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Flowering variation of a young Scots pine (*Pinus sylvestris* L.) clonal seed orchard based on years

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Abstract

In this research, it was tried to determine the male and female flower yield in young clonal seed orchard (YCSO) with the expectation that the flowering variation would be decreased among years, clones and ramets thanks to aging. The measurements were made on five ramets for each of 30 clones in four years in a Scots pine (*Pinus sylvestris* L.) seed orchard. The orchard, originated from the Araç-Dereyayla seed stand in Kastamonu, was established in 1995 by using two years-old grafts in Kastamonu, located northwestern Black Sea region of Türkiye. The examined characters were number of male (NMF), and female flowers (NFF). When the four-year of data from the YCSO was evaluated, mean values from NMF and NFF among the clones were significant and variation of the ramets was high. The coefficient of variation (*Cv*) from the NMF of the clones gradually decreased with the subsequent years (Cv_{2006}=147.54%, Cv_{2010}=59.04%), whereas the *Cv* in the NFF did not show any decrease with the same years. In addition, there were significant differences among the years as to the NMF, and NFF. Over the four years, the NMF was significantly lower than that of the NFF, and even in some years the NFF was doubled compared with the NMF (NMF_{2006}=65.9; NMF_{2007}=314.29; NMF_{2008}=427.85; NMF_{2010}=115.73 & NFF_{2006}=123.80; NFF_{2007}=604.68; NFF_{2008}=394.62; NFF_{2010}=196.72). Abundant flowering periods seen in natural stands were also observed in the male and female flowers of the clonal seed orchards. For the studied seed orchard high variation both among the clones and the ramets indicates the high selection capacity in the breeding programs. The bigger variation among the ramets confirm that the genotypes have responded against the heterogeneity of growing area in seed orchard or the ramets have not reached the optimum flowering period. These results have shown the importance of the practices which increase the flowering yield and effectiveness of fertilization in YCSO.

Keywords: Scots pine, Ramet, Clone, Variation, Flowering, Clonal seed orchard

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1. Introduction

Scots pine (*Pinus sylvestris* L.) is one of the most common and important tree species in Türkiye due to usefulness of its wood to many commercial uses. Also, for its great importance in Turkish forestry, this species is classified as an economically important tree species in the "*National Tree Breeding and Seed Production Program*" (Koski & Antola, 1993). The stands of this species are approximately 1,421,061 hectares in Türkiye. Of this area, 64% is productive forest (crown closure is 11-100%), and 34% is unproductive forest (crown closure is under 10%) (URL-1, 2022).

These unproductive areas have been evaluated as potential areas for artificial regeneration, rehabilitation, and restoration practices to diminish the wood supply deficit and for supplying the other forest functions to the community in Türkiye. In this context, clonal seed orchards, on the one hand, are one of the ex-situ conservation tools of forest gene resources, and on the other hand, they are very important tools for establishing plantations with high genetic gain with improved-high quality reproductive material. Presently, demand for Scots pine seeds and seedlings in Türkiye is obtained mainly by domestic production, but currently only 9.2% of the Turkish seed demand in Scots pine is supplied from current 105.8 ha of seed orchards, 20 Scots pine seed orchards (Cengiz, 2003; Öztürk & Yıldız, 2022). As to some projections, the need of Scots pine seed is 715 kg per year (Bilir et al., 2007). Only definite amount of this seed demand was supplied from the seed orchards in Türkiye. However, most of these seed orchards are still rather young (Bilir & Ulusan, 2008).

New orchards have been established to cover the demand of improved seed for afforestation according to the national seed and tree-breeding program of Türkiye (Koski & Antola, 1993; Bilir et al., 2008). Seed orchards have the potential to increase forest production and offer a reliable seed supply, their role and function has recently been compiled. They are by far the most important outlets to forestry of breeding programs and they can be useful in gene conservation. Investment in seed orchards is often by the most cost-efficient way of increasing future forest production (Bilir et al., 2008). For that reason, it is desirable to improve the function of seed orchards. However, there are some problems in seed production on these seed orchards (Sıvacıoglu & Ayan, 2008). In spite of the fact that most ortets originate from phenotypically selected seed stands, seed orchards are becoming gradually more important (Bilir et al., 2006). High and genetically improved seed yield from the seed orchards is an integral part in the success of a tree breeding program. Moreover, clonal seed orchards are one of the important seed sources for forest plantations. Moreover, these seed sources constitute an important link between tree breeding and plantation forestry. In recent years, considerable progress has been made to better understand the reproductive biology of conifers in seed orchards (Kang, 2001; Bilir et al., 2006; Çelik & Ayan, 2009; Ayan & Çelik, 2009; Sıvacıoğlu et al., 2009; Sıvacıoğlu & Ayan, 2010; Yiğit et al., 2010). In addition, many studies have been conducted in recent years on the flowering yield, fertilization variation of clones/ramets in both natural and artificial populations such as seed stands, clonal seed orchards, and their effects on breeding studies (Kang & Lindgren, 1998; Nikkanen & Ruotsalainen, 2000; Ayan et al., 2005; Çılgın et al., 2007; Şevik et al., 2010).

