

## FERTILITY VARIATION OF *Melaleuca cajuputi* subsp. *cajuputi* AND ITS IMPLICATION IN SEED ORCHARD MANAGEMENT

Noor K. Kartikawati\*<sup>1</sup>

<sup>1</sup>Biotechnology and Forest Tree Improvement Research and Development Institutes, Jl. Palagan Tentara Pelajar Km.15, Purwobinangun, Pakem, Sleman, Yogyakarta, Indonesia

Received: 16 February 2015, Revised: 16 September 2016, Accepted: 19 September 2016

FERTILITY VARIATION OF *Melaleuca cajuputi* subsp. *cajuputi* AND ITS IMPLICATION IN SEED ORCHARD MANAGEMENT. Information about fertility variation of flowering trees in seed orchard including determining the quality of seed production and estimating the genetic diversity are still lacking. This paper evaluates fertility variation, effective population size and genetic diversity among cajuput trees in seedling seed orchard at Paliyan, Gunungkidul for optimizing seed orchard management. A total of 160 trees were observed in three flowering periods of 2011-2013. The fertility based on the number of flowers and fruits were registered for each tree at the age of 12, 13 and 14 years. Results show that there are similar patterns of fertility after three years observation. Sibling coefficients ( $\Psi$ ) which show fertility variation during three flowering periods are 1.39, 1.25 and 1.43 respectively. They show deviation from random mating, because of individual imbalance for producing flowers and fruits. However, the number of fertile trees was comparatively higher at 2011. More than 15 families of effective population size were recorded each year and supported more than 75% individuals in seed orchard to contribute flowers and seeds. High value of genetic diversity was calculated based on fertility variation (0.965, 0.967 and 0.957, respectively). Fertility variation led to consequence on seed deployment, including seeds of fertile families which should be collected equally and mixed to compose equal proportion of seeds and to avoid domination of highly fertility families and genetic drift. Silvicultural treatments in seed orchard management were indispensable to promote fertility uniformity and to increase effective population size in seed orchard for obtaining maximal genetic gain.

Keywords: *Melaleuca cajuputi*, seed orchard, fertility variation, effective population size, genetic diversity

*VARIASI FERTILITAS Melaleuca cajuputi subsp. cajuputi DAN IMPLIKASINYA TERHADAP MANAJEMEN KEBUN BENIH. Informasi tentang variasi fertilitas pembungaan di kebun benih meliputi penentuan kualitas produksi benih dan perkiraan keanekaragaman keturunan genetik masih jarang tersedia. Penelitian ini bertujuan untuk mengevaluasi variasi fertilitas, ukuran populasi efektif dan keragaman genetik yang dihitung berdasarkan nilai koefisien sibling ( $\Psi$ ) di kebun benih kayu putih di Paliyan, Gunungkidul untuk mengoptimalkan pengelolaan kebun benih ke depan. Sejumlah 160 pohon induk di kebun benih dievaluasi berdasarkan jumlah pohon berbunga, jumlah bunga, jumlah buah selama 3 periode pembungaan (2011-2013). Hasil penelitian menunjukkan bahwa variasi fertilitas ( $\Psi$ ) berturut-turut adalah 1,39, 1,25 dan 1,43. Hal ini mengindikasikan terjadi penyimpangan dari perkawinan secara acak karena ada variasi fertilitas dari pohon-pohon di kebun benih. Pengamatan dalam 3 periode pembungaan menunjukkan pola yang cenderung sama, namun jumlah individu berbunga terbanyak terjadi pada tahun 2011. Ukuran populasi efektif ( $N_p$ ) diperoleh 15, menunjukkan 15 famili yang didukung oleh 75% pohon-pohon di kebun benih berkontribusi terhadap pembungaan. Keragaman genetik yang dihitung berdasarkan variasi fertilitas menunjukkan angka yang cukup tinggi yaitu diatas 0,9 dalam 3 periode pengamatan. Sebagai konsekuensi akibat variasi fertilitas adalah dalam penyebaran benih perlu dilakukan pencampuran benih dari semua famili yang memproduksi benih dalam proporsi yang seimbang agar tidak terjadi dominasi famili-famili tertentu dan terjadinya damparan genetik (genetic drift). Tindakan manipulasi lingkungan perlu dilakukan untuk mendorong terjadinya keseragaman fertilitas dalam kebun benih sehingga seluruh potensi genetiknya dapat dinikmati.*

*Kata kunci:* Melaleuca cajuputi, kebun benih, variasi fertilitas, ukuran populasi efektif, keragaman genetik

\* Corresponding author: aticka\_kart@yahoo.com

## I. INTRODUCTION

*Melaleuca cajuputi* subsp. *cajuputi* or cajuput is one of *Melaleuca* species producing essential oil, mainly 1.8 cineole. It grows naturally in Northern Territory, Western Australia and East Timor (Brophy, Craven, & Doran, 2013). Cajuput plantation is the main source of cajuput oil industry in Indonesia. Breeding program of cajuput carried out by Biotechnology and Forest Tree Improvement Research and Development Institute, Yogyakarta, in collaboration with Commonwealth Scientific and Industrial Research Organization (CSIRO) Australia has established seed orchards in Paliyan, Ponorogo and Cepu. The seed orchards are expected to produce genetically improved seed in an adequate quantity to improve productivity of cajuput plantation. The expected genetic gain 10% of 1.8 cineole content and 21% of oil yield (Susanto, Doran, Arnold, & Rimbawanto, 2003) has been predicted, based on assumption of 100% effective population size. However, variation of fertility and mating system might reduce genetic gain, diversity and vigor of seed crop (Kamalakkanan, Varghese, & Lindgren, 2007).

