Optimizing breeding strategy of *Melaleuca cajuputi* subsp. *cajuputi* for a multiple-trait selection: considering the economic weight of traits for oil yield productivity

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**Abstract.** *Melaleuca cajuputi* breeding in Indonesia is entering the advanced generation cycle and improvements have been achieved for oil concentration and 1.8 cineole-content. In commercial plantations, the total oil yield is an important factor to ensure the sustainability and continuity of oil production. This variable is calculated based on oil concentration, survival rate, and leaf biomass. However, to date, biomass productivity is maintained through silviculture practices rather than genetics. Therefore, genetic improvement for other traits related to leaf biomass is necessary. This study aimed to optimize the breeding strategy of *M. cajuputi* for a multiple-trait selection using the economic weight of traits related to oil yield. The economic weight was derived by combining selection results in the past generation breeding population and the assessment in genetic gain trials. The study revealed that leaf biomass should be prioritized as selection criteria for oil concentration in the advanced generation breeding based on the current baseline of the achieved gain. The implication of the economic weight to further generation breeding selection for improving oil yield productivity is that the major traits affecting the oil yield should be incorporated simultaneously for selection in the breeding strategy of *M. cajuputi*. The leaves biomass could be more weighted than other traits in constructing the index for the multiple-trait selection considering the correlation among the three traits observed.

1. **Introduction**

*Melaleuca cajuputi* comprises three subspecies. Subsp. *cajuputi* occurs naturally in the Maluku Islands of Indonesia, particularly in the islands of Buru, Seram and Ambon, Western Australia and Northern Territory; subsp. *cumingiana* grows in Sumatra, south-western Kalimantan, Myanmar, Malaysia, Thailand, and Vietnam; and subsp. *platyphylla* can be found in southern Papua, Aru and Tanimbar Indonesia, south-western part of Papua New Guinea, and northern Queensland Australia [1]. *M. cajuputi* subsp. *cajuputi* is the source of cajuputi oil widely used as liniment and inhalant to treat colds, stomach aches, insect bites and other ailments. In this paper, the term *M. cajuputi* is used to refer to subsp. *cajuputi*. Natural stands of *M. cajuputi* in Maluku are managed to optimize oil production. This species had been well domesticated in Indonesia and is widely cultivated outside the natural ranges, such as in Java, Sumatra, Sumbawa, Kalimantan, and Papua. Indeed, the major percentage of cajuput oil produced in Indonesia comes from those plantations.

Breeding works have been carried out to increase the productivity of the *M. cajuputi* plantation [2]. Three important traits to improve productivity are oil yield, cineole content and leave biomass. Leave
biomass is the main product from *M. cajuputi* trees for producing oil through a distillation process. In plantation, the trees are coppiced at around 1 meter to collect leaves and stimulate leave sprouting. This paper discusses the strategy for multiple trait selections to optimize breeding, particularly by using economic weight. Multiple-trait breeding is a concept in breeding aiming to produce a maximum of economically useable products per tree per hectare [3]. In the case of *M. cajuputi* breeding, it is intended to produce maximum oil. Tree breeder is rarely faced with a situation where improvement in only a single trait is desired. The *M. cajuputi* breeding program also faces such a situation in which improvement in oil yield alone is not the best option. Therefore, practically all applied tree breeding programs use multiple traits in evaluating and selecting plus trees.

2. Material and Methods
A review of the cajuput breeding program was made based on analyzing from research reports of some genetic trials in Gunungkidul, Ponorogo, Cepu and Gundih, Central Java, reviewing and referencing related to multiple traits. The traits observed in the research report related to the genetic improvement resulted from the breeding program. Therefore, breeding evaluation achievement needs to be done to optimize genetic gain and impact the economic value.