Differences in growth and other characters in seed orchards will affect the seed crop. The economy of seed orchards is dependent on a high seed production, which is convenient to collect. Thus, the evaluation of the clonal variation for more effective orchards is a very urgent issue for Turkish foresters.

This study aims at finding out how flowering varies in “young” clonal seed orchards (YCSO) within years depending on the clones and ramets. The hypothesis of the study; in YCSO thanks to aging, flowering variation among years, clones and ramets was assumed to decrease.

2. Material and Methods

2.1. Material

This study was carried out in Tekçam clonal seed orchard [(CSO), (Nu of CSO: 151)] located in Taşköprü, Kastamonu (41036' N, 35005' E, 1160 m). The orchard was established in 1995 and comprises 1987 ramets of 30 clones from selected plus trees in Araç-Dereyayla seed stand (Figure 1). The seed stand is located in the “first main breeding zone, and second sub-breeding zone” within the natural distribution area of the Scots pine in Türkiye (URL2, 2008). Grafts were two years old at the time of establishment and were planted with a spacing of 6 x 6 m. Commercial cone harvesting started in 2003 when the grafts were 10 years old. Until 2014, no artificial pruning has been done in the orchard.

2.2. Methods

The female and male flowers measurements were performed in 2006, 2007, 2008, and 2010 on five ramets, chosen randomly, from each of 30 clones. The numbers of male and female flowers produced by the clones in the orchard over a period of four years were determined. Clonal variation in female and male flower production has been found in some studies conducted in clonal seed orchards of other pine species by Keskin (1999), Kang (2000), Zhuowen (2002) and Bilir et al. (2002), it was evaluated as an important criterion in terms of deciphering the differences among clones. Data were subjected to one-way analysis of variance for year and clone factor. Variables were tested for normality and homogeneity of variances, and logarithmic transformation for counting were made. Duncan's multiple range test was used to identify the homogenous groups of treatments for each factor. Basic statistics for the data (arithmetic mean, minimum-maximum values, standard deviation, standard error, coefficient of variation, range, etc.) were defined. Moreover, a correlation analysis was done to measure the relationship between the coefficient of variation and the average flowering.

Figure 1. Geographical location of the study area (Tekçam *Pinus sylvestris* L. clonal seed orchard in Kastamonu-Taşköprü, Türkiye)

3. Results

3.1. Female flower productivity of the clones

When the clones were compared in terms of their averages of female flower productivity for a four-year period (2006, 2007, 2008 and 2010), it was the Clone 29 which had the highest number of flowering with 720.94 female flowers. Clone 5, however, had the lowest number of flowering with 188.56 female flowers. Besides, in terms of female flower productivity, Clone 3 which had a high average of female flower productivity had the highest standard deviation of 702.535 and lowest standard deviation of 150.713 (Table 1).

It was determined that the variation of the clones increased when the female flower productivity of the clones went up, and that this relation could best be defined with a polynomial equation, and that the correlation coefficient between the number of female flowers and variation was 0.942 (Figure 2).

According to the results of Duncan's multiple test, 30 clones in the Scots pine clonal seed orchard were observed to have formed 5 (five) homogenous groups in terms of female flower productivity. The highest female flower productivity as for the four-year averages was achieved in the Clones 29, 3, 20, 4 and 30, respectively (Table 2). The range was calculated as 532.38.

