Effects of geoclimatic factors on the variability in *Pinus pinea* cone, seed, and seedling traits in Turkey native habitats

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**Abstract**

**Background:** Stone pine (*Pinus pinea* L.) is harvested for its edible kernels. It is an important forest tree species, and also plays an important role in afforestation. Successful afforestation activities strongly depend on the quality of seeds and seedlings. Five eastern native populations of stone pine were analyzed, in terms of their geographical and climatic parameters, in order to identify their cone, seed, pine nut, and seedling traits. With this regard, we aimed to contribute to the reforestation, yield, and breeding of this species.

**Results:** All native populations occur in the sub-humid bioclimatic zone, according to Emberger’s bioclimatic classification, ranging 74.7–63.2 of Emberger’s Q Index value (Q), which synthesizes humidity and temperature, with lower values indicating more arid conditions. With regard to geographical factors, altitude was found to have the greatest effect on cone diameter and seed weight, while there was a weak correlation of cone, seed, and pine nut dimension with longitude and latitude. In terms of climatic factors, Q and mean of the maximum temperature of June, July, and August (ME) were found to be the main drivers behind significant effects in cone, seed, and pine nut morphometric traits. The most negative effect on seed number cone−1 (r: −0.897), pine nut number cone−1 (r: −0.923), pine nut yield (r: −0.903), and pine nut weight (r: −0.878) was Q, whereas cone diameter, seed length, and seed weight were positively correlated with the ME (r: 0.939, r: 0.889, and r: 0.866, respectively). Cone and seed diameter and seed weight increased with decreasing summer drought index (r: −0.806, r: −0.846, and r: −0.866, respectively). Rainfall in the June of the third year prior to harvest time (RJ) had a negative effect on the percentage of sound seeds per cone. Annual rainfall in the third year prior to harvest time (AR) significantly affected pine nut weight (r: 0.889), although rainfall during summer had a negative effect. The empty seed ratio of the different populations ranged from 20 to 68%, while considering all populations, the empty seed ratio was 46%.

**Conclusions:** The study suggests that the Q Index and ME should be taken into account to determine cone to pine nut yield. With this regard, plantations of stone pine should be established on sub-humid areas and Q values should not exceed 75.

**Keywords:** Bioclimatic zone, Emberger, Mediterranean, Stone pine, variation

**Background**

Broad range of ecological conditions could create a variety of ecotypes (Huang 1993). This could contribute to the differences in characteristics of species such as cone, seeds, and shape which are important parameters related to improving seed yield. A better understanding of cone-yield variation patterns would facilitate many aspects of management (Mutke et al. 2005). Many studies have assessed cone and seed morphology of conifer populations regarding geographic and climatic variation (Şefik 1964; Kaya and Işık 1997; Mutke et al. 2005; Calama and Montero 2007; Liu et al. 2013; Loewe-Muñoz et al. 2020). Climatic variables such as annual

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and autumn thermal oscillation, spring hydric index, and spring and summer precipitation are directly linked with cone production (Calama et al. 2007; Loewe-Muñoz et al. 2016).

Stone pine is one of the commercially and ecologically important forest trees. Nuts constitute the most important non-wood product obtained from Mediterranean forests (Calama et al. 2016); it is one of the most characteristic species in the Mediterranean basin, with its umbrella shape and its use in horticulture, being cultivated for ornamental purposes. The cones are harvested for their edible kernels (pine nuts), which have been used as a food item since Paleolithic times (Mutke et al. 2005). Stone pine nuts are among the most expensive nuts; this high price makes them attractive as a crop. The species performs well in poor or eroded soils; its resistance to pests and diseases reduces the need for cultivation practices, and its high resistance to drought makes it a good candidate for conversion to a horticultural crop. Around the Mediterranean Sea, there are about 700,000 ha of stone pine-dominated forests, stretching from the Atlantic coast in Portugal to the shores of the Black Sea and Mount Lebanon (Mutke et al. 2012). Its distribution is mainly concentrated in Spain (450,000 ha), Portugal (90,000 ha), Turkey (50,000 ha), and Italy (40,000 ha) (Pereira et al. 2015).

Stone pine is characterized by high phenotypic plasticity and adaptability, but low genetic variability (Loewe-Muñoz et al. 2016). Genetic variation is generally considered to be an important adaptation to environmental conditions. Genetically depauperate, but geographically widespread, species are rare, and no other abundant and widespread plant species has as little genetic diversity as the stone pine. However, the species does have a considerable amount of variation in its adaptive traits (Vendramin et al. 2008).

