

Flowering and seed production of sandalwood (*Santalum album*; *Santalaceae*) along environmental gradients in Gunung Sewu Geopark, Indonesia

YENI W.N. RATNANINGRUM^{1,✉}, ENY FARIDAH², SAPTO INDRIOKO³, ATUS SYAHBUDIN⁴

¹Laboratory of Forest Seed Technology, Faculty of Forestry, Universitas Gadjah Mada. Jl. Agro, Bulaksumur, Sleman 55281, Yogyakarta Indonesia.
Tel./Fax.: +62-274-550543. ✉email: yeni.wnr@ugm.ac.id

²Laboratory of Forest Tree Improvement, Faculty of Forestry, Universitas Gadjah Mada. Jl. Agro, Bulaksumur, Sleman 55281, Yogyakarta, Indonesia.

³Laboratory of Plant Physiology, Faculty of Forestry, Universitas Gadjah Mada. Jalan Agro, Bulaksumur, Sleman 55281, Yogyakarta, Indonesia.

⁴Laboratory of Dendrology and Ethnobotany, Faculty of Forestry, Universitas Gadjah Mada. Jl. Agro, Bulaksumur, Sleman 55281, Yogyakarta, Indonesia.

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Abstract. Ratnaningrum YWN, Faridah E, Indroko S, Syahbudin A. 2016. Flowering and seed production of sandalwood (*Santalum album*; *Santalaceae*) along environmental gradients in Gunung Sewu Geopark, Indonesia. *Nusantara Bioscience* 8: 180-191. We observed flowering and seed production of sandalwood populations along environmental gradients in Gunung Sewu Geopark, Indonesia, in 2013 to 2014 flowering periods. We observe flowering phenology with regard to floral initiation, phases and periods, and measured Pollination Effectiveness and Reproductive Success, following modified methods of Owens et al and Ghazoul. Flowering and seed production varied with altitude and climatic conditions, and altered by changes in rainfall, temperature and soil moisture. Sites with lower altitude, lower rainfall, highest temperature, lowest relative humidity, and lowest soil moisture flowered earlier and shorter. Flowering delayed and prolonged by increasing altitude, temperature, and humidity. At all sites, flowering delayed and prolonged in rainy compared to dry season. Lower populations produced more flowers in rainy season, however, higher abortion resulted in very low seed production. Dry season produced less flowers but higher reproductive success was achieved, resulting in more fruits. The lowest altitude performed highest reproductive outputs. More marked differences among sites were observed in the dry season. Flowering and seed production was also observed to be temperature sensitive. Both drop and increase on temperature resulted in the alteration of flowering frequency and reproductive outputs. Sandalwood in Gunung Sewu grows naturally in association with tropical monsoon vegetation, i.e. acacia, cajuputi, teak, and mahogany. The conservation of this endangered species, particularly under wider range of landscapes, should be maintained with a different strategy according to the degree of plant sensitivity to environmental features.

Keywords: Biodiversity conservation, environmental gradients, Gunung Sewu Global Geopark Network, reproductive biology, sandalwood

INTRODUCTION

Flowering phenology varies greatly with climate zone over broad geographical ranges (Bertin 2008; Liberato et al. 2011; Richardson et al. 2013). Climate affects the sequence of different reproductive stages from flowering to seed production (Alizoti et al. 2010). Phenology alteration along altitudinal gradients have been considered to be the altitudinal roles on temperature alteration (Bertin 2008; Sabat et al. 2011). Hence, it is important to determine the correlation of climate and phenology under different climatic regimes and altitude, since it has major implications for many ecological and evolutionary aspects including conservation of biodiversity (Bertin 2008; Del-Cacho et al. 2013; Richardson et al. 2013). Altitudinal changes in plant phenology have been well-documented in temperate regions (Phillips et al. 2011; Shiping et al. 2014), however, only very limited results were available from warmer areas such as tropical, semi-arid, and arid regions (Dreyer et al. 2006; Lesica and Kittelson 2010; Dalglish et al. 2012; Haukka et al. 2013). Observation on plant

phenology has mostly been conducted on humid-temperate climates including the Arctic, sub-Antarctic, Mediterranean (Bertin 2008; Sabat et al. 2011) and Western American regions (Phillips et al. 2011). However, only very limited results were available from warmer areas such as tropical, semi-arid, and arid regions (Dreyer et al 2006; Lesica and Kittelson 2010; Dalglish et al 2012; Haukka et al 2013), particularly in the East Asia (Guo et al 2013; Richardson et al 2013; Sommer et al 2013). For tropical forests itself, several works have only been accomplished in Amazonia and Central Africa; while the effects of climate conditions on plant phenology in Southeast Asia are still highly unknown (Richardson et al 2013; Del-Cacho et al 2013). One of critical gaps that are in need of further research is the environmental roles controlling phenology in tropical and arid regions (Richardson et al. 2013).

In temperate regions, onset of flowering was earlier at lower elevations due to higher temperature, that in many cases is correlated to higher soil moisture (Del-Cacho et al 2013). Flowering initiation has also been delayed at both high latitudes and altitudes (Phillips et al. 2011; Shiping et

al. 2014). However, the role of climatic factors and altitude may differ for warmer zones such as tropical and arid regions. For those warmer zones, climate impacts will vary with the degree to which the site water balance is influenced (Bertin 2008; Liberato et al. 2011; Richardson et al. 2013). Several studies in the tropics indicated strong impact of rainfall, temperature and soil water status on flowering (Alizoti et al. 2010; Del-Cacho et al. 2013; Richardson et al. 2013; Sommer et al. 2013). In the arid and semi-arid regions of Africa, temperature decreases but soil moisture increases with the increment of altitude (Dreyer et al. 2006; Lesica and Kittelson 2010; Dalglish et al. 2012; Haukka et al. 2013). At both tropical and arid regions, rising temperatures and reduced rainfall in dry season will extremely decline soil water status, resulting in the earlier and shorter flowering period (Dreyer et al. 2006; Haukka et al. 2013; Sommer et al. 2013).

An economic-important species native to South-eastern parts of Indonesia, *Santalum album* Linn. (Santalaceae) (Harbaugh and Baldwin 2007) - Herein after referred to sandalwood - has undergone significant degradation due to high demand on its pricey wood and oil, and therefore classified as the threatened species (IUCN 2009). Despite significant degradation on its origin in the South-eastern parts of Indonesia, new landraces occurred in Gunung Sewu Area of Java island, the central part of Indonesian archipelagos. The origin of these landraces is still in debates. The oldest herbarium specimens of sandalwood in Java islands (dated by year 1853) was collected from Imogiri Sub-district, and another specimen (dated by year 1960) was found in Nglipar Sub-district; both were part of Gunung Sewu. First introduction of sandalwood in Playen Sub-district, the Middle Zone of Gunung Sewu, was conducted in 1967 using seedlings originated from Timor island (Indrioko and Ratnaningrum 2015). Most of studies considered Timor and Sumba islands as the origin of these Gunung Sewu landraces, in which the genetic materials might be introduced to Java during the Dutch occupation period in 1800's.