3.2. Male flower productivity of the clones

When compared for their four-year averages of male flower productivity, the Clone 27, with 728, 67 flowers, exhibited the highest flowering; the Clone 1, on the contrary, had the lowest flowering with 44.81 male flowers. Furthermore, the standard deviation was the highest (646.901) for Clone 27 which had a high average of the male flower productivity, whereas the standard deviation was the lowest (54.856) for Clone 1 in terms of male flower productivity (Table 3).

It was determined that the variation of the clones increased when the male flower productivity of the clones increased, and that this relation could best be defined with a polynomial equation, and that the correlation coefficient between the number of male flowers and variation was 0.946 (Figure 3).

According to the results of Duncan's multiple test, 30 clones in the Scots pine clonal seed orchard were observed to have formed 8 (eight) homogenous groups in terms of male flower productivity (Table 4). In terms of male flower productivity, the clones exhibited much more variations. The range of male flower productivity for four years was calculated as to be 683.86.

When four-year averages of male and female flower productivity within the clones were reviewed, it was found out that the clone factor had a statistically significant effect on male and female flower productivity (Table 5), and also that both male and female flower productivity showed significant statistical differences within years (Table 5).

Table 1. Female flower productivity averages of the clones for a four-year period and basic statistics

Number of clone	N	Mean	Standard Deviation	Standard Error	Minimum	Maximum
1	18	289.94	305.243	71.946	3	988
2	17	227.06	177.312	43.004	3	620
3	17	694.12	702.535	170.390	52	2640
4	18	501.44	498.264	117.442	3	1864
5	18	188.56	189.935	44.768	1	569
6	16	276.63	274.274	68.568	4	1048
7	18	352.56	297.005	70.005	9	1086
8	18	325.78	312.944	73.762	44	1380
9	18	274.06	313.043	73.785	22	1210
10	18	265.11	261.766	61.699	22	848
11	18	325.44	305.254	71.949	36	1240
12	18	252.33	170.527	40.193	8	579
13	18	327.78	282.653	66.622	11	1198
14	18	344.33	273.000	64.347	4	880
15	18	307.33	289.869	68.323	64	1280
16	18	268.56	265.844	62.660	18	1180
17	18	414.22	206.851	48.755	81	850
18	18	305.00	250.500	59.043	8	1020
19	16	417.75	468.467	117.117	4	1578
20	18	541.83	507.137	119.533	6	1720
21	18	430.39	441.703	104.110	3	1528
22	18	290.33	227.598	53.645	27	784
23	18	220.17	197.612	46.578	1	675
24	18	272.00	235.357	55.474	23	1011
25	18	276.17	258.911	61.026	3	825
26	17	231.47	150.713	36.553	57	546
27	18	198.89	159.487	37.592	45	586
28	17	413.29	367.077	89.029	36	1341
29	18	720.94	675.203	159.147	120	2127
30	16	480.13	440.494	110.124	1	1441
Total	530	346.96	359.001	15.594	1	2640

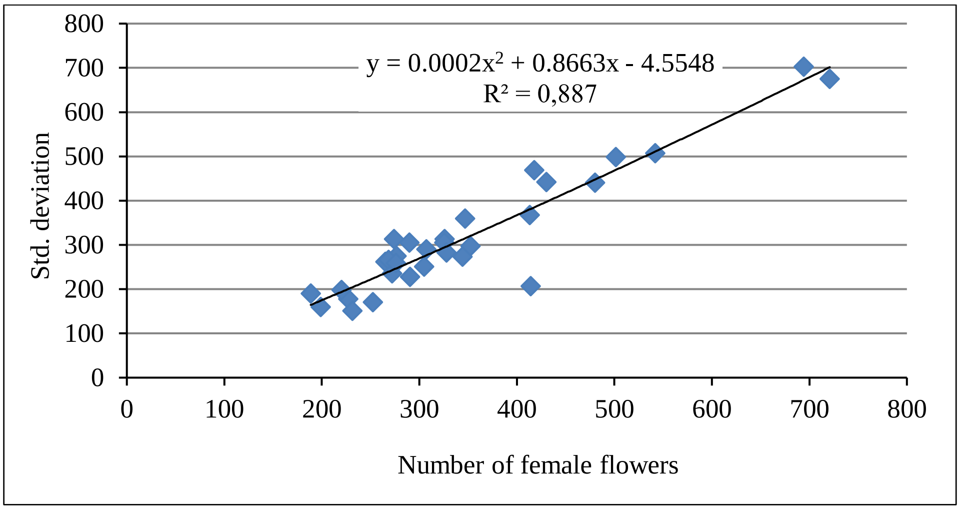

Figure 2. The relationship between the average productivity of female flowers and standard deviation

