

Cotyledon Number Variation in Some *Calabrian Pine*, *Crimean Pine* and *Lebanon Cedar* Populations in Turkey

Mahmut D. AVŞAR

Department of Forest Engineering, Faculty of Forestry, Kahramanmaraş Sütçü İmam University, 46060 Kahramanmaraş, Turkey

Corresponding Author
e-mail: mdavsar@ksu.edu.tr

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Abstract

In this study, cotyledon number variation in seedlings obtained from four populations of each of *Calabrian pine* (*Pinus brutia* Ten.), *Crimean pine* (*Pinus nigra* Arn. subsp. *pallasiana* (Lamb.) Holmboe) and *Lebanon cedar* (*Cedrus libani* A. Rich.) was investigated. The mean cotyledon number varied between 8.76-9.24 in *Calabrian pine*, 9.64-10.49 in *Crimean pine* and 8.84-9.43 in *Lebanon cedar* by the populations. There was statistically significant difference ($P < 0.05$) among the populations of these species in terms of cotyledon number. Cotyledon number varied between 7-12 in *Calabrian pine*, 6-15 in *Crimean pine* and 5-12 in *Lebanon cedar*. The general mean cotyledon number and cotyledon number variation were the highest in *Crimean pine*, followed respectively by *Lebanon cedar* and *Calabrian pine*. In tree improvement activities, the mentioned variations among different populations of these species should be considered.

Key words: *Pinus brutia*, *Pinus nigra* subsp. *pallasiana*, *Cedrus libani*, Cotyledon number, Population

INTRODUCTION

Cotyledons are one of the organs forming embryo of the seeds. Cotyledons, known as seed leave, have important roles in seeds. Cotyledons protect plumule in seeds and during germination, absorb nutrition from endosperm or stores nutrition in the seeds with no endosperm [1]. Cotyledons are food store and provide the energy needed to seedling during the period for elongating the roots of seedling in soil and making primary leaves [2].

The number of cotyledons is one of the plant seedling characteristics. While this number is 1 or 2 in Angiosperms, it varies between 2 and 18 in Gymnosperms [2]. Because cotyledon number may vary by genera and species especially in Gymnosperms; it may sometimes be used for separation of genera and species during the seedling phase. In addition, cotyledon number is one of the characters affected at least by site conditions. In many studies [3, 4, 5, 6, 7, 8], it was determined that cotyledon number is a character of which family heritability is respectively high.

Calabrian pine (*Pinus brutia* Ten.), *Crimean pine* (*Pinus nigra* Arn. subsp. *pallasiana* (Lamb.) Holmboe) and *Lebanon cedar* (*Cedrus libani* A. Rich.) are economically important forest tree species of Turkey. These species cover large areas in Turkey and have been widely used for reforestation activities. It was determined that there was a genetically positive relationship between cotyledon number and the first year height growth of the seedlings in *Calabrian pine* [5, 4, 7], *Crimean pine* [3, 6] and *Lebanon cedar* [8]. When considering the importance of fast height growth for early years in struggle with ground cover, the selection of populations and families with higher cotyledon numbers has been important for reforestation success.

In this study, cotyledon number variation in seedlings obtained from seeds of different populations of *Calabrian pine*,

Crimean pine and *Lebanon cedar* was investigated. Thus, both variations within populations and differences among populations were evaluated in terms of cotyledon number in seedlings of these species.

MATERIALS AND METHODS

In the study, the seeds of four populations of each of *Calabrian pine*, *Crimean pine* and *Lebanon cedar* were used (Table 1). The populations are located in the eastern part of the Mediterranean Region of Turkey. The seeds were obtained from natural populations of the mentioned species and in different years. 1000-seed weights of the seeds were determined according to ISTA rules [9].

Table 1. Some information about the populations and used seeds by tree species

Tree Species	Population	Elevation	Collection	1000-Seed
		(m)	Year	Weight (g)
Calabrian Pine	Kahramanmaraş-Suçatı	800	2003	61.78
Calabrian Pine	Andırın-Kesim	400	2001	50.89
Calabrian Pine	Antakya-Uluçınar	390	2000	53.12
Calabrian Pine	Antakya-Yayladağı	480	2000	54.45
Crimean Pine	Göksun-Göksun	1550	2002	27.25
Crimean Pine	Göksun-Büyükçamurlu	1650	2005	27.72
Crimean Pine	Andırın-Akifiye	1450	2002	37.87
Crimean Pine	Pos-Soğukoluk	1200	2005	29.37
Lebanon Cedar	Kahramanmaraş-Başkonuş*	1250	2003	--
Lebanon Cedar	Kahramanmaraş-Hartlap	1400	2002	95.24
Lebanon Cedar	Kahramanmaraş-Çağlayançerit	1300	2002	82.41
Lebanon Cedar	Göksun-Göksun	1450	2002	82.28

* The seeds were obtained from newly germinated seeds on the ground.

