論 文 の 内 容 の 要 旨

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平成 17 年度博士課程　進学
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論文題目　Study on genetic diversity and mating system in a seedling seed orchard of *Pinus merkusii*
(メルクシマツ 実生採種園における遺伝的多様性と交配様式に関する研究)

Pinus merkusii is the only pine that distributes across the equator and has a feature of year round reproduction. It is one of the important tree species in Indonesia for wood materials and resin production. Its genetic improvement was started in the mid 1970th with the establishment of seedling seed orchard (SSO) by planting selected families from artificial plantations in thirteen districts of Java Island, Indonesia.

Panmictic equilibrium is a key factor to make a breeding strategy in success by transforming parental genetic diversity to the filial generation. Moreover, synchronization of flowering among parent trees affects on quantity and quality of produced seed by influencing mating system. Thus, study on flowering characteristics of *P. merkusii* is important to ensure the sustained production of high quality seed crops under its distinguishing flowering character. However, there was no previous study dealing with the relationship between mating system and genetic diversity of *P. merkusii*.

This study aimed (1) to assess the status of mating, the level of genetic diversities maintained in the parental population and in the offspring population were determined and (2) to clarify the seasonal variations in the mating system that may influence on the productivity and the genetic diversity of the seed crops, seasonal differences in flowering and mating were assessed in the SSO. All the researches in this thesis were based on the data and materials from the SSO at Jember, East Java, Indonesia.

This thesis was composed by six chapters. The first chapter is a general introduction, where background of this study, taxonomic and biological features of *P. merkusii*, reviews on genetic diversity and mating system in seed orchards, and purposes of this study were described. A breeding plan for *P. merkusii* in Indonesia and the SSO used in this thesis were explained in Chapter 2.

In Chapter 3, development of microsatellite markers for *P. merkusii* to study genetic diversity and mating system was described. Ten microsatellite markers were successfully isolated by a dual-suppression-PCR technique. Of ten microsatellite loci isolated, five were codominant and polymorphic, two were monomorphic, two were multiband and one locus was not amplified. The number of alleles observed for each locus ranged from 3 to 6. The value of H_E ranged from 0.389 to 0.728. Three loci were deviated significantly from HWE, due to an excess of homozygotes. Null-alleles were expected to be present at these loci. On the other hand, closely related individuals present in SSO may also be responsible for these HWE deviations. No evidence for linkage disequilibrium was detected for these loci.

In Chapter 4, the level of genetic diversity maintained in the SSO and in the produced offspring was determined using five microsatellite markers. And the mating status in the SSO was discussed based on the results.

The genetic diversity parameters were quite similar among districts where mother trees in the SSO were selected, and F_{ST} value among districts was very low (0.008). These results indicate that there was a high degree of genetic uniformity among districts of parent sources. Our data support the suggestion in a previous study that the trees in the SSO originate from materials with limited genetic diversity.

Allelic richness was higher in the offspring populations (mean=3.90) than in the parental populations (mean=3.48) in every block. H_O and H_E were higher in the offspring (mean=0.601 and 0.545, respectively) than in the parental populations (mean=0.471 and 0.489, respectively) in most of the blocks. The F_{IS} values did not significantly deviate from zero both in parental and offspring population. Significant linkage disequilibrium was only observed in the parental populations (mean=2.00). The effective population size calculated was higher in the offspring populations (mean=606.22) than in the parental populations (mean=486.53). The F_{ST} values among parental populations and offspring populations were 0.038 and 0.029 respectively. These findings suggest that seed production in the SSO may be nearly panmictic and that genetic exchange freely occurs among the parent trees. Thus, high genetic diversity is still maintained in the offspring generation, in spite of the genetic similarity of trees in the SSO.

Seasonal variations in mating system in the SSO were studied in Chapter 5, which was composed by two sub chapters.

In the first sub chapter, number of female and male flowers were assessed in the three different seasons, abundant female flowers but few male flowers in August (62.2 vs. 4.5), similar numbers of female and male flowers were observed in November (37.9 vs. 28.4) and low numbers of both female and male flowers in March (35.3 vs. 5.4). The numbers of pollen grains observed were low in August (1.2±0.7) and March (1.3 ±1.5) and much higher in November (3.4±6.1). These results show that the abundance of pollen roughly corresponded to the frequencies of male flowers. The number of seeds per cone was higher in March than in both November and August. The number of filled seeds per cone was high in both November and March, but low in August. Since disproportionate flowerings can lead to insufficient pollen, and thus adversely affect the number of filled seeds by increasing the selfing rate. Insufficient pollen lead poor quality pollen grains can make substantial contributions to fertilization, thereby yielding weak progeny.