2.1. Oil productivity growth: genetic improvement perspectives
Productivity could be defined as a measure of the production effectiveness process by comparing the amount of input and the yield of products. The productivity in *M. cajuputi* industry includes a combination of the two successive main steps of production. First is the plantation as a resource for producing the raw materials, and the second is the distillation as the manufacturing process for further oil production from the raw materials.

In plantations, high productivity is commonly attained through improving the quality of planting materials and cultivation techniques. Therefore, improving planting materials through genetic improvement would be an important factor due to its long-lasting impacts and cumulative changes in the performance of target traits. Therefore, higher genetically improved materials could significantly contribute to *M. cajuputi* oil production. However, the absolute values might vary slightly over time and planting location due to differences in climates, soil, and seasons.

Genetic improvement of *M. cajuputi* has been targeted for two oil properties, that is, 1.8-cineole and oil concentration (%) [2, 4]. It has been reported that such improvement of both traits, assuming the same standard of cultivation and amount of leaves production, accounted for 60 % of the average oil productivity growth rate per unit of plantation area on one cycle of harvesting time. It revealed that the breeding selection index weighted by oil concentration (%) significantly affects the increase of total *M. Cajuputi* oil production and, subsequently, its profits. Furthermore, oil production could also be improved by increasing leaves production by manipulating the tree density. Interestingly, this productivity growth could be maintained over the years since re-harvesting leaves could be practiced many times at 6-9 months intervals and each planted tree's rotation age (>25 years).

The effects of genetic improvement on oil productivity could be realized quickly at 18-24 months after planting. Under this perspective, it is suggested that the *M. cajuputi* oil productivity growth could then be determined from the progress of genetic improvement under the same traits weights for selection. Whatever the cultivation practices are adopted, the annual oil productivity growth would be stable and controllable under a measure of genetic improvement. Fewer frequencies of cultivation practices are needed in the plantation since once the tree is planted, it could be harvested many times over the years to produce the oil, leading to the effectiveness of genetic-based technology on *M. cajuputi* oil productivity higher. Therefore, genetic improvement of *M. cajuputi* has been an important source of oil productivity in the plantation.

The importance of genetic factors on oil productivity has also been realized in some other essential oil species. For example, a study on *M. alternifolia* reported that under various genetic selection strategies from first-generation seedling seed orchard (SSO), selected provenance and clonal seed orchard (CSO) provided an estimated average improvement in yield over the industry standard of
43%, 55% and 83%, respectively [5]. In other species, the breeding of lemongrass could increase oil content by around 31% per generation after five cycles of breeding compared to unimproved planting materials [6]. Those great improvements proved that genetic manipulation through breeding would become essential for improving oil productivity for the essential oil species.

2.2. Role of silviculture and breeding

Silviculture and breeding are two activities that cannot be separated. According to [7], tree breeding covered selective breeding, delivery systems for genetic gain, arrangement for the deployment of improved breeds or clones to site and silvicultural regimes. In contrast, silviculture involves raising or keeping site productivity by using fertilizer and controlling vegetation competition, controlling pests and disease, optimizing site potential and growth of target trees, and enhancing wood quality by handling stocking. Implementation of improved seed in operational plantation needs silvicultural technique to increase plantation productivity. There is a formula to describe the tree performance in tree improvement, i.e., \( P = G + E \), where \( P \) is a phenotype, a performance tree that expresses genetic and environment. The environment includes the silviculture technique to support the tree performance for an optimum product.

The breeding program of \( M. \) cajuputi has produced improved seed with the gain of 10 – 26% for oil content and 2-17% for 1.8 cineole [4]. Therefore, appropriate silviculture techniques should be applied in plantation management to obtain maximum genetic gain. Some silviculture techniques that had been used in the \( M. \) cajuputi plantation are:

a. Spacing.
Leaves biomass was influenced by environmental factors such as spacing. In the agroforestry system, the commonly used spacing for \( M. \) cajuputi is 4x2 m or 3x2 m and farmers use the land around \( M. \) cajuputi trees for crop planting. However, in monoculture planting, spacing 2x1 m is used. The spacing arrangement will determine the number of trees per ha (density), affecting the final biomass yield. More expansive space will give more place to grow, but a limited number of trees per ha. In contrast, narrow space increases competition among trees and may cause suboptimal growth of the trees.

