 2007).

In the discussions about inbreeding and genetic diversity in clonal seed orchards focusing on the clone numbers (Koski, 1980; Lindgren, 1974; Moran, Bell & Matheson, 1980), it is often assumed that all clones are represented by close-to-equal numbers of fertile ramets (Jonsson, Ekberg & Eriksson, 1976; Hodge & White, 1993; Xie, Woods, & Stoehr, 1994; Kang & Lindgren, 1998). However, it is generally acknowledged that clones are quite often represented by differing numbers of ramets in actual clonal seed orchards (Schoen, Denti & Stewart, 1986; Muona and Harju, 1989; Bilir and Ayan, 2005). Together with variation in fertility, this variability in ramet number will be reason for unequal gamete contributions by clones in the orchards. In case of unrelated and non-inbred clones, the effective number of clones in an orchard will be equal to the status number of the seed orchard (Lindgren and Kang, 1997; Lindgren and Mullin, 1998). Further, it can also be regarded as the status number of the seed crop from an ideal seed orchard where clones are non-inbred and not related, and where gamete contributions are proportional to the ramet number (Lindgren and Mullin, 1998).

In this study, to determine the effective clone number based on clone and ramet number, and to evaluate of ramet variation on gene diversity in a clonal seed orchard of Anatolian Black Pine was aimed. Moreover, the findings were evaluated to shed light on future and present management of clonal seed orchards.

Material and Method

The material seed orchard was established by Research Institute of Forest Tree Seeds and Tree Breeding on 13 ha of area, located at Hanönü (Kastamonu) Turkey in 1993 with 2039 grafts of 30 clones, taken out from chosen trees in Kastamonu-Karadere seed stand. Grafts were 1 year-old at the time of set up and planting space was 8 m x 8 m. First cones were harvested in 2003. In 2008, the existed ramets were counted firstly for determining the ramet number for the clones. Later, all cones on the ramets were counted and CGP (clone contribution to gene pool) was analysed.

The effective clone number (Nc) for the studied orchard was determined by using the variation coefficient (CV) for the ramet numbers representing each clones. The Nc value is determined as Nc=N (census number) /(CV^2+1). In case of unrelated, non-inbred parents, parental fertility in commensurate with number of ramets and moreover inclusion of all parents, effective clone (N_C) number turn into orchard status number (N_S) (Kang et al. 2001, Lindgren & Mullin 1998).

There is a relation between gene diversity (GD) with effective clone numbers as, GD=1-0.5/N_C (Lindgren and Kang, 1997) or with status number (N_S) as, GD = 1- 1/(2*N_S). Moreover, this relation can be stated also with the relative effective clone number (N_r) and clone numbers (N) as, (GD=1-0.5/N*N_r). The highest level of gene diversity of seed material for a certain clone number is acquired whenever all parents join to the gene pool of the seed orchard at an equal level (Kang et al., 2001). Thus situated variation coefficient (CV) among clones for ramet numbers take on an important task in seed crop gene diversity. To confront the census number (N) and the clone effective number (Nc) in clonal seed orchard, mostly their relation as, N_r = N_C /N was used (Kang et al., 2001; Kang and Lindgren, 1998; Bilir and Ayan, 2005; Lindgren and Kang, 1997).

Analyses of variance were performed for cone numbers. To data of cone number, one-way variance analysis applied. Thus, normality/homogeneity tests and logarithmic transformation were done. Variation coefficient within the clones (CV_C) and grafts (CV_G) in percent was determined as the ratio of standard deviation of the clones/grafts to arithmetic means.

Results

There is large differences for living number of ramets among clones. For instance; in 2008, there is 8 times differences between lowest (clone 22, 8 ramets) and highest (clone 2, 66 ramets) number of ramets per clone. In the establishment phase, there was also 7 times difference between clone 22 (12 ramets) and clone 2 (85 ramets) (Table 1).

