Uluslararası Tarım Araştırmalarında Yenilikçi Yaklaşımlar Dergisi
International Journal of Innovative Approaches in Agricultural Research 2023, Vol. 7 (3), 356-370
https://doi.org/10.29329/ijiaar.2023.602.10

Review article

Microsatellite Markers: The Efficient Method for the Determination of Pollen Contamination in Conifer Seed Orchards

Behiye Banu Bilgen [a, *] & Nuray Kaya [b]

[a] Department of Agricultural Biotechnology, Faculty of Agriculture, Tekirdağ Namık Kemal University, Tekirdağ, Türkiye
[b] Department of Biology, Faculty of Science, Akdeniz University, Antalya, Türkiye

Abstract

Seed orchards are specialized forest plantations of genetically superior candidate parents selected to produce genetically superior seeds and/or seedlings. Pollen contamination is one of the most important factors affecting the yield, adaptation, and genetic quality of seeds produced from seed orchards in forest tree breeding programs. Potential pollen from forests surrounding the seed orchard is a major concern in tree breeding because it contributes to the loss in genetic gains expected from seed orchard crops. Microsatellite markers are among the most effective markers that are frequently used for creating genetic maps of many species, determining genetic diversity, identifying genetic diseases, population genetic studies, linkage analysis, fingerprint analysis, genotyping, and parental identification. In this study, a bibliometric analysis was performed to quantitatively and qualitatively evaluate the articles published in the last 25 years on seed orchards and pollen contamination. Searching the Web of Science (WOS) with the criteria of "forest trees" and "seed orchards" revealed that 820 articles were published in the last 25 years. It is seen that 77 of these articles are related to pollen contamination. Canada, China, Japan, Sweden, and the USA have been the top contributors to research on pollen contamination in seed orchards of forest trees in the last 25 years, respectively. According to the data obtained, it has been shown that the genetic contamination level of forest tree species in seed orchards is generally between 5% and 90%. It has been determined that microsatellite markers are more widely used in recent years to determine the degree of pollen migration and genetic contamination. It was concluded that studies on pollen contamination were carried out in only two Turkish red pine orchards in Türkiye, which has a total of 189 seed orchards, the majority of which belong to conifers, and that similar studies should be planned in other seed orchards.

Keywords: Bibliometric analysis, Pollen contamination, Seed Orchards, Simple Sequence Repeats.

Received: 22 June 2023 * **Accepted:** 25 July 2023 * **DOI:** https://doi.org/10.29329/ijiaar.2023.602.10

* **Corresponding author:**

Bilgen Behiye Banu is an associated professor in the Department of Agricultral Biotechnology at Tekirdağ Namık Kemal University in Tekirdağ, Turkey. Her research interests include the the Plant Biotechnology, Plant Genetic Diversity, Plant Genetic Resources, Molecular Markers and Marker Assisted Selection.
Email: bbilgen@nku.edu.tr

INTRODUCTION

Biological diversity includes all plant and animal species that spread in a country, especially those that have a very important place in agriculture, forestry, animal husbandry, medicine, pharmacy, and industry, both economically and in terms of use. However, due to reasons such as rapid population growth, unplanned urbanization, industrialization, forest fires, air pollution, agricultural land acquisition, global warming, erosion, and misuse of our natural resources, the distribution areas of living species are endangered, the structure of gene pools is changing, and their evolutionary potential is lost, so our biological richness is rapidly depleting. These factors finally damage biodiversity. Conservation of biological diversity, especially the protection of forest ecosystems with rich biodiversity, is of primary importance in terms of both ecological, aesthetic, and economic aspects, as well as preventing the extinction of endangered species.

Our forests are one of our self-renewing biological richness. Forest ecosystems are different units of nature that can vary over very short distances. In mountainous regions where forest ecosystems exist in Türkiye, especially climate, soil, and biological environmental factors change over short distances and more frequently. Gene pools and gene combinations are different due to different environmental factors and selection pressures in neighboring populations of the same species. Because of this, different races and sub-races with different fitness values may occur living in short distances. The existence of different races or sub-race at these short distances has been demonstrated by the studies concluded in many native and foreign tree species (Bradshaw, 1972; Hamann et al., 1998; Işık, 1999a, b; Ohsawa & Ide 2008).

The determination of genetic diversity is significant to be used within the framework of sustainability principles. Sustainable management of forests is possible with studies to be carried out at every step, from the gene level to the ecosystem level. In order to increase productivity in forest trees, it is necessary to determine and improve the genotypes that are fast-growing and resistant to biotic and abiotic factors. Genetic diversity is the main resource for establishing genetic breeding programs (Sütcü et al., 2022). Species with high genetic diversity should be determined for breeding studies to reach the desired goal. In the establishment of new forests, natural forest populations, seed stands, seed plantations, or seed orchards, whose genetic diversity has been determined, based on morphological data or at the molecular level, are used.

The main purpose of this review is to examine the pollen contamination studies carried out on seed orchards in approximately 25 years by using microsatellite (SSR) markers, which is one of the effective molecular markers, and to compare them with the studies conducted in our country.

CONIFER SEED ORCHARDS

Conifer seed orchards are specialized forest plantations of selected and genetically superior candidate parents to obtain genetically superior seeds and seedlings for use in forestry studies (Buiteveld et al., 2001; Zhuowen, 2002; Funda & El-Kassaby, 2012; Bilgen & Kaya 2014, 2016). Conifer seed orchards are plantations where the pollen flow from outside the orchard is reduced or destroyed, which consists of selected clones and generations, which are subjected to special operations to produce easy and abundant forest tree seeds (Kang et al., 2001a, 2004). For this reason, seed orchards are established by using trees that are assumed to be genotypically superior to natural forest populations as rootstocks (Zobel & Talbert, 1984). Seed source is very important for afforestation. The superior genetic characteristics of the seeds to be used in afforestation can ensure that the genetic gain expected from the forests to be established with these seeds is high. For this purpose, primarily seeds obtained from seed orchards are used in afforestation. In order to obtain the superior species and breeds required for use in forestry and afforestation studies, it is of great importance to select and bring together genetically superior individuals and establish seed orchards. These are plantations that are isolated from other pollen sources, and for obtaining frequent, abundant, easy, high genetic and physiological value seeds, and are subjected to special care and management (El-Kassaby et al., 1989; Di-Giovanni & Kevan, 1991; Kang et al., 2004).