The genetic structure of seed orchard offspring is determined by several factors and processes, namely flowering synchrony, fertility variation, mating system components (inbreeding/outbreeding, pollen dispersal within the seed orchard, and pollen contamination from outside sources), composition and size of the maternal population and viability selection operating

on zygotes. The reproduction dynamic in seed orchard (flowering time, fertility variation, pollen dispersal, and mating system) is an important consideration in management of seed orchard to deliver good quality of seed. In reality, asynchrony phenology, fertility variation and pollen contamination are very common in seed orchard, both in conifer and broad leaves species.

Knowledge about fertility variation is important for seed orchard managers, especially with respect to seed production output, and also for prediction of gene diversity (Bila, Lindgren & Mullin, 1999; Kang & Mullin, 2007). Most seed orchard's fertility variation studies have shown that there are fertility variation among trees in a seed orchard, ranged from 1.0 to 41.7 (Kang, Bila, Harju, & Lindgren, 2003). Variation of fertility can influence the genetic gain and genetic diversity of the offspring, hence not all genetic potential is heritable to the offspring.

However, information about fertility variation in seed orchard has not been always available including in cajuput as the species of interest in this study. Therefore, a research was carried out with the main objective to evaluate fertility variation, effective population size and genetic diversity among trees in the seedling seed orchard of cajuput at Paliyan, Gunungkidul.

## II. MATERIAL AND METHOD

### A. Seed Orchard

The research was carried out at the seedling seed orchard at Paliyan, Gunungkidul, Yogyakarta (at 7° 59'10.4"S latitude, 110°29'10.8



Figure 1. Location of Paliyan Seedling Seed Orchard

°E longitude and 150 m altitude, annual rainfall 2,000 mm) (Figure 1). It was established in 1998 using Randomized Complete Block Design with 19 families (hal-sib) consisting of 10 tree plots (line plots) per family and 10 blocks as replication (Table 1). The seed orchard covers an area of 0.5 ha with initial spacing of 3m x 1.5m. After final thinning, this seed orchard consisted of 160 individuals.

### B. Assessment of Flowering

Flower and fruit production were recorded during three flowering periods (2011-2013). An assembled bamboo ladder was used to climb to the top of the tree for data collection (Figure 2). The number of primary, secondary and tertiary branches was counted in each sample tree. The number of flowers in one inflorescence was recorded based on 10 samples collected from inflorescences in each tree. The total numbers of flower and fruit of each tree were obtained by extrapolating the count made on fruits in top crown, middle and lower crown (Bila et al., 1999; Kang & Lindgren, 1999). Observation was conducted at peak flowering.

### C. Variation in Fertility

Cajuputi flowers was categorized as hermaphrodite. Male and female fertility of a tree is measured by the estimated number of flowers and fruits respectively produced by the tree. Gender fertilities were calculated as the number of reproductive structures (flowers and fruits) produced by a tree relative to the total structures produced by all trees. It was computed based on the estimated number of flowers (assumed to be equal to male fertility) and fruits (assumed to be equal to female fertility) for each tree. Total fertility of a tree ( $\bar{p}_i$ ) was taken as the average of the male ( $m_i$ ) and female ( $f_i$ ) fertilities of each tree. Sibling coefficient ( $\Psi$ ) is the probability that two genes randomly drawn from the gamete gene pool originates from the same parent compared to the probability if the parents have equal representation (Kang & Lindgren, 1999). Sibling coefficient is a measure used to describe fertility variation among the trees in a population. It is calculated from the number of families in the orchard ( $N$ ) and individual fertility ( $p_i$ ) of each family as the following:



Figure 2. Picture of branches of *Melaleuca cajuputi* subsp. *cajuputi*

Table 1. Detail information of cajuputi families using as material in Paliyan SSO

