Fertility variation, seed collection and gene diversity in natural stands of Taurus cedar (Cedrus libani)

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Abstract
Seed stand is a natural forest population that is one of the essential seed sources for global seed supply. Individual fertility is an ability to produce progeny to next generation. Prediction of fertility variation is useful for seed quality, ecosystem management and gene conservation. Fertility variation and gene diversity in seed stands of Taurus cedar were estimated based on the difference in strobilus production between female and male parents. A total of 50 trees were randomly chosen from each stand, and female and male strobili were counted for three consecutive years. The mean of female strobili ranged from 31 to 150, and that of male ranged between 77 and 828. The variation in strobilus production was subjected to estimation of female and male fertility variation. The total fertility variation ($\Psi$) was estimated from the female and male fertility variation. The $\Psi$ in a good crop year was smaller than in a poor year. The effective number of parents ($N_p$) was calculated based on the $\Psi$, which varied from 34.7 to 44.2. When strobilus productions were pooled across 3 years or populations, the $N_p$ increased. Equal cone harvest could mitigate the fertility variation among individuals but caused loss of seed production. Mixing seeds from different years could also decrease the fertility variation and increase the gene diversity of seeds. However, a balance between the size of $N_p$ and the number of pooling years should be carefully considered for maintaining the gene diversity in the natural stands.

Keywords Forest reproductive material · Effective population size · Strobilus production · Parental balance curve · Equal cone harvest

Introduction
Taurus cedar (Cedrus libani A. Rich), also called as Lebanon cedar, is a valuable timber species and quite striking part of flora in the landscape. It is endemic to elevated mountains around the Eastern Mediterranean in Lebanon, Syria, and Turkey. Taurus cedar is classified as one of the economically important species for Turkish forestry and the “National Tree Breeding and Seed Production Programme” (Koski and Antola 1993) because of its valuable wood product and the largest natural distribution on the Taurus Mountains in southern Turkey (Boydak 2003).

Taurus cedar forests cover about 463,000 ha according to Turkish forest inventory, while Boydak (2003) reported that the suitable plantation area for the species was 600,000 ha. Annual seed production is about 139 tones based on forestry inventory between 2012 and 2018 from the seed stands of Taurus cedar (General Directorated of Forestry 2015). The seeds are produced from 21 seed stands selected in 1313.5 ha core area and 2791.9 ha total area included as protection zones. A total of 8 seed orchards (54.1 ha) were established but they are not productive yet because of young age (General Directorated of Forestry 2020).

Seed stands are one of the important seed sources, and they are essential for global seed production programs. The primary objective of seed stands is to produce seeds as fast as possible, but they can also act as breeding and conservation populations. Seed stands have been designated and utilized to produce seeds for reforestation in many countries, and become an important global seed source (Zobel and
The seed stands are one of the most common and cost-effective means of making available a stable supply of seeds for artificial plantation.

Genetic quality of seed stands and the vitality and performance of the resulting seeds depend on many factors, including individual fertility, flowering synchronization, mating system and gene flow (Muller-Starck and Ziehe 1984; Zobel and Talbert 2003; Bilir 2011). In any seed stands, some trees produce more flowers, pollen or seeds than others. The seeds from seed stands are often derived from a limited number of parents. It is reasonable to assume that the seed lot from one crop year does not represent the total gene pool of the respective seed stand. It is common that a small portion of the seed parents contribute a disproportionately large amount to the progeny. This unequal contribution leads to an increase in genetic relatedness and gene diversity loss in the resultant seeds.

Estimation of fertility variation is one of the important procedures of breeding and conservation programs, which is defined as a difference in the ability to give progeny (i.e., reproductive success) among individuals (Bila 2000; Kang 2001). It is a useful tool for different purposes such as gene conservation, seed production programs, managing forest genetic resources and evolutionary and physiological study. Many studies have been carried out on fertility variation among genotypes in managed populations (Kang et al. 2003; Bilir et al. 2005; Kamalakannan et al. 2015; Bilir and Ozel 2017; Yazici and Bilir 2017). Knowledge of fertility variation estimated on the basis of reproductive characters is getting importance because of its easy, cheap and light survey. The fertility variation should be quantified, and its impacts on the population should also be evaluated and mitigated for maintaining gene diversity (Kang et al. 2003). However, theoretical development on fertility variation and its related genetic parameters has been limited in natural forest populations.

