IMPORTANCE OF EFFECTIVE CLONE NUMBER IN SEED ORCHARDS: A COMPARATIVE STUDY ON SEVEN CONIFER SPECIES IN TURKEY

ZNAČAJ EFEKTIVNOG BROJA KLONOVA U KLONSKIM SJEMENSKIM PLANTAŽAMA: KOMPARATIVNO ISTRAŽIVANJE ZA SEDAM CRNOGORIČNIH VRSTA U TURSKOJ

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Abstract

The Mediterranean Basin is one of the major plant diversity centers in the northern hemisphere. The Eastern Mediterranean Basin is also a hotspot region of gene diversity for conifer species. In this study, Turkey's conifer seed orchards were investigated for their effective number of clones. The mean census number of clones (N) was estimated 33.12. The mean effective number of clones (N_c) was calculated as 27.59. The mean relative effective number of clones ($N_r = N_c/N$) was 0.827. The estimated proportional gene diversity was found 0.973, with a range from 0.922 to 0.983. Thus, considerable attention should be given to use nearly equal ramet numbers during seed orchard establishment and management operations. Threats such as climatic change, fire, disease and insects should be considered during seed orchards establishment. High number of populations from wide range of species should be sampled and seed orchards should be established locally depending on ecological requirements of species. This is also essential for sustainable management of forest genetic resources. Information both from phenotypic selection and molecular genetic analysis should be used to establish future seed orchards.

KEY WORDS: climatic change, effective clone number, gene diversity, ramet variation, seed orchard

Introduction
Uvod

The Mediterranean Basin is one of the 233 ecological regions and five Mediterranean bioclimatic areas (California-Baja California, Chile, South Africa, Australia and Mediterranean Basin) based on its plant biodiversity (Leone and Lovreglio 2004, Underwood et al. 2009). It is not only one of the world's major centers for plant diversity, but also has a relatively high number of forest tree species. More than 100 tree species can be found on the Mediterranean Basin that occupies about 1.8% of worldwide forest lands (Fady-Welterlen 2005). One of the important components of plant diversity in the Mediterranean Basin is conifer species. The conifer flora of the Mediterranean region, main species being *Pinus brutia* Ten., *Pinus halepensis* Mill., *Pinus pinaster* Ait. and *Pinus nigra* Arnold, contains 10 pine species that cover totally 13.10⁶ ha area which represents 5% of the total Mediterranean Basin area (Leone and Lovreglio 2004).

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Gene diversity of conifer species in the Mediterranean Basin decreases along east-west gradient (Fady and Conord 2010). The eastern Mediterranean Basin, especially Greece and Turkey, has high species and gene diversity than other parts of Mediterranean Basin due to heterogeneous features such as biogeography, microclimates, biotic and abiotic factors (Grivet et al. 2009, Medail and Diadema 2009).

Turkey is a biologically diverse region and has great variability in topography and climate due to its geographical location joining two continents (Ciplak 2003). Thus, this variability has given Turkey a diverse range of ecosystems. Turkey, surrounded by seas of different ecological properties on 3 sides, with altitudes ranging from sea level to above 5000 m resulting in a variety of climatic conditions through the country, has a biological wealth incomparable to any of the neighboring countries. It has several distinct biogeographic regions, each having its own endemic species (totally over 30%) and natural ecosystems providing major flyways for millions of migratory birds with about 21 million ha of forested land which constitutes 27.2% of total land area of the country (Anonymous 2006, Ture and Bocuk 2010). In Turkey, *P. brutia* and *P. halepensis* forests cover approximately 5.5 million ha and total conifer forests have about 10 million ha area (Anonymous 2006). However, forest lands both in Turkey and in all over the world are under threat of climate change, tourism and urban development, population growth, human activity, grazing, pollution, fire and habitat loss (Kaya and Raynal 2001, Isik 2011).

Conserving genetic diversity is essential for a sound forest ecology and management practices. Genetic diversity within species plays important role in resistance and adaptability of forests. Today's forests as well as their genetic diversity would probably be maladapted to future climate. According to The Intergovernmental Panel on Climate Change (IPCC) held in 2007, global average surface temperatures will rise about 1.8 to 4.0 °C during the 21st century, and up to 30% of the world's species will be at increased risk of extinction (Maciver and Wheaton 2005, StClair and Howe 2011). Changes in climatic events such as annual rainfall patterns, decrease in precipitation, reduction in soil moisture and decrease in water availability have strong effects on forest ecology and managements (De Dios et al. 2007, Ravindranath 2007, D'Amato et al. 2011). Productivity of pine forests is related to climatic, geological and topographic parameters, and also successional stage (Vila et al. 2003). When the rates of change of the future climate exceed the historical rates, rate of forest losses from fires, insects, diseases, population and urbanization are expected to change significantly. Forest management strategies will need to be prepared in a proactive adaptive manner with taking consideration of threats of climate change for tomorrow's forests (Maciver and Wheaton 2005, D'Amato et al. 2011).