No Family	Seedlot	Oil content (W/W % DW)	Cineole (%)	Provenance name	Latitude (°S )	Longitude (°E )	Altitude (m)
1	MM2033	3,88	47	Ratagelombeng, Buru	03°08'33"	126°54'36"	40
2	MM2054	2,53	62	Masarete, Buru	03°22'38"	127°08'12"	20
3	MM2057	2,45	59	Masarete, Buru			
5	MM2060	2,84	59	Masarete, Buru			
25	MM2064	1,01	32	Masarete, Buru			
8	BVG2913	3,35	47	Waipirit, Seram	03°19'43"	128°20'20"	10
9	BVG2919	3,02	57	Pelita Jaya, Seram	03°03'00"	128°08'00"	100
10	BVG2920	2,79	52	Pelita Jaya, Seram			
11	BVG2923	1,82	67	Pelita Jaya, Seram			
12	BVG2936	2,82	64	Cotonea, Seram	03°37'02"	128°18'40"	60
13	BVG2937	1,82	54	Cotonea, Seram			
14	BVG2941	2,93	60	Cotonea, Seram			
18	BVG2973	2,32	59	Suli, Ambon			
19	DL786	2,78	59	Wangi, NT Australia	13°09'00"	130°35'00"	30
20	DL1705	2,96	55	Port Keats, NT Australia	14°14'02"	129°31'11"	5
21	DL1787	4,85	47	Beagle Bay, W Australia	16°58'33"	122°40'04"	10
22	DL1797	3,47	52	Beagle Bay, W Australia			
23	DL1803	3,59	58	N Broome, W Australia	17°46'00"	122°16'00"	10
24*	Gundih ptn			Gundih Cent. Java	07 11'07	110 54'19"	60

$$\psi = N \sum_{i=1}^N p_i^2 \tag{1} \text{ Lindgren (2006):}$$

A maternal sibling coefficient ( $\psi_f$ ) and a paternal sibling coefficient ( $\psi_m$ ) can be given as (Kang & El-Kassaby, 2002):

$$\psi_f = N \sum_{i=1}^N f_i^2 \tag{2}$$

$$\psi_m = N \sum_{i=1}^N m_i^2 \tag{3}$$

**D. Co-ancestry and Effective Population Size**

Group Co-ancestry ( $\Theta$ ) is the probability that two genes chosen at random from a gene pool are identical by descent. If the trees are non-related and non-inbred, all pair co-ancestries are equal to zero and all self co-ancestries are equal to 0.5. Group Co-ancestry is calculated using the methods from Lindgren and Mullin (1998) in Varghese, Nicodemus, Nagarajan, and

$$\Theta = 0,5 \sum_{i=1}^N p_i^2 \tag{4}$$

The effective population size ( $N_p$ ) is independent of how parents are related or inbred, the status number depends on the relatedness of the parent. It is practical to have a unique term for effective number based on fertility variation among parents only.  $N_p$  is equivalent to the status number ( $N_s$ ) of a seed orchard where families are unrelated and non-inbred (Lindgren & Mullin, 1998).  $N_p$  is calculated as follows (Kang & Lindgren, 1999):

$$N_p = \frac{N}{\psi} \tag{5}$$

**E. Genetic Diversity**

Genetic diversity is the function of the group co-ancestry and can be calculated in the

orchard relative to a reference population. The reference population (natural forest) has zero group co-ancestry as it is considered to have an infinite number of unrelated individuals (Varghese et al., 2006). The genetic diversity ( $GD$ ) is calculated as follows:

$$GD = 1 - \Theta \dots\dots\dots(6)$$

### III. RESULT AND DISCUSSION

#### A. Variation of Fertility

Observation during three flowering periods in cajuput seedling seed orchard showed significant number of flower, fruit and fruitset which contributed 143 (89%) individuals in 2011; 113 (70%) individuals in 2012 and 121 (76%) in 2013. Fertility variation, effective population size and genetic diversity in three flowering seasons are shown in Table 2.

If each individual in cajuput seed orchard had the same fertility value ( $\Psi$ ), it would be equal to 0.00625. In fact, the fertility value of individuals ranged from 0 to 0.031. This indicates unequal contribution among individuals in seed production. The same trend was obtained in fertility observation in

consecutive year. Dominant individuals in seed production impacted on genetic variation as a majority of seed produced from seed orchard. The observation in 3 successive years of flowering periods showed low variation of total fertility ( $\Psi$ )=1.25 – 1.47) while female fertility variation ( $\Psi_f$ ) varied between 1.59 – 1.71 Male fertility ( $\Psi_m$ ) showed varied 1.40 to 1.75, almost similar to female fertility. These indicate balance in fertility between female and male, as available pollen was sufficient for fertilizing the ovule.

Although fertility variation and number of trees producing flower decreased between 2011 and 2012, the fruiting ability increased to 15%, which was possibly due to the climatic factors. The total precipitation and the rainy days in each year would have an impact on fertility of trees. Lower precipitation in 2012 (1,904 mm) compared to precipitation in 2011 (2,163mm) (Dinas Tanaman Pangan dan Perikanan, 2012, 2013) was received in location during the observation periods. Therefore, pollinator activity might be more intensive in 2012 and fertilization could be optimized. During the same periods, it showed low deviation of the

Table 2. Average of diameter, height, flower, fruitset, fertility variation, effective population size and genetic diversity of cajuput seedling seed orchard at Paliyan, Gunungkidul

Remarks	Observation in 2011	Observation in 2012	Observation in 2013
Age (yr)	12	13	14
Average diameter (cm)	14.3	14.9	15.4
Average height (cm)	7.80	8.01	8.75
Average flower per tree	2116	1622	1967
Average fruit per tree	1293	825	1063
Average fruitset	0.53	0.68	0.61
Number of trees	160	160	160
Fertile trees (%)	89	70	76
Male fertility variation ( $\Psi_m$ )	1.545	1.405	1.75
Female fertility variation ( $\Psi_f$ )	1.710	1.599	1.63
Total fertility variation ( $\Psi$ )	1.392	1.250	1.47
Coancestry ( $\theta$ )	0.035	0.033	0.043
$N_s$ ( Status number)	15.1	14.9	15.4
Genetic diversity	0.965	0.967	0.957

daily average of air temperature (24°C in the morning to 30°C in the afternoon) and air humidity (70% in the morning and decreased to 56% in the afternoon) (Dinas Tanaman Pangan dan Perikanan, 2012, 2013). This low deviation did not influence pollinator activities.