Gunung Sewu, a mountainous limestone zone spanning three GeoAreas, comprises approximately 1300 km² of width. Previous geographical processes that started from about 1.8 million years ago - involved tectonic movements, volcanic activities, and seawater erosion - has derived this area into various landscape structures differed in altitude, elevation, soils, and microclimate conditions (Simanjuntak 2002). Flora and fauna of Gunung Sewu are still highly unknown (Haryono and Suratman 2010), and to the best of our knowledge, none have explored this experimentally. Sandalwood in Gunung Sewu extended along geographical gradients under various types of landscapes. This research represented the first attempt to determine the flowering and seed production of three sandalwood populations along geographical gradients in Gunung Sewu, Indonesia, in 2013 to 2014 flowering periods. This work will hopefully contribute to the arrangement of more comprehensive scheme on protecting geological features along with conserving biodiversity in Gunung Sewu.

MATERIALS AND METHODS

Study area

Geographically, Gunung Sewu Geopark areas stretched 85 km west-east in southern Java covers three GeoAreas of Gunungkidul (Yogyakarta), Wonogiri (Central Java), and Pacitan (East Java) with approximate area of 1300 km². Recently, Gunung Sewu possessed two seasons. Rainy season occurred from October to March and the dry season took place from April to September. As it is adjacent to Indian Ocean, Gunung Sewu areas experienced two types of climate - *Aw*, the semi-arid to arid type, characterized by a long drought; and *Am* which is representing an intermediate condition between the tropical and sub-tropical climate (Simanjuntak 2002; Haryono and Suratman 2010).

Gunungkidul GeoArea of Gunung Sewu is geographically derived into three zonations: (i) Northern Zone, the highland of Batur Agung, 300m to 800m asl; (ii) Middle Zone, the lowland of Ledok Wonosari, 150m to 300m asl; and (i) Southern Zone, the Gunung Sewu Karst at 0m to 200m asl (Simanjuntak 2002). New landraces of sandalwood occurred at various type of landscapes in Gunungkidul GeoArea, and were divided into three groups (Table 1; Figure 1). *The first group* was the Northern Zone, consisted of Nglanggeran and Sriten populations in the highland parts, and Bejiharjo population in the lowland parts. *The second group* was the lowland of Middle Zone, consisted of Bleberan and Ngingrong populations. This Middle Zone was also considered as the center of sandalwood in Java Island, given that these landraces surrounded by several *ex situ* conservation areas within less than 10 km in range. These conservation areas, totally comprised more than 30 Ha of width, were established since year 1967 and consisted of more than 40 provenances originated from Timor, East Nusa Tenggara and Java islands of Indonesia. *The third group* was the karst region of Southern Zone and were consisted of Semugih, Pucanganom, Petir and Botodayakan populations.

We compared one sandalwood population (the lowland basin of Bleberan) in the Middle Zone, one (the highland of Nglanggeran) in the Northern Zone, and one more (the karst area of Petir) in the Southern Zone of Gunung Sewu region. These sites are separated by more than 20 km, are having different altitude, and are experienced different climatic regimes with clear ecological differences.

Study of species

Sandalwood is a long-lived, yearly flowering perennial found on semi-arid and tropical region throughout south-eastern and middle parts of Indonesia. It is a sub-shrub attaining a height of about 12 m and a girth of 10 to 30 cm, mainly vegetatively reproduced by root suckers, particularly under marginal condition. Generally, sandalwood in Indonesia flowered twice a year: at the beginning of dry season on May to September, and at the beginning of rainy season on November to March; with 4 to 5 months of flowering period (Ratnaningrum and Indrioko 2014, 2015).

Table 1. The characteristics of study sites

Population and width	Altitude; latitude & longitude	Climate type	Soil and rock units	Habitat characteristics
Nglangge-ran; 79.3 ha	710m to 750m asl; 7°50'28" S to 110°32'55" E	Am type	Dominated by latosols with volcanic and sediment rocks, some with deeper solum.	Sandalwood first documented in 1970's, recently occurred in groups of stands across the Nglanggeran mountain regions, in association with the tropical mountain vegetation. Habitat dominated by the association of naturally regenerated mahogany, <i>Gliricidia</i> sp, and several <i>Garcinia</i> and <i>Eugenia</i> families.
Bleberan; 52.9 ha	150m to 170m asl; 7°50'8" S to 110°50'75" E	Intermediate between Aw and Am types	Dominated by association of red Mediterranean and black grumusols with limestone rocks, mostly with deeper solum.	Sandalwood first documented in 1970's along the catchment area of the ancient subterranean Oya River at the lowland basin of middle zone. Located in the center of sandalwood in Java Island, surrounded by several <i>ex situ</i> conservation areas which also share the same river. The plant association consisted of more diverse vegetation including teak, mahogany, <i>Gliricidia</i> sp, <i>Schleicera</i> sp, cajuputi, and acacia.
Petir; 78 ha	70m to 100m asl; 7°50'60" S to 110°60'15" E	Aw type	Dominated by latosols with limestone rocks. Solum was deeper at the basins, very shallow at the limestone-rocky hills.	Sandalwood first documented in 1960's in karst hilly areas, recently covering more than twenty open dry-rocky hills. Adult plants were mostly derived from root suckers. In this open-undulating areas, sandalwood is grown in association with dry rocky-limestone vegetation including acacia and cajuputi, but more dominated by shrubs such as grasses and <i>Eupatorium</i> sp.