A census (minimum) number of adult trees should be required to form approved seed stands in the category “selected” for wind-pollinated tree species in the scheme of forest reproductive materials (OECD 1974). For example, a number of 40 adult trees, an age of 40 years and an area of 0.25 ha are required as the minimum for Douglas-fir, while a minimum area of 2.5 ha is required for Norway spruce and Scots pine (Wojacki et al. 2019). The census number does not give sufficient genetic information, while the effective number would give better information for the characteristics of reproductive materials (Wright 1931; Falconer and MacKay 1996; Lindgren and Mullin 1998).

An effective population size is one of the key parameters in conservation and population genetics (Allendorf et al. 2013). The concept of effective population size is central to ecological, quantitative and evolutionary genetics (Charlesworth and Charlesworth 2010) and plant breeding (Falconer and Mackay 1996). It quantifies the magnitude of genetic drift and inbreeding in a real population. A substantial number of extensions to the basic theory and predictions have been made such as the inbreeding effective population size, the variance effective population size (Wright 1931), the selection effective population size (Lynch 2007) and the status number (Lindgren and Mullin 1998).

The main purposes of the present study are (1) to survey the female and male strobilus production for three consecutive years in three seed stand populations of Taurus cedar, (2) to estimate the female and male fertility variation among individuals based on the flowering survey, (3) to estimate the effective number of parents in order to monitor gene diversity of seeds harvested from the seed stands, and (4) to discuss its effect on forest management practices (i.e., gene diversity manipulation) for supplying reproductive materials in the natural seed stands of Taurus cedar.

Materials and methods

Flowering survey

Three seed stands were surveyed for flowering at three different locations where the species have optimal natural distributions from southern part of Mediterranean region in Turkey (Table 1). The Senirkent and Egirdir populations ($P_1$ and $P_2$) are tectonic, tectono-karstic basin, and the soil types of the region are reddish brown Mediterranean soils, brown forest soils and chestnut soils. The average annual temperature and precipitation of Senirkent ($P_1$) are 12 °C and 556 mm. The winter months are much rainier than the summer months in Senirkent. In Egirdir ($P_2$), the summers are warm, dry and clear and the winters are very cold. The temperature varies from −2 to 30 °C. The Kizildag

### Table 1 Geographic information (latitude, longitude and altitude), age, size, mean DBH and stand density of the seed stands of Taurus cedar

| Population   | Latitude (N) | Longitude (E) | Altitude (m) | Age (year) | Size (ha) | Mean DBH (cm) | Density (trees/ha) |
|--------------|--------------|---------------|--------------|------------|-----------|---------------|------------------|
| Senirkent ($P_1$) | 38° 05' 23"  | 30° 42' 20"  | 1600         | 53         | 77.0      | 22.0          | 800              |
| Egirdir ($P_2$)   | 37° 44' 47"  | 30° 49' 21"  | 1600         | 113        | 48.9      | 36.0          | 650              |
| Kizildag ($P_3$)  | 37° 51' 00"  | 31° 19' 10"  | 1550         | 144        | 23.0      | 50.0          | 450              |
population \( P_2 \) is Taurus orogenic belt mostly and involves limestones. The soil type of the region is brown forest soils at Inner Anatolia subalpine zone, and the annual temperature and rainfall are 20 °C and 400–600 mm. The \( P_3 \) region gets humid air masses especially during growth period originating from Mediterranean Sea (Atalay 1987).

Fifty healthy individual trees were randomly chosen from each seed stands. The numbers of female \( (N_♀) \) and male \( (N_♂) \) strobili were surveyed for three consecutive years (2016–2018) in three seed stands. The number of female strobili was counted entirely from the individuals. The production of male strobilus was estimated by using number of male strobili in a branch sampled from each direction and multiplying the branch number of each individual. We did not collect data of flowering time or female receptivity to describe the overlapping of flowering periods. Pollen contamination was ignored in the study.

The coefficients of variation (CV) for female and male fertility were calculated and applied to estimate the fertility variation in female and male parents, respectively (see Eqs. 2-a and 2-b). The individual contribution (fertility) to strobilus production was proportionally calculated from the surveyed flowering data in each population.