Seed orchards are basically established to achieve four significant purposes. These are;

a) To obtain seeds with a high genetic and physiological value that will enable the formation of trees with superior genetic characteristics,

b) To obtain trees that can adapt to certain regions with their superior genetic characteristics,

c) To obtain genetically superior seeds, which enable the formation of trees with the desired genetic characteristics, in greater quantity and more economically,

d) To ensure the protection of individuals belonging to species or races with superior genetic characteristics (El-Kassaby et al., 1989; Ertekin, 2012; Bilgen & Kaya, 2014).

Seed orchards are a very important tool for tree breeders to change the genetic structure of forest populations in the desired direction and to domesticate populations in nature according to our purpose. Seed orchards are resources that ensure the collection of sufficient quality seeds for afforestation works. In this way, individuals carrying the genes we want are brought together in the seed orchards and a special gene pool is created by preventing the mixing of undesired genes among them. There are basically two types of seed orchards. The first of these is a vegetative or clonal seed orchard, and the second is a seedling seed orchard. While clonal seed orchards are established using selected clones of a single parent tree (grafting, cuttings, layering, tissue culture, etc.), seedling seed orchards are established by planting seedlings grown from seed in a pattern (Tunçtaner, 2007).

The number of clones to be used in the establishment of seed orchards has an important place in ensuring genetic diversity. If the number of clones to be used is large, genetic diversity may be high. If a small number of clones are used, rare alleles in the source population may be lost because of sampling and this may cause a decrease in genetic diversity (Bilir et al., 2004). The number of clones that should be found in seed orchards usually varies between 20 and 50 clones. An average of 30 clones is used in the establishment of first-generation seed orchards. In addition to the number of clones the seed orchard has, the number of ramets belonging to each clone in the orchard has an important place in terms of the functions of the seed orchards. Because the differences in the number of ramets belonging to each clone may cause unequal contribution to the production of female flowers, male flowers, and gametes by the clones in the orchard (Kang et al., 2001b). The number of ramets belonging to the clones is important in terms of the participation rate of the clones in the gene pool in the seed product and the amount of product. To obtain high gene diversity in the seed, it should be preferred to establish seed orchards by using the same number of ramets belonging to each clone or as close to each other as possible.

The first clonal seed orchard was established on the island of Java/Netherlands in 1880 for the *Cinchona ledgeriana* species (Feilberg & Soegaard, 1975; Ertekin, 2012). In Türkiye, the first seed orchards were established in 1964 in Istanbul-Belgrad Forest with black pine and yellow pine species by the Istanbul University Faculty of Forestry Department of Silviculture and Afforestation (Tunçtaner, 2007). Until 2023, 189 seed orchards were established for different forest trees in different regions of Türkiye by the Republic of Türkiye, Ministry of Agriculture and Forestry (Table 1) (OATIAM, 2023).

Table 1. Seed orchards established in Türkiye

Species name	**Number**	**Total Area (Ha)**
Pinus brutia (Kızılçam)	84	689.8
Pinus nigra (Karaçam)	54	483.7
Pinus sylvestris (Sarıçam)	21	113
Picea orientalis (Ladin)	9	34.9
Pinus pinea (Fıstıkçamı)	7	93.6
Cedrus libani (Sedir)	7	46.4
Pinus halepensis (Halepçamı)	2	9.5
Juniperus phoenicea (Finike Ardıcı)	1	1.1
Pinus pinaster (Sahilçamı)	1	3.3
Sorbus torminalis (Akçaağaç Yapraklı Üvez)	1	4.2
Liquidambar orientalis (Sığla)	1	3.1
Ziziphus jujuba (Hünnap)	1	3.1
Overall Total	189	1485.7

Pollen contamination from individuals outside the seed orchard and self-pollination is known as potential problems with pollination in seed orchards. If there is a problem with pollination, one or more of the above conditions may be adversely affected. Studies on determining the mating system and estimating the pollen contamination rate in seed orchards are increasing day by day (Adams & Birkes, 1989). Accurate estimation of pollen pollution is of great importance for determining genetic gain in the orchard, developing seed orchard management strategies to reduce pollen pollution, and evaluating the effectiveness of seed orchards (Torimaru et al., 2009).

MICROSATELLITE (SIMPLE SEQUENCE REPEAT, SSR) MARKERS

Nowadays, as a result of the development of various molecular genetic markers by scientists in population genetic studies, DNA-based genetic markers (such as RFLP, PCR-RFLP, AFLP, RAPD, SCAR, SRAP, and SSR) are used instead of morphological and protein (isoenzyme) markers. One of the fastest and most effective methods used in studies at the DNA level to determine genetic diversity, mating system, and pollen contamination is to use the diversity of microsatellites or simple sequence repeats (SSRs). Microsatellites are polymerase chain reaction (PCR) based genetic markers based on the detection of differences in the number of repeats in the intra-gene and/or inter-gene regions under investigation. To determine the microsatellite polymorphism, it is amplified by PCR using primers complementary to the flanking regions of the repetitive region or locus studied, and the obtained fragments are analyzed electrophoretically (Bandelj et al., 2004; Varshney et al., 2005; Vieira et al., 2016; Kocaman et al., 2020).

Although microsatellites were originally designed for the human species, over time they have become a powerful tool in molecular research with plant and animal species. Microsatellites are frequently used to create genetic maps of many species, determine genetic diversity and genetic diseases, population genetic studies, linkage analysis, fingerprint analysis, genotyping, and parental determination. It also provides useful information in determining the gene flow patterns and the rate of genetic drift (Buiteveld et al., 2001; Balloux & Lugon-Moulin, 2002; Heywood & Iriondo, 2003; Bandelj et al., 2004; Slavov et al., 2004; Varshney et al., 2005; Oliveira et al., 2006; Vieira et al., 2016).