Kang et al. (2003) reported that a sibling coefficient of 2 would be typical in good or moderate flowering years in mature seed orchard. Recent study on cajuput seed orchard in Paliyan, Gunungkidul revealed fertility variation of 1.39. This indicates that there was a deviation of 1.39 times from random mating to inbreeding. The deviation from random mating might be due to flowering asynchrony, family arrangement, and combining abilities or pollen contamination; so that it changed the allele frequency in a population (Kang & Mullin, 2007). The sibling coefficient ( $\Psi$ ) expresses the increase in the probability that sibs or relatives occur in comparison to the ideal situation where families have equal fertility. Codesido and Fernandez-Lopez (2014) mentioned if there was a perfect positive correlation ( $r = 1$ ) between gender fertilities, the sibling coefficients for maternal, paternal and total fertility were the same ( $\Psi = \Psi_f = \Psi_m$ ). On the other hand, if there was a perfect negative correlation ( $r = -1$ ), the sibling coefficient for total fertility equals one ( $\Psi = 1$ ) and  $\Psi_f = \Psi_m$ . In dioecious, species female

fertility and male fertility are usually unbalanced. When the difference in fertility between genders is large, it may significantly affect the gene diversity of the offspring in seed orchard (Ertekin, 2010; Kang, & Kim, 2012). In this study, observations in three consecutive years showed low variation of fertility in cajuput seed orchard at Paliyan, Gunungkidul, and indicated similarity of fertility, included between female fertility and male fertility. In general, fertility variation tends to be small and effective large number of good seed production. Assessment in three successive years (2011- 2013) indicated high seed production and low fertility variation.

Fertility of trees may change from time to time. The previous research showed that fertility of trees is an interaction of 2 major factors. The first factor is genetics (Gömöry, Bruchanik, & Paule, 2000) and environment, which includes precipitation, temperature, and days length (Lesica & Kittelson, 2010; Giménez-Benavides, García-Camacho, Iriondo, & Escudero, 2010). The second factor is seed orchard management practices, including top pruning, fertilizer and hormone application for flower stimulation (Cherry, Anekonda, Albrecht, & Howe, 2007). Fertility variation also occurs in seed orchard of some species, such as *Eucalyptus camadulensis* and *E. tereticornis* (Kamalakkannan et al., 2007), *Chamaecyparis*

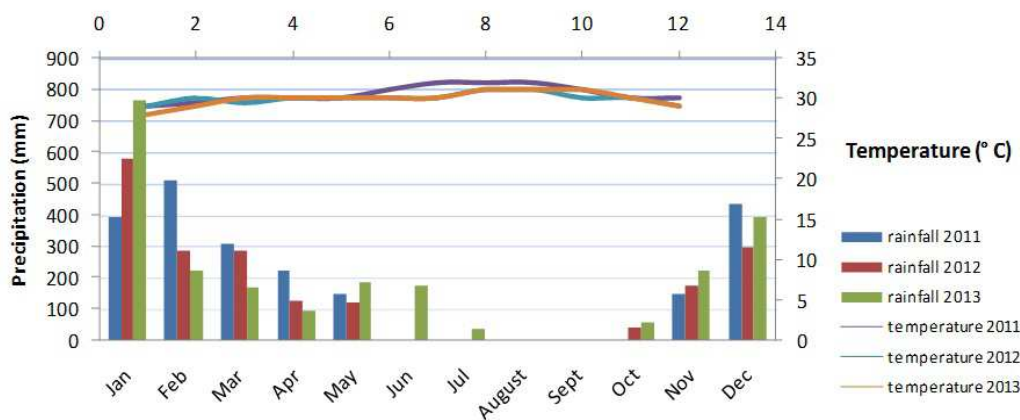


Figure 3. Profile of precipitation and temperature at Paliyan 2011-2013

*obtusata* in Korea (Kang & Mullin, 2007); *Tectona grandis* in India (Varghese, Kamalakannan, Nicodemus, & Lindgren, 2008); *Tectona grandis* in Cepu, Indonesia (Sumardi, 2011) and some pines (Bilir, Kang, & Ozturk, 2002; Bilir, & Temiraga, 2012).

