

Effects of carbon and nitrogen content on seed germination of calabrian pine (*Pinus brutia*) populations

Efectos del contenido de carbono y nitrógeno en la germinación de semillas de poblaciones de pino de Calabria (*Pinus brutia*)

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SUMMARY

Calabrian pine (*Pinus brutia*) seeds collected from fifteen populations were analyzed for their carbon (C) and nitrogen (N) content. Moreover, germination percentages (GP) and mean germination times (MGT) of the seeds were evaluated based on their carbon and nitrogen concentrations, C/N ratio, total carbon mass (CM), and total nitrogen mass (NM). Except for C/N ratios, variables showed significant differences among populations. The highest seed carbon concentration was 54 %, and the lowest was 50 %. Seed nitrogen concentrations varied from 2.6 % to 3.2 %. The highest correlations were found between the GP of chilled filled seeds (GPFS) and the combined concentrations of carbon and nitrogen ($R^2 = 0.65$) and the GPFS and concentrations of carbon ($R^2 = 0.63$), both being significant. The relationships between germination parameters and combined concentrations of carbon and nitrogen as well as CM and NM traits were generally higher than any found in individual evaluations, except for the concentrations of carbon.

Key words: calabrian pine, seed, carbon, nitrogen, germination, chilled seed, Turkey.

RESUMEN

Semillas de pino de Calabria (*Pinus brutia*) recolectadas de 15 poblaciones fueron analizadas en su contenido de carbono (C) y nitrógeno (N). Además fueron evaluados los porcentajes de germinación (GP) y tiempos medios de germinación (MGT) de las semillas según sus concentraciones de carbono y nitrógeno, relación C/N, masa total de carbono (CM) y masa total de nitrógeno (NM). Excepto en las relaciones C/N, las variables mostraron diferencias significativas entre las poblaciones. La mayor concentración de carbono de las semillas fue de 54 %, y la más baja fue de 50 %. Las concentraciones de nitrógeno en las semillas variaron de 2,6 a 3,2 %. Las correlaciones más altas se encontraron entre el GP de las semillas estratificadas (GPFS) y las concentraciones combinadas de carbono y nitrógeno ($R^2 = 0,65$), y entre los GPFS y concentraciones de carbono ($R^2 = 0,63$), ambas significativas. Las relaciones entre los parámetros de germinación y las concentraciones combinadas de carbono y nitrógeno, así como los rasgos CM y NM fueron generalmente mayores que cualquiera de las evaluaciones individuales, a excepción de las concentraciones de carbono.

Palabras clave: pino de Calabria, semillas, carbono, nitrógeno, germinación, semillas estratificadas, Turquía.

INTRODUCTION

Calabrian pine (*Pinus brutia* Ten.) is a dominant tree species in the eastern part of the Mediterranean Region, whereas aleppo pine (*Pinus halepensis* Mill.) is more common in the western part. Both of these pine species have been the primary focus of reforestation in the Mediterranean basin. Its fast growth, adaptation to drought and useful wood also increased the interest in calabrian pine at international scale and it became an interesting subject for many investigations (Boydak *et al.* 2006). Calabrian pine is one of the important native forest tree species of Turkey. It usually grows in pure stands and is also a valuable source of timber production. In addition,

it provides soil stabilization and serves as a wildlife habitat (Boydak 2004). Among native forest-tree species, it shows the highest distribution area, accounting for 27% of Turkey's total forest area (5.9 million hectares) (Anonymous 2012).

Seed reserve materials play a vital role in the life strategies of plants (Soriano *et al.* 2011), and seed germination is also affected by the use and storage of these reserve materials (Bewley 1997, Borisjuk *et al.* 2004, Fait *et al.* 2006). Furthermore, the nitrogen (N) and carbon (C) contents of seeds are considered to be reliable indicators (Toorop *et al.* 2012) among seed reserve materials. The nitrogen and protein ratios of seeds have a substantial effect on the plant efficacious use of nitrogen as well as seed quality

and nutrition (Masclaux-Daubresse and Chardon 2011). Moreover, during the first stage of seed germination, an adequate supply of nitrogen is necessary in order to satisfy the amino acid requirements of the seed needed for protein synthesis (Soriano *et al.* 2011).

