

POLLEN DISPERSAL PATTERNS EBONI LASITAE PROVENANCE BASED ON SIMPLE SEQUENCE REPEATS (SSR)

Muhammad Restu*, Gusmiaty, and Siti Halimah Larekeng

Biotechnology and Tree Breeding Laboratory, Fac. of Forestry, Hasanuddin University,
Jl. Perintis Kemerdekaan Makassar, Indonesia 90245

*Corresponding author: tueid@yahoo.com

Phone: 0811443515

ABSTRACT

Parentage analysis has been used to evaluate pollen dispersal in ebony (*Diosphyros celebica* Bakh.). The objectives of this research were to evaluate (i) the dispersal of pollen, and (ii) the distance of pollen travel in ebony Lasitae provenances. The finding of this activities should be beneficial to support breeding of this ebony tree. There were 14 progeny arrays were harvested from 3 female parents. There were 32 ebony trees surrounding the female parents were analyses as the potential male parents for the progenies. Ebony trees were mapped according to their GPS position. All samples were genotyped using four SSR marker loci. Parentage analysis was done using CERVUS version 2.0 software. Results of the analysis indicated the evaluated markers were effective for assigning candidate male parents to all evaluated seedlings. There is no specific direction of donated pollen movement from assigned donor parents to the female ones. The donated pollens could come from assigned male parents in any directions relative to the female parent positions. Pollen dispersal pattern of ebony female parent occurred in outcrossing pollination among its different donated trees. Based on the progenies analysis, ebony female parent is a dominantly outcrossing pollination species. Distances of pollen travel reach to 18 until 269 meter.

Key words: Ebony, ssr marker, pollen dispersal.

INTRODUCTION

IUCN Red List of Threatened Species categorized ebony as one of the vulnerable species (BPTH Sulawesi 2003). Restu (2007) have indicated that genetic diversity of five ebony provenances showed low genetic diversity level as compared to other forest plants. Ebony provenance tends to increase homozygosity or self-pollinate at 95.4% of genetic diversity from diversity within populations. The pollen dispersal study of ebony has currently not been published so that it is deemed necessary for studying pollen dispersal in ebony. The result of this study was to provide important genomic and molecular information that contributes to ebony breeding program based on genetic resources conceptual conservation in the near future.

MATERIALS AND METHODS

Time and Location of Research

This research was conducted during the period of February up to April 2015. The field activities were at the Lasitae Forest provenance, Barru District, South Sulawesi, Indonesia. The research site was at the following GPS location: S4 26.380 E119 38.920 (Fig.1). The laboratory activities were done at Biotechnology and Tree Breeding Faculty of Forestry, Hasanuddin University, Makassar, Indonesia.

Selection of Parents and Progeny Arrays

There were 32 ebony trees in the field research site. Only 3 female parents with 3-5 progeny was analyzed. The location of the female and the assigned male parents were plotted in the map of adult individuals generated by Garmin MapSource GPS mapping software version 76C5x.

Genotyping of Parents and Progenies

DNA isolation was conducted using the CTAB method (Sambrook and Russell 2001) with modification (Larekeng *et al.* 2015). SSR marker at 17 loci (Liang *et al.* 2015) were evaluated for their polymorphism 4 polymorphic loci were selected. To generate markers, PCR amplifications were conducted using the following reaction mixtures: 2 µl of DNA; 0.625 µl of primers, 6.25 µl PCR mix (KAPA Biosystem), and 3 µl ddH₂O. Amplifications were conducted using the following steps: one cycle of pre-amplification at 94 °C for 5 minutes, 35 cycles of amplification steps at 94 °C for 15 seconds (template denaturation), annealing temperature for 15 seconds (primer annealing), and 72 °C for 5 seconds (primer extension), and one cycle of final extension at 72 °C for 10 minutes as suggested by KAPA Biosystem kit.

The generated SSR markers were separated using Superfine Resolution Agarose 3% (Seng *et al.* 2013) using TAE 0.5x buffer (Brody and Kern 2004) and

stained using gel red staining. The electrophoregrams were visualized over the UV transluminescence table and recorded using digital camera. The recorded pictures were used to determine the genotype of the evaluated samples.

Identification of the Candidate Male Parents and Pattern of Pollen Dispersal

Identification of the assigned male parent was done by analyzing genotype of progeny and the respective female parent versus the genotype of all adult trees in the selected samples. Parentage analysis using the genotype of progenies, female parents, and potential male parents was done using CERVUS version 2.0 software (Marshall *et al.* 1998). The progeny and female parent genotype were compared with those of other adult trees and the assigned male parent was selected based on the output of CERVUS version 2.0 analysis results (Marshall *et al.* 1998).