The assessment of fertility variation in 3 successive years indicated that flowering time and duration may depend on a large extent of temperature increase, and mainly on water availability. In the first year of assessment (2011), the stand which received rainfall 2,163 mm/year (Dinas Tanaman Pangan dan Perikanan, 2012), showed longer duration and more abundant of flowering production compared to the second assessment (2012) which had rainfall of 1,904 mm/year (Dinas Tanaman Pangan dan Perikanan, 2013). While in 2013, the stand received rainfall 2,317 mm/year (Dinas Tanaman pangan dan Perikanan, 2013). Profile of precipitation and temperature at Paliyan is shown in Figure 3. The same phenomenon was also occurred in *E. camadulensis* at Panampally, India (Varghese, Kamalakannan, Harwood, Lindgren, & McDonald, 2009), which was flowering more abundantly in wet season. Young, Boshier, and Boyle (2000) mentioned that in some cases, flowering in environment with higher humidity appeared to be delayed. However, it was followed by prolonged flowering time and more abundant of flowers. In contrast, rainfall could hamper the pollination and fertilization success through decreasing pollinator activity and stabilizing fertilization process. Baskorowati, Moncur, Cunningham, Doran, and Kanowski (2010) reported that fertilization in *Melaleuca alternifolia* occurred 4 days after pollination. As *M. cajuputi* has close relationship with *M. alternifolia*, they are likely to have similar timing phenomenon of fertilization. This study revealed that peak flowering occur during very dense precipitation, with 24 days rainfall in a month.

The site condition is influenced by not only plant growth, but also fertility of individuals. Establishment of seed orchard is usually located in a similar site to plantation distribution of

the species, such as establishment of cajuput seed orchard in Paliyan, Gunungkidul. It has similar micro site to the extensive plantation of cajuput established by Forest District of Gunungkidul. The seed orchard is located 150 m above sea level at 7°59'10.4"S and 110°29'10.8"E. The local climate is categorized as Type C according to Schmidt and Fergusson, with the precipitation of 2,100 mm per year, and soil texture is categorized as clay loam. The genetic materials for establishing cajuput seed orchard are originated from Buru Island, Ambon Island, Seram Island, Western Australia, Northern Australia and Gundih. Similar soil texture and microclimate in Gundih (Central Java) and Paliyan (Gunungkidul) supported that seedlots from Gundih were more adaptive and produced more flowers and fruits abundantly in comparison to the others. On the contrary, seedlots from Western Australia and Northern Australia were difficult to adapt to different climate and geographical condition. Of the nineteen seedlots in seed orchard studied, seedlot no 24 (from Gundih) has the best fertility, while seedlot no 21 (from Australia) has the worst fertility.

Domestication process of a species led to changes in physiology of plant, including the fertility. Individuals with high value of fertility tend to be selected for domestication, so that seed orchard or artificial plantation should have low variation of fertility. Varghese et al., (2009) reported that *Eucalyptus camaldulensis* in Panampally, India, increased fertility in the first generation of seed producing area after domestication process. Fertility variation among individuals influences the different contribution of gametes. Individuals with higher value of fertility contributed more gametes. Flowering as representation of fertility value of each individual leads to changes in pattern and level of genetic variation within population through fruit and seed production (Young et al., 2000).

## B. Genetic Diversity and Effective Population Size

The observation during three flowering periods showed variation number of flowering

individuals in peak flowering time. Examination conducted in 2011 showed that out of 160 trees in the seed orchard, 143 trees contributed to seeds production during peak flowering period. It indicated that 89% trees took part in seed production. On the contrary, examination conducted in 2012 resulted in slightly decreasing number to only 113 (70%) flowering individuals during peak flowering time. However, observation in 2013 resulted in increasing number of flowering trees.

Genetic diversity predicted based on the fertility on three observation periods is categorized to high value ( $GD=0.96$ ). It is a valuable asset for development of the next generations of seed source. Genetic diversity of seeds produced by seed orchard is influenced by many factors, including fertility variation among individuals, flowering synchrony, mating system, compatibility among individuals, and pollen contamination from outside of seed orchard (Lai et al., 2010); (Machanská, Bajcar, Longauer, & Gömöry, 2013). The maximum genetic diversity within seed orchard ( $GD=1$ ) will be achieved if all individuals in seed orchard contributed to produce seed equally. This assumption is virtually never fulfilled and is commonly observed that seed orchard produced seeds disproportionately due to small portion of flowering trees.

Kartikawati, (2015) reported flowering synchrony at Paliyan seedling seed orchard in 2011-2012 tend to synchrone. About 70-80% mother trees in the seed orchard producing flower at the same time. Furthermore, Kartikawati, Naiem, Hardiyanto, and Rimbawanto (2013) observed mating system di Paliyan seedling seed orchard in 2011 mentioned that there is random mating in that seed orchard. However, this research indicates that fertility rate among individual trees is uneven due to domination of several families. Uneven contribution was possibly due to fertility variation and flowering asynchrony in seed orchard (Gates & Nason, 2012). Kang, Lindgren, and Mullin (2001) also confirmed that variation of fertility lead to increase inbreeding

and reduce diversity in produced seeds. Therefore, genetic diversity of seed orchard is often not equal to produced seed at any time. The most important factors that determine the genetic diversity of seeds from seed orchards is the quality of flowering and flowering synchrony (Chen & Hsu, 2011). Early and late flowering individuals tend to be pollinated by related individuals or even individuals outside seed orchard with synchronous flowering phenology.