This difference both establishment phase and in 17 years-old, could stem from epibiot-hipobiot incompatibility and environmental factors. In 17 years period, the highest MP (mortality percentage) observed for clone 29 (50 %), whereas the lowest for clone 6 (18.18 %) (Table 1).

All clones combined, the orchard had a mean of 122.4 cones with range among the clones from 32.8 (clone 28) to 189.8 (clone 13). The CV_G values varied from 66.8 to 129.9 while the variation coefficients among the clones (CV_C) is 31.1. The bigger CV_G values than CV_C indicates the bigger variation within the clones than among the clones in the orchard for cone number. The analysis of variance showed that there was significant differences among clones at 0.001 probability level for CGP (Table 1). The CGP varied 0.83-5.46% among the clones. The top of 5 clones (20, 21, 10, 15, 13) had 24.82 % of all CGP (Table 1).

The CV values varied from 22.3 % in 1993, to 27.1 % in 2008. As to the data, there is a negative significant ($p<0.001$) relation (r=-0.998) between CV of ramet number and Nc (Nc=-0.14CV+31.82). Thus, Nr values varied from 0.95 in 1993 to 0.93 in 2008 (Table 2). In 1993, each clone represent with a mean of 69.6 grafts, whereas this value decrease to 48.0 in 2008. Total graft number varied 2089 to 1441, from 1993 to 2008 (Table 2).

Discussion

In Turkey, the existed clonal seed orchards were commonly established using ramets of phenotypically selected plus trees, without information on breeding value (Tunçtaner, 2007; Hodge & White, 1993). It is often considered preferable to use an equal number of ramets per clone whenever possible, and there was no reason not to see equal ramet representation as an aim in the first cycle of seed orchards (Kang et al., 2001). In the process of orchard establishment, however, unintentional variation in the number of ramets for selected clones occurs. For example, in the studied orchard, ramet numbers varied from 12 to 85 in the establishment phase. One of the major reason of this variation may be graft availability. Once planted, grafts may die for different reasons such as unfavourable site growing conditions, graft incompatibility, different kinds of biotic or abiotic injuries. In the studied orchard, mostly this variation originated from unfavourable site conditions.

Table 1. Ramet numbers (RN), total cone number (TCN), contribution of clones to gene pool of orchard (CGP) and descriptives for studied orchard.

Clone number	RN 1993	RN 2008	MP	TCN	CGP[4] %		Mean	SE	Min-Max	SD_G	CV_G%
1	81	62	23.46	7240	4.26	bcde[2]	[1]116.8	11.9	1-340	93.9	80.4
2	85	66	22.35	7351	4.32	bcde	111.4	15.8	0-680	128.7	115.5
3	79	59	25.32	5771	3.39	efgh	97.8	16.1	0-720	123.4	126.2
4	80	51	36.25	5785	3.40	efgh	113.4	11.1	0-300	79.3	69.9
5	77	56	27.27	6277	3.69	defg	112.1	12.5	0-348	93.6	83.5
6	77	63	18.18	7321	4.31	bcde	116.2	10.5	0-440	83.6	71.9
7	75	53	29.33	5298	3.12	fghi	99.9	13.2	3-480	96.5	96.6
8	45	30	33.33	4733	2.78	ghij	157.7	24.4	0-520	133.6	84.7
9	73	56	23.29	7127	4.19	cde	127.2	15.2	0-520	114	89.6
10	80	60	25.00	8153	4.80	abc	135.8	13.1	0-428	101.2	74.5
11	58	47	18.97	5085	2.99	fghij	108.2	12.1	3-292	83.3	77.0
12	36	28	22.22	4705	2.77	ghij	168	21.2	15-414	112.2	66.8
13	73	49	32.88	9288	5.46	a	189.5	18.3	0-480	128.5	67.8
14	75	47	37.33	5158	3.03	fghij	109.7	14.4	0-396	99.2	90.4
15	73	49	32.88	8873	5.22	ab	181.1	18.3	0-560	128.3	70.8
16	59	28	52.54	4539	2.67	ghij	162.1	21.9	11-560	116.2	71.7
17	75	41	45.33	5357	3.15	fghi	130.6	16.1	6-464	103.5	79.2
18	77	54	29.87	4987	2.93	ghij	92.3	9.9	0-260	73.1	79.2
19	69	45	34.78	3973	2.34	ij	88.3	10.4	0-260	69.9	79.2
20	80	56	30.00	7810	4.59	abcd	139.4	15.4	0-460	115.4	82.8
21	78	58	25.64	8077	4.75	abc	139.3	15.3	0-460	116.9	83.9
22	12	8	33.33	1410	0.83	l	176.2	56.7	13-408	160.5	91.1
23	62	38	38.71	7214	4.24	bcde	189.8	22.8	0-620	141.1	74.3
24	74	41	44.59	3542	2.08	jk	86.4	17.5	0-540	112.2	129.9
25	75	54	28.00	6798	4.00	cdef	125.9	15.1	0-620	110.6	87.8
26	76	50	34.21	2325	1.37	kl	46.5	6.2	0-132	43.9	94.4
27	77	57	25.97	6246	3.67	defg	109.6	11	0-340	83.3	76.0
28	79	54	31.65	1773	1.04	l	32.8	5.2	0-152	38.7	118.0
29	54	27	50.00	3479	2.05	jk	128.8	21.8	0-380	113.6	88.2
30	75	54	28.00	4305	2.53	hij	79.7	7.3	0-196	53.6	67.3
Mean	69.6	48.0		5666.6	3.33		122.4	7.0	32.8-189.8	38.1	31.1
F value							[3]14.578***				