While the seeds of *Calabrian pine* and Crimean pine were directly taken to germinate, the seeds of *Lebanon cedar* (except Kahramanmaraş-Başkonuş population) were taken to germinate after cold-moist pretreatment for 30-day [10] at +4 oC in the refrigerator in order to overcome seed dormancy. The seeds were placed on filter paper in glass petri dishes (diameter 9 cm) and germinated under the conditions of room temperature (23±3 oC) and normal light. The seeds were moistened adequately during germination treatment. Since the seeds were obtained at different years, germination treatments were also conducted at different times. After the radicle considerably extended and the cotyledons were evident, cotyledon numbers were determined on 100 seedlings randomly selected for every population of the each species and some statistical values of these were calculated.

One-way analysis of variance (ANOVA) was applied to determine whether there was a statistically significant difference in terms of cotyledon number among the populations of each species and Duncan's multiple range test was applied to determine different groups [11]. In all statistical analyses, a confidence level of P=0.05 was used for statistical significance; and the analyses were carried out by using SPSS 11.5 package.

RESULTS AND DISCUSSION

The results obtained in the study and discussions relating to these were given separately by the tree species below.

Calabrian Pine

The mean cotyledon number varied between 8.76 and 9.24 by the populations and the general mean cotyledon number was 8.95 in *Calabrian pine* (Table 2). The results of one-way ANOVA showed that there was statistically significant difference in terms of cotyledon number among *Calabrian pine* populations (F=4.862; P=0.002). According to the results of Duncan's multiple range test, the populations separated into two different groups (Table 2). Thus, the highest cotyledon number was in Kahramanmaraş-Suçatı population and there was no statistically significant difference among the other three populations.

The mean cotyledon number in the various *Calabrian pine* populations of Turkey varied between 7.67-9.49 [12], 8.15-9.19 [13], 7.70-9.28 [14], 8.14-8.80 [5], 8.00-8.30 [4] and 8.44-8.93 [7]. It was reported that there was statistically significant difference in terms of cotyledon number among *Calabrian pine* populations in some other studies [12, 13, 14, 5, 4, 7], as well.

Cotyledon number of *Calabrian pine* varied between 7 and 12 and the most encountered cotyledon number was 9 with a proportion of 38.25% (Table 3). Cotyledon number in *Calabrian pine* was reported to be 6-11 [15] and 4-12 [12].

Crimean Pine

The mean cotyledon number varied between 9.64 and 10.49 by the populations and the general mean cotyledon number

was in Andırın-Akifiye population and there was no statistically significant difference among the other three populations.

Table 2. Statistical values of cotyledon numbers in Calabrian pine populations and the results of Duncan's multiple range test

Population	Mean*	SE	SD	Min.	Max.	CV (%)
Kahramanmaraş-Suçatı	9.24b	0.09	0.93	8	11	10.10
Andırın-Kesim	8.76a	0.08	0.83	7	10	9.48
Antakya-Uluçmar	8.89a	0.10	1.00	7	12	11.29
Antakya-Yayladağı	8.91a	0.09	0.93	7	11	10.47
General	8.95	0.05	0.94	7	12	10.51

*Means followed by the different letters are significantly different ($P<0.05$)

Table 3. Percent distribution of cotyledon numbers in Calabrian pine populations

Population	Cotyledon Number					
	7	8	9	10	11	12
Kahramanmaraş-Suçatı	-	25	35	31	9	-
Andırın-Kesim	6	31	44	19	-	-
Antakya-Uluçmar	4	36	34	20	5	1
Antakya-Yayladağı	3	33	40	18	6	-
Mean	3.25	31.25	38.25	22.00	5.00	0.25

Table 4. Statistical values of cotyledon numbers in Crimean pine populations and the results of Duncan's multiple range test

Population	Mean*	SE	SD	Min.	Max.	CV (%)
Göksun-Göksun	9.64a	0.11	1.11	7	12	11.56
Göksun-Büyükçamurlu	9.66a	0.13	1.27	6	13	13.10
Andırın-Akifiye	10.49b	0.14	1.40	8	15	13.38
Pos-Soğukoluk	9.85a	0.11	1.09	8	12	11.02
General	9.91	0.06	1.27	6	15	12.79

*Means followed by the different letters are significantly different ($P<0.05$)

The mean cotyledon number in the various Crimean pine populations of Turkey varied between 8.00-8.40 [3], 9.12-9.35 [6], 7.41-7.61 [16] and 8.48-8.86 [17]. The mean values in this study were higher than the values obtained in the previous studies. It was reported that there was statistically significant difference in terms of cotyledon number among Crimean pine populations [17]; there was no statistically significant difference in some other studies [3, 6, 16].