b. Fertilizer
\( M. \) cajuputi needs nutrients for supporting biomass growth. Harvesting biomass periodically causes nutrient cycling to not return to the soil, so adding nutrients to the soil needs to be applied. Organic fertilizer of ± 0.5 kg is added to each \( M. \) cajuputi tree once per year. In the early wet season, chemical fertilizer such as NPK 25-7-7 is provided as much as 10-20 gr for each tree.

c. Irrigation
Commonly, in \( M. \) cajuputi plantation in Java (Perhutani), irrigation is not applied due to high cost. Fortunately, cajuput is relatively adaptive to dry climate, although irrigation may still be necessary when rainfall is below 1000 mm year\(^{-1}\), as experienced by \( M. \) cajuputi plantation in Bima NTB. This plantation adopted water irrigation for \( M. \) cajuputi plantation to stimulate growth.

d. Site matching
\( M. \) cajuputi is relatively easy to grow in many soil types, and even it can grow in marginal soil. Cajuput also can grow on peatland. In some cases, this species is planted in land reclamation of mining land, and it performs well. However, land with an altitude of over 700 m above sea level is not suitable to support cajuput growth.

3. Result and Discussion

3.1. Current status of breeding: achievement and challenges

The breeding program of \( M. \)elaleuca cajuputi subsp. cajuputi began in 1995. Collaboration between the Centre for Forest Biotechnology and Tree Improvement (CFBTI) and Commonwealth Scientific
and Industrial Research Organisation (CSIRO) Australia aimed to improve the productivity of *M. cajuputi*, mainly in oil properties (1.8 cineole and oil content) [2]. This program started with exploration in the natural distribution of *M. cajuputi* in Buru island, Ambon island, Ceram, and Mollucas island. Some 256 mother trees, leaves and seeds were collected. Screening for oil properties has resulted in 20 families and is used for the genetic material of progeny trials. The first progeny trial was established in Paliyan, Gunungkidul, in 1998, consisted of 20 families, ten tree plots and ten replicates. First, the selection was conducted based on growth and oil properties and retaining the best trees in each plot. Next, this progeny trial was converted as a seedling seed orchard. Finally, control pollination between plus trees in Paliyan Seedling Seed Orchard (SSO) was conducted for establishing a full-sib progeny trial. Other first progeny trials were established in Ponorogo, Cepu, and Gundih in 2000 with 50-83 families. These progeny trials have been converted to seedling seed orchards as well.

Clonal seed orchard established in 2007 in Playen Gunungkidul and Sleman, consisted of 30 vegetative materials of superior trees in Paliyan SSO, Ponorogo SSO, Gundih SSO. The second-generation progeny trial established in 2009 in Gunungkidul consisted of 65 families in three tree plots and four replicates.

A genetic gain trial to verify the achievement of the breeding program has been established and evaluated. It showed that gain of 1.8 cineole 2-17% was better than that of unimproved seed and 10-26% for oil content [2]. However, another trait (leaves biomass) evaluated in genetic gain showed no significant difference between improved seed and unimproved seed. This phenomenon is acceptable because the breeding program focused on improving 1.8 cineole and oil content. The scheme of *M.cajuputi* breeding strategy is described in Figure 1.