Seed orchards have important role in afforestation and reforestation strategies. They are established with the aim of obtaining high genetic quality seeds and seedlings compared to phenotypically or genotypically selected stands (Buiteveld et al. 2001, Zhuowen 2002, Gomory et al. 2003, Hansen 2008). In other words, seed orchards, containing genetically superior trees, are expected to provide genetically and physiologically high value seeds frequently and also they are isolated from undesirable pollen sources (El-Kassaby et al. 1989, Di-Giovanni and Kevan 1991, Kang et al. 2004). The establishment of seed orchards requires time, money, expertise, investment and collaboration of concerned organizations (Di-Giovanni and Kevan 1991). The composition and breeding values of parent genotypes, pollen contamination rate, mating system components and the distribution of maternal and paternal gametes in orchard are main factors that affect the genetic quality of seeds produced (Stoehr et al. 1998, Kang et al. 2001a, Gomory et al. 2003). Thus, seed orchards are kept to produce genetically improved forest tree plantations.

The basic objectives of forest tree breeding activities are establishment of new forest by taking advantage of the genetically superior features of natural forest, raising quality and quantity of wood raw material per unit area and providing resistance to pests such as insects and fungi. Forest tree breeders want to get maximum breeding progress and high genetic diversity from establishment new seed orchards (Hosius et al. 2000). Panmictic reproduction is required for production of genetically superior seeds in the seed orchard and obtaining maximum gene diversity based on a given number of clones (Harju and Muona 1989, Stoehr et al. 1998, Kang et al. 2001a, Funda et al. 2009, Alizoti et al. 2010). If seed orchards do not reproduce panmictically, some reduction in expected genetic gains will be observed (Harju and Muona 1989, Funda et al. 2009). Gene diversity is important for the sustainability of forest ecosystems and the adaptability of seeds to some environmental factors such as climate change and diseases. Fertility variation and pollen flow from outside stands have various undesirable effects on gene diversity of seed orchard crops (Kaya et al. 2006, Bilir et al. 2008). The number of clones used in establishment of seed orchards has an important role in ensuring gene diversity, maximizing roguing possibility and minimizing inbreeding (Lindgren and Prescher 2005). Using equal number of ramets per clones is an important approach in orchard establishment. Unequal production of female flowers, male flowers and gametes might occur due to variation in the number of ramets per clones. It is often preferred to use an equal number of ramets per clone but unintentional variation in the number of ramets occurs during establishment. Graft availability, graft incompatibility, dying of grafts due to unfavorable growing conditions, biotic and abiotic factors, and mislabeling of grafts are main

reasons of ramet number variations (Kang et al. 2001a). It is generally known that actual seed orchards have often different numbers of ramets per clone. Both variation of ramet number per clones and fertility variation cause unequal gamete contributions in the orchards (Muona and Harju 1989, Kang et al. 2001b).

The aims of this study were (1) to estimate the effective clone numbers for seed orchards in each of the conifer species in Turkey, (2) to discuss the causes and implications of the variation in ramet number among the seed orchards, (3) to determine the relationship between effective clone number and gene diversity. It is expected that the results from this study will contribute establishment and management of present and future seed orchards.

Material and methods
Materijal i metode

Tree breeding studies were started in 1964 in Turkey. The National Tree Breeding and Seed Production Program (NT-BSP) were implemented in 1994 (Koski and Antola 1993). *Pinus brutia* Ten.*, P. nigra* Arnold*, P. sylvestris* L.*, Cedrus libani* A. Richard and *Fagus orientalis* Lipsky were determined as target species. In different regions of Turkey, 172 seed orchards of seven major timber conifer species (*P. brutia*, *P. nigra*, *P. sylvestris*, *P. pinea* L., *P. halepensis* Mill., *Picea orientalis* (L.) Link. and *C. libani*) were established by Turkish Ministry of Forestry and Water Works, Forest Tree Seeds and Tree Breeding Research Directorate by 2011 (OATIAM 2011). Currently 159 Gene Conservation Forests for conifers have been set aside with about 25198.4 ha land area, by the Turkish Forest Tree Seeds and Tree Breeding Research Directorate for the conifer species in the country. In this study, variation in ramet number among clones in all clonal seed orchards of conifers in Turkey was estimated. The number of ramets per clone for the studied seed orchards was checked after establishment to determine whether thinning occurred or not.