When microsatellite markers are compared with other markers (isoenzymes, AFLP, RAPD, RFLP, STR, etc.), it is seen that polymorphism is high, that is, allele diversity is high. This makes microsatellites advantageous not only in studies such as gene flow and parental determination in plant population genetics but also in different studies on natural plant populations. In addition, microsatellites can easily be used for living species with low genetic diversity. The dispersed presence of microsatellites in the genome, the very high reproducibility level of microsatellite analyses, and the small amount of DNA samples (1.5-50 ng) sufficient for analysis indicate that microsatellites are safe markers (Oliveira et al., 2006; Semagn et al., 2006). Microsatellites are inherited codominant, that is, homozygous and heterozygous genotypes can be easily distinguished from each other without genetic crossovers (Bandelj

et al., 2004; Oliveira et al., 2006; Semagn et al., 2006). During microsatellite analysis, different PCR products can be mixed and loaded onto the same gel, or multiplex PCR can be set up using several microsatellite reagents at the same time. Thus, a great gain is achieved in terms of time, labor and money. In addition to these features, it is one of the biggest advantages that markers found and/or designed for a species can also be used for nearby species (Oliveira et al. 2006). The mutation rate in microsatellite loci varies according to living species (10^{-2}-10^{-6} nucleotides/loci/generation). It is higher than other loci in the same species and/or in the same genome (Li et al., 2002; Ribeiro et al., 2002; Oliveira et al., 2006).

Microsatellite analysis also has some disadvantages. One of these disadvantages is that "null" alleles are encountered during SSR analyses. These alleles are not amplified, so they do not appear in the gel. As a result, they may cause under-evaluation of heterozygotes. However, this is not a common situation (Varshey et al. 2005). Another important problem in SSR analysis is that DNA polymerase inadvertently yields products of different sizes during amplification in the analysis of mono- and di-nucleotide repeats. These products are often less intense than the desired or work area product and are overlooked. However, if there is overlap in different products (desired and accidentally reproduced) belonging to heterozygous individuals, it becomes difficult to separate the desired region product. This problem can be solved by using an internal standard with a known band size (Oliveira et al. 2006).

Identifying and isolating microsatellite regions, sequence analysis, and testing of markers is an expensive process that requires time and expertise. We can list the most effective methods of obtaining new primers as follows: a) identifying suitable primers by scanning the sources, b) designing new primers using the sequences in the database, and c) working with a research laboratory specialized in the field that develops primers (Varshney et al., 2005). For example; Vendramin et al., (1996) developed 20 pairs of SSR primers from the chloroplast genome of *Pinus thunbergii* Parl., and the designed SSR primers were used for molecular genetic analyses in many tree species close to this species (Navascues et al., 2006; Naydenov et al., 2005a; Naydenov et al., 2005b; Naydenov et al., 2006; Terrab et al., 2006; Myers et al., 2007, Kaya et al., 2008, Dzialuk et al., 2009, Soto et al., 2010; Kurt et al., 2012; Bilgen & Kaya, 2014; Urbaniak et al., 2019; Sheller et al., 2021).

Chloroplast microsatellite markers (cpSSR) are useful in genetic fingerprinting and analysis of gene flow. To determine the mating system, paternity and pollen distribution, it is necessary to know the genotype of the mother tree and the different seeds (son progeny) of the mother tree and even the genetic structure of the pollen sources surrounding them (father). For this purpose, it is important to select genetic markers that show a high degree of polymorphism (Austerlitz et al., 2004). If the appropriate genetic marker is selected, it can be determined whether the pollen comes from within the population (local) or outside the population (foreign), from which pollen source and in what proportion (Ennos, 1994).

It is important to determine the haplotype identities of each clone in the seed orchard in terms of chloroplast microsatellite (cpSSR) loci, as well as the haplotype identities of the individuals in the natural population outside the seed orchard, and the frequencies of the haplotypes in determining the level of pollen contamination in a particular seed orchard by chloroplast microsatellite analysis. After the genotypes of the parents are determined, the genotypes of the embryos of a certain number of seeds formed on the individuals in the seed orchard are determined in terms of all studied loci and compared with the genotypes of the parents. The aim here is to determine whether a seed is produced entirely by fertilization of the gametes of individuals in the seed orchard. If an allele not found in the orchard is detected in the genotype of the embryo of a seed, then this seed is clearly a product of foreign pollen (foreign father, contamination). Based on this information, a minimum estimate of genetic contamination is made.

POLLEN CONTAMINATION AND MOLECULAR MARKER STUDIES IN THE LAST 25 YEARS

We performed a bibliometric analysis to assess quantitatively and qualitatively what published articles of the last 25 years related to seed orchard and pollen contamination. It has been determined that 820 articles have been published in the last 25 years in the search made with the criteria of "forest trees" and "seed orchards" in the Web of Science (WOS). It is seen that 77 of them are related to pollen contamination. The countries that contributed the most research on pollen contamination in the seed orchards of forest trees during 1997-2022 were Canada, China, Japan, Sweden, and the USA, respectively (Figure 1).