Table 2. Results of Duncan's multiple test as to number of female flowers

Number of clone	N	Subset for alpha = 0.05				
		1	2	3	4	5
5	18	188.56				
27	18	198.89	198.89			
23	18	220.17	220.17	220.17		
2	17	227.06	227.06	227.06		
26	17	231.47	231.47	231.47		
12	18	252.33	252.33	252.33		
10	18	265.11	265.11	265.11	265.11	
16	18	268.56	268.56	268.56	268.56	
24	18	272.00	272.00	272.00	272.00	
9	18	274.06	274.06	274.06	274.06	
25	18	276.17	276.17	276.17	276.17	
6	16	276.63	276.63	276.63	276.63	
1	18	289.94	289.94	289.94	289.94	
22	18	290.33	290.33	290.33	290.33	
18	18	305.00	305.00	305.00	305.00	
15	18	307.33	307.33	307.33	307.33	
11	18	325.44	325.44	325.44	325.44	
8	18	325.78	325.78	325.78	325.78	
13	18	327.78	327.78	327.78	327.78	
14	18	344.33	344.33	344.33	344.33	
7	18	352.56	352.56	352.56	352.56	
28	17	413.29	413.29	413.29	413.29	
17	18	414.22	414.22	414.22	414.22	
19	16	417.75	417.75	417.75	417.75	
21	18	430.39	430.39	430.39	430.39	
30	16		480.13	480.13	480.13	480.13
4	18			501.44	501.44	501.44
20	18				541.83	541.83
3	17					694.12
29	18					720.94
Sig.		0.104	0.056	0.056	0.058	0.064

Table 3. Male flower productivity averages of the clones for a four-year period and basic statistics

Number of clone	N	Mean	Standard Deviation	Standard Error	Minimum	Maximum
1	16	44.81	54.856	13.714	3	188
2	18	301.56	237.643	56.013	8	760
3	18	376.06	352.944	83.190	11	1260
4	18	330.33	368.626	86.886	6	1480
5	18	221.94	203.511	47.968	7	607
6	17	222.00	230.345	55.867	6	644
7	18	223.78	241.144	56.838	2	828
8	18	203.33	233.737	55.092	18	865
9	18	290.50	282.742	66.643	27	1080
10	18	356.50	268.528	63.293	71	1024
11	18	200.00	224.248	52.856	6	714
12	18	100.00	138.993	32.761	3	596
13	16	169.38	157.095	39.274	6	483
14	16	52.69	84.013	21.003	1	328
15	18	124.83	145.389	34.269	1	598
16	16	293.94	195.326	48.831	14	674
17	18	458.83	287.970	67.875	85	1120
18	18	204.72	229.354	54.059	5	782
19	15	86.67	103.668	26.767	3	339
20	18	131.33	112.899	26.610	4	347
21	18	120.72	186.080	43.859	9	736
22	18	326.06	281.476	66.345	8	1012
23	18	180.61	178.680	42.115	1	520
24	18	198.78	227.877	53.711	7	782
25	17	141.35	134.706	32.671	7	498
26	17	169.53	159.474	38.678	33	610
27	18	728.67	646.901	152.476	87	2160
28	17	536.29	473.388	114.813	74	1624
29	17	229.65	255.899	62.065	2	762
30	18	247.33	290.620	68.500	2	785
Total	524	244.69	292.524	12.779	1	2160