Seed development represents the carbon sink of the plant (Wardlaw 1990, Way *et al.* 2010), and accumulation of carbon in seeds is directly related to the photosynthesis mechanism (Borrel *et al.* 2001, Gregersen *et al.* 2008, Masclaux-Daubresse and Chardon 2011). Additionally, in higher quality seeds, increased levels of carbon fixation occur in conjunction with the accumulation of increased levels of other nutrients, including nitrogen (Huxman *et al.* 1999).

Literature contains a limited number of studies that have focused on the carbon and nitrogen content and carbon/nitrogen ratios of forest tree seeds in Turkey (Gül 1992, Matthaus and Özcan 2011, Caliskan and Makineci 2014) and the relationship between these ratios and seed germination.

Hence, the main aims of this study were: (1) to compare carbon and nitrogen content, carbon/nitrogen ratio, total carbon mass, and total nitrogen mass in the seeds of fifteen different calabrian pine populations, and (2) to evaluate the relationships between carbon and nitrogen content of seeds and germination parameters.

In relation to the aims, the specific hypotheses of the study are: 1) H_0 : The seed lots of different calabrian pine populations have no variation in carbon and nitrogen concentrations, carbon/nitrogen ratio, carbon mass, and nitrogen mass. 2) H_0 : The carbon and nitrogen contents of seeds have no effect on germination parameters.

METHODS

Seed material. Cones were collected from fifteen calabrian pine seed stands by the Turkish Ministry of Forest and Water Affairs, Forest Tree Seeds and Tree Breeding Research Directorate. In each seed stand, cones were collected from more than 100 mature trees. After removed from the cones, all the seeds were then combined into single seed lots representing each population. The geographic locations of the seed stands are given in table 1 and figure 1.

Screens were used to clean and separate every seed lot from the twigs, needles and other particles, and damaged and insect-infested seeds were eliminated by hand selection (Schmidt and Thomsen 2003, Karrfalt 2008). The remaining seeds were stored at 3 °C (± 1 °C). In each clean seed lot, randomly selected seeds were subjected to germination tests as well as carbon and nitrogen analyses. Furthermore, 1,000 - seed weights (g) were determined based on eight replications of 100 seeds (ISTA 1999). Since calabrian pine seeds have chilling requirements (Boydak *et al.* 2006, Şefik 1964, 1965, Aslan and Uğurlu 1986, Skordilis and Thanos 1995, Thanos 2000) the seeds were chilled at +3 °C (± 1 °C) for 45 days.

Germination tests. Germination tests were performed in petri dishes measuring 9 cm in diameter on two layers of filter paper saturated with deionized water and were carried out using four replicates of 50 seeds each at a constant temperature of 20 °C (± 0.5 °C). Seeds were considered to be germinated when geotropism occurred in the radicles and a growth of more than 5 mm in length from the seed coat was seen. Experiments were checked every day over a 28-day period, and the germinated seeds were then removed from the petri dishes. Next, the ungerminated seeds were subjected to a cut test to determine the proportion of sound and empty seeds in every replication of each treatment. Germination data were expressed in terms of germination percentages (GP) [the germination percentage of the total seeds in the control group (GPC) (unchilled), the germination percentage of filled seeds in the control group (GPFC) (unchilled), the germination percentage of the total number of pretreated seeds (GPS) (chilled), and the germination percentage of pretreated filled seeds (GPFS) (chilled)] and mean germination times (MGT) [the mean germination time of the seeds in the control group (MGTC) (unchilled) and the mean germination time of the chilled seeds (MGTS)]. MGTs were calculated using the following formula [1] (Bewley and Black 1994) in which t represents the time in days starting from the day the tests began, and n is the number of seeds which completed germination on day t . In this equation, lower MGT values indicate quicker germination:

$$MGT = \sum(t \times n) / \sum n \quad [1]$$

Carbon and nitrogen analyses. The seed samples (30 seeds for each replication) were ground, and the Dumas dry combustion method was utilized to determinate carbon and nitrogen concentrations using a LECO Truspec 2000 CN analyzer (LECO Corporation, St. Joseph, MI, USA) (Leco 2000) with 10 replicates for each population. Carbon mass (CM) and nitrogen mass (NM) were then calculated from the carbon-nitrogen concentration and seed mass.