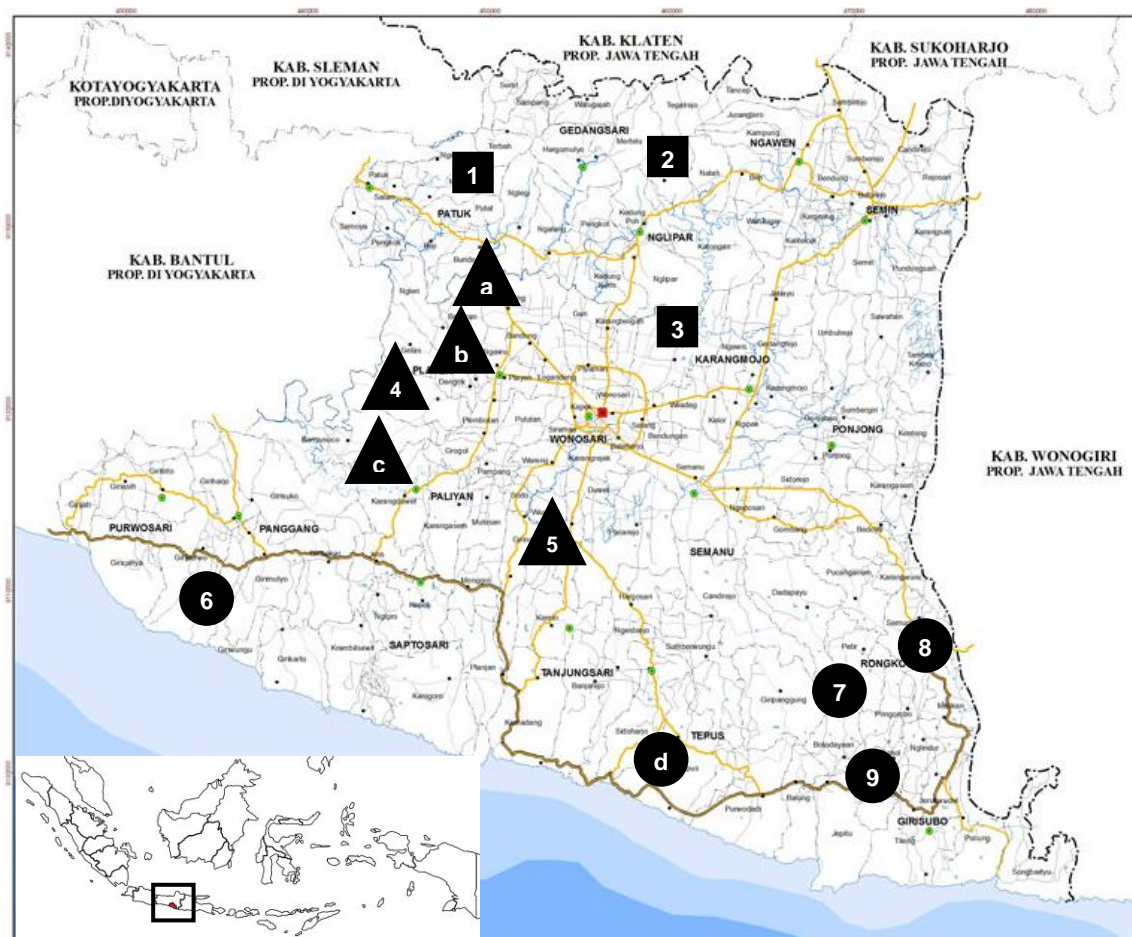


Figure 1. Study sites, sandalwood landraces in the Gunungkidul GeoArea. The first group (Northern Zone): Nglanggeran (1), Sriten (2) and Bejiharjo (3); the second group (Middle Zone): Bunder (a), Wanagama (b), Banyusoco (c), Bleberan (4), and Mulo (5); and the third group (Southern Zone): Pucanganom (6), Petir (7), Semugih (8), Botodayakan (9) and Tepus (d) populations, respectively. The Arabic numbers represent natural landraces, while the alphabetic fonts represent *ex situ* conservation areas, respectively. Within each zone, sandalwood populations are marked by squares (Northern Zone), triangles (Middle Zone) and circles (Southern Zone) shapes, respectively. Study was carried out in Nglanggeran (1), Bleberan (4), and Petir (7) landraces which were representing each of groups in geographical zone

Procedures

Flowering phenology observation

Observation on flowering and seed production was conducted during two years (from 2013 to 2014) flowering periods following modified methods of Owens et al (2001) and Ghazoul (1997). To observe flowering phenology, 9 flowered individuals were randomly selected at each site (total N 27). Each of selected individuals were separated 5 to 10 m in distance. Ten inflorescences on each of 9 selected individuals were marked and the flowering phenology parameters (floral initiation, flowering phases and flowering period) were then observed daily during the flowering period. A single inflorescence and a single flower from each individual were photographed daily.

Measuring pollination effectiveness and reproductive success

To measure geographical variation in seed production, we regularly monitored ten randomly chosen flowering plants in each population (total N 30), in which reproductive traits were then measured following modified methods of Owens et al (2001). We recorded the number of inflorescences produced by each individual and the total number of flowers produced on ten randomly chosen inflorescence per individual. To measure reproductive parameters (Pollination Effectiveness, *PE* and Reproductive Success, *RS*), the flowers, fertilized flowers, and mature seeds were counted and the value of *PE* and *RS* were then calculated. Pollination Effectiveness (*PE*) - represents the ability of reproductive organs to pollinate, fertilize and produce a young fruit - was measured by dividing the number of *Fertilized flower* to *Flower*. Reproductive Success (*RS*) - corresponds to the ability of reproductive organs to form mature seeds - was measured following the formula, $RS = (Fruit/Flower) \times (Seed/Ovule)$.

Collecting the data of environmental parameters

The data of monthly rainfall is obtained from the weather station belongs to Local Government of Gunungkidul District. The daily 12: 00 h temperatures and relative humidity were used to calculate average monthly temperatures and relative humidity. The soil water status was measured in every two months following Gravimetric method (Poerwowidodo 1992). Soil was sampled at 10 cm in depth where the soil-rhizosphere interaction has intensively occurred. At all sites, every three points of shaded and opened area were marked in which three replications of soil samples per point were then taken. A 10 gram of composite soil was dried at 105°C for 48 h. Soil moisture was then measured following the formula: soil moisture (%) = (fresh weight - dry weight)/dry weight x 100%.

RESULTS AND DISCUSSION

Fluctuation on climatic conditions

Petir, the lowest population (70m to 100m asl) located at karst area with soil dominated by latosols with limestone rocks. This population experienced *Aw* climatic type characterized by lowest rainfall (2035 mm yearly rainfall

on less than 6 rainy months), which was consequentially resulting in the highest temperature (39.67°C), lowest relative humidity (33.81%) and lowest soil moisture (27.93%), respectively.

Bleberan, the middle altitude population (150m to 170m asl) located in the catchment area of the ancient subterranean river, Oya River, where soils were derived from the association of red Mediterranean and black grumusols with limestone rocks. This population located in the Middle Zone of Gunungkidul, a basin area that was receiving abundant of rainfall (2547 mm yearly rainfall on 2 to 6 rainy months). Therefore, even it possessed the intermediate of *Am* and *Aw* climatic type with high temperature (34.8°C) and light intensity, it was able to maintain high soil moisture (33.3%) and relative humidity (59.25%).

Nglanggeran, the highest population (710m to 750m asl) is the ancient volcano dated from Middle Miocene and is influenced by volcanic activities. Soils dominated by latosols with volcanic and sediment rocks. This population possessed *Am* climatic type, received most abundant rainfall (2579.5 mm yearly rainfall on more than 6 rainy months) and therefore is having lowest temperature (31.89°C), highest relative humidity (69%) and highest soil moisture (33.85%), respectively.

This study recorded fluctuation on climatic condition during 2012 to 2014 flowering periods (Figure 2). Change on the pattern of rainfall was observed during year 2013. Normally, Gunung Sewu areas experience dry season in May to October and receive rain from November to April. In the middle of rainy season in February to March 2013, rain was extremely declined, and Petir which was receiving no rain (0 mm rainfall) suffered a drought for the whole 2 months. This lowest altitude population undergo the most extreme temperature increment (41.8°C), that was resulting in the considerable reduction on soil moisture (24.92%). This drought period has also occurred at Bleberan, the middle altitude population. However, the latter population was located along the catchment area of the Oya River, which helps it maintain high soil moisture and relative humidity. Hence, Bleberan showed no significant responses to the change of micro-climate due to the drought events.

Extremely changes on daily temperature was also observed from late June to July 2014 when there was extremely drop on temperature due to the effect of cold winter wind from Australian continents. A 4°C to 7°C of temperature reduction was observed at all sites during three to five weeks period. This extreme temperature reduction, however, did not cause significant changes on the monthly relative humidity and soil water status.

Flowering phenology

Sandalwood flowers arranged in inflorescences consisting of 20 to 40 single flowers (Figure 3). There were six flowering phases that were started with (1) flower buds initiation and development prior to anthesis (4 to 7 weeks), followed by (2) early anthesis that is coincident with the pollen maturity (2 to 4 days), and (3) fully anthesis that is coincident with stigma receptivity (5 to 7 days), that was followed by (4) the formation and development of

pollinated flowers into young fruit (5 to 10 days), (5) the enlargement of young fruit into its maximum size (4 to 6 weeks), and (6) the development of fruit until maturity (3 to 4 weeks) (Figure 4).