Besides, seed production of trees is key to regeneration of forests and to successful conservation management (Gonçalves and Pommerening 2012). Successful restoration and rehabilitation in Mediterranean areas greatly depends on the quality of the seeds and seedlings used (Boydak and Çalışkan 2014). Significant degradation of the vegetation across large areas of the Mediterranean Basin has called for rehabilitation using native coniferous and broadleaved species (Boydak et al. 2006). Stone pine has been used in a variety of remedial ways, such as for ecological restoration, watershed and soil protection, the stabilization of dunes, and afforestation in urban areas (Mutke et al. 2012; Boydak and Çalışkan 2014, 2015; Çalışkan and Boydak 2017). The first dune afforestation project using Pinus pinea is thought to have been by the Romans along the Turkey-Antalya (Köprüçay) coastline, in an effort to stop mass movement and to prevent invasions of their agricultural land (Boydak and Çalışkan 2014).

On the other hand, Mutke et al. (2005) stated that further efforts will be required in order to accurately estimate the impacts of temperature rise and changes in rainfall patterns on cone development yield in stone pines. In the last few decades, efforts have been made in Spain, Turkey, and Portugal to domesticate the species, including intensive planting, reproductive propagation by grafting selected clones for cone production (Mutke, cited in Calama et al. 2016), and by employing irrigation and fertilization techniques (Calama et al. 2007; Loewe-Muñoz et al. 2017). Despite this, the main crop of cones collected and marketed are from either natural forests or afforestations (Mutke et al. 2012).

There is limited literature concerning variations among native populations of the species, in terms of cone, seed, germination, and seedling traits, and their correlation with climatic variables (Baskin and Baskin 1998; Ganatsas et al. 2008). The aims of the present study were to determine (a) the variation in cone, seed, and seedling morphological traits between, and among, the eastern stone pine populations (specifically, in Turkey); and (b) the relationships among climatic and geographical variables with cone and seed traits. With this regard, the present study will contribute to the reforestation, yield, and breeding of this species.

Materials and methods
Seed material collection
Natural stands of stone pine were selected from five populations across Turkey (Fig. 1, Table 1). Mature cones were collected in December 2016 from 15 to 25 randomly selected, mature trees in each population, which were growing at a distance of at least 50–100 m from each other, in order to capture a range of genetic variation (Ürgenç 1982). Ten cones were collected per tree, each placed in separate, labeled cloth bags (to prevent mildew). The cones were transported to the laboratory where they were stored at room condition until analyzed (Buru et al. 2016). The seeds were then manually extracted from 50 randomly selected cones from each of the five populations.

In Table 1, the coefficient ‘S’ explains the severity of the summer drought, with higher values indicating weak summer drought conditions. The coefficient ‘Q’ refers to climatic humidity and temperature, with lower values indicating more arid conditions. The coefficient ‘m’ represents the duration of the frost cycle, with higher values indicative of a shorter cycle (Dirik 2008).

We used Emberger’s equation, which is commonly used for the Mediterranean region, to categorize the bio-climate (Daget et al. 1988), the pluviothermic quotient (Q) calculated as:
\[
Q = \frac{2000 \cdot P}{(M + m + 546.24) \cdot (M - m)}
\]

where \( P \) is the mean annual rainfall (in mm), \( M \) is the mean of the maximum temperatures in the hottest month (in °C), and \( m \) is the mean of the minimum temperatures in the coldest month (in °C). The summer drought index (\( S \)) was calculated as \( S = \frac{\text{PE}}{\text{ME}} \), where \( \text{PE} \) is the sum of the rainfall (in mm) in June, July, and August, and \( \text{ME} \) is mean of the maximum temperatures in June, July, and August.

**Cone and seed measurements**
Fifty randomly selected cones, collected from 16 to 25 families in each population, were completely dissected to

**Table 1** Some geographical and climatic characteristics of the five stone pine (Pinus pinea) populations across their natural distribution in Turkey

| Populations          | Latitude Longitude | P (mm) | M (°C) | m (°C) | PE (mm) | ME (°C) | \( S^2 \) Summer Drought Index \( ^a \) | \( Q^2 \) Humidity category \( ^a \) | Climate types \( ^b \) |
|----------------------|--------------------|--------|--------|--------|---------|---------|----------------------------------------|----------------------------------------|------------------|
| K.Maraş-Önsen        | 37° 30' 27" 36° 40' 59" | 742    | 36.6 1.5 | 7.4    | 40.3    | 0.18    | 2                                      | 72.39 Sub-humid               | Semi-arid        |
| Muğla-Yatağan        | 37° 23' 11" 27° 50' 22" | 633    | 36.4 2.2 | 31.5   | 39.7    | 0.79    | 2                                      | 63.41 Sub-humid               | Semi-arid        |
| Aydın-Koçarlı         | 37° 42' 35" 27° 41' 18" | 609    | 36.9 4.2 | 13.8   | 40.5    | 0.34    | 2                                      | 63.38 Sub-humid               | Semi-arid        |
| İzmir-Kızak           | 39° 14' 45" 27° 09' 50" | 612    | 34.0 3.3 | 26.5   | 39.0    | 0.68    | 2                                      | 68.18 Sub-humid               | Semi-arid        |
| Çanakkale-Kirazlı      | 40° 1' 12" 26° 35' 36" | 605    | 31.2 3.4 | 38.6   | 34.7    | 1.11    | 3                                      | 74.71 Sub-humid               | Semi-arid        |