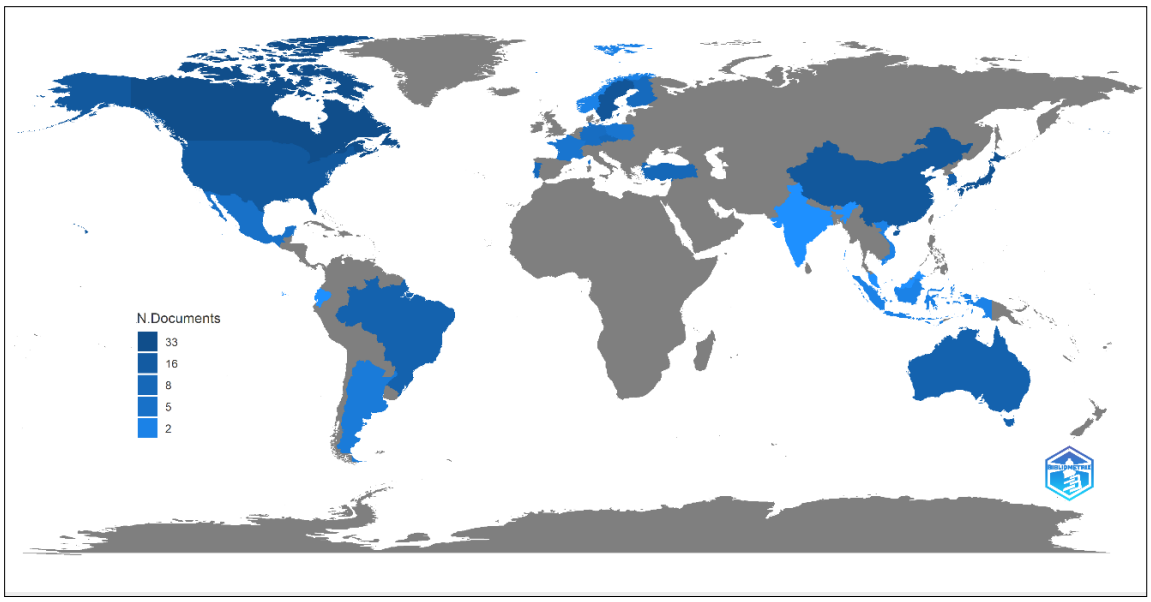

Figure 1. Most productive countries by number of publications.

Türkiye is also one of the countries that make an important contribution to world science on pollen contamination in seed orchards. When we look at the most used keywords in the published articles over

time, it is clear that the frequency of use of the word microsatellite marker (SSR) tends to increase (Figure 2). When we look at the keywords used together in the articles, three main clusters stand out: pollen contamination, mating system, and reproductive phenology (Figure 3).

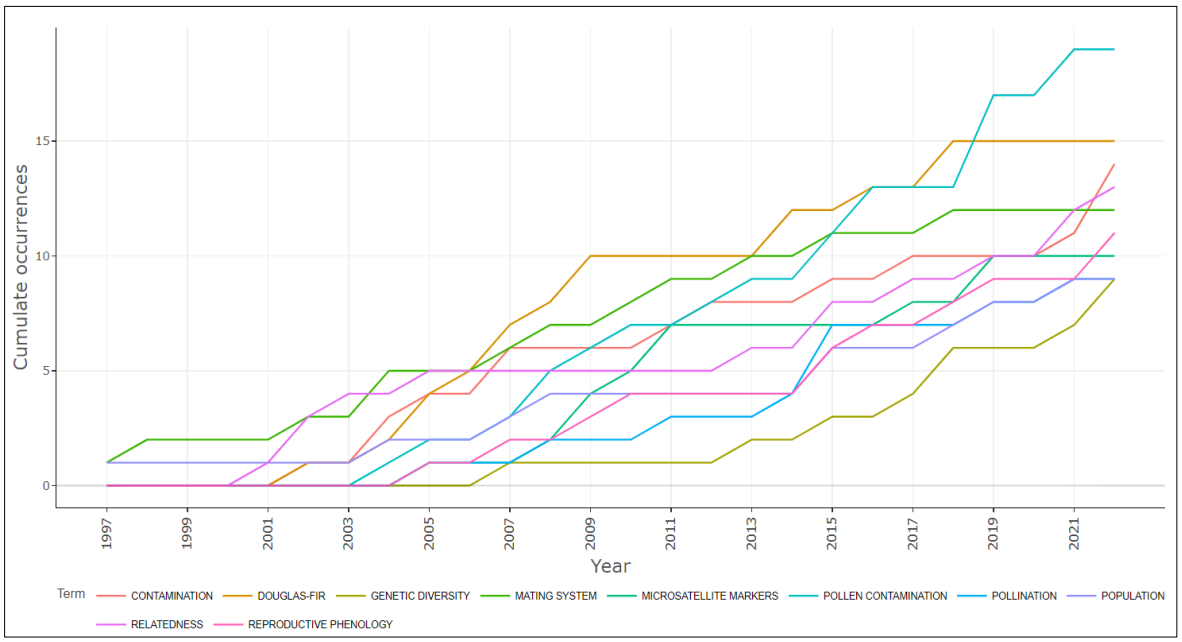

Figure 2. Cumulative changes in frequency of keywords used by authors over time (1997-2022).

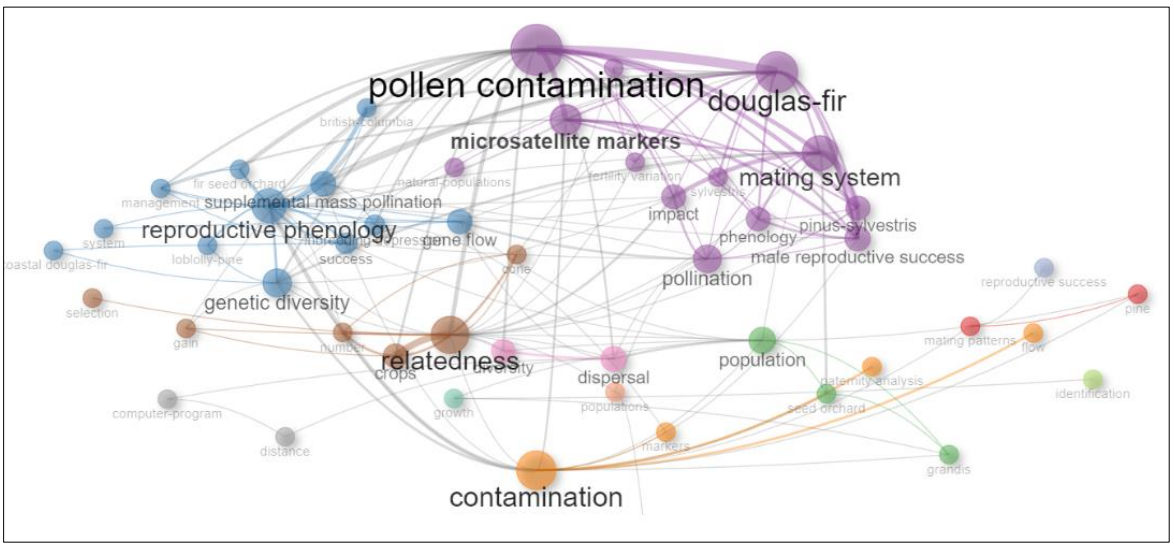

Figure 3. Co-occurrence network of the most frequent authors' keywords for the 25-year period (1997-2022). The distance between items demonstrates relative strength and topic similarity. Keywords belonging to the same cluster appear with the same color. The font and circle size of each keyword is proportional to the number of documents in which a keyword occurs; lines indicate co-occurrence links between terms, while line width is indicative of the link strength between two terms.