Average number of ramets per orchards, average coefficient of variation for ramets in orchards (C.V%), average census number of clones in orchards (N), average effective number of clones based on variation in the number of ramets (N_c) and the relative effective number of clones (N_r) were calculated for all seed orchards according to Kang et al. 2001b. The effective number of clones, N_c, was calculated based on the variation of ramet numbers among clones;

$$N_c = \frac{n_{total}^2}{\sum_{i=1}^{N} n_i^2}$$

where n_{total} is the total number of ramets in the seed orchard; n_i is the number of ramets of clone i; and N is the census number of clones in the seed orchard (Kang et al. 2001b).

The relative effective number of clones (N_r) was used to compare the census number (N) and the effective number of clones (N_c) in the seed orchards. Relationships between N_c and N and, between N_c and age of orchards were determined using Pearson's correlation coefficient (r). The N_c in an orchard is equivalent to the status number of the seed orchard when clones are non-inbred and unrelated (Lindgren and Mullin 1998, Kang et al. 2001b). The estimated proportional gene diversity (GD = 1–1/2N_c) for all seed orchards were calculated according to Kang et al. (2001b).

Results
Rezultati

When all the seed orchards of conifers are considered, the clone number ranged from 8 [*P. sylvestris* seed orchard, National code (ND) 136] to 152 (*P. sylvestris* seed orchard, ND 160). Most of the seed orchards were established with about 30 clones. Large differences for total living number of ramets among orchards and number of ramets per clone were observed. The minimum ramet number was observed in a *P. nigra* seed orchard (ND 41), having 75 ramets from 10 clones on total of 0.2 ha area. The maximum ramet number was observed also in *P. nigra* seed orchard (ND 80 and 140) having 3225 ramets from 30 clone on 20.6 ha area. The average seed orchard size was 6.46 ha (ranging from 3.46 to 11.72 ha). The average number of ramets per orchards was 1141.6 (Table 1). Spacing between ramets in the seed orchards varies between 4x4 m and 10x10 m, most being 8x8 m spacing.

Pinus brutia: There were 64 seed orchards. The number of clones ranged from 10 (ND 22) to 146 (ND 155). The number of ramets in the seed orchards ranged from 229 (ND 33) to 2790 (ND 38). The smallest seed orchard (ND 2) was 1.0 ha in size whereas the largest one (ND 16) was 18.6 ha. The ages of seed orchards were between 7 and 36 years. Spacing between ramets in most seed orchards were 8x8 m (smallest 5x5 m, largest 10x10 m).

Pinus nigra: There were 56 seed orchards. The number of clones ranged from 10 (ND 41) to 120 (167). The number of ramets in the seed orchards ranged from 75 (ND 41) to 3225 (ND 80 and 140). The smallest seed orchard (ND 41) was 0.2 ha in size whereas the largest one (ND 73) was 20.7 ha. The ages of seed orchards were between 2 and 48 years. Spacing between ramets in most seed orchards were 8x8 m (smallest 4x4 m, largest 8x8 m).

Pinus sylvestris: There were 21 seed orchards. The number of clones ranged from 8 (ND 136) to 152 (ND 160). The number of ramets in the seed orchards ranged from 94 (ND 87) to 2287 (ND 178). The smallest seed orchard (ND 87) was 0.2 ha in size whereas the largest one (ND 160) was 9.9 ha. The ages of seed orchards were between 8 and 48 years. Spacing between ramets ranged between 5x5 m and 7x7 m.