The distance between the known female parent and the assigned male parent was calculated using the Garmin MapSource GPS mapping software version 76C5x software.

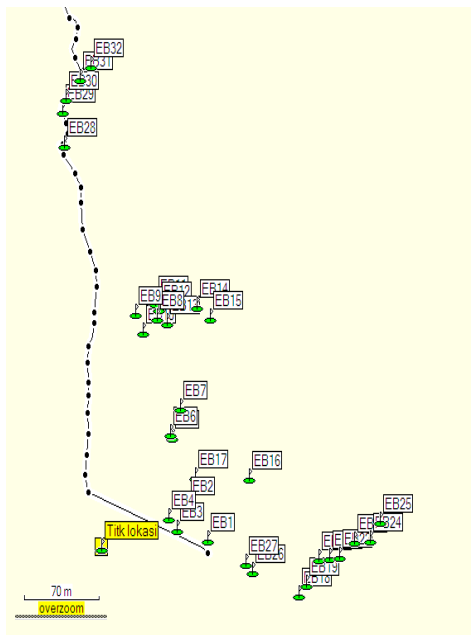


Figure 1. Map of study site with the existed ebony at Lasitae provenance, Barru District, South Sulawesi, Indonesia. The marks (EB) in this map indicated the male and female ebony positions of the ebony provenances.

RESULTS AND DISCUSSION

Genotyping of Parents and Progeny Arrays

Four SSR loci are used in Table 1. An example of the polymorphic marker generated by either the selected SSR 8917 (DC591591) and *ssr* DK29 (DQ097497) primer pairs producing polymorphic markers is presented in Fig. 2. Mean number of alleles per locus is 3.5 and mean PIC for all marker loci was 0.398.

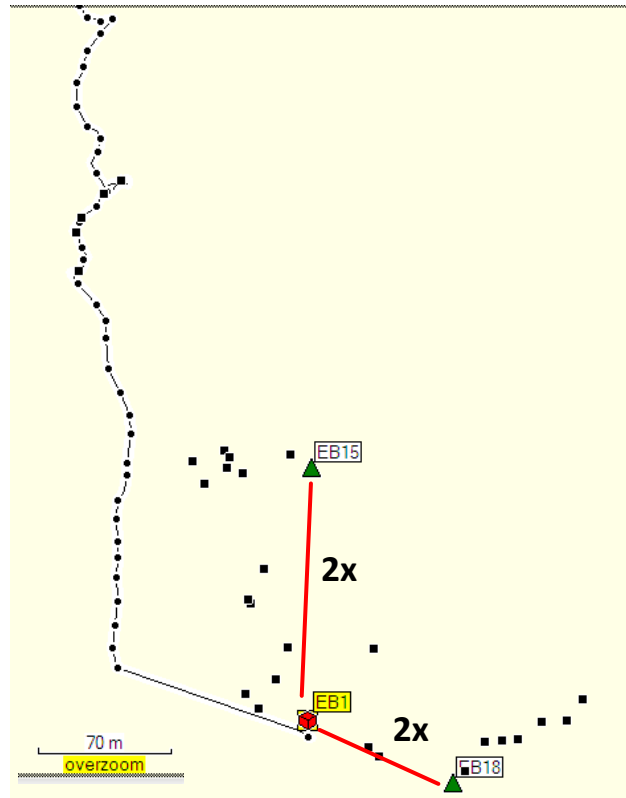


Figure 3. Pollen dispersal pattern EB1 female parent at Lasitae provenance, Barru district, South Sulawesi.