If all individuals had relatively the same fertility values, the genetic diversity of produced seed will be equal to one. The genetic diversity of produced in cajuput seed orchard at Paliyan was equal to 0.96. It indicated a reduction in the genetic diversity of 0.04. The uneven fertility in seed orchard of *E. tereticornis* leads to reduce 4.2% of genetic diversity in the second generation and 4.4% in the third generation (Varghese, Ravi, Gu Son, & Lindgren, 2003). Kang et al. (2005) also confirmed that decreasing genetic diversity in seed orchard from a generation to generation was due to fertility variation. However, the genetic diversity of cajuput seed orchard in Paliyan is categorized as high. The high genetic diversity will support future adaptability of the species.

Effective population size as a parameter which is closely correlating with the genetic and genotypic diversity of the seed orchard output is an important indicator of seed orchard function. Parent effective population size ( $Np$ ) of cajuput seed orchard in Paliyan was calculated based on fertility variation data which showed high values of parent effective population size in both flowering seasons observation of 2011 ( $Np=15.1$ ), 2012 ( $Np=14.9$ ) and 2013 ( $Np=15.4$ ). It indicated that there were 15 families in cajuput seed orchard that contributed relative evenly in gamet availability.

Effective population size influences generative reproduction of a certain species, which is closely related to flowering, pollination, and fertilization aspects. Kartikawati et al. (2013) reported that supported by a large number of effective population size, panmixia random



mating occur in *M. cajuputi* seed orchard. These factors will significantly determine seed production success, in term of its quality and quantity. A large effective population size allows to maintain genetic diversity. On the contrary, small effective population size increases the chances of inbreeding or selfing, and causes inbreeding depression. If effective population size was less than ten, the decreasing of genetic diversity on the next generation will mainly be influenced by genetic drift and very small fertility variation (Kang et al., 2001). Consequently, the decrease in seed genetic diversity will have a direct effect on the planted forest genetic diversity.

### C. Implications for Optimizing Seed Orchard Management

Evaluation results on fertility variation, effective population size, and genetic diversity are essential to scientifically support the management of cajuput seed orchard at Gunungkidul. In general, based on the performance of cajuput seed orchard, including a lot of fertile trees (more than 75%), low variation of fertility (1.25 – 1.47), high genetic diversity of offspring (0.96), and large effective population size ( $N_{ep}=15.4$ ), are highly recommended to harvest the seeds and deploy them in an operational scale of plantation. The observation on *Eucalyptus nitens* stand in South Africa with more than 40% of flowering trees resulted better growth performance of their offspring (Swain, Verry, & Laing, 2013).

The fertility variation of cajuput seed orchard indicated deviation from random mating by 1.39 times (observation in 2011), 1.25 times (observation in 2012) and 1.47 times (observation in 2013). The deviation was influenced by individual imbalance of flowering and fruiting productivity. Although the deviation is categorized as low, improvement of seed orchard management is important in order to promote fertility balance of individuals in the cajuput seed orchard.

Branch and top pruning of cajuput is essential in order to shape wide and low tree canopy and

to promote growth of lateral branch to increase flowering and fruiting, so that seed production could be developed. It has been applied in several plants (Lawande, Haldankar, Dalvi, & Parulekar, 2014). Fertilizing and hormone might be applied to stimulate and preserve flowering. Hormone application to stimulate flowering using paclobutrazole was proven to increase fertility and the number of capsules in some species of *Eucalyptus* (Varghese et al., 2009). This method could be applied to cajuput with adjusted-dose use.

The observation in three flowering periods resulted fertility variation and difference number of trees contributed to produce seeds. Management strategy should be adopted to anticipate fertility variation in difference flowering times. Seed harvesting should be maintained in the same volume for each tree to avoid the domination of certain families and to prevent genetic drift. The seeds domination of certain families stimulates the accumulation of genetically related seeds and the reduction of genetic diversity. Another strategy is to mix the seed crops of different harvesting times in order to maintain genetic diversity and effective population size in seed orchards.

### IV. CONCLUSION

The observation on flowering and fruiting time during 2011, 2012, and 2013 showed that most individuals (80-89%) in seed orchards contributed to produce seeds with fertility variation of 1.39, 1.25 and 1.47 respectively. The genetic diversity remains high (0.96) for three observation periods, with relative stable effective population.

Silvicultural treatments such as fertilization, branch pruning, and hormone paclobutrazole may be applied subject to prior experimental results. They are essential to homogenize the fertility rate and to obtain genetic improvement as expected. Fertility dynamics should be observed in some flowering periods to detect certain families that either are dominant or do not contribute in producing seeds. Management

strategy in the application of genetically improved seed should be adjusted in order to maintain (even to increase) the genetic diversity of the planted forest.

## ACKNOWLEDGEMENT

The author is very grateful to Prof. Moh. Na'iem, Dr. Eko Bhakti Hardiyanto, and Dr. Anto Rimbawanto for their invaluable comments and supports. The author would also like to express gratitude to Sunaryanto and Sunar for their help and support on data collection.