Table 1. Information about coefficient of variation among seed orchards (C.V. %), effective number of clones (N_e) based on variation in the number of ramets and the relative effective number of clones (N_r); the estimated proportional gene diversity (GD) (standard deviation is in parenthesis)

Tablica 1. Koeficijent varijacije između sjemenskih plantaža (CV %), učinkoviti broj klonova (N_e) temeljen na na različitom broju rameta i relativnog efektivnog broja klonova (N_r), procijenjena proporcionalna genetska raznolikost (GD) (standardno odstupanje prikazano je u zagradama)

Species (Vrsta)	Number of orchards (Broj plantaža)	*Avg. size (ha) (Prosječna veličina)	Avg. number of ramets/orchard (Prosječan broj rameta / plantaža)	Avg. C.V. (%) for ramets (Prosječni C.V. (%) za ramete)	Avg. N (Prosječni N)	Avg. N_e (Prosječni N_e)	Avg. N_r (Prosječni N_r)	GD (genska raznolikost)
Pinus brutia	64	7.07 (4.11)	1100 (650.16)	41.69 (22.68)	41.83 (30.56)	32.89 (18.31)	0.844 (0.13)	0.981 (0.009)
Pinus nigra	56	8.05 (5.03)	1245.4 (789.22)	33.28 (16.90)	32.05 (15.29)	28.16 (10.98)	0.892 (0.09)	0.980 (0.009)
Pinus sylvestris	21	5.21 (2.08)	1080.8 (516.87)	32.34 (10.79)	39.38 (34.32)	35.55 (31.63)	0.903 (0.06)	0.980 (0.014)
Pinus pinea	5	11.72 (7.39)	1587.4 (1255.44)	28.68 (26.96)	36.80 (15.21)	32.45 (11.58)	0.897 (0.13)	0.983 (0.005)
Pinus halepensis	2	4.10 (1.80)	389 (154.15)	41.58 (22.05)	15 (7.07)	6.60 (1.59)	0.523 (0.35)	0.922 (0.019)
Cedrus libani	12	5.59 (2.43)	1403.8 (729.69)	31.06 (14.74)	34.58 (7.87)	31.03 (5.97)	0.906 (0.08)	0.983 (0.002)
Picea orientalis	9	3.46 (2.14)	1185 (842.53)	45.48 (21.90)	32.2 (6.67)	26.43 (6.16)	0.823 (0.12)	0.980 (0.004)
Overall mean (Ukupni srednji)		6.46 (2.81)	1141.6 (376.82)	36.30 (6.47)	33.12 (8.77)	27.59 (9.02)	0.827 (0.13)	0.973 (0.022)

*Average

Pinus pinea: There were five seed orchards. The number of clones ranged from 30 (ND 104, ND 105, ND 106 and ND 107) to 64 (ND 197). The number of ramets in the seed orchards ranged from 270 (ND 106) to 3068 (ND 105). The smallest seed orchard (ND 106) was 1.7 ha in size whereas the largest one (ND 105) was 19.5 ha. The ages of seed orchards were between 1 and 25 years. Spacing between ramets in most seed orchards was 8x8 m, except ND 197 which was 10x10 m.

Pinus halepensis: There were only two seed orchards. ND 108 has 10 clones and 280 ramets in 2.3 ha area with 9x9 m spacing. ND 109 has 20 clones and 498 ramets in 5.9 ha area with 8x8 m spacing. The ages of seed orchards were 29 and 24 years, respectively.

Picea orientalis: There were nine seed orchards. The number of clones in most seed orchards was 30, except ND 121 which has 50 clones. The number of ramets in the seed orchards ranged from 342 (ND 146) to 3121 (ND 121). The smallest seed orchard (ND 146) was 0.9 ha in size whereas the largest one (ND 121) was 7.5 ha. The ages of seed orchards were between 12 and 22 years. Spacing between ramets in most seed orchards was 5x5 m, except ND 110 and ND 152 which was 6x6 m.

Cedrus libani: Seed orchards number was 12. The number of clones varies between 30 and 55 (ND 186). The number of ramets ranged between 584 (ND 200) and 2781 (ND 118). The size of establishment area ranged between 2.1 (ND 200) and 10.0 ha (ND 118). The ages of seed orchards were between 1 and 22 years. Spacing between ramets was 5x5 m and 6x6 m.

The average number of related statistics and their corresponding standard deviations for each species are presented in Table 1. The average coefficient of variation for ramet numbers among all seed orchards (C.V. %) was calculated to be 36.30. The mean census number (N) and effective number of clones (N_e) were 33.12 and 27.59, respectively. The largest difference between N and N_e value was observed in *P. halepensis* (56%), represented by only two seed orchards. The smallest difference between N and N_e value was observed in *P. sylvestris* (9.7%), represented by 12 seed orchards (Table 1). Most of the seed orchards (76.3%) had effective number of clones between 5 and 30. One sixth of them had N_e values ranging between 31 and 60. Also, in 11 (% 6.5) seed orchards N_e value was estimated to be higher than 61.