At the first phase of development, reproductive shoots emerged, elongated and gradually transformed into floral buds, that was continue developed prior to anthesis (Figure 4 (1)). The second phase was the early anthesis in which the perigonium - a fusion of sepals and petals structure - began to bloom, white in color. At this phase, the yellowish anthers dehiscence and spread the yellow mature pollen (Figure 4 (2)). In the third phase, perigonium gradually changed from reddish white into maroon, along with the receptivity of the stigma, while the anthers gradually shrinkage and pollen started to lose viability (Figure 4 (3)). The successful pollination and fertilization will result in the fourth phase: formation and development of pollinated flowers into green-small fruits (Figure 4 (4)). At the fifth phase, fruits continue enlarged into maximum size (Figure 4 (5)). At the last phase, the developing fruits gradually changed its color from red to dark purple as a sign of maturity (Figure 4 (6)).

Individuals at the same site performed similarly on flowering phenology with regard to floral initiation, flowering period and flowering frequency. However, floral initiation and flowering period differed among sites (Figure 5). Flowering buds were initiated first at Petir. This lowest altitude received lowest rainfall which was consequentially resulting in the highest temperature, lowest relative humidity, and lowest soil moisture. This lowest altitude population also performed shorter flowering period, there was only 3.5 months needed to develop flower buds into mature fruits. Bleberan population that is located in a slightly higher altitude performed moderate level of flowering. At this middle altitude population, flower buds emerged somewhat later and flowering period was longer: 4 months was needed to produce mature fruits. Bleberan located in the Middle Zone of Gunungkidul basin area that was receiving the most abundant of rainfall. This population also located along the catchment area of the

Oya River, which helps to maintain high soil moisture and relative humidity. Flowering initiation was delayed and prolonged with the increase of altitude, moister and cooler conditions. At the highest altitude of Nglanggeran population, flowering was only started about three weeks later, and it took 5 months to complete the flowering period. This highest altitude population experienced lower temperature, higher relative humidity, and higher soil moisture.

At all population, flowering was also delayed and prolonged in rainy season compared to those of dry season. In lower altitude populations, first flowering buds were emerged about two or three weeks after the first rain, and 4 to 4.5 months were needed to complete the flowering period. At higher altitude population, flowering initiation was only occurred about 2 months after the first rain, with more than 5 months flowering period. Such differences of flowering period between seasons was observed due to the difference on the duration of first (floral buds initiation and development), fifth (young fruit enlargement) and sixth (fruit maturity) phases. No differences were found on the second, third and fourth phases.

Flowering in Petir population behaved differently as a response of drought from February to March 2013. In this middle of rainy season, this lowest altitude population received a very limited rainfall and undergo extreme temperature increment, that was resulting in the considerable reduction on soil moisture. This drought took place at the fifth and sixth phase of fruit development. Mass fruit abortion occurred as the drought started in the early days of February. This fruit abortion was immediately followed by mass production of new flower buds, replacing the aborted fruits. This additional flowering performed shorten duration to be only 3.5 months, and succeed to produce mature fruits. This drought period has also occurred at the middle altitude population Bleberan. However, this population was able to maintain high soil moisture and relative humidity, and therefore possessed no significant responses on flowering behavior due to the drought condition.

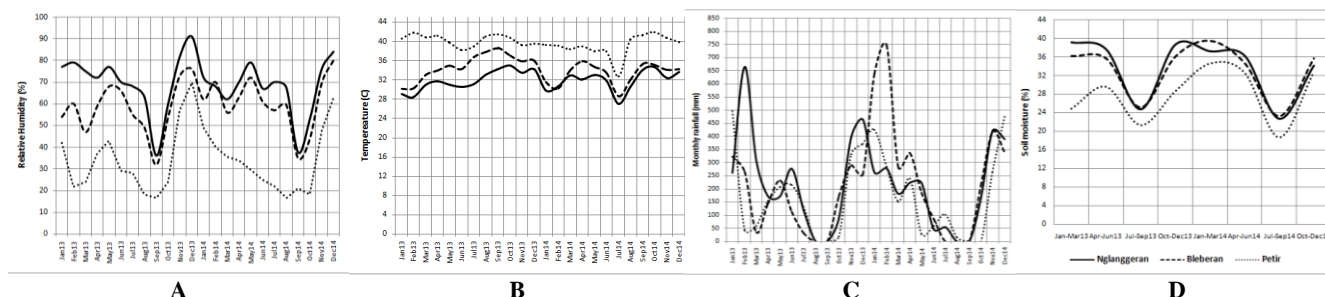


Figure 2. Fluctuation on environmental condition in three sandalwood populations at Gunung Sewu areas from 2013 to 2014 flowering periods. The y ordinate indicates the monthly rainfall (mm), relative humidity (%), soil moisture (%) and monthly temperature ($^{\circ}\text{C}$), respectively. The Nglanggeran, Bleberan and Petir population are each represented by solid, dashed and dotted line, respectively. The horizontal x line represents the months during year 2013 to 2014.

Flowering frequency was also altered from late June to July 2014 due to the extremely drop on temperature as the effect of cold winter wind from Australian continents. A 4°C to 7°C of temperature reduction was observed at all sites during three to five weeks period, at the fifth or sixth phase of fruit developments. In coincidence with this developing fruit, new flower buds abruptly emerged in

response to the extreme temperature reduction. However, this new addition of flowering was failed to produce fruit. Mass flowers abortion of this additional flowering period was observed during the second or third phase, indicating the failure on both pollination and fertilization processes. Whilst, some of fruits from the former period still continue to develop into maturity.



Figure 3. Inflorescence of sandalwood flowers (left); the structures of single flower at early, fully and late anthesis phase (middle); and the floral parts: (1) ovary, beared by receptacle, (2) outer perigonium, (3) inner perigonium, (4) filament, usually covered by light-white featherlike structures, (5) anthers, consisted of pollen grains, and (6) stamen (right).