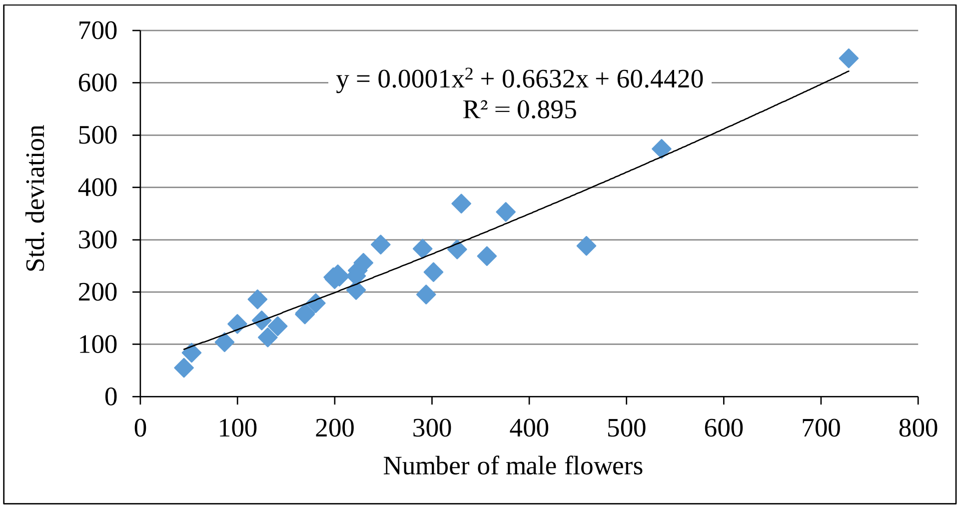

Figure 3. The relationship between the average productivity of male flowers and standard deviation

Table 4. Results of Duncan's multiple test as to number of female flowers

Number of clone	N	Subset for alpha = 0.05							
		1	2	3	4	5	6	7	8
1	16	44.81							
14	16	52.69							
19	15	86.67	86.67						
12	18	100.00	100.00						
21	18	120.72	120.72	120.72					
15	18	124.83	124.83	124.83					
20	18	131.33	131.33	131.33					
25	17	141.35	141.35	141.35	141.35				
13	16	169.38	169.38	169.38	169.38	169.38			
26	17	169.53	169.53	169.53	169.53	169.53			
23	18	180.61	180.61	180.61	180.61	180.61			
24	18	198.78	198.78	198.78	198.78	198.78			
11	18	200.00	200.00	200.00	200.00	200.00			
8	18	203.33	203.33	203.33	203.33	203.33			
18	18	204.72	204.72	204.72	204.72	204.72			
5	18	221.94	221.94	221.94	221.94	221.94			
6	17	222.00	222.00	222.00	222.00	222.00			
7	18	223.78	223.78	223.78	223.78	223.78			
29	17	229.65	229.65	229.65	229.65	229.65			
30	18	247.33	247.33	247.33	247.33	247.33			
9	18		290.50	290.50	290.50	290.50	290.50		
16	16		293.94	293.94	293.94	293.94	293.94		
2	18		301.56	301.56	301.56	301.56	301.56		
22	18			326.06	326.06	326.06	326.06		
4	18			330.33	330.33	330.33	330.33		
10	18				356.50	356.50	356.50	356.50	
3	18					376.06	376.06	376.06	
17	18						458.83	458.83	
28	17							536.29	
27	18								728.67
Sig.		0.070	0.054	0.061	0.052	0.063	0.109	0.064	1.000

3.3. Flower productivity as of years

The flower productivity for the clones and the years is given in Figure 4. The results of the analysis of variance for male and female flower productivity are given in Table 5.

Female flower productivity was investigated by not taking the clone disparities into account. The investigation revealed that there was not a constant increase in the productivity depending on age, and that there were significant decreases or increases in productivity within the years. Table 6 gives the results of Duncan's multiple test.

Male flower productivity was investigated by not taking the clone disparities into account. The investigation revealed that there was not a constant increase in the productivity depending on age, and that there were significant decreases or increases in productivity within the years. Table 7 gives the results of Duncan's multiple test.

Year-to-year flower productivity of the clonal seed orchard is given in figure 5, and the coefficient of variation is given in figure 6. The coefficient of variation (*Cv*) from the NMF of the clones gradually decreased with the subsequent years (Cv_{2006}=147.54%, Cv_{2010}=59.04%), whereas the *Cv* in the NFF did not show any decrease with the same years.

When male and female flower productivity of the clones are evaluated with respect to their share within the total annual flower production, it was found out that the share of the clones for male and female flower productivity within the total annual flower production showed differences each year.