\(^a\) Bioclimate zones according to Emberger (Daget et al. 1988)
\(^b\) Climate types according to Erinç’s precipitation efficiency formula (Erinç 1965)
determine the seed yields of the five populations. The seeds extracted from each cone were separately immersed in water to distinguish the sound (those that sank) seeds (Table 2). All the seed measurements were performed on a random sample of 10 seeds per cone (Ganatsas et al. 2008). The 1000-seed weight (1000-SW) was determined using the ISTA (1999) procedure.

Seed germination
Germination tests were performed in a growth chamber (model VT3 4034; Vötsch Industrietechnik, Germany), maintained at 15, 20, and 25 °C until no additional seed germinated was observed. Randomly selected, sound seeds, with three replications (3 × 50) for each population, were used for germination. These were placed in 11-cm-diameter glass Petri dishes that were lined with two layers of filter paper, moistened with deionized water. The germinations were assessed daily, with germinated seeds being removed from the Petri dishes when their radicles had protruded (Farelli et al. 1997; Saracino et al. 2017). At the end of the test, the ungerminated seeds were subjected to a cut test to determine the proportion of sound to empty seeds in each replication of each treatment.

Stone pine seeds were also sown in the Forest Nursery Directorate in Bahçeköy/Sarıyer/Istanbul, Turkey at the beginning of March of 2017. The nursery is located at an altitude of 126 m above sea level at 41° 10′ 56″ N, 28° 59′ 14″ E, and the climate around the nursery is humid, mesothermal, and maritime, with a moderate deficit of water in the summer months, according to Thornthwaite’s classification. The mean annual precipitation is about 1111.4 mm, and the mean annual temperature is 12.8 °C (Akburak et al. 2018).

Plastic containers (18 cm tall and 190 cm³), filled with conventional media (soil, peat, and river sand in a ratio of 2:1:1) were used for sowing the seeds. For the trial, a randomized block design, with five replications, was used (135 seeds × 5 replications × 5 populations = 3375 sound seeds used). The germinations in the nursery were

| Table 2 | List of morphometric traits of P. pinea fruits and seedlings with measurement and units |
|---------|--------------------------------------------------------------------------------------------|
| Morphometric | Abbreviation | Unit | Measurement |
| Fruit traits | | | |
| Cone diameter | CD | mm | Three measurements across the widest portion with a digital caliper |
| Cone length | CL | mm | Measured using a digital caliper |
| Cone weight | CW | g | 3-year-old cones were weighed with precision scale (accuracy of 0.01 g) |
| Seeds cone⁻¹ | SN | No. | All seeds per cone were extracted and counted |
| Empty seeds | ES | % | ES = (SN-PN) / SN |
| Seed yield | SY | % | SY = (SN × SW) / CW × 100 |
| Seed diameter | SD | mm | Three measurements across the widest portion with a digital caliper |
| Seed length | SL | mm | Measured using a digital caliper |
| Seed weight | SW | g | Weighed with precision scale (accuracy of 0.01 g) |
| Pine nuts cone⁻¹ | PN | No. | All extracted seeds per cone were immersed and sinking seeds were counted |
| Pine nut weight | PW | g | Five sound seeds and empty seeds were weighed from each cone. Calculated differences average five sound seeds weight and empty seeds weight per cone². |
| Pine nut yield | PY | % | PY = (PN × PW) / CW × 100 |
| Seedling traits | | | |
| Number of cotyledons | NC | No. | Counted in 175 seedlings (7 seedlings × 5 replications × 5 populations) |
| Height | SdL1 | cm | Measured by scale at the end of first vegetation period in 175 seedlings |
| Height | SdL2 | cm | Measured by scale at the end of second vegetation period in 175 seedlings |
| Root collar diameter | SdD1 | mm | Measured by digital caliper at the end of first vegetation period in 175 seedlings |
| Root collar diameter | SdD2 | mm | Measured by digital caliper at the end of second vegetation period in 175 seedlings |
| Lateral branches | LB1 | No. | Counted in 175 seedlings at the end of first vegetation period |
| Lateral branches | LB2 | No. | Counted in 175 seedlings at the end of second vegetation period |
| Bud set | BS | % | Observed in 175 seedlings at the end of second vegetation period¹ |

¹²Indicate ≈ 20% and 10% moisture content
²No terminal bud observed at the first year
recorded two times in a week until no further germinated was observed.