Among the articles published in the last 25 years directly related to pollen contamination in seed orchards of forest trees, the most cited and important articles were reviewed in detail. We have tried to

highlight the outstanding points in these articles. In plant populations, the way the gametes of the male and female individuals come together to form a new individual is called the mating system. The mating system in forest tree populations is seen in two ways. The first is selfing, and the other is outcrossing. If the female flower of an individual is pollinated by the pollen of the same individual (or another ramet of the same clone), this event is called self-fertilization. In seed orchards, self-fertilization can occur in two ways. The first is the fertilization of female flowers in the seed orchard with pollen belonging to other clones in the seed orchard, and the second is the fertilization of female flowers in the seed orchard with the pollen of individuals from natural populations near the seed orchard (Kaya, 2005).

Seed orchards are expected to produce seeds that are both genetically good and reflect the genetic diversity of the clones in the orchard. To determine whether this expectation can be realized or not, the three important elements of the mating system in the orchard are the self-fertilization rate of the clones in the orchard, the rate of seeds formed due to pollen from the natural trees outside the orchard, and the rate of fertilization of different clones in the orchard are used.

It is desirable to fertilize the female flowers of an individual (ramet) belonging to a clone in the orchard with pollen emitted from the male flowers of an individual belonging to a different clone in the same orchard. Pollen from low genetic trees outside the seed orchard pollinates the female flowers in the seed orchard composed of superior trees is called pollen contamination. As a result of genetic contamination by pollen, undesirable genotype seeds can be formed due to the mixing of pollen from the seed orchard and the forests established with these seeds are genetically low quality (Wheeler & Jech, 1986). In addition, although pollen pollution contributes to genetic diversity, it reduces the genetic gain to be obtained from the seed orchard because of selection (Kang et al., 2001a; Fernandes et al., 2008). If a seed orchard is close to undesirable (lower quality) populations or if the orchard is established in an area dominated by forests of the same species, the pollen pollution rate will be much higher due to the high pollen density in the air at the time of flowering (Greenwood & Rucker, 1985; Pakkanen et al. 2000). Also, if the gene pools of the two sources differ, and therefore the difference in gene frequencies increases, the consequences of pollen pollution will be more severe than expected (Sniezko, 1981).

Studies have shown that the level of genetic contamination in seed orchards of forest tree species is generally between 5% and 90%. Pollen contamination rates determined in different tree seed orchards using microsatellite markers are given in Table 2. In two *Pinus pinaster* seed orchards, pollen contamination rates were determined as 36% and 52.4% with 6 and 3 SSR loci, respectively (Plomion et al., 2001; Fernandes et al., 2008). In Türkiye, two studies have been found so far on the detection of pollen contamination (These studies were carried out within the scope of the authors' doctoral theses). In the first study conducted by Kaya et al., (2006) in Antalya-Asar *P. brutia* seed orchard, pollen pollution was determined as 85% with isozyme analysis. The second is performed by Bilgen and Kaya

(2014) with the use of cpSSR markers in Antalya *P. brutia* seed orchard and the pollen contamination rate was calculated as %39.3. Information obtained from studies on pollen contamination is important for the control of breeding populations and the development of effective gene protection strategies. Although there are 189 seed orchards in Türkiye, it has been observed that there are not enough studies to determine the pollen contamination of seed orchards, especially conifer seed orchards.

Table 2. Estimates of pollen contamination rate (m) in different tree seed orchards by microsatellite markers

Species name	Molecular marker used (locus number)	m (%)	Reference
Prosopis alba	SSR (10)	28-37	D'Amico et al., 2019
Eucalyptus urophylla	SSR (12)	11.9	Pupin et al., 2019
Larix kaempferi	SSR (17)	6.3	Chen et al., 2018
Picea abies	SSR (11)	20	Sonstebo et al., 2018
Schima superba	SSR (13)	7.01	Yang et al., 2017
Eucalyptus camaldulensis	SSR (11)	14.7	Gonzaga et al., 2016
Pseudotsuga menziesii	SSR (6)	18.4	Kess & El-Kassaby, 2015
Pinus brutia	SSR (6)	39.3	Bilgen & Kaya, 2014
Pinus koraiensis	SSR (13)	25	Feng et al., 2010
Pinus sylvestris	SSR (9)	52	Torimaru et al., 2009
Pinus pinaster	SSR (3)	52.4	Fernandes et al., 2008
Pseudotsuga menziesii	SSR (9)	35.3	Slavov et al., 2005
Pinus contorta	SSR (6)	5.5	Stoehr & Newton, 2002
Quercus robur	SSR (6)	70	Buiteveld et al., 2001
Pinus pinaster	SSR (6)	36	Plomion et al., 2001

Conclusion

The distance between the seed orchard and natural populations or populations with genetically undesirable characteristics (lower quality), the size of the orchard, the amount of pollen produced by ramets in the orchard, and the coincidence of flowering in nearby natural populations and flowering

times in the orchard are among the factors affecting the rate of pollen pollution (Di- Giovanni et al., 1996). To determine the rate of pollen pollution (contamination) in seed orchards; Different ways are used, such as the establishment of pollen traps, the application of the emasculation method of cutting male flowers formed by individuals in the seed orchard, and the use of genetic markers. Pollen traps and emasculation methods have some disadvantages compared to genetic markers. Although the emasculation method is used to determine pollen pollution, it is not fully sufficient to determine the pollution rate; it is also an expensive and time-consuming method. The establishment of pollen traps is slightly cheaper than the emasculation method. Pollen traps are useful in determining the overall rate of contamination but do not give an accurate indication of where the contamination originated (Lowe & Wheeler, 1993). Therefore, DNA markers have been used more widely in recent years to determine the degree of pollen migration and genetic pollution (Fernandes et al., 2008, Torimaru et al., 2009, Feng et al., 2010; Bilgen & Kaya, 2014; Kess & El-Kassaby, 2015; Gonzaga et al., 2016; Yang et al., 2017; Chen et al., 2018; Sonstebo et al., 2018; D'Amico et al., 2019; Pupin et al., 2019).