Statistical analyses. Prior to the statistical evaluation, the arcsine square root transformation was used for GPs, and the generalized linear model procedure was employed for the analysis of variance (ANOVA). The following model was used to calculate the ANOVA for the seed traits:

$$y_{ij}: \mu + P_i + e_{ij} \quad [2]$$

In this model, y_{ij} represents the trait of j in the replication of the i population ($j = 1, 2, 3$, and 4 for GPs and MGTs and $j = 1, 2, 3, \dots, 10$ for carbon concentrations, nitrogen concentrations, C/N ratio, carbon mass, and nitrogen mass); μ signifies the overall mean; P_i represents the effect due to the i population ($i: 1, 2, 3, \dots, 15$); and e_{ij} stands for the error.

Table 1. Seed collection sites of calabrian pine.

Sitios de colecta de semillas de pino de Calabria.

	Populations	Altitude (m)	Latitude (°N)	Longitude (°E)	Exposure
1	Pos-Soğukoluk	735	37 35 24	35 21 28	S
2	Pos-Karsantı	735	37 34 30	35 24 30	W-NW
3	MKPaşa-Çaltılıbük	250	39 58 45	28 40 54	NE
4	Marmaris-Çetibeli	60	37 00 17	28 19 42	NW
5	Muğla-Karabörtlen	680	37 05 33	28 32 22	Plain
6	Yılanlı-Boyalı	750	37 17 18	28 33 50	N-SE
7	Muğla-Gökova	270	37 00 39	28 24 30	NW
8	Durağan-Adadağı	400	41 27 13	35 06 03	N-W
9	Amasya-Destek	430	40 53 43	36 21 19	W
10	Ayvacık-Baharlar	450	39 36 24	26 34 32	N-S
11	Yenice-Yenice	300	39 56 04	27 20 57	NW
12	Geyve-Taraklı	530	40 23 12	30 25 13	N
13	Andırın-Kesim	400	37 24 18	36 23 42	E
14	Serik-Pınargözü	500	37 17 14	30 57 57	W-SW
15	Antalya-Düzlerçamı	275	36 59 45	30 33 10	Plain