[1] Values are the means of all grafts.

[2] For each trait, mean values with the same letter are not significantly different at $P < 0.05$ level;

[3] ***: significant at $P < 0.001$ and the values are transformed.

[4] *RN*=Ramet number, *MP*=Mortality percentage *TCN*=Total cone number *CGP*= contribution percentage of clone to gene pool *SE*=standard error SD_G= Standard deviation among the grafts CV_G =Variation coefficients among the grafts

Table 2. Clone and graft number, effective clone number (Nc), relative effective number of clones (Nr), variation coefficients of grafts (CV%) , Gene diversity (GD) of the studied orchard.

Year	Clone number	Graft number				Nc	Nr	CV%
		Average	Total	Min.	Max.			
1993	30	69.6	2089	12	85	28.6	0.95	22.3
2008	30	48.0	1441	8	66	27.9	0.93	27.1

Also, the genetic differences among clones may contribute to this mortality causing variation in the number of living ramets for individual clones in the studied orchard. As seen on Table 1, the mortality percentage varied from 18.18 to 52.54 among the clones. Also, the ramet number variation may arise also from non-genetic reasons such as different grafters, root-stock quality, storage facilities. If grafts die soon after planting, they may be replaced by reserve ramets that may represent clones where initial grafting was successful or which was not used for the intended seed orchard.

The effective number of clones (Nc), based on the variation in the number of ramets among clones, describes the gene pool of the orchard. However, when we are interested in the gene diversity of the seed orchard crop, incorporation of fertility variation among clones with variation in the number of ramets will give a better prediction of it. Also, Nc will be more informative concerning gene diversity of the seed crop than is the census number (N). It was developed status effective number (Ns) in connection with fertility variation in seed orchards. Loss in gene diversity is inversely proportional to the effective number of clones (Kang & Lindgren, 1998). On average, the effective number of clones was calculated to be 28.6 in 1993, 27.9 in 2008. The census number (30) of clones differs from the effective number of clones in the studied clonal seed orchard.

There is no big difference on relative effective number of clones (Nr) between 1993 and 2008 (0.95 and 0.93). But, what is important here is the reduction of the contribution of some clones to the gene pool.

It is beneficial and more desirable in future orchards than at present to intentionally use an unequal number of ramets per clone. So, the clones that contribute most to breeding value of the seed orchard are permitted to contribute more to the diversity loss.

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