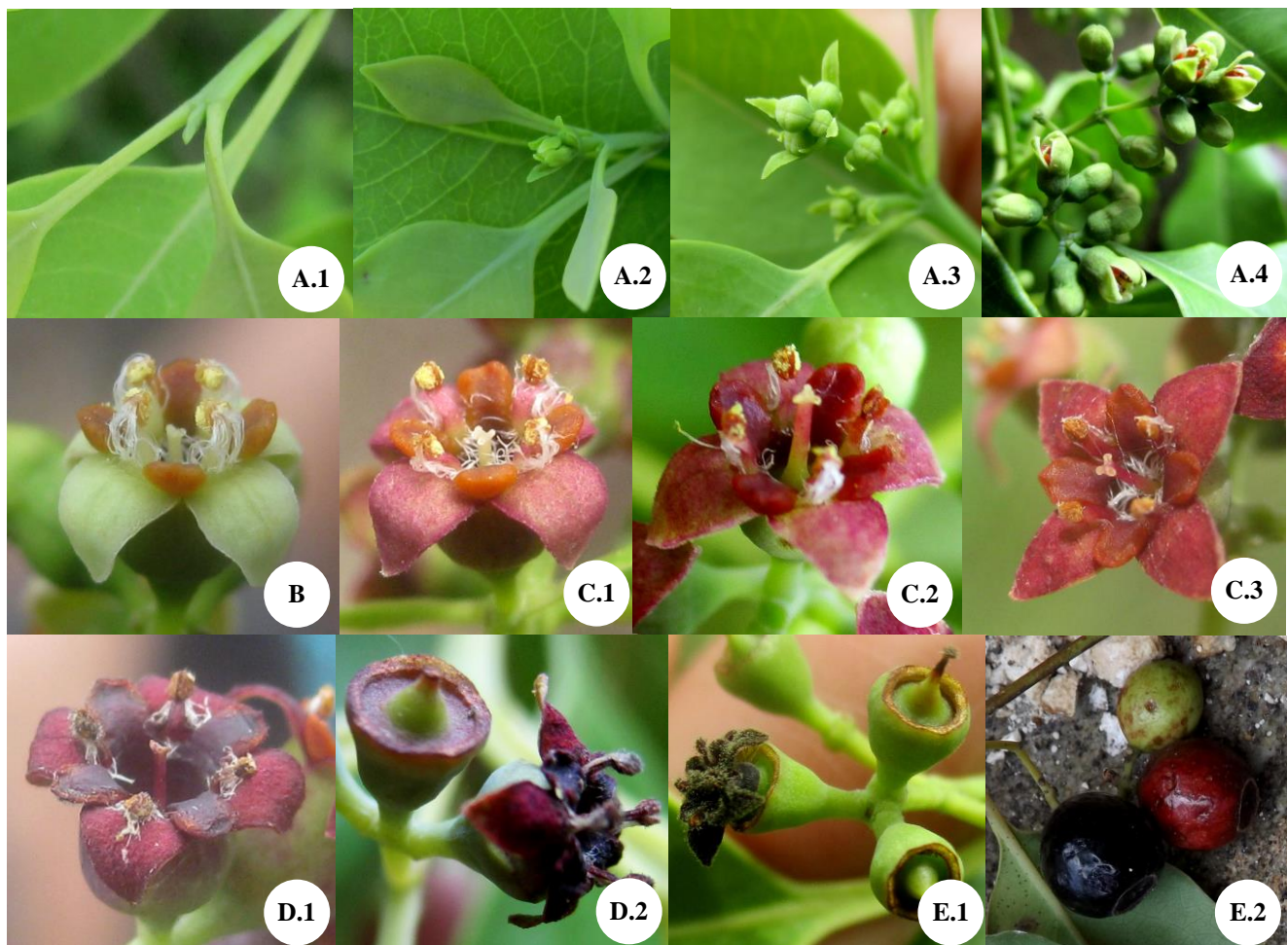


Figure 4. Six flowering phases of sandalwood from flower buds initiation to fruit maturity. A. Flower buds initiation and development prior to anthesis (4-7 w), B. Early anthesis, coincident with pollen maturity (2-4 d), C. Fully anthesis, coincident with stigma receptivity (5-7 d), D. The formation and development of pollinated flowers into young fruit (5-10 d), E. The enlargement of young fruit into its maximum size (4-6 w), and (6) the development of fruit until maturity (3-4 w)

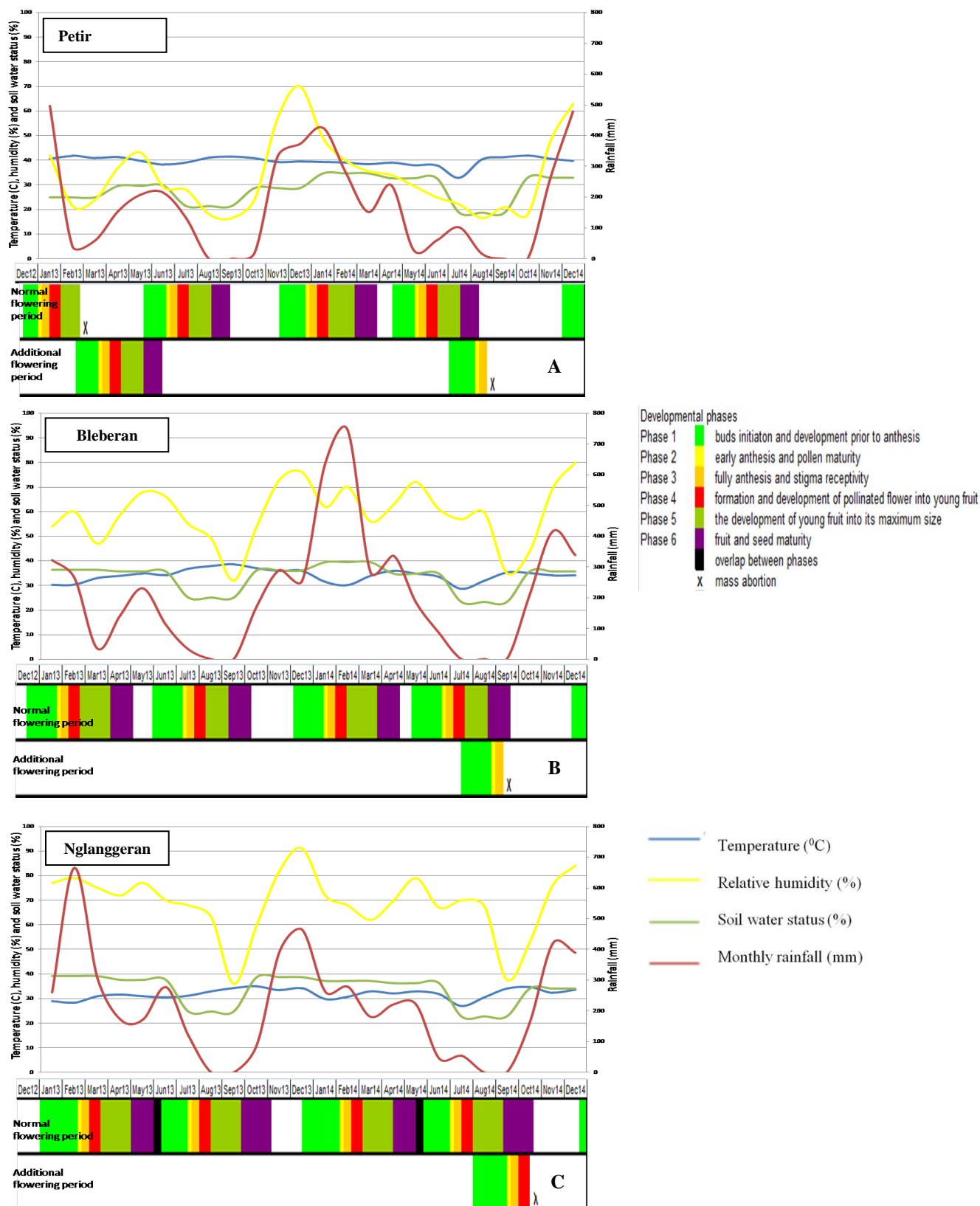


Figure 5. Flowering phenology in three sandalwood populations at Gunung Sewu areas: Petir (A), Bleberan (B) and Nglanggeran (C) from 2013 to 2014 flowering periods. The primary y ordinate indicates the relative humidity (%), soil moisture (%) and monthly temperature (°C), each represented by yellow, green and blue lines, respectively. The secondary y ordinate indicates the monthly rainfall (mm) represented by red line. The horizontal x line represented the months during year 2013 to 2014. Flowering phenology derived into six developmental phases that is marked by different colors. The first row represented the regular flowering period within a year, while second row is the addition of new flowering period due to the extreme changes on climatic condition. The X marks showed the mass flowers or fruits abortion that was resulting in the incomplete flowering phase

Early responses to this drop of temperature were expressed by sandalwood in lowest altitude population, which has no previous experience to be exposed to such extremely cold condition. Petir and Bleberan population showed immediately responds by producing large amount of new flower buds, right after the drop of temperature in the early days of July. However, mass flower abortion occurred during the second phase in the middle of August. The highest altitude population Nglanggeran, that used to be exposed to such condition of cold, responded later by producing new flower buds only by the end of July, coincident with the fruit maturity of former period. This new flowers aborted mostly during the third phase at the early days of September.