Relationship between N_e and N in each of the clonal seed orchards in Turkey were presented in Figure 1. For the seed
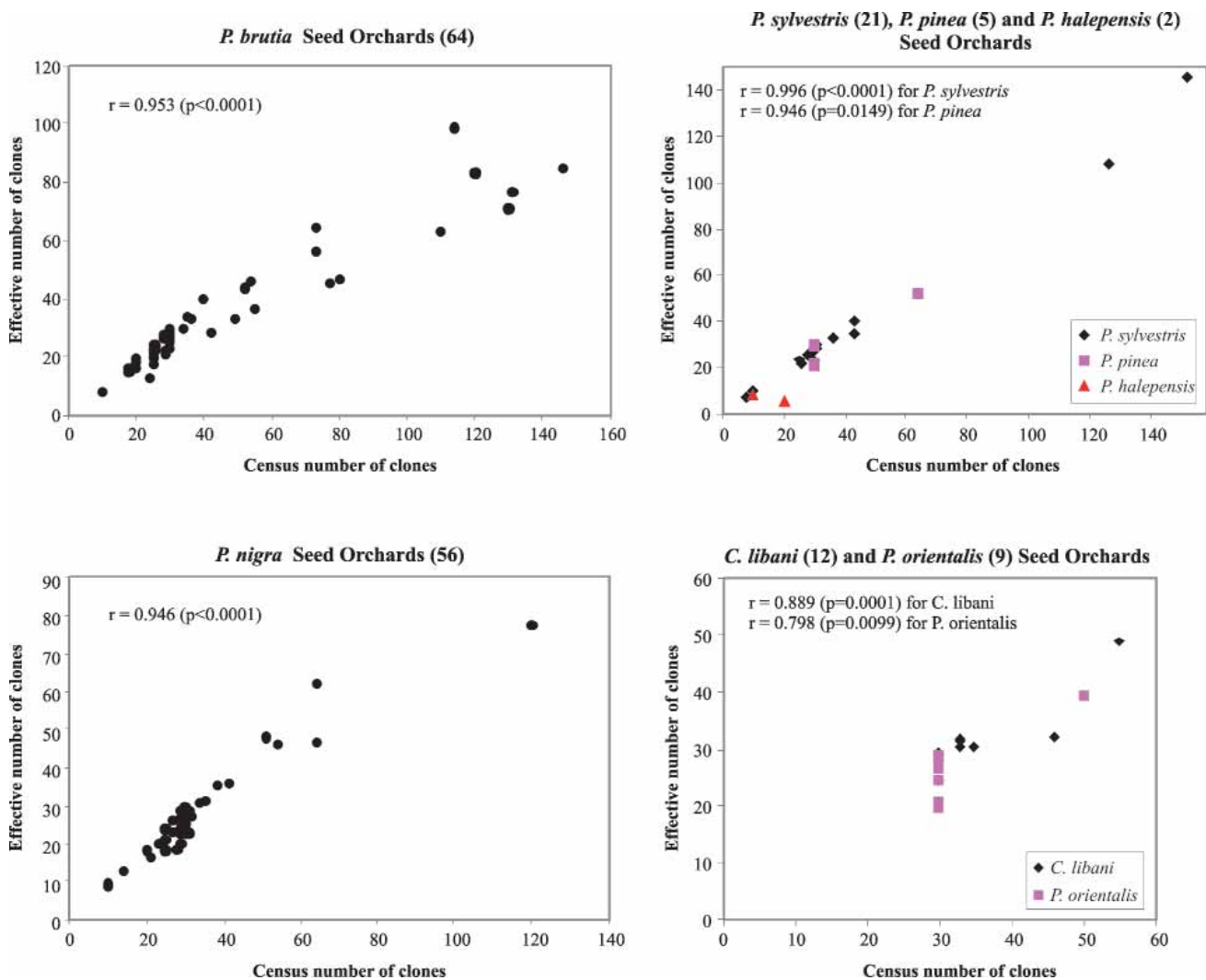
Figure 1. Relationship between effective number (N_c) and census number of clones (N) in clonal seed orchards for each of the seven conifer species in Turkey (pooled data for a given species). Number of seed orchards for each species is shown in parenthesis next to species name