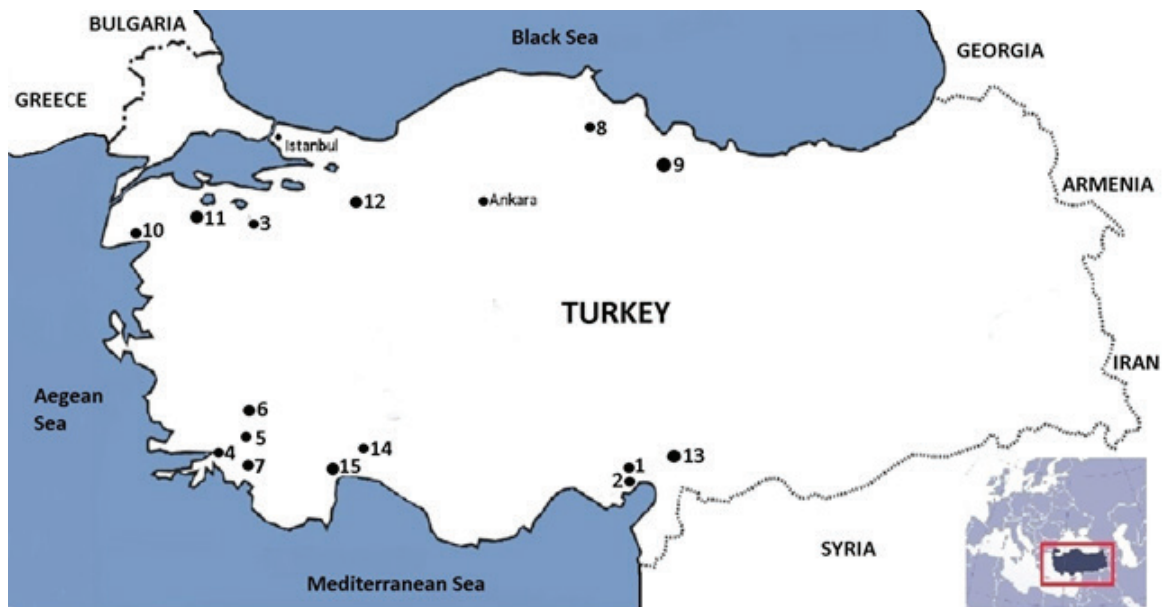


Figure 1. The location of calabrian pine populations.

Localización de las poblaciones de pino de Calabria.

In addition, Duncan's post hoc test was used to determine the significant differences among means ($P < 0.05$). Furthermore, the magnitude of the variation (as a percentage of the total variation) due to population and error was evaluated for each trait using the restricted maximum likelihood method, and the coefficient of variation was then determined based on the overall mean and total variance

for each trait. Correlations were also calculated to examine the relationship among carbon concentrations, nitrogen concentrations, carbon/nitrogen ratio, carbon mass, and nitrogen mass along with germination parameters. In addition, suitable regression models were used to evaluate the significant relationships between germination parameters and the chemical traits of seeds.

RESULTS

The lowest seed carbon concentration (50 %) was found in the Antalya-Düzlerçamı population, whereas the highest concentration (54 %) was in Ayvacık-Baharlar (table 2). ANOVA results revealed significant ($P = 0.0001$) differences among the calabrian pine populations with regard to the carbon concentration of seeds. Furthermore, according to the results of the Duncan test, the Ayvacık-Baharlar, Andırın-Kesim, and Antalya-Düzlerçamı populations were ranked individually while the others were ranked in different groups (table 2). The seed nitrogen concentrations of the populations varied from 2.6 % in Yılanlı-Boyalı and Andırın Kesim to 3.2 % in Muğla-Gökova and Antalya-Düzlerçamı, and these concentrations demonstrated statistically significant differences ($P = 0.0126$) among populations (table 2). However, C/N ratios showed no significant ($P = 0.0883$) differences, with ratios of between 15.7 in Antalya-Düzlerçamı and 21.8 in Andırın-Kesim (table 2). Maximum carbon mass and nitrogen mass were 34.5 g in Durağan-Adadağı and 2.0 g in Pos-Karsantı and Ayvacık-Baharlar, respectively (table 2); while minimum carbon mass and nitrogen mass were 26.4 g in Yenice-Yenice and 1.4 g in Yılanlı-Boyalı and Andırın-Kesim, respectively (table 2).

Significant differences ($P = 0.0001$) related to GPC, GPFC, GPS, GPFS, MGTC and MGTS were also found among the different calabrian pine populations. For example, GPC ranged from 19.5 % in Amasya-Destek to 85 % in Antalya-Düzlerçamı while the MGTC ranged from 12.8 in Yenice-Yenice to 18.2 in Amasya-Destek. Furthermore, the lowest MGTS rate of 10.0 was found in the Muğla-Gökova population, while the highest rate of 14.4 was located in Andırın-Kesim (table 2). Additionally, the seed weights of the calabrian pine populations ranged from 38.4 g in Andırın-Kesim to 55.5 g in Serik-Pınargözü, and the mean seed weight of all of the populations was 47.8 g (table 2).