### Genetic variation and parental balance

The following ANOVA model was used to analyze the difference in strobilus production among populations and years by the SAS software (SAS Inst. Inc. 1988):

\[
Y_{ijk} = \mu + F_i + B_{j(i)} + FB_{(ij)} + e_{ijk}. \tag{1}
\]

where \( Y_{ijk} \) is the observation from the \( k \)th tree of the \( j \)th population in the \( i \)th year, \( \mu \) is the overall mean of strobilus production, \( F_i \) is the effect of \( i \)th year, \( B_{j(i)} \) is the effect of the \( j \)th population in the \( i \)th year, \( FB_{(ij)} \) is the effect of the interaction between \( i \)th year and \( j \)th population, and \( e_{ijk} \) is the random error.

The correlation between female and male strobilus production was analyzed by the Pearson’s product moment correlation coefficients. The correlation coefficients \( r \) were used for estimation of the total fertility variation (see Eq. 3).

The parental balance curve was used to characterize high or low productive individuals for female and male strobili. Individual trees were ranked from high to low for the production of female and male strobilus production, and then, cumulative percentage calculations were plotted against the total number of individuals in the studied population (Chaisurisri and El-Kassaby 1993). Female and male genetic contributions can be explained by the parental balance curves, and the cumulative percentage curve is often used to quantify fertility variation in forest populations (Griffin 1982; Kang 2001).

### Theoretical frameworks on fertility variation and effective number

#### Fertility variation estimation

Female and male fertilities of the \( i \)th individual were defined as the ability to produce female and male strobili, respectively, and estimated as the relative proportion of female and male strobilus production to the entire population (Muller-Sturck and Ziehe 1984). The variations of female and male fertility \( (\psi_f \) and \( \psi_m ) \) among individuals, also called female and male gametic fertility variations, were estimated by the coefficient of variation (CV) for female and male strobilus production suggested by Kang and Lindgren (1998) as:

\[
\psi_f = N \sum_{i=1}^{N} f_i^2 = CV_f^2 + 1 \tag{2-a}
\]

\[
\psi_m = N \sum_{i=1}^{N} m_i^2 = CV_m^2 + 1 \tag{2-b}
\]

where \( N \) is the census number, \( f_i \) is the fertility as female of the \( i \)th individual, \( m_i \) is the fertility as male of the \( i \)th individual, and \( CV_f \) and \( CV_m \) are the coefficients of variation in female and male strobilus production among individuals in the studied population, respectively.

Combined fertility variation of both female and male leads to the total fertility variation, designated by a symbol \( \Psi \) (called sibling coefficient). Correlated relation between female and male strobilus production in monoeocious plants like conifers is an important consideration to predict gene diversity of seed crops. Under the correlation \( r \) between female and male fertility variation, the sibling coefficient \( (\Psi) \) of combined fertility variation is drawn as (Kang and El-Kassaby 2002):

\[
\Psi = N \sum_{i=1}^{N} P_i^2 = N \sum_{i=1}^{N} \left( \frac{f_i + m_i}{2} \right)^2 = 0.25N \sum_{i=1}^{N} \left( f_i^2 + m_i^2 + 2f_im_i \right)
\]

\[
= 0.25(\Psi_f + \Psi_m) + 0.5 \left[ 1 + r\sqrt{(\Psi_f - 1)(\Psi_m - 1)} \right] \tag{3}
\]

where \( N \) is the census number; \( P_i \) is the total fertility as an whole parent of \( i \)th individual; \( f_i \) and \( m_i \) are the fertilities as female and male parents of the \( i \)th individual; \( \Psi_f \) and \( \Psi_m \) are the fertility variations of female and male parents; \( r \) is the correlation coefficient between female and male strobilus production in the population, respectively.

Sibling coefficient \( (\Psi) \) expresses the probability that two alleles drawn randomly from the gamete gene pool originate from the same parent compared to the probability when the parents have equal representation (Kang and Lindgren 1998; Kang et al. 2003). Fertility variation (sibling coefficient) is
a standardized measure that does not depend on the census number \((N)\) of parents, but only on how variable their fertility is. Thus, the fertility of each parent is given as a fraction of all gametes.