Declaration of Interest Statement

The authors declare no conflict of interest.

REFERENCES

Adams, W.T., & Birkes, D.S. (1989). Mating patterns in seed orchards. In: Proceedings of 20th Southern Forest tree Improvement Conference, June 26-30, 1989, Charleston, South Carolina.

Austerlitz, F., Dick, C.W., Dutech, C., Klein, E.K., Oddou-Muratorio, S., Smouse, P.E., & Sork, V.L. (2004). Using genetic markers to estimate the polen dispersal curve. Molecular Ecology, 13: 937-954.

Balloux, F., & Lugon-Moulin, N. (2002). The estimation of population differentiation with microsatellite markers. Molecular Ecology, 11: 155-165.

Bandelj, D., Jakse, J., & Javornik, B. (2004). Amplification of fluorescent-labelled microsatellite markers in olives by a novel, economic method. Acta agriculturae Slovenica, 83(2): 323-329.

Bilgen, B.B., & Kaya, N. (2014). Chloroplast DNA variation and pollen contamination in a *Pinus brutia* Ten. clonal seed orchard: implication for progeny performance in plantations. Turkish Journal of Agriculture and Forestry, 38: 540-549.

Bilgen, B.B., & Kaya, N. (2016). Use of nSSR markers for determination of clonal identity and genetic structure in a *Pinus brutia* Ten. clonal seed orchard. Fresenius Environmental Bulletin, 25(9): 3687-3693.

Bilir, N., Kang, K.S., Zang, D., & Lindgren, D. (2004). Fertility variation and status number between a base population and a seed orchard of *Pinus brutia*. Silvae Genetica, 53: 161-163.

Bradshaw, A.D. (1972). Some of the evolutionary conseqences of being a Plant. In: M. Dobzhansky K. Hecht and W.C. Stere (Eds.). Evolutionary Biology. Appl. Century Crofts, 25-47, New York.

Buiteveld, J., Bakker, E.G., Bovenschen, J., & de Vries, S.M.G. (2001). Paternity analysis in a seed orchard of *Quercus robur* L. and estimation of the amount of background pollination using microsatellite markers. Forest Genetics, 8 (4): 331-337.

Chen, X., Sun, X., Dong, L., & Zhang, S. (2018). Mating patterns and pollen dispersal in a Japanese larch (*Larix kaempferi*) clonal seed orchard: a case study. Sci. China Life Sci. 61: 1011-1023. https://doi.org/10.1007/s11427-018-9305-7

D'Amico, I., Juan, C.V., Beatriz, O.S., Ewens, M., & Bessega, C. (2019). Pollen contamination and mating patterns in a *Prosopis alba* clonal orchard: impact on seed orchards establishment. iForest, 12: 330-337. https://doi.org/10.3832/ifor2936-012

Di-Giovanni, F., & Kevan, P.G. (1991). Factors affecting pollen dynamics and its importance to pollen contamination: a review. Canadian Journal of Forest Research, 21: 1155-1170.

Dzialuk, A., Muchewicz, E., Boratynski, A., Montserrat, J.M., Boratynska, K., & Burczyk, J. (2009). Genetic variation of *Pinus uncinata* (Pinaceae) in the Pyrenees determined with cpSSR markers. Plant Systematics and Evolution, 277: 197-205.

El-Kassaby, Y.A., Rudin, D., & Yazdani, R. (1989). Levels of outcrossing and contamination in two *Pinus sylvestris* L. seed orchards in Northern Sweden. Scandinavian Journal of Forest Research, 4: 41-49.

Ennos, R.A. (1994). Estimating the relative rates of pollen and seed migration among plant populations. Heredity, 72: 250-259.

Ertekin, M. (2012). Genetic diversity of seed orchard crops, The molecular basis of plant genetic diversity, Prof. Mahmut Caliskan (Ed.), ISBN: 978-953-51-0157-4, InTech, http://www.intechopen.com/books/the-molecular-basis-of-plant-genetic-diversity/genetic-diversity-of-seedorchard-crops.

Feng, F.J., Sui, X., Chen, M.M., Zhao, D., Han, S.J., & Li, M.H. (2010). Mode of pollen spread in clonal seed orchard of *Pinus koraiensis*. Journal of Biophysical Chemistry, 1(1): 33-39.

Feilberg, L., & Soegaard, B. (1975). Historical review of seed orchards, in. Seed orchards, Forestry Commission Bulletin, No.54, pp. 1-8, London, England.

Fernandes, L., Rocheta, M., Cordeiro, J., Pereira, S., Gerber, S., Oliveira, M.M., & Ribeiro, M.M. (2008). Genetic variation, mating patterns and gene flow in a *Pinus pinaster* Aiton clonal seed orchard. Annals of Forest Science, 65: 706p1-10.

Funda, T., & El-Kassaby, Y.A. (2012). Seed orchard genetics. CAB Reviews, 7: 13.

Gonzaga, J.M.S., Manoel, R.O., Sousa, A.C.B., Souza, A.P., Moraes, M.L.T., Freitas, M.L.M., & Sebbenn, A.M. (2016). Pollen contamination and nonrandom mating in a *Eucalyptus camaldulensis* Dehnh seedling seed orchard. Silvae Genetica, 65: 1-11. https://doi.org/10.1515/sg-2016-0001

Greenwood, M.S., & Rucker, T. (1985). Estimating pollen contamination in Loblolly pine seed orchards by pollen trapping. Proc. 18th Southern Forest Tree Improv. Conf., May 21-23, 1985.

Hamann, A., El-Kassaby, Y.A., Koshy, M.P., & Namkoong, G. (1998). Multivariate analysis of allozymic and quantitative trait variation in *Alnus rubra*: geographic patterns and evolutionary implications. Canadian Journal of Forest Research, 28: 1557-1565.