Table 5. Analysis of variance table for female and male flower productivity as of years and the clones

Variance Source	Sum of Squares	df	Mean Square	F	Sig.
Number of female flowers of clones as to four years average					
Between Groups	8890264.552	29	306560.847	2.585	<0.001
Within Groups	59288080.693	500	118576.161		
Total	68178345.245	529			
Number of male flowers of clones as to four years average					
Variance Source	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	10761363.107	29	371081.486	5.393	<0.001
Within Groups	33991840.425	494	68809.394		
Total	44753203.532	523			
Number of female flowers as of years					
Variance Source	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	19447319.576	3	6482439.859	69.971	<0.001
Within Groups	48731025.670	526	92644.535		
Total	68178345.245	529			
Number of male flowers as of years					
Variance Source	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	11803362.909	3	3934454.303	62.092	<0.001
Within Groups	32949840.623	520	63365.078		
Total	44753203.532	523			

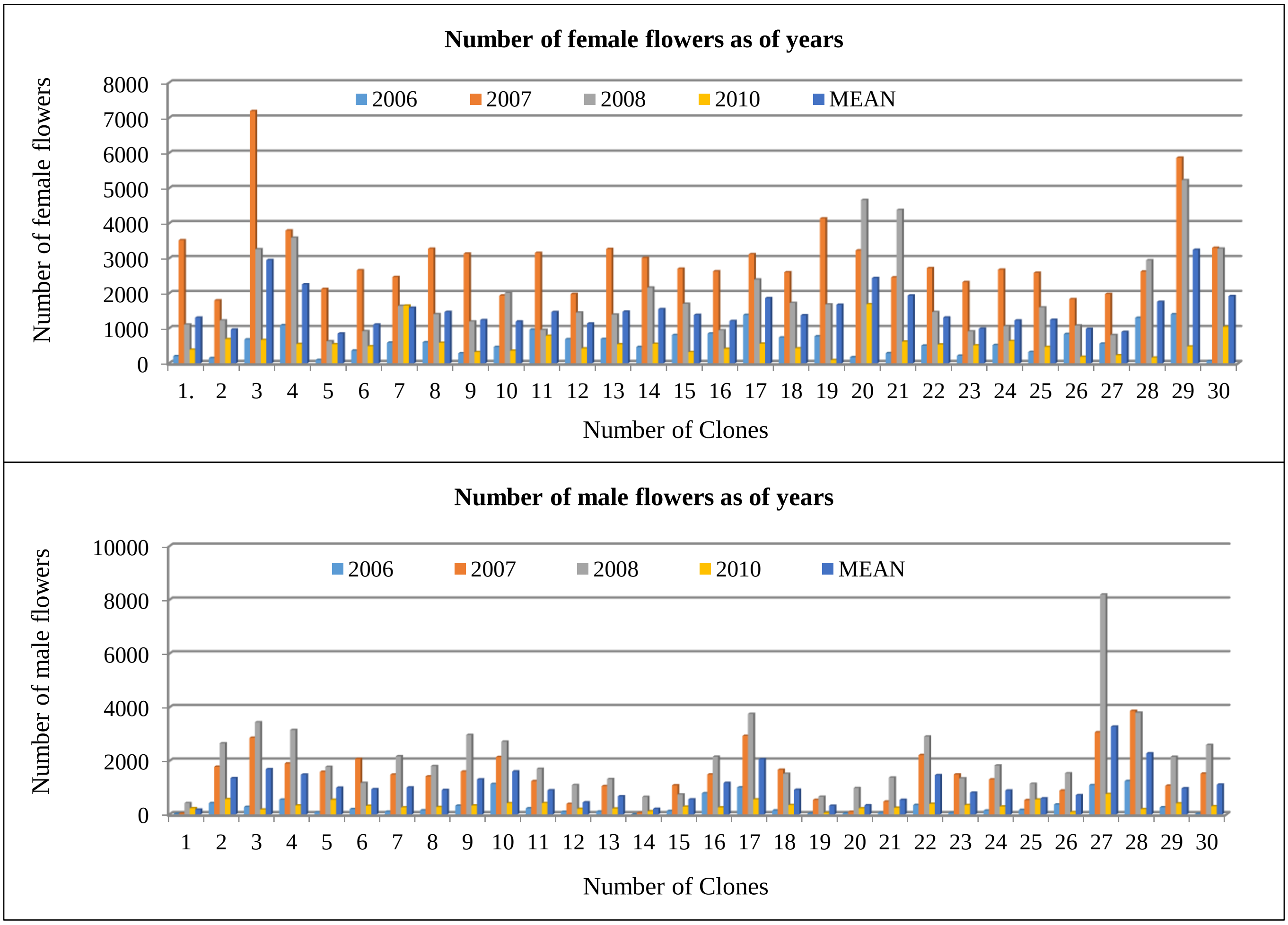

Figure 4. Flower productivity of the clones as of years

Table 6. Inter-year variations of female flowers and the results of the Duncan's multiple test