The germination percentage (GP) and mean germination time (MGT) for laboratory and field conditions (FGP and FMGT) were calculated thus:

\[
GP \text{ or } FGP(\%) = \frac{G}{T} \times 100
\]

where \(G\) is the total number of germinated seeds and \(T\) is the total number of seeds; and

\[
MGT \text{ or } FMGT = \frac{\sum (t \times n)}{\sum n}
\]

where \(t\) is the number of days from the beginning of the test and \(n\) is the number of germinated seeds on day \(t\) (Bewley and Black 1994).

Statistical analysis
All statistical analyses were carried out using SPSS (2010) software. The GP and FGP data were arcsine transformed. The GP, FGP, MGT, and FMGT data of the five populations were analyzed using ANOVA. The following ANOVA models were used:

\[
Y_i = \mu + \phi_i + \epsilon_{ik}
\]

where \(Y_i\) is the observed value of the cone, seed, and seedling traits; \(\mu\) is the overall mean; \(\phi_i\) represents the effect of population; and \(\epsilon_{ik}\) is the effect caused by error. The populations were excepted as fixed factors; and

\[
Y_{ik} = \mu + \phi_i + T_k + \Phi_{ik} + \epsilon_{ik}
\]

where \(Y_{ik}\) is the observed value of the germination traits; \(\mu\), \(\phi_i\), \(T_k\), \(\Phi_{ik}\), and \(\epsilon_{ik}\) are the overall mean, population effect, temperature effect, and error, respectively. The populations and temperatures were excepted as fixed factors. Duncan's post hoc test was used to determine homogeneous subgroups. Pearson's correlation coefficients between the cone, seed and seedling traits, germination values, and geographical and climatic variables were determined.

Results
The ANOVA results for the cone and seed traits showed significant differences among the five native populations from Turkey. The range of variation in the cone diameter (CD), cone length (CL), and cone weight (CW) ranged from 48–87 mm, 58–150 mm, and 82–435 g, respectively. The seed diameter (SD), seed length (SL), and 1000-SW varied between 6.5 and 10.9 mm, 11.8 and 22.4 mm, and 589 and 903 g, respectively. The pine nut yield (PY) was low in all the populations, ranging from 22.4 mm, and 589 and 903 g, respectively. The pine nut yield (PY) was low in all the populations, ranging from 22.4 mm, and 589 and 903 g, respectively. The cone diameter (CD), cone length (CL), and cone weight (CW) had a significant (the strongest) positive effect on cone size and seed size. CD and CL had a significant (the strongest) positive effect on CW (\(r: 0.885\) and \(r: 0.849\), respectively). SD and SL significantly affected the SW (\(r: 0.921\) and \(r: 0.866\), respectively). SN increased with increasing cone size. PY was negatively related to ES. In addition, PY had a significantly positive correlation with cone size, including CW and SL (Table 6).

The 1000-SW increased with increased altitude (\(r: 0.866\)). SL and SD positively correlated with altitude (\(r: 0.866\)).
Table 3  Cone and seed morphometric traits of five stone pine (P. pinea) populations in Turkey

| Parameters | Populations | 1 K.Maraş-Önsen | 2 Muğla-Katrançlı | 3 Aydın-Koçarlı | 4 İzmir-Koza | 5 Çanakkale-Kirazlı | Mean |
|------------|-------------|-----------------|------------------|----------------|--------------|-----------------|------|
| CD         | 77.2 (65–85)b | 74.5 (56–85)b   | 79.1 (71–87)b    | 78.4 (64–86)b | 63.6 (48–75)b | 74.6 (61–84) |
| CL         | 95.1 (74–114)b | 103.2 (59–126)c | 102.4 (82–150)c | 105.0 (72–127)d | 79.6 (58–98)b | 97.1 (69–123) |
| CW         | 238.5 (145–335)b | 264.6 (103–408)c | 294.4 (122–424)d | 289.2 (132–435)cd | 167.8 (82–279)a | 250.9 (117–376) |
| SD         | 9.2 (7.5–10.9)b | 8.5 (7.0–10.1)b | 8.7 (7.0–10.3)b | 8.9 (6.9–10.8)b | 8.2 (6.5–9.8)a | 8.7 (7.0–10.4) |
| SL         | 17.8 (13–21.6)b | 17.9 (14–22.7)b | 18.9 (15.5–22.2)b | 18.7 (14.9–22.4)c | 15.6 (11.8–19.4)a | 17.8 (14–21.5) |
| 1000-SW    | 902.7 (0.4–1.3)a | 754.1 (0.4–1.1)b | 844.2 (0.5–1.2)c | 885.9 (0.4–1.3)c | 589.4 (0.3–0.9)b | 795.3 (0.8) |
| SN         | 40 (4–82)a     | 54 (8–103)a     | 75 (26–118)a     | 55 (21–113)a   | 25 (3–56)a    | 50 (12–94)     |
| SY         | 13.2 (3–24)b   | 12.9 (4–22)b    | 18.5 (11–32)d    | 15.6 (9–26)c   | 8.1 (1–15)a   | 13.7 (6–24)   |
| ES         | 60 (21–89)b    | 20 (2–69)b      | 21 (2–71)b       | 68 (27–98)c    | 60 (20–88)b   | 46 (14–83)   |
| PN         | 17 (1–51)b     | 44 (5–96)f      | 60 (24–109)d     | 21 (1–82)b     | 10 (2–31)a    | 30 (7–74)     |
| PW         | 0.19 (0.05–0.34)a | 0.22 (0.01–0.46)ac | 0.23 (0.01–0.62)c | 0.23 (0.05–0.35)f | 0.18 (0.05–0.36)a | 0.21 (0.03–0.43) |
| PY         | 1.37 (0.15–4.90)a | 3.53 (0.19–9.99)b | 4.47 (0.27–9.74)d | 1.55 (0.11–7.11)a | 1.20 (0.12–3.61)ab | 2.42 (0.17–7.07) |