## REFERENCES

- Baskorowati, L., Moncur, M.W., Cunningham, S.A., Doran, J.C., & Kanowski, P.J. (2010). Reproductive biology of *Melaleuca alternifolia* (Myrtaceae) 2. Incompatibility and pollen transfer in relation to the breeding system, (1984), 384–391.
- Bila, A.D., Lindgren, D & Mullin, T.J. (1999). Fertility variation and its effect on diversity over generation in a teak plantation (*Tectona grandis* L.f). *Silvae Genetica*, 48, 109–114.
- Bilir, N., & Temiraga, H. (2012). Fertility Variation and Status Number in Clonal Seed Orchard of *Pinus sylvestris*. *Pakistan Journal of Biological Sciences*, 15(22), 1075–1079. <http://doi.org/10.3923/pbjs.2012.1075.1079>
- Bilir, N., Kang, K. S., & Ozturk, H. (2002). Fertility variation and gene diversity in clonal seed orchards of *Pinus brutia*, *Pinus nigra* and *Pinus sylvestris* in Turkey. *Silvae Genetica*, 51(2-3), 112–115.
- Brophy, J., Craven, L.A., & Doran, J. C. (2013). Melaleucas. Their botany, essential oils and users. (-, Ed.). Canberra: *ACLAR Monograph No. 156*. Australian Centre for International Agricultural Research.
- Chen, Y. Y., & Hsu, S. B. (2011). Synchronized reproduction promotes species coexistence through reproductive facilitation. *Journal of Theoretical Biology*, 274(1), 136–144. <http://doi.org/10.1016/j.jtbi.2011.01.013>
- Cherry, M. L., Anekonda, T. S., Albrecht, M. J., & Howe, G. T. (2007). Flower stimulation in young miniaturized seed orchards of Douglas-fir (*Pseudotsuga menziesii*). *Revue Canadienne De Recherche Forestiere*, 37(1), 1–10. <http://doi.org/10.1139/X06-199>
- Codesido, V., & Fernandez-Lopez, J. (2014). Juvenile radiata pine clonal seed orchard management in Galicia (NW Spain). *European Journal of Forest Research*, 133(1), 177–190. <http://doi.org/10.1007/s10342-013-0757-3>
- Dinas Tanaman Pangan dan Perikanan. (2012). Data curah hujan Kabupaten Gunungkidul. Gunungkidul.
- Dinas Tanaman Pangan dan Perikanan. (2013). Data curah hujan Kabupaten Gunungkidul. Gunung Kidul.
- Doran, J.C., Rimbawanto, A., Gunn, B.V., & Nirsatmanto, A. (1998). Breeding Plan for *Melaleuca cajuputi* subsp. *cajuputi* in Indonesia. (-, Ed.) (- ed.). Indonesia: CSIRO Forestry and Forest Product, Australia Tree Seed Centre and Forest Tree Improvement Research and Development Institute.
- Ertekin, M. (2010). Clone fertility and genetic diversity in a Black Pine seed orchard. *Silvae Genetica*, 4(December 2008), 145–150.
- Gates, D. J., & Nason, J. D. (2012). Flowering asynchrony and mating system effects on reproductive assurance and mutualism persistence in fragmented fig-fig wasp populations. *American Journal of Botany*, 99(4), 757–768. <http://doi.org/10.3732/ajb.1100472>
- Giménez-Benavides, L., García-Camacho, R., Iriando, J. M., & Escudero, A. (2010). Selection on flowering time in Mediterranean high-mountain plants under global warming. *Evolutionary Ecology*, 25(4), 777–794. <http://doi.org/10.1007/s10682-010-9440-z>
- Gömöry, D., Bruchanik, R., & Paule, L. (2000). Effective Populatinumber estimation of three Scots pine (*Pinus sylvestris* L.) seed orchards based on an integrated assessment of flowering, floral phenology, and seed orchard. *Forest Genetics*, 7(1), 65–75. Retrieved from [http://www.tuzvo.sk/files/fg/volumes/2000/FG07-1\\_065-075.pdf](http://www.tuzvo.sk/files/fg/volumes/2000/FG07-1_065-075.pdf)
- Kamalakkanan, R., Varghese, M., & Lindgren, D. (2007). Fertility variation and its implications on relatedness in seed crops in seedling seed orchard of *Eucalyptus camaldulensis* and *E. tereticornis*. *Silvae Genetica*, 56(6), 253–259.
- Kang, K.S., & Kim, C.S. (2012). Clonal fertility