If there is no correlation between female and male fertility, Eq. (3) is simplified as:

\[
\Psi = 0.25(\Psi_f + \Psi_m) + 0.5
\]  

(4)

Collecting equal proportions of seeds from each individual, called equal seed harvesting, is often proposed to mitigate the fertility variation among individuals. When the equal seed harvesting among individuals is imposed in a seed stand population, Eq. (4) is then described as \(\Psi = 0.25\psi_m + 0.5\), since the female fertility does not have variation. However, note that the equalizing female fertility should be primarily set to the most productive parents, which can lead to loss of total seed output. For example, 30% equalizing means that 30% of the most productive parents would give the same female fertility by setting the seed production of the least fertile parent within the 30%.

**Effective number of parents**

The effective number of parents \((N_p)\) is the number of individuals in which an idealized population would produce the same number of sibs (offspring) as the real population (Kang 2001). The effective number of parents is one of the concepts of effective population size that comes from the transition (Kang 2001). The effective number of parents is calculated based on the total fertility variation \(\Psi\), female \(\psi_f\) and male \(\psi_m\) fertility variations, respectively. The relative effective number of parents \((N_r)\) and the relative effective numbers of female and male parents, \(N_{f}^{(f)}\) and \(N_{m}^{(m)}\), were connected to the \(N_p\), \(N_{f}^{(f)}\) and \(N_{m}^{(m)}\) as (Kang et al. 2003; Bilir 2011; Kamalakannan et al. 2015):

\[
N_p = \frac{\Psi}{N}, \quad N_f(f) = \frac{\Psi_f}{N}, \quad N_p(m) = \frac{\Psi_m}{N}
\]  

(5)

\[
N_i = \frac{N_p}{N}, \quad N_i(f) = \frac{N_f(f)}{N}, \quad N_i(m) = \frac{N_p(m)}{N}
\]  

(6)

where \(N\) is the census number in a population, \(\Psi\) is the total fertility variation, and \(\psi_f\) and \(\psi_m\) are the fertility variations of female and male parents in the studied population.

**Results**

**Strobilus production and parental balance**

The numbers of female and male strobili varied among populations and years, and also between individuals within a population or a year (Table 2). The mean female strobili ranged from 31 in 2017 to 150 in 2018 in the Kizildag population \((P_1)\), and that of male strobili varied between 77 in 2017 \((P_1)\) and 828 in 2018 \((P_3)\). The Egirdir population \((P_2)\) was poorest in female and male strobilus production among the seed stand populations for all studied years (on average 55 for \(N_{f}^{(f)}\) and 98 for \(N_{m}^{(m)}\)). The average of female and male strobilus production was highest (on average 109 for \(N_{f}^{(f)}\) and 514 for \(N_{m}^{(m)}\)) in the \(P_3\) population. On average (pooled) of 3 years across populations, the year 2016 was good female production year, while the year 2017 was poor year (Table 2). On the other hand, the male strobilus production was highest in 2018 and poorest in 2017.

| Year | Population 1 | Population 2 | Population 3 | Pooled |
|------|--------------|--------------|--------------|--------|
| Mean | CV | Range | Mean | CV | Range | Mean | CV | Range | Mean | CV |
| 2016 | \(N_0\) | 120 | 0.746 | 40–420 | 75 | 0.687 | 25–322 | 147 | 0.496 | 36–323 | 114 | 0.417 |
| | \(N_2\) | 396 | 0.721 | 45–870 | 105 | 0.536 | 35–300 | 626 | 0.832 | 60–2020 | 376 | 0.497 |
| 2017 | \(N_0\) | 43 | 0.556 | 20–135 | 54 | 0.586 | 13–140 | 31 | 0.519 | 9–75 | 43 | 0.412 |
| | \(N_2\) | 77 | 0.561 | 25–218 | 84 | 0.496 | 26–225 | 88 | 0.362 | 15–205 | 83 | 0.288 |
| 2018 | \(N_0\) | 46 | 0.549 | 18–115 | 37 | 0.595 | 20–135 | 150 | 0.481 | 22–342 | 77 | 0.383 |
| | \(N_2\) | 351 | 0.793 | 50–950 | 105 | 0.843 | 22–550 | 828 | 0.865 | 80–2080 | 428 | 0.651 |
| Pooled | \(N_0\) | 70 | 0.523 | 33–193 | 55 | 0.396 | 20–143 | 109 | 0.353 | 30–204 | 78 | 0.877 |
| | \(N_2\) | 275 | 0.662 | 58–608 | 98 | 0.390 | 39–228 | 514 | 0.574 | 140–1351 | 295 | 1.407 |
The coefficient of variation (CV) of strobili production changed over years and varied among populations (Table 2), and the CV values between female and male were not much larger than expected in natural seed stands (Bilir and Ozel 2017). When pooled, the CVs were decreased for female and male fertility, compared to a single year or population. The value of CV ranged from 0.288 in 2017 (male) to 0.651 in 2018 (male) when three populations were combined in each year, and varied from 0.353 in $P_3$ (female) to 0.662 in $P_1$ (male) for pooled 3 years in each population (Table 2). However, the CV values increased (0.887 and 1.407) for grand mean of 3 years and three populations, implying that female and male fertility variation among individuals could increase when the entire strobilus production was completely pooled.