Flowering plasticity was recorded in this study. At all sites, flowering frequency and period were turning back into its normal behavior, twice flowering a year, as the climate was returned into normal condition.

Seed production

Generally, lower populations Petir and Bleberan produced more abundant flowers in rainy season. However, higher abortion in rainy season resulted in very low seed production at both sites. In dry season, both sites produced less flowers, but the pollination and reproductive success were higher and more fruits were produced. In contrary to other sites, the highest population Nglanggeran showed no differences in flowers and seed production at both rainy and dry season. Nglanggeran generally produced the most abundant flowers among sites, but the highest abortion rate resulted in the lowest fruit production. The highest reproductive output was achieved at the lowest altitude population.

No significant differences observed on the number of flowers (ranged from 30 to 46 single flowers per inflorescence) among sites (Figure 6). However, flowers abundance varied with season: rainy season always produces more flowers (37 to 63 single flowers) compared to those of dry season (12 to 47 single flowers per inflorescence), respectively. More marked differences were observed in the dry season, when only a half portion of flowers produced at the lower sites Petir (12 to 29 single flowers) and Bleberan (16 to 24 single flowers) compared to those of highest site Nglanggeran (30 to 47 single flowers per inflorescence), respectively.

Variation on pollination and reproductive success resulted in the different seed sets among sites (Figure 7). Despite the less flower production, lower altitude populations produced more fruits, that was represented by the higher value of PE and RS, respectively. In average, lowest population Petir possessed highest reproductive outputs (27.3% PE and 5.8% RS), followed by Bleberan (22.7% PE and 2.6% RS) and Nglanggeran (9.7% PE and only 0.7% RS), respectively. Less flowers were produced in the dry season at all sites, however, it resulted in more fruits, that was represented by higher PE (10.1% to 40%) and RS (0.3% to 10.5%), respectively. Rainy season produced more flowers, but contradict resulted in fewer fruits which were represented by lower PE (6.5% to 21%) and RS (0.3% to 2.9%), respectively. Highest flowers and

fruits abortion occurred at the highest population Nglanggeran, particularly in rainy season, resulted in a very low PE and RS.

Discussion

The effects of environmental differences on flowering phenology of sandalwood

Flowering behavior of sandalwood may correspond to the alteration on soil humidity due to the fluctuation of temperature and rainfall. We proposed several aspects of climate components that may contribute to the flowering phenology of sandalwood in several populations along environmental gradients of Gunung Sewu.

Temperature and rainfall. Earlier and shorter flowering period was observed at the lower altitude population which experienced lower rainfall, a condition that consequentially resulted in higher temperature, lower relative humidity, and lower soil moisture. Flowering initiation was delayed with longer period by increasing altitude, moister and cooler conditions. Similar findings were observed on sandalwood planted at *ex situ* conservation in Wanagama Forest Station that was also a part of Gunung Sewu Geopark (Ratnaningrum and Indrioko 2015). Provenances originated to the arid regions of Indonesia flowered earlier with shorter period compared to those of tropical regions. A landrace that was collected from Lawu, a tropical highland mountain in Central Java, flowered latest with longest period. Similar results were also reported with *Oxalis* species in the arid region of South Africa which started flowering earlier and achieved higher seed set at the lowest altitude in warmer and drier condition. Also, flowering was delayed by increasing altitude, moister and cooler conditions (Haukka et al. 2013).

In tropical region, flowering is more affected by rainfall through its role in controlling soil water status and relative humidity (Alizoti et al. 2010; Haukka et al. 2013; Sommer et al. 2013). Increasing temperatures and changing patterns of precipitation have had considerable effects on the timing of plant phenology (Dreyer et al. 2006; Lesica and Kittelson 2010), and other factors such as water and solar radiation may modify its effects (Grab and Craparo 2011). The earlier and shorter flowering was strongly associated with a warmer climate (Dreyer et al. 2006; Dahlgren et al. 2007; Lesica and Kittelson 2010; Grab and Craparo 2011; Phillips et al. 2011; Dalgleish et al. 2012; Haukka et al. 2013; Shiping et al. 2014), and the trend toward earlier flowering has frequently been found to be associated with warmer temperatures. High temperatures may speed growth through all stages of development, and therefore shortening the duration of stages (Sommer et al. 2013; Shiping et al. 2014).

In this study, flowering was also delayed and prolonged in rainy compared to dry season at all sites. In the tropics, the fluctuation of rainfall may have considerable impacts on flowering and seed production (Del-Cacho et al. 2013). Tropical forests are differentiated by the amount and seasonal variability of temperature and site water balance, thus variation in phenology tend to be driven by the duration of dry season (Bertin 2008; Liberato et al. 2011).

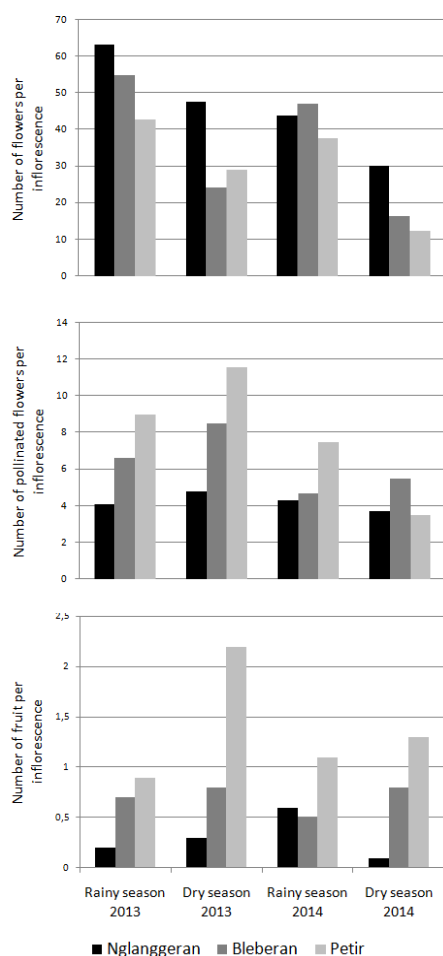


Figure 6. Number of flowers (above), young fruits (middle) and mature fruits (below) per inflorescence on three of sandalwood populations in Gunung Sewu during the rainy and dry season in 2013 to 2014 flowering periods. The y ordinate indicates the number of flowers (above), pollinated flowers (middle) and mature fruits (below), each population represented by different color of bars. The horizontal x line represents the rainy and dry season within 2013 to 2014 flowering periods

The warmer and drier years have compressed the flowering periods of sandalwood in this study. Same results were observed in *Oxalis* in South Africa that warmer and drier years have shortened flowering (Dreyer et al. 2006). Same findings were also achieved in sandalwood in Wanagama, another site of Gunung Sewu, where a prolonged rainy season in 2009 to 2010 resulted in a later initiation and longer flowering period. The prolonged rainy season also resulted in a shortened period of stigma and pollen longevity (Ratnaningrum and Indrioko 2015). The extremely increment of rainfall might have consequential effects on the drop of temperature that was, in turn, resulting in the shorter stigma longevity (Owens et al. 2001). Many studies suggested that flowering behavior in semi-arid regions occurred as a response to rainfall coinciding with higher temperatures (Haukka et al. 2013), while some other studies oppositely revealed that the beginning of flowering depended on the onset of first rains after a dry summer, as well as a drop in daily temperature (Dreyer et al. 2006).