The highest correlation was found between GPFS and the combined concentrations of carbon and nitrogen ($R^2 = 0.65$). This was followed by GPFS and concentration of carbon ($R^2 = 0.63$); both of these relationships were significant. The relationships between the germination parameters in the combined concentration of carbon and nitrogen and the combined traits of carbon mass and nitrogen mass were generally higher than those found in the individual evaluations, except for the concentration of carbon. The regression models with $P < 0.05$ are given below (table 3).

$$(GPC): y = 534.103 - X_1 * 9.297; (X_1 = \% C); R^2 = 0.34; S_e = 16.63; P = 0.024$$

$$(GPC): y = 448.688 - X_1 * 10.141 + X_2 * 43.744; (X_1 = \% C, X_2 = \% N); R^2 = 0.50; S_e = 15.08; P = 0.017$$

$$(GPS): y = 464.007 - X_1 * 7.9998; (X_1 = \% C); R^2 = 0.47; S_e = 10.80; P = 0.005$$

$$(GPS): y = 424.868 - X_1 * 8.386 + X_2 * 20.045; (X_1 = \% C, X_2 = \% N); R^2 = 0.53; S_e = 10.55; P = 0.010$$

$$(MGTS): y = 24.429 - X_1 * 4.136; (X_1 = \% N); R^2 = 0.30; S_e = 1.19; P = 0.034$$

$$(GPFC): y = 774.402 - X_1 * 13.722; (X_1 = \% C); R^2 = 0.50; S_e = 17.29; P = 0.003$$

$$(GPFC): y = 693.536 - X_1 * 14.520 + X_2 * 41.415; (X_1 = \% C, X_2 = \% N); R^2 = 0.60; S_e = 16.10; P = 0.004$$

$$(GPFC): y = 185.073 - X_1 * 10.786 + X_2 * 116.405; (X_1 = \text{carbon mass}, X_2 = \text{nitrogen mass}); R^2 = 0.44; S_e = 19.12; P = 0.031$$

$$(GPFS): y = 659.148 - X_1 * 11.564; (X_1 = \% C); R^2 = 0.63; S_e = 11.29; P = 0.0004$$

$$(GPFS): y = 630.839 - X_1 * 11.844 + X_2 * 14.498; (X_1 = \% C, X_2 = \% N); R^2 = 0.65; S_e = 11.41; P = 0.002$$

According to results, the amount of variation due to the populations was higher for germination parameters than for seed chemical traits (table 4), and the coefficient of variation of GPs and MGTs ranged from 22.1 - 28.3 and 5.4 - 6.0, respectively. The coefficient of variation of carbon concentration, nitrogen concentration, carbon/nitrogen ratio, carbon mass, and nitrogen mass varied between 4.3 and 22.7 (table 4).

DISCUSSION

The results of this study demonstrated that all seed parameters, except for carbon/nitrogen ratio, differed significantly among the investigated calabrian pine populations. With regard to the relationships between carbon and nitrogen contents of seeds and germinations traits, the highest correlation existed between GPFS and the combined concentration of carbon and nitrogen followed by the correlation between GPFS and concentration of carbon. As our results showed, the seed carbon concentration had a stronger effect on GP and MGT parameters than that posed by the seed nitrogen concentration and carbon/nitrogen ratios. These results demonstrate that factors other than those related to the populations themselves may play a role on the observed variations in these population differences. For example, the nitrogen supply of the trees might have a direct effect on the nitrogen concentration of their seeds (Munier-Jolain *et al.* 2008, Masclaux-Daubresse *et al.* 2010, Masclaux-Daubresse and Chardon 2011). Moreover, our results indicated that carbon is a more stable parameter in seeds, whereas nitrogen is characteristically more variable. For instance, in contrast to carbon, nitrogen in the seeds of growing plants is largely obtained through the recycling of nitrogen sources that are assimilated prior

Table 2. Duncan test of calabrian pine populations in terms of carbon concentrations, nitrogen concentrations, carbon mass, nitrogen mass, C/N ratio, GPC, GPFC, GPS, GPFs, MGTC, MGTS. GPC is the germination percentage of the total seeds in control (unchilled); GPFC is the germination percentage of filled seeds in control (unchilled); GPS is the germination percentage of the total seeds (chilled); GPFs is the germination percentage of filled seeds (chilled). MGTC is the mean germination time of the seeds in control (unchilled); MGTS is the mean germination time of the seeds (chilled). Means in the same column followed by the same lowercase letter are not significantly different ($P < 0.05$).