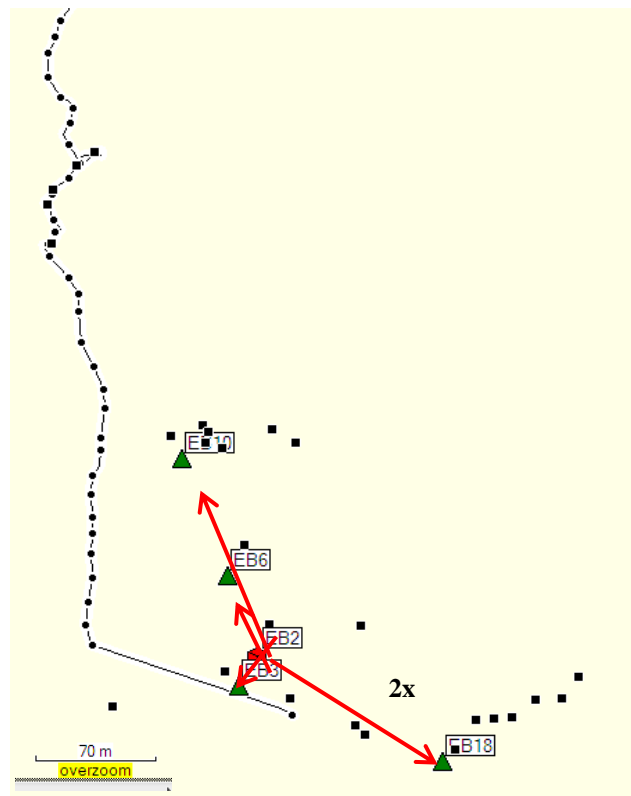


Figure 4. Pollen dispersal pattern EB2 female parent at Lasitae provenance, Barru district, South Sulawesi.

Table 1. SSR loci was used for pollen dispersal ebony *Lasitae* provenance (Liang *et al.* 2015)

No	Locus name/genbank accession no.	Repeat motif	Primer sequence (5'-3')	Tm (°C)	Allele size (bp)
1	1430 DC588341	(GAG)5	F: TCA GTA AAG CTG CGG GCA TC R: ACG GTT CTC CTG ATC CTC ACG	56	190 – 250
8	8917 DC591591	(AT)10	F: ACA CGT TCA GTA CCA GGA GGG A R: AGT ACC ACA AAC CAC CAG TGG	55	166 – 197
9	9004 DC591297	(GCAGGA)3	F: GCC ACA AAC TTC ACA GAG GAC C R: AGG CGA GTG CGA GTA AGA CGA A	55	251 – 272
16	ssrDK29 DQ097497	(CCTTT)8	F: ATCATGAGATCAGAGCCGTC R: CACGTTAACGTTACGGAACA	53	112 – 152

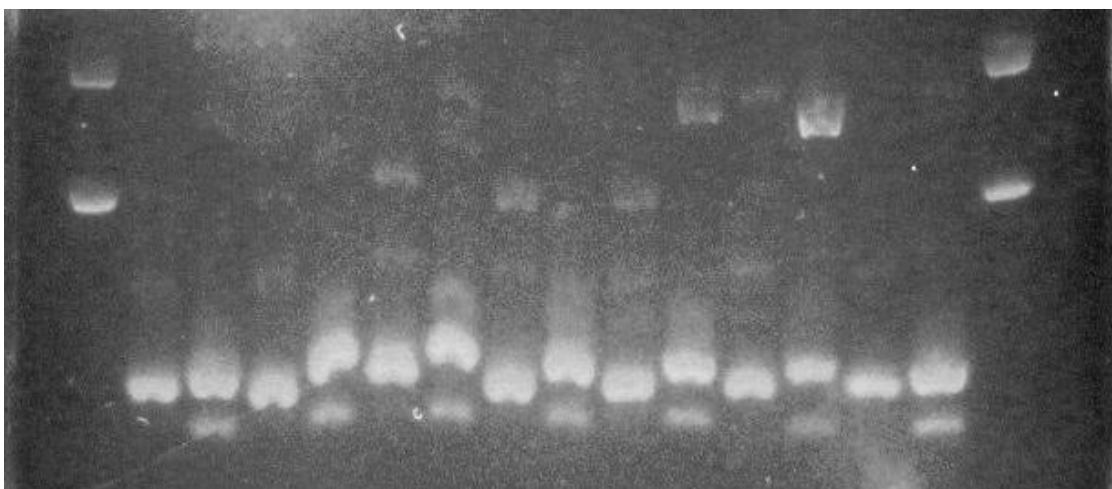
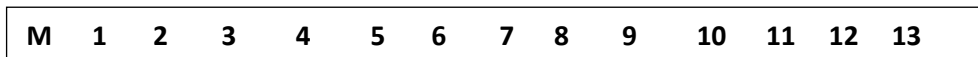


Figure 2. Polymorphism of SSR markers generated by PCR of the genomic DNA sample # 1-14 with a pair of 8917 (DC591591) and ssrDK29 (DQ097497). M: 100 bp DNA ladder markers. Odd numbered columns to sample dna for locus 8917, even-numbered columns to sample dna for locus ssrDK29.