Slika 1. Odnos između učinkovitog (N_c) i utvrđenog broja klonova (N) u klonskim sjemenskim plantažama kod sedam vrsta četinjača u Turskoj (podaci su objedinjeni na razini vrste). Broj klonskih sjemenskih plantaža za svaku vrstu prikazan je u zagradi
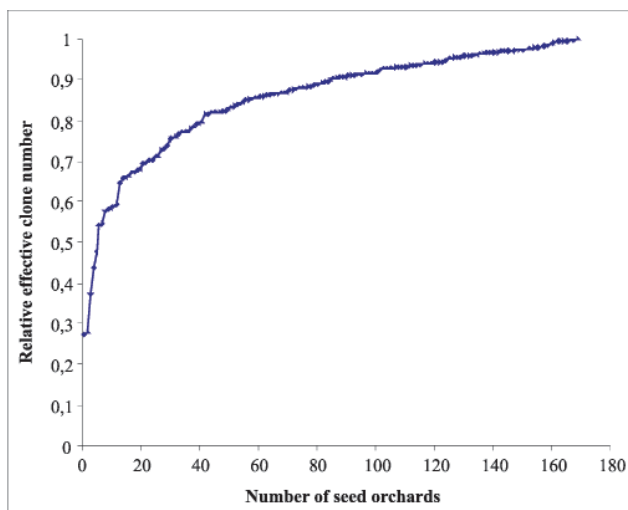

Figure 2. Distribution of relative effective number of clones (N_r) in 169 clonal seed orchards of conifers in Turkey

Slika 2. Distribucija relativnog efektivnog broja klonova (N_r) u 169 klonskih sjemenskih plantaža četinjača u Turskoj

orchards of each species except for that of *P. halepensis*, Pearson's correlation coefficient between N and N_c was high and significant. Pearson's correlation coefficients varied from 0.798 (p < 0.0099) (*Picea orientalis* seed orchards) to 0.996 (p< 0.0001) (*P. sylvestris* seed orchards) among the species (Figure 1). Pearson's correlation coefficients between age and N_c (varied from −0.38 to −0.93) showed that there was negative correlation, which means that the older the orchard was, the smaller the effective number of clones. The average r value between age and N_c was calculated as −0.47 (p< 0.00001).

Distribution of N_r in 169 clonal seed orchards of conifers in Turkey was shown in Figure 2. The N_r among seed orchards within species and among species was different. When all the seed orchards are considered, the mean N_r value was calculated as 0.827 (Table 1). For all the seed orchards analyzed, 5 orchards had N_r value less than 0.5, and 157 seed orchards had N_r value higher than 0.6 (Figure 2).

The estimated proportional gene diversity was in the range of 0.922 to 0.983 (mean 0.973) among the studied seed orchards (Table 1). There was positive correlation between gene diversity and N_c value (r = 0.953, p = 0.0001), and also between gene diversity and N_r value (r = 0.977, p = 0.0001).

Discussion
Rasprava

Plus trees, selected based on their desirable phenotypic characteristics, are used for establishment of clonal seed orchards (Kang et al. 2001b). Genetic thinning and selective harvesting may change clonal ramet proportion in orchards, while, at the same time, they can improve the genetic quality of seed orchards (Kang et al. 2001a, Prescher et al. 2008). Both ramet number variation and ramet fertility of clones contribute to the gene pool in a seed orchard. Therefore, important attention should be given on ramet number variation during seed orchard establishment and management.

In this study, overall mean effective number of clones (N_c) based on variation in the number of ramets was estimated as 27.59. Furthermore, *P. sylvestris* seed orchards had the highest N_c value (35.55), followed by *P. brutia* seed orchards (32.89) and finally, *P. halepensis* seed orchards with the lowest value (6.6). Kang et al. (2001b), working on a 255 conifer clonal seed orchards in Finland, Korea and Sweden, estimated the mean effective number of clones (N_c) as 66, ranging from 10 to 421. Prescher et al. (2008) reported N_c values in *Picea abies* seed orchard as 19.99 and 22, before and after thinning, respectively.

The N_r was 0.827, with a range from 0.523 to 0.906 in this study. Kang et al (2001b) estimated overall average N_r value as 0.74; *Picea abies* seed orchards in Sweden had the highest, *Pinus koraiensis* seed orchards in Korea had the lowest value. Varghese et al. (2006) reported N_r value in two clonal seed orchards of *Tectona grandis* in southern India as 0.582 and 0.120, respectively. The seed orchard with high N_r value had relatively more number of fertile ramets and better sexual symmetry, both contributing to the higher gene diversity. Results of our study indicate that seed orchards of conifer forest trees in Turkey have relatively high effective number of clones. This implies that Forest Tree Seeds and Tree Breeding Research Directorate have an efficient program for seed orchards establishment and management.