There was fluctuation of female and male strobilus production among years and populations (Fig. 1). In seed stands of Taurus cedar, the production of female (seed) and male (pollen) strobili seemed to be low, especially in the poor flowering year. The observed parental balance curves for female and male strobili production deviated significantly from the ideal situation (equal production among individuals) in all seed stands (Fig. 2). It was clearly shown that the female and male parents contributed unequally to the gamete gene pool. Thus, specific individuals may consistently produce high or low amounts of strobili based on their genetic tendencies. The most abundant five individual trees (10% of total) produced 29.9% and 21.3% of female and male strobili in 2016, 23.3% and 23.2% in 2017, and 22.0% and 25.0% in 2018 in the $P_1$ population. These were the similar trend as in the $P_2$ and $P_3$ populations (Fig. 2).

The significant difference in female and male strobilus production among populations and years was supported by the analysis of variance (ANOVA). The ANOVA for female and male strobilus production showed highly significant differences ($p < 0.01$) among populations and years (Table 3).

The Pearson’s correlation coefficients showed positive and significant relationships between female and
male strobilus production for all populations within a year ($r$= 0.491, 0.454 and 0.581) (Table 4). The correlation coefficients ($r$) between female and male strobilus production were applied to estimate the total fertility variation ($\Psi$) and the effective number of parent ($N_p$) (see Eq. 3).

**Fertility variation and effective number of parents**

Fertility variations of female ($\psi_f$) and male ($\psi_m$) parents varied among populations and years (Table 5). The $\psi_f$ ranged from 1.23 ($P_3$ in 2018) to 1.55 ($P_1$ in 2016) and the $\psi_m$ ranged 1.13 ($P_1$ in 2017) to 1.73 ($P_1$ in 2018), showing that the difference of fertility between female and male was not so large. The variation also indicated that the fertility variation among individuals in a good crop year was smaller than in a poor year (Table 5).

The total fertility variation ($\Psi$) ranged from 1.13 to 1.44 across populations and years (Table 6). The $\Psi$ was smallest in the population $P_3$ in 2017 and largest in the population $P_2$ in 2018. This was inversely mirrored to the effective number of parents ($N_p$), and thus, the $N_p$ was smallest (69% of census) in the population $P_2$ in 2018 and largest (88%) in the population $P_3$ in 2017 (Table 6).

**Table 3** Analysis of variance for female ($N_♀$) and male ($N_♂$) strobilus production among years and populations in the seed stands of Taurus cedar

| Source Variable | df | Sum square | Mean square | F | P |
|-----------------|----|------------|-------------|---|---|
| Year ($Y$)      | $N_♀$ | 2 | 379,562 | 189,781 | 70.90 | *** |
|                 | $N_♂$ | 2 | 10,369,646 | 5,184,823 | 48.70 | *** |
| Population ($P$) | $N_♀$ | 2 | 235,498 | 117,749 | 43.99 | *** |
|                 | $N_♂$ | 2 | 13,055,300 | 6,527,650 | 61.31 | *** |
| $Y \times P$   | $N_♀$ | 4 | 306,182 | 76,546 | 28.60 | *** |
| interaction     | $N_♂$ | 4 | 7,263,984 | 1,815,996 | 17.06 | *** |
| Error           | $N_♀$ | 441 | 1,180,390 | 2677 |
|                 | $N_♂$ | 441 | 46,954,148 | 106,472 |
| Total           | $N_♀$ | 450 | 4,839,589 |
|                 | $N_♂$ | 450 | 116,923,598 |