Prueba de Duncan para las poblaciones de pino de Calabria en términos de concentraciones de carbono y nitrógeno, masa de carbono y nitrógeno, relación C/N, GPC, GPFC, GPS, GPFs, MGTC, MGTS. GPC: porcentaje de germinación de las semillas en el total de control (no estratificado); GPFC: porcentaje de germinación de semillas en control (no estratificado); GPS: porcentaje de germinación del total de semillas (estratificadas); GPFs: porcentaje de germinación de semillas llenas (estratificadas); MGTC: tiempo medio de germinación de las semillas en el control (no estratificado); MGTS: tiempo medio de germinación de las semillas (estratificadas). Medias en la misma columna seguidas por la misma letra minúscula no son significativamente diferentes ($P < 0.05$).

Populations	Carbon (%)	Nitrogen (%)	C/N	Carbon mass (g)	Nitrogen mass (g)	GPC	GPFC	MGTC	GPS	GPFs	MGTS	Seed Weight (g)
1 Pos-Soğukoluk	52.4abcde	3.0abc	17.6a	29.2bc	1.7abc	49.0def	59.8cde	17.0abc	46.5b	58.1cde	12.2cd	47.7de
2 Pos-Karsanti	51.0cdef	3.0abc	17.5a	34.0a	2.0a	42.0f	52.5de	17.4ab	48.5b	63.0cd	13.1bc	45.9d
3 MKPaşa-Çalrılibük	52.7abc	2.8abc	18.9a	29.1bc	1.6bc	56.0cd	62.2c	18.0a	43.0bc	46.7def	14.1ab	47.0d
4 Marmaris-Çetibeli	53.3ab	3.0abc	18.0a	32.2ab	1.8ab	50.5de	58.7cde	16.6abcd	41.0bc	48.2cdef	10.3gf	50.4fg
5 Muğla-Karabörtlen	52.6abcd	3.1ab	17.3a	30.6ab	1.8abc	49.0def	51.0e	15.9bcde	41.0bc	42.3ef	10.3gf	46.5d
6 Yılanlı-Boyalı	52.6abcd	2.6c	20.5a	28.3bc	1.4c	34.5g	41.1f	15.3cde	30.5c	36.3f	12.0de	43.6c
7 Muğla-Gökova	53.2ab	3.2ab	16.8a	31.4ab	1.9ab	58.5c	63.6c	14.9de	38.0bc	40.9ef	10.0g	52.2g
8 Durğan-Adadağı	53.3ab	2.9abc	18.5a	34.5a	1.9ab	24.0ih	27.9g	16.8abc	50.0b	59.5cde	13.3abc	51.5g
9 Amasya-Destek	52.6abcd	2.8abc	19.2a	31.3ab	1.7abc	19.5i	23.2g	18.2a	31.0c	36.9f	12.6cd	50.8g
10 Ayvacık-Baharlar	54.1a	3.1ab	17.6a	33.8a	2.0ab	28.0h	31.1g	14.7ef	31.0c	35.2f	12.5cd	48.9ef
11 Yenice-Yenice	50.6def	3.1ab	16.6a	26.4c	1.6abc	52.5ef	75.0c	12.8f	47.5b	64.2abc	12.3cd	40.8b
12 Geyve-Taraklı	50.5ef	2.8bc	18.3a	28.6bc	1.6bc	73.0b	93.6b	15.2bcde	69.5a	84.8ab	13.1bc	48.6ef
13 Andırın-Kesim	50.8cdef	2.6c	21.8a	26.5c	1.4c	42.5f	60.7cd	17.0abc	45.5b	65.9bcd	14.4a	38.4a
14 Serik-Pmargözü	51.3bcdef	3.0abc	17.4a	31.9ab	1.9ab	83.0a	97.6a	14.1ef	75.5a	87.8ab	10.6ef	55.5h
15 Antalya-Düzlerçamı	50.3f	3.2a	15.7a	29.0bc	1.9ab	85.0a	96.6a	15.4cde	71.0a	81.6a	12.2de	48.9ef
Mean	52.1	3.0	18.1	30.5	1.7	49.8	59.6	16.0	47.3	56.8	12.2	47.8

Table 3. Relationships between the chemical traits and germination parameters in calabrian pine seeds.
Relaciones entre características químicas y parámetros de germinación en semillas de pino de Calabria.