Year	N	Mean	Std. Error	Std. Deviation	Min.	Max.	Year	N	Subset for alpha = 0.05		
									1	2	3
2006	146	123.8	10.08	121.76	1	528	2006	146	123.8		
2007	149	604.7	32.08	391.56	22	2640	2010	86	196.7		
2008	149	394.6	31.20	380.9	14	2127	2007	149		394.6	
2010	86	196.7	18.18	168.63	45	1260	2008	149			604.7
Total	530	346.96	15.59	359.0	1	2640	Sig.			1.000	1.000

Table 7. Inter-year variations of male flowers and the results of the Duncan's multiple test

Year	N	Mean	Std. Error	Std. Deviation	Min.	Max.	Year	N	Subset for alpha = 0.05		
									1	2	3
2006	147	65.91	8.02	97.25	1	512	2006	147	65.9		
2007	143	314.29	21.45	256.49	3	1624	2010	85	115.7		
2008	149	427.85	31.47	384.1	6	2160	2007	143		314.3	
2010	85	115.73	7.41	68.32	3	417	2008	149			427.9
Total	524	244.69	12.78	292.52	1	2160	Sig.		0.120	1.000	1.000

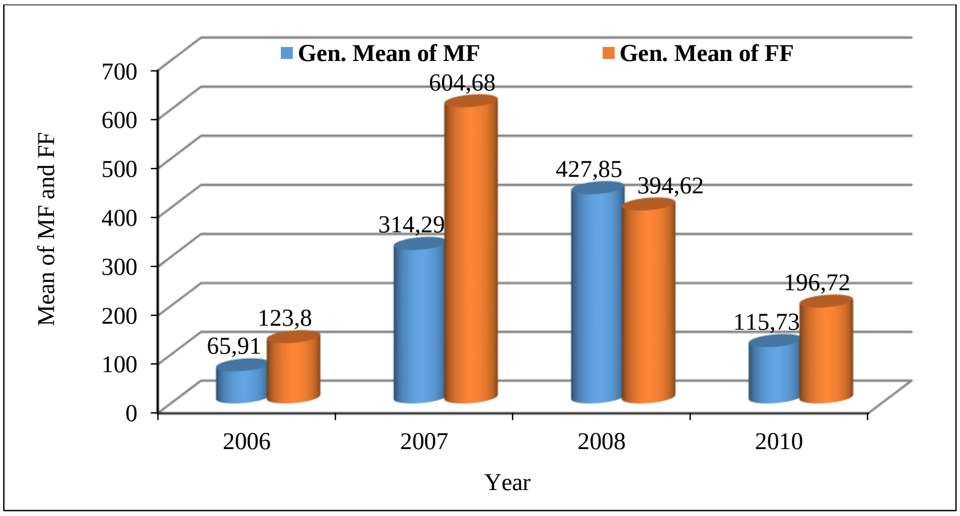

Figure 5. Production of male and female flowers of the clonal seed orchard as of years