***Indicates statistically significant difference at the probability level of 0.01

**Table 4** Pearson’s correlation coefficients ($r$) among female ($N_♀$) and male ($N_♂$) strobilus productions based on pooling of three populations within a year

| Year | $N_♀$ | $N_♂$ | $N_♀$ | $N_♂$ | $N_♀$ | $N_♂$ | $N_♀$ | $N_♂$ |
|------|-------|-------|-------|-------|-------|-------|-------|-------|
| 2016 | -     | 0.491**| -     | -     | -     | -     | -     | -     |
| 2017 | 0.159NS| -0.126NS| -     | -     | -     | -     | -     | -     |
| 2018 | 0.268**| 0.207* | 0.454**| -     | -     | -     | -     | -     |

NS Indicates non-significant

* And ** indicate statistically significant at the probability levels of 0.05 and 0.01

**Table 5** Female ($\psi_f$) and male ($\psi_m$) fertility variation and the female ($N_p^{(f)}$) and male ($N_p^{(m)}$) effective number in the seed stand populations for the three consecutive years

| Year | $P_1$ | $P_2$ | $P_3$ |
|------|-------|-------|-------|
|      | $\psi_f$ | $\psi_m$ | $N_p^{(f)}$ | $N_p^{(m)}$ | $\psi_f$ | $\psi_m$ | $N_p^{(f)}$ | $N_p^{(m)}$ | $\psi_f$ | $\psi_m$ | $N_p^{(f)}$ | $N_p^{(m)}$ |
| 2016 | 1.56   | 1.52   | 32.1   | 32.9   | 1.47   | 1.29   | 34.0   | 38.8   | 1.25   | 1.69   | 40.1   | 29.5   |
| 2017 | 1.31   | 1.31   | 38.2   | 38.0   | 1.34   | 1.25   | 37.2   | 40.1   | 1.27   | 1.13   | 39.4   | 44.2   |
| 2018 | 1.30   | 1.63   | 38.4   | 30.7   | 1.35   | 1.71   | 36.9   | 29.2   | 1.23   | 1.75   | 40.6   | 28.6   |
| Pooled | 1.27   | 1.44   | 39.2   | 34.8   | 1.16   | 1.15   | 43.2   | 43.4   | 1.12   | 1.33   | 44.5   | 37.6   |

*, f and m represent female and male, respectively
Equal cone harvest simulation

The census number (N) to collect cones could be taken to achieve satisfactory gene diversity of seeds when the fertility variation among individuals is qualified in a population (Kang et al. 2003). The practice of equal cone harvest was simulated for a good crop year (2018) in Kizildag population (P3). The equalizing of female fertility was preferentially applied to the most fertile female parents. When equal cone harvest was proportionally increased, the effective number of parents also increased (Fig. 3). By equalizing the most fertile parent, the effective number of parents increased from 38.1 (no simulation) to 42.1; on the other hand, the loss of total seed crop was 85% (Fig. 3).

The simulation of equal cone harvest, however, resulted in loss of seed-cone production. Thus, a balance in proportion of cone harvest (census number), production of cone (seeds) and effective number of parents (gene diversity) should be considered for the implementation of equal cone harvest. In the simulation, the overrepresented female parents were the first concern for equalizing of female fertility in the seed stand of Taurus cedar.

Discussion

Strobilus production variation

The quantity of strobilus and coefficient of variation (CV) showed a large difference among populations and years, and also between individuals within a population (Table 2). This was evident from the yearly fluctuation in strobilus production (Fig. 1) and ANOVA results (Table 3). Bilir and Ozel (2017) reported that the averages of female and male strobili were 120 and 560 in a 55-year-old natural population and a large variation in strobilus production among individuals was found. A large difference in strobilus production was found among individuals and years within a population and among populations in the same species (Yazici and Bilir 2017), and in other forest tree species (Bila 2000; Bilir et al. 2005; Kamalakannan et al. 2015; Park et al. 2017). Boydak (2003) reported that a good crop year in natural stands of the same species was once in two or 3 years. It should be emphasized that selecting populations and harvesting years are important for collecting seeds and managing seed stand populations. There could be genetical (Eriksson et al. 1973) and environmental factors such as tree form, growth, resistance, slope, aspect, altitude and age (Bilir 2011; Yazici and Bilir 2017) for selecting and managing the seed stands. The production of cones, flowers, pollen, fruits and seeds has been used to estimate fertility variation among individuals in many plant species (Savolainen et al. 1993; Yazici and Bilir 2017) for selecting and managing the seed stands. The progress of a seed production program depends on a plentiful delivery of viable seed. The final seed yields may be influenced by breakdown of any one of the processes of pollination, pollen grain germination, pollen tube growth, fertilization and embryo development (Brown 1971; Sarvas 1962). For maximum yield of seed, the female strobili must be pollinated when they are fully receptive to pollen. Empty seeds and low viability may result from a breakdown in embryogeny, and a further loss of seeds occurs by premature abscission of seed cones.