![Figure 1. The scheme of *M. cajuputi* breeding strategy.](image)

3.2. Relationship of biomass and oil concentration to oil yield: product equilibrium

In commercial plantations, the oil yield is an important factor to ensure the sustainability and continuity of total oil yield production. This parameter is calculated as a combination of oil concentration and leaf biomass. Therefore, it is accepted that the oil yield is primarily affected by oil concentration and leaf biomass [8-10]. The increase in oil concentration leads to an increase in oil...
production per unit of biomass. Genetic improvement has been considered as the only technique to increase oil concentration. Genetic improvement and silvicultural practices could increase leaves biomass. However, silvicultural practices are mainly used in the conventional leaf biomass production of \textit{M. cajuputi}, such as the arrangement of tree spacing to modify the tree density per unit area. However, this approach could not increase oil productivity continuously due to the maximum limit of the tree's biological character to grow. Allometric study in \textit{M. cajuputi} showed that the biomass production was affected by height, the diameter of stem and canopy [11]. On the other hand, the increase in tree density diminishes the growth of stem diameter and canopy. Another study in blue and oil mallee revealed that although the trees could be planted at densities between 3300 and 9000 plants/ha, the optimum biomass and oil production density was found in 5000 plants ha$^{-1}$[12].

Figure 2 shows an oil yield equilibrium of \textit{M. cajuputi}. It illustrates the growing trend of oil yield based on the silvicultures represented by tree density changes and genetic improvement as the target on breeding selection, assuming the same quantity of leaf per tree. Without any involvement of genetic improvement, the trend change of oil yield will appear in line with the change of tree density. The increase of tree density will increase total leaves production until the maximum space limit to optimal tree growth. After that, it declines continuously. Subsequently, the oil yield will also be in line with the rate of genetic improvement on oil concentration in which the increase of oil yield reflects the gain. However, oil yield will decline when the higher rate of genetic improvement stimulates the effect of adverse correlation between the oil concentration and leaf production per tree.

The growth trends of oil yield based on tree density and genetic improvement show a different feature among the two factors. In general, the genetic improvement will significantly affect the increase in oil yield than the ordinary plantation without improved seed. In addition, the peak of oil yield due to the changes of genetic improvement could be reached in smaller density conditions than that of the ordinary plantation. Therefore, it suggests that genetics is an essential factor in increasing the oil yield of \textit{M. cajuputi}. However, the continuous decline of growth trend from the two factors after the peaks will reach a balance point, indicating that any further increases of genetic improvement on oil concentration will affect oil yield compared to the ordinary plantation.

![M. cajuputi oil yield equilibrium](image)

**Figure 2.** The growth trends of \textit{M. cajuputi} oil yield based on tree density and genetic improvement on oil concentration.

### 3.3. Improvement of breeding strategy: economic weight consideration on genetic selection

The current genetic breeding program for \textit{M. cajuputi} has been focused on genetic selection for improving oil concentration. On the other hand, leaf biomass as another important trait in determining the oil yield was manipulated by changing tree density per unit area rather than genetics. However, an adverse correlation between these two traits should be considered since the increase of oil concentration could reach the maximum limit of oil yield. Then there is a decline in leaf production.
Therefore, genetic improvement for traits related to the leaf biomass is necessary to tackle this problem since the increase of biomass could be maintained if genetic resources are available.

From a forest tree perspective, the productivity of a tree or plantation at the end-product of industry and manufacturing is assessed by a unit that combines measures of the tree's traits, such as a unit of tree volume or oil yield. Improving productivity based on this unit through a breeding program means that the genetic improvement of trees should be established involving multiple traits. This can be designed as either based on index selection or by independent culling in one generation of breeding [13, 14]. However, the effectiveness of these designs to the expected genetic gain of the target traits could differ. Many studies reported that the index selection that combines the merits from some traits into one unit of value index could provide a more precise picture in selecting the genetic either in the level of individual tree or family. In this design, the weight of each trait is necessary to calculate the value index. In addition, the estimates of genetic parameters involving the heritability and genetic correlation among the target of traits used in the index would be an important consideration.