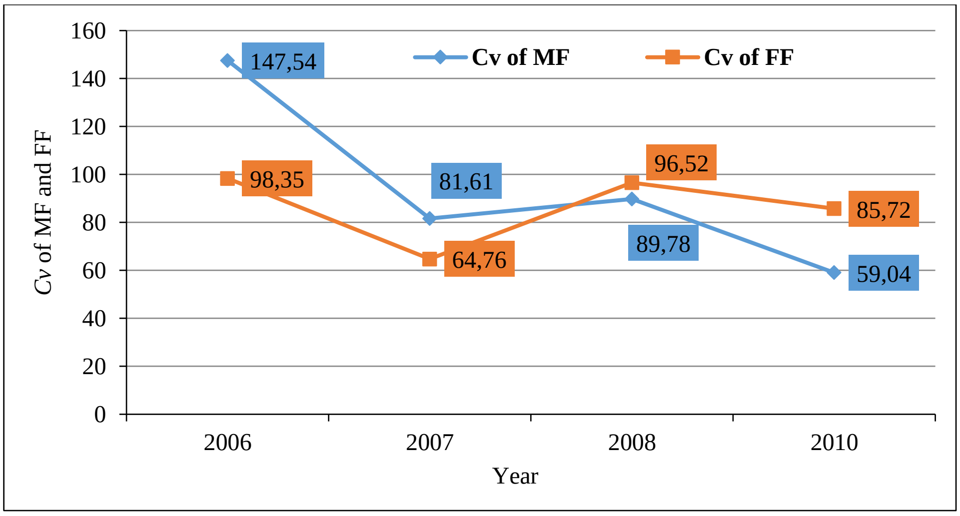

Figure 6. Change of *Cv* as of years for male and female flowers

4. Conclusion and Discussion

Variation in cone production within the clones (5-465) is greater than among the clones (33.4-287.8) as it is shown by El-Kassaby et al. (1989) for *Pseudotsuga menziesii*. Also Sıvacıoğlu and Ayan (2008) reported that significant differences existed between the clones in all traits (number of fertile and infertile scales, cone volume, cone number, filled and empty seed number, seed efficiency and 1000 seed weight) in 13 year-old Scots pine (*Pinus sylvestris* L.) clonal seed orchard in Taşköprü-Kastamonu. Clonal variation in both seed and cone production capability will not affect genetic composition of seed orchard seeds, but also the balance between maximizing genetic gain and maintaining seed production capacity at the time of seed orchard rogueing (Ying & Illingworth, 1985). In addition, balancing the ramet numbers representing the clones in the clonal seed orchards will increase the effective number of clones, as well as reduce the establishment cost of clonal seed orchards, as well as facilitate the organization of facilities (Bilir & Ayan, 2005; Ulusan & Bilir, 2008; Öztürk & Yıldız, 2022).

In this study, four-years data from Scots pine YCSO was evaluated, mean values from NMF and NFF among the clones were significant and variation of the ramets was high. The coefficient of variation from the NMF of the clones gradually decreased with the subsequent years whereas the *Cv* in the NFF did not show any decrease with the same years. Also there were significant differences among the years as to the NMF, NFF. Over the four years, the NMF was significantly lower than that of the NFF, and even in some years the NFF was doubled compared with the NMF. Çelik and Ayan (2009) stated that the flowering phenology of the clones examined were; the development of male and female flowers in terms of time, differences among clones and years, even it has been determined that these differences may occur even the same clone, among the ramets. Intra-clone differences are related to the position in which the graft material (epibiot) is taken from the ortet. Similar findings have been noted by researchers working in various clonal seed orchardss (O'Reilly et al., 1982; Boes et al., 1991; Matziris, 1993, 1994, 1997, 1998; Nikkanen & Ruotsalainen, 2000; Nikkanen, 2001; Ertekin, 2006; Ertekin & Tunçtaner, 2009).

For the studied seed orchard high variation both among the clones and the ramets indicates the high selection capacity in the breeding programs. The bigger variation among the ramets confirm that the genotypes have responded against the heterogeneity of growing area in seed orchard or the ramets have not reached the optimum flowering period. These results have shown the importance of the practices which increase the flowering yield and effectiveness of fertilization in YCSO. In the same time, the data of the periodical and comparative experiments in the same orchard, will support the breeding perspectives of this species, and these results will serve as a sample for similar evaluations in other species.

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Conflict of interest

The authors declare that there is no conflict of interest.

Declaration

This article was produced within the scope of the Master's thesis titled "Effect of zeolite with Added Fertilizer on Flowering and Cone Production in Seed Orchards" prepared by Canan BERBER ÜNAL and Master's thesis titled "Flowering Phenology in Taşköprü-Tekçam Scots pine (*Pinus sylvestris* L.) Clonal Seed Orchard" by Durmuş Ali ÇELİK under the supervision of Prof. Dr. Sezgin AYAN at the Institute of Science of Kastamonu University. In addition, the data of this article were presented as oral and poster presentations, separately, in two different conferences at "*International Forestry Conference on Forestry Science and Practices for the Purpose of Sustainable Development of Forestry*" – 20 Years of the Faculty of Forestry in Banja Luka, 1-4 November, 2012, Banja Luka, Bosnia-Herzegovina, and "*IUFRO Forest Tree Breeding Conference*", 25-29 August, 2014, Prague, Czech Republic.

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