Variable	Parameter	Carbon %	Nitrogen %	Carbon/Nitrogen	Carbon mass	Nitrogen mass	%C-%N	Carbon mass - nitrogen mass
GPC	R ²	0.34	0.10	0.24	0.10	0.00	0.50	0.38
	S _e	16.63	19.32	17.78	19.35	20.39	15.08	16.78
	P-value	0.0236	0.2426	0.0638	0.2514	0.9037	0.0165	0.0596
MGTC	R ²	0.05	0.16	0.20	0.05	0.01	0.24	0.26
	S _e	1.53	1.43	1.40	1.53	1.56	1.42	1.40
	P-value	0.4233	0.1344	0.0910	0.4389	0.7724	0.1904	0.1631
GPS	R ²	0.47	0.03	0.14	0.01	0.02	0.53	0.17
	S _e	10.80	14.64	13.75	14.73	14.71	10.55	14.05
	P-value	0.0048	0.5668	0.1692	0.6721	0.6568	0.0103	0.3218
MGTS	R ²	0.08	0.30	0.26	0.06	0.16	0.35	0.18
	S _e	1.36	1.19	1.22	1.38	1.30	1.19	1.33
	P-value	0.2970	0.0343	0.0518	0.3716	0.1367	0.0763	0.2940
GPFC	R ²	0.50	0.05	0.16	0.19	0.01	0.60	0.44
	S _e	17.29	23.96	22.45	22.10	24.48	16.10	19.12
	P-value	0.0030	0.4315	0.1337	0.1040	0.7796	0.0039	0.0306
GPFS	R ²	0.63	0.00	0.06	0.05	0.00	0.65	0.18
	S _e	11.29	18.51	17.98	18.06	18.53	11.41	17.45
	P-value	0.0004	0.8831	0.3847	0.4199	0.9513	0.0018	0.3014

Table 4. Estimation of variance components and coefficient of variation (CV).
Estimación de los componentes de la varianza y coeficiente de variación (CV).

Characters	Overall mean	Variance components (%)		CV (%)
		Population	Error	
Nitrogen (%)	3.0	10	90	13.9
Carbon (%)	52.1	22	78	4.3
Carbon/Nitrogen	18.1	6	94	21.9
Carbon mass	30.5	24	76	14.9
Nitrogen mass	1.7	12	88	22.7
GPC	49.8	95	5	27.6
GPFC	59.6	95	5	28.3
MGTC	16.0	61	39	5.4
GPS	47.3	69	31	22.1
GPFS	56.8	68	32	23.7
MGTS	12.2	82	18	6.0

to seed formation (Cliquet *et al.* 1990, Patrick and Offler 2001, Masclaux-Daubresse and Chardon 2011). Our findings, which identified the relationship between seed germination parameters and seed carbon and nitrogen concentration, were similar to those of Frenne *et al.* (2011), who found that the seed carbon concentration had a stronger effect on GP and MGT than the effect observed in seed nitrogen concentrations and C/N ratios. They also determined that there was a relationship of $R^2 = 0.69$ between the seed germination rate and seed carbon concentrations and a relationship of $R^2 = 0.11$ between the seed germination rate and seed nitrogen concentrations.