- variation and its effect on the effective population size in the seed orchard of diocious species, *Fraxinus rhynchophylla*. *Silvae Genetica*, 61, 79–85.
- Kang, K. ., & Lindgren, D. (1999). Fertility variation among clones of Korean pine (*Pinus koraiensis* S. et Z.) and its implications on seed orchard management. *For. Genet*, 6(3), 191–200. Retrieved from [http://www.tuzvo.sk/files/fg/volumes/1999/FG06-3\\_191-200.pdf](http://www.tuzvo.sk/files/fg/volumes/1999/FG06-3_191-200.pdf)
- Kang, K. S., Bila, A.D., Harju, A.M., & Lindgren, D. (2003). Estimation of fertility variation in forest tree populations. *Forestry*, 76(3), 329–344. <http://doi.org/10.1093/forestry/76.3.329>
- Kang, K.S., & El-Kassaby, Y.A. (2002). Considerations of correlated fertility between genders on genetic diversity: The *Pinus densiflora* seed orchard as a model. *Theoretical and Applied Genetics*, 105(8), 1183–1189. <http://doi.org/10.1007/s00122-002-1064-4>
- Kang, K.S., El-Kassaby, Y.A., Chung, M.S., Kim, C. S., Kang, Y. J., & Kang, B. S. (2005). Fertility variation and genetic diversity in a clonal seed orchard of *Cryptomeria japonica*. *Silvae Genetica*, 54(3), 104–107.
- Kang, K.S., Lindgren, D., & Mullin, T. J. (2001). Prediction of genetic gain and gene diversity in seed orchard crops under alternative management strategies. *Theoretical and Applied Genetics*, 103(6-7), 1099–1107. <http://doi.org/10.1007/s001220100700>
- Kang, K. S., & Mullin, T. J. (2007). Variation in clone fertility and its effect on the gene diversity of seeds from a seed orchard of *Chamaecyparis obtusa* in Korea. *Silvae Genetica*, 56(3-4), 134–137.
- Kartikawati, N.K. (2015). Indeks overlap dan sinkroni pembungaan dalam kebun benih kayuputih Paliyan, Gunungkidul. *Jurnal Pemuliaan Tanaman Hutan*, 9(2), 103–115.
- Kartikawati, N. ., Naiem, M., Hardiyanto, & Rimbawanto, A, (2013). Improvement of seed orchard management based on mating system of cajuputi trees. *Indonesian Journal of Biotechnology*, 18(1), 26–35.
- Lai, B.S., Funda, T., Liewlaksaneeyanawin, C., Kl, J., Van, A., Annette, V. N., ... Jack, W. (2010). Pollination dynamics in a Douglas-fir seed orchard as revealed by pedigree reconstruction. *Annals of Forest Science*.
- Lawande, K.E., Haldankar, P.M., Dalvi, N.V., & Parulekar, Y. R. (2014). Effect of Pruning on Flowering and Yield of Jamun cv. Konkan Bahadoli. *Journal of Plant Studies*, 3(1), 114–118. <http://doi.org/10.5539/jps.v3n1p114>
- Lesica, P., & Kittelson, P.M. (2010). Precipitation and temperature are associated with advanced flowering phenology in a semi-arid grassland. *Journal of Arid Environments*, 74(9), 1013–1017. <http://doi.org/10.1016/j.jaridenv.2010.02.002>
- Lindgren, D., & Mullin, T. . (1998). Relatedness and status number in seed orchard crops. *Canadian Journal of Forest Research*, 28, 276–283.
- Machanská, E., Bajcar, V., Longauer, R. ., & Gömöry, D. (2013). Effective population size estimation in seed orchards: A case study of *Pinus nigra* Arnold and *Fraxinus excelsior* L./ *F. angustifolia* Vahl. *Genetika*, 45(2), 575–588. <http://doi.org/10.2298/GENSR1302575M>
- Sumardi. (2011). Fertility variation and effective population size. *Journal of Forest*, 7(7), 66–79.
- Susanto, M., Doran, J., Arnold, R., & Rimbawanto, A. (2003). Genetic variation in growth and oil characteristic of *Melaleuca* subsp. and potential for genetic improvement. *Journal of Tropical Forest Science*, 15(3), 469–482.
- Swain, T. L., Verry, S. D., & Laing, M. D. (2013). A comparison of the effect of genetic improvement, seed source and seedling seed orchard variables on progeny growth in *Eucalyptus nitens* in South Africa. *Tree Genetics and Genomes*, 9(3), 767–778. <http://doi.org/10.1007/s11295-013-0593-0>
- Varghese, M., Ravi, N., Gu Son, S., & Lindgren, D. (2003). Variation in Fertility and its impact on Genetic Diversity in a seedling seed orchard of *Eucalyptus tereticornis*. In *Proceeding of Symposium Eucalyptus Plantation Research, Management and Development* . Singapore: World Scientific Publishing Co.Pte.Ltd.
- Varghese, M., Nicodemus, A., Nagarajan, B., & Lindgren, D. (2006). Impact of fertility variation on gene diversity and drift in two clonal seed orchards of teak (*Tectona grandis* Linn. f.). *New Forests*, 31(3), 497–512. <http://doi.org/10.1007/s11056-005-2178-8>
- Varghese, M., Kamalakannan, R., Nicodemus, A., & Lindgren, D. (2008). Fertility variation and its impact on seed crops in seed production areas and a natural stand of teak in southern

- India. *Euphytica*, 160(1), 131–141. <http://doi.org/10.1007/s10681-007-9591-3>
- Varghese, M., Kamalakannan, R., Harwood, C.E., Lindgren, D., & McDonald, M.W. (2009). Changes in growth performance and fecundity of *Eucalyptus camaldulensis* and *E. tereticornis* during domestication in southern India. *Tree Genetics and Genomes*, 5(4), 629–640. <http://doi.org/10.1007/s11295-009-0215-z>
- Young, A., Boshier, D., & Boyle, T. (2000). *Forest conservation genetics: principles and practises*. Collingwood: CSIRO Publishing.