The main reasons for variations in ramet number are graft availability and incompatibility, growth conditions and mislabeling of ramets. Variation in the number of ramets per clones in seed orchards may result unequal production of female flowers, male flowers and gametes. Equal contribution of clones to the formation of the progeny is necessary because the production of genetically superior seeds in the seed orchard through panmictic reproduction is important

(Kang et al. 2001a, Lindgren and Prescher 2005). If seed orchards do not reproduce panmictically, some reduction in the expected genetic gains will occur. The quality of a seed produced in seed orchards is determined by many factors, including the rate of pollen contamination from outside sources, degree of self-fertilization, and equality of the clones (in other words ramet number variation) as pollen donors. The level of contribution varies significantly among clones in the seed orchard that have variations in ramet number (Kaya et al. 2006, Funda et al. 2009). El-Kassaby and Cook (1994) studied the within and among clonal variation in reproductive energy and reproductive success, and their impact on the genetic diversity of cone/seed crop in a Douglas fir seed orchard. In that study, the average number of seed-cone producing ramets per clone was ranging between 1 and 15, they observed significant correlation (p≤0.05) between clone size (variable number of seed-cone bearing ramets per clone) and both seed-cone crop size and filled seed yield. Nikkanen and Ruotsalainen (2000) reported that the status number is important measure for describing the genetic diversity of the seed orchard crop. They found that the status number of studied Norway spruce seed orchard was 56 (84% of the number of clones in the orchard) when the ramet number variation per clone was considered.

The mean estimated proportional gene diversity was 0.973 among the seed orchards studied. We found positive correlation between gene diversity and N_c value, and also between gene diversity and N_r value. Gene diversity increases as N_c or N_r increases. Our result in this study is compatible with other studies on conifer seed orchards (Kang and Lindgren 1998, Kang et al. 2001b). Kang and Lindgren (1998) estimated gene diversity in *P. densiflora*, *P. thunbergii* and *P. koraiensis* seed orchards by total strobili production as 0.993, 0.991 and 0.987, respectively. Kang et al (2001b) calculated the estimated mean gene diversity as 0.992 in 255 conifer clonal seed orchards. Differences among clones in fertility and ramet number affect genetic composition of seed orchards and may cause loss in genetic diversity (Kang and Lindgren 1998).

Kang et al. (2003) estimated genetic gain after the implementation of %45 and %70 genetic thinning in a *Pinus densiflora* clonal seed orchard. They reported genetic gain as 0.07% at initial establishment of orchard, 6.3% at 1st genetic thinning and 11.6% at 2nd genetic thinning. Genetic diversity was estimated in a 13-year old *Quercus serrata* breeding seed orchard under three selection (roguing) methods (Kang et al. 2007). They calculated genetic diversity loss for individual, family and family + within family selection as 1.05%, 1.72% and 1.29%, respectively. Kang et al. (2007) concluded that genetic gain from seed orchards can be manipulated by using different selection methods, but strong genetic thinning might result in a decrease of genetic diver-

sity. Icgen et al. (2006) reported that the majority of genetic diversity present in natural populations of *P. brutia* appears to be captured in seed orchards and plantations. Also, the results of our study showed that all studied seed orchards have high proportion of gene diversity (Table 1). As a result, different selection methods and management strategies should be applied on seed orchards depending on related species to get maximum genetic gain and maintain high level of genetic diversity.

Kang and Mullin (2007) studied effect of variation in effective clone number on the genetic diversity of *Chamaecyparis obtusa* seed orchard in Korea over a four-year period. They indicated that gene diversity in seed crop would increase if seeds collected from different years are pooled. Varghese et al. (2006) estimated gene diversity in two 25-years old clonal seed orchards of *Tectona grandis* in southern India as 0.943 and 0.792, respectively. They reported that improvement in diversity level were inadequate in the second seed orchard, and suggested some measures such as intentional adjustment of ramet numbers of each clone, that can help to reduce the loss of gene diversity during domestication process. The seed orchards in our study are all first generation seed orchards; and management and improvement studies for future seed harvests from the seed orchards are in progress in Turkey (Koski and Antola 2003). However, genetic diversity parameters of seed orchards in this study showed parallelism with previous studies (Godt et al. 2001, Kang et al. 2001b, 2003). Therefore, phenotypic selection, related species fertility and genetic analysis studies should be considered to establish second generation seed orchards.