Cotyledon number of Crimean pine varied between 6 and 15 and the most encountered cotyledon number was 10 with a

proportion of 29.50% (Table 5). Gülcü [17] also determined that cotyledon number varied between 5-13 in Crimean pine. **Lebanon Cedar**

The mean cotyledon number varied between 8.84 and 9.43 by the populations and the general mean cotyledon number was 9.12 in *Lebanon cedar* (Table 6). The results of one-way ANOVA showed that there was statistically significant difference in terms of cotyledon number among *Lebanon cedar* populations ($F=5.953$; $P=0.001$). According to the results of Duncan's multiple range test, the populations separated into two different groups (Table 6). Thus, the highest cotyledon number was in

Göksun-Göksun population and there was no statistically significant difference among the other three populations.

Table 5. Percent distribution of cotyledon numbers in Crimean pine populations

Population	Cotyledon Number									
	6	7	8	9	10	11	12	13	14	15
Göksun-Göksun	-	1	13	35	29	16	6	-	-	-
Göksun-Büyükçamurlu	2	1	9	38	26	17	5	2	-	-
Andırın-Akifiye	-	-	9	13	28	30	14	3	2	1
Pos-Soğukoluk	-	-	10	29	35	18	8	-	-	-
Mean	0.50	0.50	1.025	2.875	2.950	2.825	0.25	1.25	0.50	0.25

Table 6. Statistical values of cotyledon numbers in Lebanon cedar populations and the results of Duncan's multiple range test

Population	Mean*	SE	SD	Min.	Max.	CV (%)
Kahramanmaraş-Başkonuş	9.06a	0.10	1.01	5	11	11.18
Kahramanmaraş-Hartlap	8.84a	0.10	1.04	6	12	11.78
Kahramanmaraş-Çağlayancerit	9.13a	0.10	0.99	7	12	10.86
Göksun-Göksun	9.43b	0.09	0.95	8	12	10.03
General	9.12	0.05	1.02	5	12	11.16

*Means followed by the different letters are significantly different ($P < 0.05$)

Table 7. Percent distribution of cotyledon numbers in Lebanon cedar populations

Population	Cotyledon Number							
	5	6	7	8	9	10	11	12
Kahramanmaraş-Başkonuş	1	-	1	29	34	29	6	-
Kahramanmaraş-Hartlap	-	1	8	25	45	15	5	1
Kahramanmaraş-Çağlayancerit	-	-	3	21	47	20	7	2
Göksun-Göksun	-	-	-	16	39	33	10	2
Mean	0.25	0.25	3.02	2.75	41.25	24.25	7.0	1.25

The mean cotyledon number in the various Lebanon cedar populations of Turkey varied between 7.67-8.62 [18] and 8.45-8.73 [8]. The mean values in this study were higher than the values obtained in the previous studies. Bilir [18] and Gülbaba et al. [8] also reported that there was statistically significant difference in terms of cotyledon number among Lebanon cedar populations.

Cotyledon number of Lebanon cedar varied between 5 and 12 and the most encountered cotyledon number was 9 with a proportion of 41.25% (Table 7). Cotyledon number in Lebanon cedar was determined to be 5-13 [19], 6-11 [18] and 8-12 [20].

By the tree species, the general mean cotyledon number was the highest in Crimean pine (9.91), followed by Lebanon cedar (9.12) and Calabrian pine (8.95), respectively. The highest variation (CV) in cotyledon number was also Crimean pine (12.79%), followed by Lebanon cedar (11.16%) and Calabrian pine (10.51%), respectively. Cotyledon number varied between 7-12 in Calabrian pine, 6-15 in Crimean pine and 5-12 in Lebanon cedar. Thus, it is understood that separating these species in the seedling phase is not possible according to cotyledon numbers only.

That there were statistically significant differences in terms of cotyledon number in Calabrian pine, Crimean pine and

Lebanon cedar populations mainly indicates that there were also genetic differences among the populations. These differences among the populations should be considered in the tree improvement activities of the species.

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