Means in columns followed by the same letters are not statistically different at a 0.05 significance level using the Duncan post hoc test. For abbreviations, please see Table 2. Minimum and maximum values are presented in parentheses.

Meanation period on cone production. They also pointed out

Discussion
Cone traits
The mean CW of 251 g is consistent with reported ranges of 227–304 g, based on 3-year surveys (Gonçalves and Pomerening 2012), and 250–350 g (Mutke et al. 2011), in their native habitat, while studies on populations in non-native habitats have indicated high mean CWs of 495 g (Loewe-Muñoz et al. 2018) and 521 g (Loewe-Muñoz et al. 2019). In the present study, the average CL (9.7 cm) and CD (7.5 cm) are smaller than those reported by Loewe-Muñoz et al. (2018), which were 11.3 cm (CL) and 9.4 cm (CD), but were bigger than those from southern Greece (CL 8.3 cm and CD 6.8 cm) (Ganatsas et al. 2008).

Stone pine cones have a 3-year reproductive development cycle, which begins when the female strobili (conelets) burst in the spring of the first year, with pollination occurring a few weeks after that. Strobilus fertilization, however, takes place only in the spring/summer of the third and final year of cone maturation, when the nutritious, edible seeds are formed (Valdivieso et al. 2017). It is expected that a reduction in rainfall will have a negative effect on cone production. Calama and Montero (2007) indicated that spring and summer rainfall has a positive impact on cone production, meaning cone number per tree, CW, and PY. A decrease in stone pine seed production could also result from heavy rains during pollination (Parlak et al. 2013). In our study, rainfall in the June of the third year prior to the cone-collecting year (RI) (Mutke et al. 2005), during pollination, is a climatic factor that might cause a decrease in the sound seed ratio. This supports the findings of Parlak et al. (2013), who highlighted the importance of the pollination period on cone production. They also pointed out
Fig. 2 Percentages of sound versus empty seeds among, and between, the five natural populations of *P. pinea* across Turkey.
that non-productive areas located at altitudes below 500 m were identifiable by a thermal oscillation of 22 °C and a higher frequency of days with temperatures below 10 °C, early and late frosts, high relative humidity, and fog. Annual rainfall in the third year prior to harvest time (AR) had a positive impact on CW, as also reported by Loewe-Muñoz et al. (2016). AR showing inter-annual variation has also been positively related to cone yield (Loewe-Muñoz et al. 2020).

The climatic factors Q and ME were found to be the main drivers behind the stone pine cone, seed, and pine nut morphometric traits. The most significant limiting factors to annual cone yield appeared to be water stress during different stages of cone development and the negative effect of hot midsummers on cone setting (Mutke et al. 2005). Loewe-Muñoz et al. (2016) suggested that the selection of sites for fruiting should ensure minimum average spring temperatures above 7 °C, annual and autumn thermal oscillations below 12 °C, and a high spring rainfall, except during the male flowering period. In the final year, losses in seed yield (with up to 50% of the seeds being empty) have been reported from Portugal, Spain, Italy, and Turkey (Mutke et al. 2014), but in Chilean plantations, the percentage of damaged/empty seeds was relatively very low, at around 9% (Loewe-Muñoz et al. 2019). We found that the overall sound seed ratio was 54%, ranging from 32 to 80%. This ratio could change in the future because of stand