Genetic diversity parameters of seed orchards were shown not to be significantly different from seed stands and plantations (El-Kassaby and Ritland 1996, Godt et al. 2001, Icgen et al. 2006). Even, the percentages of polymorphic loci in seed orchards were slightly higher than natural stands and plantations (Icgen et al. 2006). These results suggest that large proportion of genetic diversity found within natural populations are captured in seed orchards through practicing phenotypic selection and implementing effective number of clones (Godt et al. 2001, Icgen et al. 2006). Results of this study suggest high proportion of gene diversity in conifer seed orchards in Turkey. However, reduced allelic richness and heterozygosity in seed orchards were also reported (Cheliak et al. 1988, Stoehr and El-Kassaby 1997, Rajora, 1999). Such contrasts may be attributed to the sampling, management practices (especially roguing of seed orchard) and mating system of the species in question (Icgen et al. 2006, Lindgren et al. 2009). These characteristics should be considered to establishment of seed orchards in the future.

Certain conditions should be achieved to obtain expected level of genetic gain from an open-pollinated seed orchards: (a) Seed orchards should be isolated from unwanted pollen sources, (b) Number of clones and ramets per clone in a seed orchard should be high enough depending on species (from 20 to 50 clones), and all clones should contribute relatively equal proportion of male and female flowers, (c) Pollen dispersal period and female receptivity should occur at the same time as much as possible, and (d) Inbreeding and mating among close relatives should be avoided (Fast et al. 1986, El-Kassaby et al. 1989, Kang et al. 2001a). Orchard-management techniques such as selective seed harvesting and genetic thinning (roguing) are used to improve genetic quality of seed orchard crops. In selective seed harvest, selection is applied to seed parents, whereas in roguing selection is applied to both seed and pollen parents. Genetic thinning maximizes the genetic gain from seed orchard crops and effective clone number in orchards (Kang et al. 2001a, Moriguchi et al. 2008, Prescher et al. 2008). The results of this study and previous studies about seed orchards which are discussed above showed that clone number, ramet number, fertility variation of related species, distribution range of related species and different management strategies should be considered to establish tomorrow's seed orchards.

Conclusions
Zaključci

Future's seed orchards should be established with phenotypic selection as well as populations' molecular genetic analysis (especially analysis of adaptive genes). Genetic gain and genetic diversity balance should be considered when establishment of first and/or second generation seed orchards. To capture and manage high levels of genetic variation in seed orchards, high number of populations, covering a wider range of environments and marginal populations of species, should be systematically sampled and genetically analyzed. According to the genetic analysis results, and considering threats such as climate change, seed orchards are established on a wide range of species distribution area depending on their provinces and local growing conditions.

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Sažetak

Mediteranski bazen jedan je od glavnih središta biljne raznolikosti u Sjevernoj hemisferi. Istočni Mediteran također je područje iznimne genetske raznolikosti četinjača. Istraživanjem su obuhvaćene klonske sjemenske plantaže Turske s ciljem utvrđivanja efektivnog broja klonova. Srednji broj klonova (N) procijenjen je na 33,12. Srednji efektivni broj klonova (N_c) iznosi 27,59, a srednji relativni efektivni broj klonova ($N_r = N_c / N$) je 0,827. Procijenjeni proporcionalni genetski diverzitet iznosio je 0,973, s rasponom od 0,922 – 0,983. Stoga je potrebno voditi računa da se koristi gotovo podjednaki broj rameta tijekom osnivanja i održavanja klonskih sjemenskih plantaža. Prijetnje poput klimatskih promjena, požara, bolesti i kukaca moraju se uzeti u obzir kod osnivanja plantaža. Potrebno je uzorkovati velik broj populacija sa šireg područja, a klonske sjemenske plantaže najbolje je osnivati lokalno uvažavajući ekološka obilježja četinjača. Ovime se također osigurava održivo gospodarenje šumskim genetskim bogatstvom. Kod osnivanja budućih klonskih sjemenskih plantaža nužno je pribaviti podatke o fenotipskoj selekciji kao i rezultate molekularne genetičke analize potencijalnih klonova.

KLJUČNE RIJEČI: klimatske promjene, efektivni broj klonova, genetska raznolikost, varijabilnost rameta, sjemenska plantaža