Table 6 Total fertility variation (Ψ) and the effective number of parent (Np) in the seed stand populations for three studied years

| Year | P1 | Np | P2 | Np | P3 | Np | Pooled* |
|------|----|----|----|----|----|----|--------|
| Ψ    |    |    | Ψ  |    | Ψ  |    |        |
| 2016 | 1.40| 35.8 (0.72) | 1.25| 40.1 (0.80) | 1.32| 38.0 (0.76) | 1.59| 94.4 (0.63) |
| 2017 | 1.26| 39.6 (0.79) | 1.21| 41.2 (0.82) | 1.13| 44.2 (0.88) | 1.22| 123.3 (0.82) |
| 2018 | 1.27| 39.5 (0.79) | 1.44| 34.7 (0.69) | 1.31| 38.1 (0.76) | 1.91| 78.4 (0.52)   |
| Pooled | 1.27 | 39.5 (0.79) | 1.10 | 45.5 (0.91) | 1.13 | 44.2 (0.88) | 2.03 | 221.5 (0.49)   |

Parentheses indicates the relative effective number of parents (Np) to the census number (N)

*Note that the census number (N) increased when populations were pooled across a year

Fig. 3 Balancing the proportion of equal cone harvest, relative production of cones and effective number of parents in a seed stand of Taurus cedar simulated for a good crop year (2018) in Kizildag population (P3)
Positive and significant correlations were found between female and male strobilus production (Table 4). This result was also reported in the same species (Yazici and Bilir 2017) and in the similar conifer species (Kang and Lindgren 1998; Kang and El-Kassaby 2002; Bilir et al. 2005). The correlation could be used for different purposes such as estimating total fertility variation and the effective number of parents as Eq. (3) in the present study.

Fertility variation and effective number of parents

Female and male fertility variations varied among populations and years (Table 5, Fig. 1). When pooled across years in a population, the female and male gametic fertility variations ($\psi_f$ and $\psi_m$) decreased (Table 5). When across years, the total fertility variation ($\Psi$) reduced, but the effective number ($N_p$) increased (Table 6), indicating that pooling seeds from 3 years could increase the gene diversity of seeds from the Taurus cedar seed stands.

On the other hand, mixing cones of all populations across years increased the $\Psi$ value, meaning that fertility variation increased compared to a single population (Table 6). When pooling seeds of three populations, it should be noted that the census number ($N$) increased to 150 and thus, the $N_p$ was larger than a single population. However, the relative effective number of parents ($N_p$) decreased. Therefore, balancing the effective number of parents (gene diversity) and the number of populations and years should be carefully considered for management of the seed stands of Taurus cedar. This result might be due to the boosted strong positive correlation (covariation) among populations and years by pooling strobilus productions (see Eq. 3 and Fig. 3).

Sibling coefficient ($\Psi$) expresses how fertility varies among parents as the increase in the probability that sibs occur compared to the situation where parents have equal fertility (Kang 2001). The $\Psi$ cannot be smaller than one. If $\Psi = 1$, all individuals have the equal fertility, and $\Psi = 2$ means that the probability that two individuals share a parent is twice as high, compared to when the parental fertility is equal across the population. As a heuristic rule of thumb, Kang et al. (2003) suggested that the $\Psi$ equals 3 in natural seed stands and 2 in managed populations as seed orchards. Total fertility variation ($\Psi$) was 2.03 in totally pooled populations and years, which was smaller than the thumb rule (Table 6).