The effects of germination on the chemical composition and biochemical constituents of seeds vary greatly with the plant species, seed varieties or cultivars and germination conditions (Gallardo *et al.* 2002). Proteins are necessary for seed germination, and they accumulate after ripening under seed-drying conditions, resulting in the release of dormancy (Gallardo *et al.* 2002, Chibani *et al.* 2006). Among the most important parameters that control the process of seed dormancy are changes associated with protein and hormones (Finch-Savage and Leubner-Metzger 2006, Finkelstein *et al.* 2008, Graeber *et al.* 2012, Miransari and Smith 2014). In addition, a group of proteins was discovered that inhibit protein degradation and regulators during seed germination (Corre-Menguy *et al.* 2002, Martinez *et al.* 2005).

Several studies have also pointed out the role that nitrogen compounds play in stimulating seed germination. For example, they can trigger the germination of Mediterranean species with limited seed reserves such as the Aleppo pine (*Pinus halepensis*) (Thanos and Rundel 1995, Broncano *et al.* 1998). In another study, Bedi *et al.* (2009) found significant correlations between GP and nitrogen (protein) content of seeds. Chandrasiri *et al.* (1987) also identified an increase in nitrogen that was associated with etiolated germination, and Lee and Karunanithy (1990) pointed out that the protein content was 21 % higher in dehulled, germinated soya beans than in ungerminated seeds (as described by Bau *et al.* 1997). Another study by Ellis and Marshall (1998) also determined that nitrogen fertilization influences seed quality, and Gardarin *et al.* (2011) demonstrated that seed protein content had no significant influence on the germination speed parameters that were measured in 25 weed species. Ene and Bean (1975) also found a correlation between the nitrogen content of ryegrass seeds and the germination rate, and this correlation ($R^2 = 0.61$) was statistically significant ($P = 0.01$). On the other hand, Broncano *et al.* (1998) found that nitrogen supply had no significant effect on seed germination in the Aleppo pine. Frenne *et al.* (2011) reported a weak relationship between the seed germination rate and seed nitrogen content. In addition, Hara and Toriyama (1998) emphasized the existence of a negative relationship ($R^2 = 0.78$) between the seed nitrogen concentration and germination time. They also reported that proteins (nitrogen) can absorb more wa-

ter than lipids or polysaccharides, meaning that more water absorption occurs at a faster pace when there is a higher nitrogen concentration in seeds. In turn, they reasoned that this can lead to increased seed germination.

Limited literature (Gül 1992, Matthaus and Özcan 2011, Caliskan and Makineci 2014) has focused on the carbon, nitrogen contents of forest tree seeds and the relationship between these ratios and germination. We identified low or moderate relationships in the regression models in which individual carbon and nitrogen concentration were used, and these were statistically significant. As seen in these significant models, nitrogen showed a weak relationship only with MGTS ($R^2 = 0.30$), but seed carbon concentrations and carbon mass and nitrogen mass generally demonstrated a moderate correlation with GPC, GPS, GPFC and GPFS. Furthermore, we obtained stronger relationships for germination parameters, except for MGTS, with combined carbon and nitrogen concentrations and carbon mass and nitrogen mass. Similarly, Caliskan and Makineci (2014) concluded that a positive linear relationship existed between carbon and nitrogen concentrations and GP, and that there was a negative relationship between carbon and nitrogen concentrations and MGT in Mediterranean cypress (*Cupressus sempervirens* L.) seeds. They also obtained more significant results by combining seed carbon and nitrogen concentrations on the regression models to estimate GP and MGT.

CONCLUSIONS

The seed lots of different calabrian pine populations show variation in carbon concentrations, nitrogen concentrations, carbon/nitrogen ratio, carbon mass, and nitrogen mass; these traits would have effect on the germination parameters. Variables show significant differences among populations except C/N ratios. The Antalya-Düzlerçamı population have the highest seed nitrogen concentration and the highest germination percentages. The highest significant correlation there is between GPFS in chilled seeds and the combined concentration of carbon and nitrogen. The relationships of germination parameters in the combined concentration of carbon and nitrogen and combination of carbon mass and nitrogen mass traits are also generally higher. If it is to continue to have a significant role in afforestation in this region, the quality of the seeds and seedlings must be maintained. While we believe that our findings shed some light on this topic, future research is needed to ensure the sustainability of this native species.

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