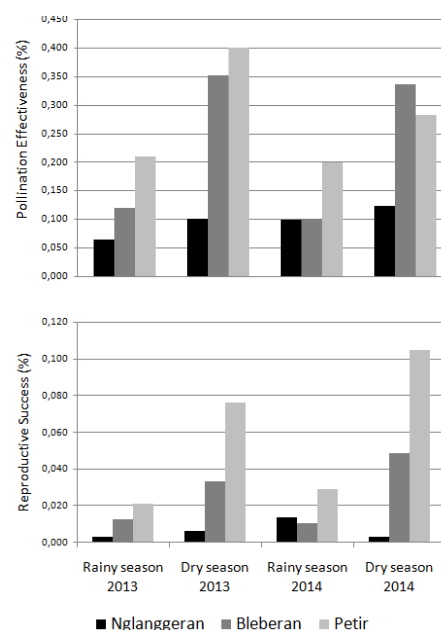


Figure 7. The value of PE (above) and RS (below) on three sandalwood populations in Gunung Sewu during the rainy and dry season in 2013 to 2014 flowering periods. The y ordinate indicates the value of PE (above) and RS (below), each population represented by different color of bars. The horizontal x line represents the rainy and dry season within 2013 to 2014 flowering periods

This study reported alteration on flowering frequency due to the extreme drop on temperature from late June to July 2014. In Wanagama, flowering frequency has also switched from twice to three times a year, with shorter period, due to an extreme temperature increment in the middle of rainy season in January 2007 (Ratnaningrum and Indrioko 2015). Sequential replacement on initial flowering due to alterations in temperature and delayed rainy season was also observed in *Oxalis* (Dreyer et al. 2006).

Mass fruit abortion, which followed immediately by mass production of new flower buds, was observed in lowest population as a response to drought from February to March 2013. In this middle of rainy season, very limited rainfall and extreme temperature increment resulted in the considerable reduction on soil moisture. Same findings were also reported with apple and pear tree in the southwestern Cape region that undergo changes in flowering pattern due to the longer drought which affected the groundwater availability (Grab and Craparo 2011). Many studies concluded that reproductive performance of the arid and semi-arid species is affected more by drought and warming environmental condition, emphasizing the importance of changes in both rainfall and temperature, and the sequential relationship between reproductive stages (Dreyer et al. 2006; Alizoti et al. 2011; Haukka et al. 2013).

Flowering time plasticity, as reported in this study, has also frequently been documented as a common adaptive feature of plants, particularly in arid and semi-arid environments (Lesica and Kittelson 2010). Generally, plants were turning back into its normal flowering behavior as the climate returned into a normal condition.

Soil moisture and soil types. Several factors in addition to temperature such as plant size (Dahlgren et al. 2007) and soil water availability (Phillips et al. 2011; Shiping et al. 2014) may cause differences in phenology. The combined effect of warming and reduced precipitation that was associated with a longer dry season was also influenced by groundwater availability (Grab and Craparo 2011). Previous studies have demonstrated that drought often shortens the duration of flowering, because flowers are open and lose water through transpiration, but limited soil moisture may slow development during bud and fruit development stages, lengthening those stages (Phillips et al. 2011; Shiping et al. 2014).

Given that rain and precipitation aspects mainly control soil moisture, temporal patterns of flowering in the tropical, arid and semi-arid region are strongly determined by rainfall seasonality, and flowering is influenced by both seasonal temperature and the onset of winter rains (Dreyer et al. 2006; Grab and Craparo 2011). These results correspond to those obtained by Dreyer et al. (2006), which suggested that warmer season and drier soils shortened flowering on the *Oxalis* species they studied. Soil temperature and slopes also influenced flowering in *Actaea spicata*. The earliest flowering observed at the warmer and drier site, on steeper and more south-facing slopes, where soil water status is lowest (Dahlgren et al. 2007). However, some other results by Haukka et al. (2013) contradicted this, since *O. pes-caprae* flowered longer in drier and warmer sites.

In line with the influence of soil water status, flowering was also affected by soil type. This effect existed due to differences in water-holding capacities of the soils (Haukka et al. 2013). On heavier soils, the wetting front is shallower, while on sandy soils, loss of water is more often to occur and seed production is more limited. Each site in this study was dominated by different soil type. In Nglanggeran population, soil was dominated by latosols with high concentration of amorphous aluminum silicates and Al-organic matter complexes, the type of soil which belongs to Andisols group (Haryono and Suratman 2010). The topsoil formation - a dark horizon consisted of high organic matter contents and large components of fine materials - tends to be stronger in cold-humid rather than warm-dry climates (Van Breemen and Buurman 2002). A very porous aggregate in Andisols enhance the ability to retain a large amount of water, that is resulting in high water retention and water binding capacity. This condition allows the soils to gain more water in rainy season and keep the humidity in dry season.

Soil in Bleberan population, the association of red Mediterranean and black grumusols with limestone rocks, was containing a high portion of smectitic clay minerals that were formed at high concentration of cations Mg and silica in the soil solution. This soil type belongs to Vertisols group, which was characteristics in strongly seasonal climates, and is commonly found in semi-arid and Mediterranean climates, tropical monsoon regions, and the catchment areas of tropical lowland rivers. In the basin areas or flat landscapes, higher clay contents and initial water contents allow better water preservation compared to

those of mound or undulating ones. Petir population characterized soils of low-rainfall regions with the accumulation of soluble minerals such as carbonates, , and chlorides that maintain the soil pH at above 7. High osmotic pressure of the soil solution due to high soluble salt was resulting in the decreasing of water availability (Van Breemen and Buurman 2002).

Altitude. The evident indication of climatically-induced changes was mostly observed at altitudinal range limits where many studies reported changes in plant reproductive events (Bertin 2008). Plant which naturally inhabits low altitude sites may express positive responses on its warmer and drier environment. Furthermore, the heat requirement of phenological events was decreased as altitude increased (Shiping et al. 2014; Phillips et al. 2011). This study conducted along elevational gradients ranged from 70 to 750 m of Gunung Sewu. Higher elevation, which correlated to the lower temperature and higher soil moisture, delayed the initial times of flowering. The flowering duration also lengthened with increasing elevation. Similar finding was also observed on annual plants along elevation gradient of Tibetan plateau (Shiping et al. 2014) and *Allium* species native to the Intermountain West region of the United States (Phillips et al. 2011).