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Table 4 Germination of \textit{P. pinea} seeds from five natural populations at 15, 20, and 25 °C and in the field

| Populations     | GP 15 °C | GP 20 °C | GP 25 °C | Field   | MGT 15 °C | MGT 20 °C | MGT 25 °C | Field |
|-----------------|---------|---------|---------|---------|-----------|-----------|-----------|-------|
| 1 K.Marə-Önsen  | 83 \(\text{ab}\) | 85 \(\text{a}\) | 12 \(\text{a}\) | 54 \(\text{a}\) | 27 \(\text{bc}\) | 13 \(\text{a}\) | 16 \(\text{a}\) | 68 \(\text{a}\) |
| 2 Muğla-Katranç | 91 \(\text{b}\)  | 81 \(\text{a}\) | 6 \(\text{a}\)  | 40 \(\text{a}\) | 17 \(\text{a}\)  | 13 \(\text{a}\) | 15 \(\text{a}\) | 69 \(\text{a}\) |
| 3 Aydın-Koşarlı  | 82 \(\text{ab}\) | 93 \(\text{a}\) | 5 \(\text{a}\)  | 49 \(\text{a}\) | 21 \(\text{ab}\) | 12 \(\text{a}\) | 18 \(\text{a}\) | 69 \(\text{a}\) |
| 4 İzmir-Kozak    | 78 \(\text{ab}\) | 86 \(\text{a}\) | 8 \(\text{a}\)  | 47 \(\text{a}\) | 29 \(\text{c}\)  | 13 \(\text{a}\) | 16 \(\text{a}\) | 69 \(\text{a}\) |
| 5 Çanakkale-Kirazlı | 68 \(\text{a}\)  | 85 \(\text{a}\) | 4 \(\text{a}\)  | 50 \(\text{a}\) | 29 \(\text{c}\)  | 15 \(\text{b}\) | 21 \(\text{a}\) | 68 \(\text{a}\) |
| Mean            | 80 A'  | 86 A'  | 7 B'  | 48 C   | 25 A      | 13 B      | 17 C      | 69 D  |

\(\text{GP}\) germination percentage, \(\text{MGT}\) mean germination time. Means in the same column followed by the same lowercase or uppercase letter are significantly different \(p < 0.05\)
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properties. The number of sound seeds is an important factor in afforestation purposes. Seed production is a limiting factor for regeneration, and it is necessary to improve seedling survival in stone pine stands (Calama and Montero 2007).

Seed traits
We found that the average SW (0.8 g) differed among the populations, and was higher than the 0.6 g determined by Mutke et al. (2011) and the 0.7 g of Ganatsas et al. (2008), while it was lower than the 0.9 g found by Loewe-Muñoz et al. (2018). The average SD and SL determined by Loewe-Muñoz et al. (2018) were 1 and 2 cm, respectively, while in the present study, they were 0.9 and 1.8 cm, respectively; these latter measurements are similar to those reported by Ganatsas et al. (2008) and Carcel et al. (2012).

Loewe-Muñoz et al. (2019) report that the mean 6-year measurement of SN in plantations in Chile was 113 cm, which is more than double that found in the present study (50), with the Çanakkale-Kirazlı population having the lowest (25). A positive correlation was found between SN and CW; this finding supports several previous studies that have indicated that heavier cones contain more seeds (Calama and Montero 2007; Bouthina et al. 2013; Loewe-Muñoz et al. 2019). In our findings, the average cone to seed yield (SY) ranged between 8.1 and 18.5%, while Loewe-Muñoz et al. (2019) reported 6-year-mean values ranging from 17.7 to 22.6%.

The 6-year measurement of PY varied from 3.6 to 5.0% in plantations in Chile (Loewe-Muñoz et al. 2019), which is approximately 1.5 times higher than was measured in our populations. We also observed that populations with larger and heavier cones had the highest PW, consistent with the findings of Loewe-Muñoz et al. (2019), who highlighted that PY depended first on PN, then on PW.

Loewe-Muñoz et al. (2019) stated that cone morphometry is not a good indicator of PY, due to the inverse relationship between CW, CD, CL, and PY, so selecting cones for size/weight would not improve PY. However, our results did not support this idea—increased CW,