Among progeny populations, N_A values were similar in November (21) and March (22) and highest in August (27). H_O was higher in August (0.384) than in both November (0.349) and March (0.314), while H_E was similar in August (0.402) and November (0.397), but low in March (0.361). The F_{IS} was lower in August (0.046) than in November (0.120) and March (0.131). The *L-D* was highest in August (6) and similar between November (2) and March (2). Insufficient pollen may cause substantial rates of pollination from distant pollen, which may explain higher N_A and the H_E value in the August was higher than for both November and March. Another caution in insufficient pollen population was an opportunity of selfing. Then we suggested that high value of significant *L-D* in August might indicate the occurrences of non-random mating. In November, near-panmictic equilibrium and minimum selfing rates could be expected due to balance in number female vs. male flowers and sufficient pollen. Thus, value of H_E in November was high. In March, limitations in both female and male flowering may lead restricted pollen dispersal contributed to the lowest level of H_E and hence high F_{IS}. Moreover, the pairwise F_{ST} values between parent population and offspring populations were significantly different; the values were 0.034, 0.031 and 0.054 in August, November and March, respectively. Low values of F_{ST} may confirm the occurrences of extent gene flow mating in August and nearly panmictic in November.

In the next sub chapter, seasonal patterns of mating system and gene flow in each season were confirmed using three models: a radius-based analysis, mixed-mating model and neighborhood model.

A radius-based analysis revealed the F_{IS} values were insignificant at radii up to 108 m, 36m and 12m for the mating in August, November and March, respectively. The *L-D* values were high in August (1-6), and low in November and March (1-2 in both cases). Changes in genetic differentiation (pairwise F_{ST}) between offspring population in radius 12m and parental populations in different radius were high in August and March, but low in November. The occurrence of substantial fertilization from distant pollen that suggested for the mating in August could be confirmed by distant radius size to get significant F_{IS}. High selfing could be confirmed by high value of *L-D*. Mating among surrounding trees in November could be confirmed by moderate radius size to get significant F_{IS}. Low value of F_{ST} could explain panmictic equilibrium in November. Restriction on gene flow for March mating could be confirmed by lowest distant to obtain significant value of F_{IS} and high value of F_{ST}.

Mixed-mating model showed that the mean multilocus outcrossing rate (t_m) was higher in November (0.962) than in both August (0.941) and March (0.956). Correlation of paternity (r_p) was highest for the August progeny (0.304) and moderate for both the November (0.205) and March progenies (0.193). Insufficient pollen in August may cause low t_m value and high r_p, in contrast sufficient pollen in November and March might cause high t_m and low r_p. Lowest value of t_m indicated high selfing in August. Indeed, highest value of r_p showed the insufficient pollen. High tm and low r_p in November and March represented the sufficient pollen in both seasons.

One of the advantages of Neighborhood model is describing effect of migratory pollen (m). Estimated selfing rates obtained by the mixed-mating model and neighborhood model were comparable. Selfing was higher in August (s=0.06) than in November (0.02) and March (0.04). Substantial fertilization from distant pollen in August could be explained by highest value of immigration pollen rate (m=0.03) than November (0.01) and March (0.02). Panmictic equilibrium in November could be confirmed by higher value of effective population size (71.42%) than August (54.08%) and March (24.68%).

Using these three methods, we succeeded in describing the real seasonal mating system in the SSO which depend on their flowering characteristics.

In Chapter 6, general discussion was conducted based on the results and some implications for the genetic improvement of *P. merkusii* were proposed.

While the parent trees in the SSO were genetically similar among seed sources, they kept moderate level of diversity. The genetic diversity in the SSO was maintained in offspring populations by nearly panmictic mating and comparatively high gene flows. However, temporal fluctuation of flowering greatly affected seed production and gene diversity of offsprings. Disproportionate flowerings might lead insufficient pollens that make weak pollen competition. In contrast, balanced number of female and male flowers might promote panmictic equilibrium. Differences in flowering characteristics influenced the mating system and patterns gene flow in spatial area in the SSO that caused differences in seed production and their genetic diversity among seasons. However, since parent trees in the SSO tend to have high outcrossing rate, so high genetic diversity of the seed crops from the SSO population may be expected for all mating season such had been examined in Chapter 4. The results in this study indicated that flowering and mating status should be considered for good seed production. In addition, reproductive phenology should be included in the criteria to consider in the layout and design of future seed orchard to enhance panmixia. The study on reproductive process of *P. merkusii* should be employed more frequent to obtain more accurate estimations on mating system in the SSO.