Heywood, V.H., & Iriondo, J.M. (2003). Plant conservation: old problems, new perspectives. Biological Conservation, 113: 321-335.

Işik, K. (1999a). Orman ağacı türlerimizde lokal ırkların önemi ve genetik kirlenme sorunları. Çevre Sorunları, Biyolojik Çeşitlilik ve Orman Gen Kaynaklarımız, TEMA Vakfı, İstanbul. 25: 137-150.

Işik, K. (1999b). Bitki gen kaynaklarımız niçin korunmalı ve planlanmalıdır? Çevre Sorunları, Biyolojik Çeşitlilik ve Orman Gen Kaynaklarımız, TEMA Vakfı, İstanbul. 25: 151-160.

Kang, K.S., Lindgren, D., & Mullin, T.J. (2001a). Prediction of genetic gain and gene diversity in seed orchard crops under alternative management strategies. Theoretical and Applied Genetics, 103: 1099-1107.

Kang, K.S., Harju, A.M., Lindgren, D., Nikkanen, T., Almqvist, C., & Suh, G.U. (2001b). Variation in effective number of clones in seed orchards. New Forests, 21: 17-33.

Kang, K.S., Lindgren, D., & Mullin, T.J. (2004). Fertility variation, genetic relatedness, and their impacts on gene diversity of seeds from a seed orchard of *Pinus thunbergii*. Silvae Genetica, 53(5-6): 202-206.

Kaya, N. 2005. Orman ağaçlarında eşleşme şekilleri. Süleyman Demirel Üniversitesi, Orman Fakültesi Dergisi, 2: 125-137.

Kaya, N., Isik, K., & Adams, W.T. (2006). Mating system and pollen contamination in a *Pinus brutia* seed orchard. New For 31: 409-416.

Kaya, Z., Skaggs, A., & Neale, D.B. (2008). Genetic differentiation of *Abies equi-trojani* (Asch.& Sint. ex Boiss) Mattf. populations from Kazdağı, Turkey and the genetic relationship between Turkish Firs belonging to the *Abies nordmanniana* Spach complex. Turkish Journal Botany, 32:1-10.

Kess, T., & El-Kassaby, Y.A. (2015). Estimates of pollen contamination and selfing in a coastal Douglas-fir seed orchard. Scandinavian Journal of Forest Research, 30(4): 266-275. https://doi.org/10.1080/02827581.2015.1012112

Kocaman, B., Toy, S., & Maraklı, S. (2020). Application of different molecular markers in biotechnology. International Journal of Science Letters, 2(2): 98-113.

Kurt, Y., González-Martínez, S.C., Alía, R., & Isik, K. (2012). Genetic differentiation in *Pinus brutia* Ten. using molecular markers and quantitative traits: the role of altitude. Annals of Forest Science, 69: 345-351. https://doi.org/10.1007/s13595-011-0169-9

Li, Y.C., Korol, A.B., Fahima, T., Beiles, A., & Nevo, E. (2002). Microsatellites: genomic distribution, putative functions and mutational mechanisms: a review. Molecular Ecology, 11: 2453-2465.

Lowe, W.J., & Wheeler, N.C. (1993). Pollen contamination in seed orchards. In: D.L. Bramlett, G.R. Askew, T.D. Blush, F.E. Bridgwater and J.B. Jett (eds). Advances in Pollen Management. pp.49-54. USDA Agric. Handb. 698. Washington, DC.

Myers, E.R., Chung, M.Y., & Chung, M.G. (2007). Genetic diversity and spatial genetic structure of *Pinus strobus* (Pinaceae) across an island landscape inferred from allozyme and cpDNA markers. Plant Systematics and Evolution, 264: 15-30.

Navascues, M., Vaxevanidou, Z, Gonzalez-Martinez, S.C., Climent, J., Gil, L., & Emerson, B.C. (2006). Chloroplast microsatellites reveal colonization and metapopulation dynamics in the Canary Island pine. Molecular Ecology, 15: 2691-2698.

Naydenov, K.D., Tremblay, F.M., Bergeron, Y., Alexandrov, A., & Fenton, N. (2005a). Dissimilar patterns of *Pinus heldreichii* Christ. populations in Bulgaria revealed by chloroplast microsatellites and terpenes analysis. Biochemical Systematics and Ecology, 33: 133-148.

Naydenov, K.D., Tremblay, F.M., Alexandrov, A., & Fenton, N.J. (2005b). Structure of *Pinus sylvestris* L. populations in Bulgaria revealed by chloroplast microsatellites and terpenes analysis: Provenance tests. *Biochemical Systematics and Ecology*, 33: 1226-1245.

Naydenov, K.D., Tremblay, F.M., Fenton, N.J., & Alexandrov, A. (2006). Structure of *Pinus nigra* Arn. populations in Bulgaria revealed by chloroplast microsatellites and terpenes analysis: Provenance tests. Biochemical Systematics and Ecology, 34: 562-574.

OATIAM. (2023). General Directorate of Forestry. Forest Tree Seeds and Tree Breeding Research Directorate. http://www.ortohum.gov.tr/

Ohsawa, T., & Ide, Y. (2008). Global patterns of genetic variation in plant species along vertical and horizontal gradients on mountains. Global Ecology and Biogeography, 17: 152-163.

Oliveira, E.J., Padua, J.G., Zucchi, M.I., Vencovsky, R., & Carneiro-Vieria, M.L. (2006). Origin, evolution and genome distribution of microsatellites. Genetics and Molecular Biology, 29(2): 294-307.

Pakkanen, A., Nikkanen, T., & Pulkkinen, P. (2000). Annual Variation in Pollen Contamination and Outcrossing in a *Picea abies* Seed Orchard. Scandinavian Journal of Forest Research, 15: 399-404.

Plomion, C., Leprovost, G., Pot, D., Vendramin, G., Gerber, S., Decroocq, S., Brach, J., Raffin, A., & Pastuszka, P. (2001). Pollen contamination in a maritime pine polycross seed orchard and certification of improved seeds using chloroplast microsatellites. Canadian Journal of Forest Research, 31: 1816-1825.