Table 5  Seedling traits of the five natural stone pine (P. pinea) populations in Turkey

| Parameters | Populations | Mean |
|------------|-------------|------|
| NC         | 1 K.Maraş-Onsen 2 Muğla-Katrançı 3 Aydın-Koçarlı 4 İzmir-Kozak 5 Çanakkale-Kirazlı | 12 (9–14) 12 (9–14) 11 (9–13) 11 (9–13) 11 (9–13) |
| SDl1       | 17.3 (6.2–23.6) 16.7 (9.6–22.0) 15.6 (5.5–22.0) 17.0 (8.7–21.0) 16.3 (10.0–23.5) | 16.6 (8.0–22.4) |
| SDl2       | 33.8 (24.6–45.7) 31.9 (22.9–39.6) 29.3 (20.5–35.5) 37.7 (19.1–52.3) 30 (18.9–39.6) | 32.6 (21.2–42.5) |
| SDl3       | 3.2 (1.9–4.9) 3.3 (2.4–4.3) 2.9 (1.7–4.2) 2.9 (1.9–3.5) 2.6 (1.2–3.8) | 3.0 (1.8–4.1) |
| SL         | 6.1 (4.6–7.0) 6.1 (4.8–7.9) 5.8 (3.4–7.7) 5.3 (3.2–7.4) 5.8 (4.1–7.5) | 5.8 (4.0–7.5) |
| LB1        | 4 (0–7) 5 (2–7) 4 (0–7) 4 (1–7) 4 (1–8) | 4 (1–7) |
| LB2        | 5 (1–11) 8 (1–13) 10 (0–19) 5 (0–14) 7 (2–13) | 7 (1–14) |
| BS         | 58 8 8 0 11 | 17 |

Means within columns following by same letters are not statistically different at 0.05 significance level in Duncan post hoc test. For abbreviations, please see Table 2. Minimum and maximum values are presented in parenthesis.

Table 6  Correlation coefficients (r values) of cone, seed, and pine nut variables of the five P. pinea natural populations across Turkey

| CW | CL | CD | SN | SW | SL | SD | SY | ES | PN | PW | PY |
|----|----|----|----|----|----|----|----|----|----|----|----|
|    | 0.849** 0.885** 0.748** 0.616** 0.765** 0.417** 0.527** −0.412** 0.646** 0.369** 0.455** |    |
|    | 0.786** 0.626** 0.590** 0.720** 0.499** 0.504** −0.390** 0.547** 0.375** 0.448** |    |
|    | 1 0.743** 0.707** 0.840** 0.533** 0.660** −0.334** 0.593** 0.303** 0.433** |    |
|    | 1 0.355** 0.651** 0.076 0.880** −0.414** 0.834** 0.176** 0.617** |    |
|    | 1 0.866** 0.921** 0.472** −0.055 0.188** 0.276** 0.109 |    |
|    | 1 0.672** 0.706** −0.235** 0.488** 0.292** 0.354** |    |
|    | 1 0.241** 0.064 −0.024 0.220** −0.046 |    |
|    | 1 −0.269** 0.667** 0.117 0.529** |    |
|    | 1 −0.796** −0.241** −0.755** |    |
|    | 1 0.209** 0.826** |    |
|    | 1 0.568** |    |
|    | 1 |    |

* and ** stating significant differences at 0.05 and 0.01 levels, respectively. For abbreviations, please see Table 2.
CD, and CL resulted in increased PY. The PY is particularly interesting because of an increase of empty and damaged seeds (Loewe-Muñoz et al. 2019), which have been associated with increased drought or phenological shifts due to climatic change (Mutke et al. 2014) and infestation with *Leptoglossus occidentalis* (Moles and Westoby 2003; Murray et al. 2004; Moles et al. 2007). Şefik (1964) determined that the SW of *Pinus brutia* (Turkish red pine) decreases from low to high altitudes, and from south to north. Also, Kaya and Işık (1997) found that the SW in *P. brutia* decreases as it ascends above sea level. Some seeds, such as those of *Picea mariana* and *Pinus banksiana*, negatively correlate with latitude and altitude, while those of *Picea glauca* have shown no correlations with latitude or altitude in shorter growing season at higher latitudes or altitudes (Moles and Westoby 2003; Murray et al. 2004; Moles et al. 2007).

Many studies have found that the seed size in many species is nonlinearly related to altitude and latitude, being dependent on low temperatures and radiation and a...
the Boreal Shield Ecozone. In the same study, in the Atlantic Maritime Ecozone, seed size in *Picea mariana* and *Picea glauca* had no relationship with latitude or altitude, while seed size in *P. banksiana* did show positive correlations with latitude and altitude (Liu et al. 2013). In the present study, we have confirmed that this non-linear relationship is not consistent within populations of stone pine, which indicate an increase in seed and cone size with increasing altitude and decreasing latitude. Furthermore, in the present study, longitude had no influence on cone or seed size. We can conclude that mean annual rainfall was not a determinant of cone or seed size in stone pine populations.

Considering that the SW decreases as elevation increases, the altitude factor should be taken into consideration for stone pine plantations being used for seed production. It should be noted that, where the soil properties are suitable, plantations above 400 m might be more productive. The impact of drought is likely to be lessened in such locations, compared to areas close to sea level, and the nutritional environment might be more suitable.