Effective population size theory has been based on the rate of change in gene frequency variance (genetic drift) and the rate of inbreeding. The effective population size ($N_e$) is defined as a hypothetical population with simplifying characteristics where genetic drift is the only factor in operation, and the dynamics of allelic and genotypic frequencies across generations depend merely on the census number ($N$) of population (Crow and Kimura 1970). The effective size of a real population is then defined as the number of individuals in an idealized population, which would give rise to the same rate of inbreeding ($N_e^{(i)}$) or the same change in the variance of gene frequencies ($N_e^{(w)}$) observed in the population under consideration (Allendorf et al. 2013), and status number ($N_s$) (Lindgren and Mullin 1998).

Effective number of parents ($N_p$) was proposed to characterize the seed from natural stands, which was based on the function of correlated fertility variation ($\Psi$) between female and male parents. The $N_p$ is the same as the status number ($N_s$) where the parents are not related nor inbred (Lindgren and Mullin 1998). The $N_s$ is the function of group ancestry ($\Theta$), while the $N_p$ is based solely on the fertility variation ($\Psi$) among members in the population of interest (Kang 2001). Both $N_p$ and $\Psi$ parameters can estimate loss of gene diversity between parents and their gametic progeny. Recently, Park et al. (2017) proposed to use the integration of fertility variation ($\Psi$) and genetic relatedness ($\Theta$) in estimating the gene diversity of seed crops.

Implication on management of seed stands

The use of bulked seeds derived from commercial harvests can influence the estimate of effective number and gene diversity. Adult trees with insufficient yield of seed cones are generally not climbed during the commercial harvest. Therefore, these adults could only contribute to the seeds as male parents. The variation in reproductive success of individual trees is amplified by this harvesting procedure (Wojacki et al. 2019). In contrast, the equal seed collection per mother tree may have an artificial balancing influence.

Seeds from seed stands must reflect the gene diversity of their parents and keep sufficient diversity for future generations. One of the management options to reduce loss of gene diversity due to fertility variation is to restrict the parental contribution to the next generation (Bila 2000; Kang et al. 2003). This restriction is more likely to be applied to the maternal contribution (e.g., equal cone harvest). The equal utilization of seeds among trees is often proposed to mitigate the effect of unbalanced contribution among parents in seed stands and orchards (Kang and Lindgren 1998; Bila 2000). By keeping the female contribution equal among individuals, the gene diversity is optimized in the seeds from seed stands. The balance between gene diversity and seed collection would be more important in genetic resource conservation of Taurus cedar.

Mixing of seeds from a few seed stands or different years was suggested as a means to mitigate the imbalance of gamete contributions among individuals (Bila 2000; Kang et al. 2003; Wojacki et al. 2019). When seeds were mixed for 3 years, the fertility variation ($\Psi$) decreased and the effective number of parents ($N_p$) increased (Table 6). However, a certain loss of gene diversity during cone
harvest seems inevitable (Fig. 3), and mixing seeds from too many populations or years might lose fertility variation and gene diversity. Thus, choice of population numbers, structural characteristics of seed stands, and spatial distribution of seed trees need to be optimized in a way that keeps the gene diversity loss to a minimum.

Stimulation of strobilus production would be necessary to increase the seed and pollen production in seed stands of Taurus cedar (Bilir and Ozel 2017; Yazici and Bilir 2017). Silvicultural thinning based on the survey of strobilus production would be proposed for spacing, aeration and pollen flow. Organic fertilizing may also be effective to produce more female strobili in the seed stands of Taurus cedar.

Conclusions

The effective number of parents could estimate the gene diversity of seeds from natural stands, which was based on the function of fertility variation between female and male parents. The fertility variation, called as a sibling coefficient (\(\Psi\)), expresses how fertility varies among parents as the increase in the probability that sibs occur compared to the situation where parents have equal fertility. Substantial variation in fertility among individuals was found in natural stands of Taurus cedar. The effective number of parents (\(N_p\)) describes the number of individuals in which an idealized population would give the same number of sibs (relatives) as the real population. It gives better genetic characteristics of seed stands and resulting seeds than the census number. Therefore, we suggest to use the \(N_p\) concept for certification of seeds from natural populations.

Equal cone harvest could increase the effective number of parents but may result in loss of seed production. If the gene diversity is of most concern in seed stands, it will be better to increase the number of trees for seed collection. Mixing seeds from different years also decreased the fertility variation among individuals and increased the gene diversity of seeds. However, a balance between the effective number of parents and the number of mixed years or populations should be carefully considered for maintaining the gene diversity of seeds from natural stands of Taurus cedar.

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Availability of data and materials The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

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