Altitude drives phenological phases mainly by its effect on temperature alteration. Flowering was earlier at lower elevations than those of higher elevations. Higher temperature that in many cases correlated to higher soil moisture has more closely occurred at lower elevations (Bertin 2008). Flowering initiation has also been delayed and possessed longer period at both high latitudes and altitudes (Phillips et al. 2011; Shiping et al. 2014). However, the environmental roles may work differs for different climatic zone, as was reported for tropical and arid regions. In many parts of arid and semi-arid regions of Africa, a temperature decreases as altitude increases, but the soil moisture was oppositely increased with the higher altitude (Dreyer et al 2006; Lesica and Kittelson 2010; Dalgleish et al. 2012; Haukka et al. 2013).

The effects of environmental differences on the reproductive outputs of sandalwood

Extreme temperature reduction in the middle of 2014 was significantly reduced the number of flowers and fruits at all sites. Particularly in lowest population Petir, drought in the middle of rainy season in 2013 resulted in the mass abortion of pollinated flowers and mature fruits. However, new flower buds emerged right after the mass fruit abortion, and was followed by the production of significant number of mature fruits, resulting in the highest RS.

Similar findings observed in *Oxalis* species in the arid region of Cape Flora, South Africa. In the dry season, the number of flowering declined with an increase in temperature. Rainy season produced more flowers, but it declined with the increase of rainfall (Dreyer et al 2006). Some other species such as sandalwood in Wanagama, the middle zone of Gunung Sewu (Ratnaningrum and Indrioko 2015) and grasses in African savanna (Dalgleish et al. 2012) performed contradictory responses, in which more abundant flower but lower fruit sets was occurred in a wet

year compared to a dry year. The alteration of flowering between years indicated that the energy allocation for flowering presumably more closely related to resource availability, such as the rainfall (Dalglish et al. 2012). The increase of 2010 yearly rainfall in Wanagama resulted in the abundance of the high flowers, but extreme reduction occurred on pollinated flowers and mature fruits, presumably due to the pollination and fertilization failure (Ratnaningrum and Indrioko 2015).

Temperature and rainfall may affect reproductive events through direct effects on both phenology and reproductive structures production. Indirect effects include the disruption of plant-pollinator interactions and changes in the size of plants and populations. Specifically, the numbers of flowers, fruits, and seeds set, as well as seed viability, may strongly correlated with water availability. This relationship between climate and reproductive performance is particularly relevant in semi-arid ecosystems, where water is the most important factor limiting plant development (Del-Cacho et al 2013).

Study in *Allium acuminatum* which naturally grow in lower elevation showed similar result that fruit set was also decreasing with increasing elevation (Phillips et al 2011). However, a high elevation *Actaea passeyi* populations behaved oppositely. Plant which naturally inhabits high altitude conditions may express positive responses on cooler and moister environment. Therefore, the higher populations may have more flowers per plant and higher fruit set in comparison to the lower elevation populations (Dahlgren et al. 2007).

This study reported extreme temperature reduction in the middle of 2014 which was significantly reduced the number of flowers and fruits at all sites. In other studies, an extreme temperature increment at the early of 2007 was also significantly reduced flowers and fruits of sandalwood in Wanagama (Ratnaningrum and Indrioko 2015). Bring altogether, these results suggested that the temperature stresses - both reduction and increment - may have significant effects on the seed production of sandalwood. The sexual reproductive phase seems to be sensitive to temperature fluctuations in every stage depend on the intensity and extension of temperature stress. Therefore, both low and high-temperature stresses during the flowering phase might be the reason for the failure of seed set (Alizoti et al. 2010; Sabat et al. 2011). The reproductive organs are especially sensitive to temperature stress (Owens et al. 2001), and genetic variation that likely reflect adaptation to temperature stress does exist at both the inter-specific and intra-specific levels (Alizoti et al. 2010).

Plants and animals in a given area have often responded at different rates to temperature change, which is likely to change patterns of interaction between plants and their pollinators (Bertin 2008; Lesica and Kittelson 2010). Insects pollinate sandalwood belong to hymenopterans, lepidopterans, and dipterans (Ratnaningrum and Indrioko 2014). Different environmental conditions due to the variation on altitude may create different suitability for the pollinators and pollination activities. Other research revealed different types, abundance, and intensity of sandalwood pollinators along altitudinal gradients in

Gunung Sewu (Ratnaningrum, unpublished data). This variation on types, abundance and intensity will consequentially affect reproductive outputs. Changes in the timing of flowering could also result in failure to produce offspring due to the lack of pollinators (Alizoti et al 2010). One of the effects of a warmer and drier climate may also be the lack of flowering synchronization, as pollen shedding might be finished before stigma receptivity, resulting in the fertilization failure (Alizoti et al. 2010; Indrioko and Ratnaningrum 2015).

Implication for conservation

Despite the diversity of geological features and biodiversity of Indonesia, less attention was paid in protecting both geo- and genetic-diversity. Previously, protected areas in Indonesia were mostly arranged based on species diversity protection, particularly under national park scheme. Therefore, it is a need to explore any possibilities that Gunung Sewu will be protected under geopark scheme. The challenges for conservation of sandalwood, particularly under geopark scheme, including how much effort should be addressed to conserve this endangered species under different landscapes as the climate becomes increasingly unsuitable. A critical initial step for conservation is to obtain big-scale inventories of the remnant populations, especially as there was a need to re-evaluate this species due to the risk of extinction (IUCN, 2009). Maintaining the natural reproductive processes within each population should be carried out with a different strategy according to the degree of plant sensitivity to environmental features. To maintain the regular flowering, it is recommended to facilitate artificial flowering stimulation. Enrichment planting with genotypes having strong reproductive fitness was also an option. Furthermore, natural pollination should be facilitated by providing suitable condition for the pollinators occurrence and pollination activities. Sandalwood is grown in association with various type of vegetation in accordance with its habitat characteristics. In tropical mountain habitat, sandalwood is associated with mahogany, *Gliricidia* sp., and several *Garcinia* and *Eugenia* families. The lowland areas consisted of more diverse vegetation including teak, mahogany, *Gliricidia* sp., *Schleicheria* sp., cajuputi, and acacia. The dry rocky-limestone habitat consisted of acacia and cajuputi, but more dominated by shrubs such as grasses and *Eupatorium* sp. Therefore, habitat-based conservation supposed to be taken into consideration.

In conclusions, flowering and seed production of sandalwood in Gunung Sewu varied with altitude and climatic conditions. Reproductive aspects of sandalwood were also observed to be environmentally sensitive. Changes in rainfall, temperature and soil water status altered flowering phenology and seed production. Floral initiation and flowering period differed among populations. Sites with lower altitude, lower rainfall, highest temperature, lowest relative humidity and lowest soil moisture flowered earlier with shorter flowering period. Flowering initiation delayed and flowering period prolonged by increasing altitude, moister and cooler

conditions. At all sites, flowering also delayed and prolonged in rainy season compared to those of dry season.

Lower populations Petir and Bleberan constantly produced more flowers in rainy season, however, higher abortion resulted in very low seed production. In dry season, generally sandalwood produced less flowers, but the pollination and reproductive success were higher and more fruits were produced. The highest reproductive output was achieved at the lowest altitude. The abundance of flowers did not differ with altitudes, however, it varied with season, in which rainy season always produce more flowers. More marked differences among sites were observed in the dry season. However, despite the less flowers, lower altitude populations produced more fruits. The highest altitude population experienced more flowers and fruits abortion at both seasons. Flowering and seed production was also observed to be temperature sensitive. Both drop and increase on temperature were resulting in the alteration of flowering frequency and reproductive outputs.

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