**Seed germination and seedling traits**

Ganatsas and Tsakaldimi (2007) found 87.2% to 100% stone pine seed germination, with a MGT of 13.4–15.6 days at 20 °C. According to Escudero et al. (2002), GP in stone pine decreased at 25 °C, and differences in the GPs among populations was more than 50%. Population effects have been found to be significant in *P. pinea* and *P. pinaster*, and in their relationship to temperature. In addition, significant interaction has been reported among population, temperature, and germination rate in *P. pinea*. However, it has also been pointed out that, unlike mountain pines (*Pinus nigra, Pinus sylvestris*), lowland pines (*Pinus halepensis, P. pinaster, P. pinea*) and *Pinus canariensis* have exhibited significant effects of temperature on germination response, and germination is higher between 15 and 20 °C than at warmer and alternating temperatures.

It has also been reported that successful seedling establishment depends on rapid germination and growth, such as in *Pinus strobus* (Parker et al. 2006) and *Pinus densata* (Xu et al. 2016). As the germination speed of a seed lot increases, the height of the seedlings from the seed lot may also increase. In other words, the lower the MGT, the taller the seedling height (Çaşlak 2006). The present study concluded that there was no significant intraspecific variation in the germinations between the field and laboratory (20 °C), although all populations showed variety in seed size, with bigger seeds germinating faster (Moles and Westoby 2004). Our results support certain studies in which it was indicated that seed weight had little effect on germination in *Picea sitchensis* (Chaisurisri et al. 1992) and *P. pinaster* (Wahid and Bou-noua 2013). Additionally, bigger seeds are prone to producing bigger seedlings in *Pinus yunnanensis* (Cai et al. 2016).

In the present study, NC exhibited significant variety across the native stone pine habitats, while Ganatsas et al. (2008) reported that this was lower in over-mature stands. Moreover, it was determined that NC positively correlated with the longitude of the origins of the stone pines. Also, there was a strong correlation between NC and BS. It could be that BS relates better to mean annual rainfall in our study, while Meng et al. (2015) indicated that annual mean temperature and mean temperature of the coldest month have a stronger relationship with BS in *P. yunnanensis* over 2 years.

A limitation of this study was that it depended on 1-year results, whereas inter-annual variations in seed production have been documented by several authors (Mutke et al. 2005; Calama et al. 2008, 2011, 2016; Loewe-Muñoz et al. 2019, 2020). Such variations could be the result of climatic factors affecting the 3-year reproductive development cycle of cones in stone pine.

**Conclusions**

The findings of this study contribute to a better understanding of native populations (with an eastern distribution) of stone pine, and should be taken into consideration in forest management especially relating to afforestation, yield, and breeding activities. Stone pine plays an important role in the rehabilitation and restoration of the Mediterranean Basin, the success of which depends on the quality of seeds and seedlings. Considering intraspecific variation, Çanakkale-Kirazlı population should be assessed within a framework of in-situ conservation due to having distinctive value in all cone and seed morphometric traits among the studied populations. The overall empty seed ratio was 46%, ranging from 20 to 68% among the populations. This dramatic decrease in seed production has been reported in previous works, and is understood to be associated with increasing drought, or phenological shifts due to climatic change and infestation with *Leptoglossus occidentalis* Heidemann. With seed weight decreasing with elevation, the altitude factor should be taken into consideration in stone pine plantations for seed production. It should be noted that, where the soil properties are suitable, plantations above 400 m may be more appropriate. In terms of climatic factors, Q and ME were found to be the main drivers behind significant effects in stone pine cone, seed, and pine nut morphometric traits. We suggest that the Q Index should be used to determine cone to pine nut yield. Moreover, stone pine plantations should be focused on sub-humid areas with Q values of less than 75. Further studies are needed to interpret these results in...
relation to the stand properties, and long-term research is needed to investigate how the stone pine will react to the novel environmental conditions of the future.

Acknowledgements
We are thankful to Turkish State of Meteorological Service for sharing meteorological data. We also thank Istanbul Forest Nursery Manager Neşet BALCI and nursery staffs for their great supports. The authors thank two anonymous reviewers and editor for their valuable comments which significantly improved the original manuscript.

Availability of data
The datasets of the current study are available from the corresponding author on reasonable request.

Authors’ contributions
All authors contributed to the study conception and design. SB and SC are performed data collection and analysis. HD revised and improved the manuscript. Final manuscript has been read and approved by all authors.

Funding
This study was supported by the Scientific Research Projects Coordination Unit of Istanbul University-Cerrahpaşa. Project No. FBA-2016-21357.

Competing interest
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Consent for publication
Not applicable.

Received: 10 August 2020 Accepted: 24 September 2020
Published online: 12 October 2020

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