Pupin, S., Sebbenn, A.M., Cambuim, J., da Silva, A.M., Zaruma, D.U.G., Silva, P.H.M., Rosse, L.N., Souza, I.C.G., Marino, C.L., & Moraes, M.L.T. (2019). Effects of pollen contamination and non-random mating on inbreeding and outbreeding depression in a seedling seed orchard of *Eucalyptus urophylla*. Forest Ecology and Management, 437: 272-281. https://doi.org/10.1016/j.foreco.2019.01.050.

Ribeiro, M.M., Mariette, S., Vendramin, G.G., Szmidt, A.E., Plomion, C., & Kremer, A. (2002). Comparison of genetic diversity estimates within and among populations of maritime pine using chloroplast simple-sequence repeat and amplified fragment length polymorphism data. Molecular Ecology, 11: 869-877.

Semagn, K., Bjornstad, A., & Ndjiondjop, M.N. (2006). An overview of molecular marker methods for plants. African Journal of Biotechnology, 5(25): 2540-2568.

Sheller, M., Ciocîrlan, E., Mikhaylov, P., Kulakov, S., Kulakova, N., Ibe, A., Sukhikh, T., & Curtu, A.L. (2021). Chloroplast DNA diversity in populations of *P. sylvestris* L. From Middle Siberia and the Romanian Carpathians. Forests, 12: 1757. https://doi.org/10.3390/f12121757

Slavov, G.T., Howe, G.T., Yakovlev, I., Edwards, K.J., Krutovskii, K.V., Tuskan, G.A., Carlson, J.E., Strauss, S.H., & Adams, W.T. (2004). Highly variable SSR markers in Douglas-fir: Mendelian inheritance and map locations. Theoretical and Applied Genetics, 108: 873-880.

Slavov, G.T., Howe, G.T., & Adams, W.T. (2005). Pollen contamination and mating patterns in a Douglas-fir seed orchard as measured by simple sequence repeat markers. Canadian Journal of Forest Research, 35(7): 1592-1603.

Sonstebo, J.H., Tollefsrud, M.M., Myking, T., Steffenrem, A., Nilsen, A.E., Edvardsen, O.M., Johnskas, O.R., & El-Kassaby, Y.A. (2018). Genetic diversity of Norway spruce (*Picea abies* (L.) Karst.) seed orchard crops: Effects of number of parents, seed year, and pollen contamination. Forest Ecology and Management, 411: 132-141. https://doi.org/10.1016/j.foreco.2018.01.009.

Sniezko, R.A. (1981). Genetic and economic consequences of pollen contamination in seed orchards. In: Proceedings, 16th Southern Forest Tree Improvement Conference; 1981 May 27-28: Blacksburg, VA. Blacksburg: Virginia Polytechnic Institute: 225-233.

Soto, A., Robledo-Arnuncio, J.J., Gonzalez-Martinez, S.C., Smouse, P.E., & Alia, R. (2010). Climatic niche and neutral genetic diversity of the six Iberian pine species: a retrospective and prospective view. Molecular Ecology, 19: 1396-1409.

Stoehr, M.U., & Newton, C.H. (2002). Evaluation of mating dynamics in a lodgepole pine seed orchard using chloroplast DNA markers. Canadian Journal of Forest Research, 32: 469-476.

Sütcü, T., Bilgen, B.B., & Tuna, M. (2022). Analysis of genetic diversity among *Onobrychis* accessions with high agronomic performance by simple sequence repeat (SSR) markers. Molecular Biology Reports, 49: 5659-5668. https://doi.org/10.1007/s11033-022-07584-x

Terrab, A., Paun, O., Talavera, S., Tremetsberger, K., Arısta, M., & Stuessy, T.F. (2006). Genetic diversity and population structure in natural populations of Moroccan Atlas cedar (*Cedrus atlantica*; Pinaceae) determined with cpSSR markers. American Journal of Botany, 93(9): 1274-1280.

Torimaru, T., Wang, X.R., Fries, A., Andersson, B., & Lindgren, D. (2009). Evaluation of pollen contamination in an advanced scots pine seed orchard. Silvae Genetica, 58(5-6): 262-269.

Tunçtaner, K. (2007). Orman genetiği ve ağaç ıslahı, Türkiye Ormancılar Derneği, Eğitim Dizisi: 4, 364 s.

Urbaniak, L., Wojnicka-Poltorak, A., Celinski, K., Lesiczka, P., Pawlaczyk, E., & Aucina, A. (2019). Genetic resources of relict populations of *Pinus sylvestris* (L.) in Western Carpathians assessed by chloroplast microsatellites. Biologia, 74: 1077-1086. https://doi.org/10.2478/s11756-019-00255-6

Varshney, R.K., Graner, A., & Sorrels, M.E. (2005). Genic microsatellite markers in plants: features and applications. Trends in Biotechnology, 23: 48-55.

Vendramin, G.G., Lelli, L., Rossi, P., & Morgante, M. (1996). A set of primers for the amplification of 20 chloroplast microsatellites in Pinaceae. Molecular Ecology, 5: 595-598.

Vieira, M.L.C., Santini, L., Diniz, A.L., & Munhoz, C.F. (2016). Microsatellite markers: what they mean and why they are so useful. Genet Mol Biol., 39: 312-328.

Wheeler, N., & Jech, K. (1986). Pollen contamination in a mature, Douglas-fir seed orchard. Proc. IUFRO Conf. Breeding, Theory, Progeny testing of seed orchards. Oct. 13-17, 1986. Williamsburg, VA.

Yang, H., Zhang, R., & Zhou, Z. (2017). Pollen dispersal, mating patterns and pollen contamination in an insect-pollinated seed orchard of *Schima superba* Gardn. et Champ. New Forests, 48: 431-444. https://doi.org/10.1007/s11056-017-9568-6

Zhuowen, Z. (2002). Pollen dispersal and its spatial distribution in seed orchards of *Cunninghamia lanceolata* (LAMB.) Hook. Silvae Genetica, 51(5-6): 237-241.

Zobel, B.J., & Talbert, J. (1984). Applied forest tree improvement. John Wiley and Sons, Inc. New York, 505 p.