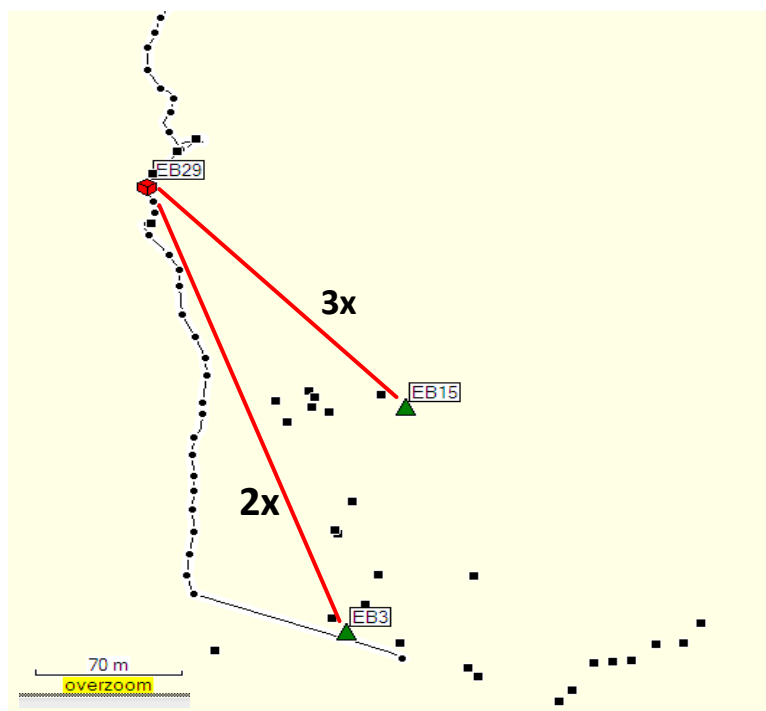


Figure 5. Pollen dispersal pattern EB29 female parent at Lasitae provenance, Barru district, South Sulawesi.

Pattern of Pollen Dispersal

As the female parent, EB1 ebony tree (Fig. 3) received 4 donated pollens from two different assigned pollen donors. Pollen dispersal distance EB15 as donor pollen tree to EB1 141 m, EB18 to EB1 is 82 m.

Ebony female parent EB2 received 5 donated pollens from four pollen donors tree. The pollen contributors are EB18 with distance pollen dispersal 109 m, EB3, EB6 and EB10 with distance 18 m, 44 m, and 110 m, respectively (Fig. 4).

As the female parent, EB29 ebony tree (Fig. 5) received 5 donated pollens from two different assigned pollen donors. Pollen dispersal distance EB15 as donor pollen tree to EB29 176 m, EB3 to EB29 is 269 m. Based on the topography provenance ebony at Lasitae, allowing for ebony flowers flown by pollinators as far as 269 m. Pollen dispersal shapes the local genetic structure of plant populations and determines the opportunity for local selection and genetic drift, but has been well studied in few animal-pollinated plants in tropical rainforests. The mating system analysis showed *O. bataua* dispersal distances were relatively large for an insect-pollinated species (mean 303 m and max 1263 m), and far exceeded nearest-neighbour distances (Ottewell *et al.* 2012). Availability of new tools, such as molecular markers, for analyzing outcrossing rate may change the previous understanding. Such changes have been shown in *Hymenaea courbaril* which was previously reported as more cross pollinated because of self incompatibility (Dunphy *et al.* 2004). However, more recent pollen dispersal studies indicated that *H. courbaril* is more self pollinated (Carneiro *et al.* 2011).

Evaluating pollen dispersal in various plant species usually use an approach based on the parent – progeny genotype (Austerlitz *et al.* 2004). Evaluations have been done in pines (Feng *et al.* 2010), *Dinizia excels* - Fabaceae (Dick *et al.* 2003), *Quercus garryana* - Fagaceae (Marsico *et al.* 2009), teak (Prabha *et al.* 2011) and kopyor coconut (Larekeng 2015). Availability of molecular markers capable of identifying genotype of parents and their progenies should assist the pollen dispersal studies. Using such markers, it should also be possible to estimate the self-pollination and outcrossing rates in a certain population (Milleron *et al.* 2012). Ebony tree research for pollen dispersal pattern must be continued. Further research and evaluation are necessary to generalize the finding since the present study is specific for the current study site.

CONCLUSION

Pollen dispersal pattern of ebony female parent occurred in outcrossing pollination among its different donated trees. Based on the progenies analysis, ebony female parent is a dominantly outcrossing pollination species. This study would be continued to further analysis with addition of SSR loci and a number of ebony trees.

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