Index selection seems to become an appropriate design for genetic selection in the breeding program of *M. cajuputi*. One study on genetic improvement for oil concentration and 1.8-cineole content estimation from a full-sib progeny trial has been reported. The gain for oil concentration increased from −1.19 to 29.67%, while the gains for 1.8-cineole content decreased from 6.19 to 0.07% [15]. This study suggested a scenario that improved 1.8-cineole content by 2.18% was the most optimal selection as genetic responses of leaf oil concentration reached 27.36%. A simultaneous improvement in both traits reached the highest and became stable when the economic weights for the index ranged from 0.3 to 4 for oil content relative to 1 for 1.8-cineole content.

Regarding the continuity of oil yield increase, weighted selection on oil concentration and leaf biomass is also necessary. A study from a genetic gain trial reported that weighted only on oil concentration in first-generation breeding under the various intensity of selection (IS) has provided a high genetic gain on oil yield compared to unimproved seed [4, 16]. However, the oil yield among the improved seed tended to decrease by 5% with the increase in IS. However, the gain for oil concentration increased about 15%. Such gain was also followed by about 15% decrease in leaf biomass. In the same study, the relationship between oil yield and oil concentration was weaker than between the oil yield and leaf biomass. It suggests that the decrease of oil yield as the increase in IS might be due to the large decrease of leaf biomass. In other words, the weighted oil concentration during the selection tends to decrease the leaf biomass. It revealed that the adverse effects of selecting the oil concentration solely to the biomass have likely occurred. From this point of view, besides the oil concentration, weighted on leaf biomass should be considered during selection to improve the breeding strategy. Another study on eucalyptus oil has pointed out that some reduction in oil yield may occur if the selection during the breeding is based solely on one trait of oil concentration [8]. In addition, index selection incorporating both whole tree biomass and leaf cineole concentration is the most effective. The impact of adverse correlation and the important technical weight differences for multiple traits in a selection index model was also commonly practiced for other breeding for a wood-based product. Studies on *Pinus radiata* [17] and *Prosopis alba* [18] reported that dynamic processes seemed to occur for gain allocated to each trait under a different scenario of weighted among the traits in multi-trait selection.

Oil distillates from leaf biomass are the main product from *M. cajuputi* trees for the industry in which the economic value from the product is considerably important. In the breeding program, the economic value was defined as "the amount by which net profit may be expected to increase for each unit of improvement in the trait" [19]. Economic weights are then measured for each target trait to construct the index value for selection. However, the most appropriate economic data for the respective trait of *M. cajuputi* is still insufficient and some of them are not available. Therefore, some considerations based on the observed genetic parameters from several previous studies could be used as a basic index approach to replace the real economic value. However, the fixed values could not be determined. Such parameters include the genetic gains obtained from previous breeding through imposing selection on a single trait of oil concentration, the correlated gain for the other two traits.
being considered, and the relationship between the oil yield and the other two oil concentration traits and leaf biomass.

Previous generations' breeding results revealed that selection on oil concentration alone yielded substantial gain on the trait concerned. Still, it yielded a relatively small correlated gain in leaf biomass and oil yield, even gradually negative when the IS increased. On the other hand, the correlation between oil yield and biomass was strong and positive, while oil yield and oil concentration were moderate. From those parameters and assuming a moderate-high heritability, improving leaf biomass through breeding could not be neglected since selection based on a single trait affects the correlated traits adversely. Using an index incorporating oil concentration and leaf biomass for selection is expected to minimize such adverse effects. In this case, weighting between the two traits should be determined to balance the combination for achieving simultaneous gains. It is suggested that leaf biomass should be put more weighted in the selection index at least four times higher than the oil concentration considering current real achievement from the breeding program and for improving the advanced generation breeding strategy. The concerns are also the benefit and continuous oil yield as a final product from the *M. cajuputi* plantation.

The study result implies that further confirmation is still necessary using actual data from a representative breeding population since a complete assessment of traits was unavailable from the previous generation breeding. However, as suggested in this study, the coefficient weight could be used as a simulation baseline in constructing index selection to determine the best combination and achieve simultaneous gains for the three traits concerned: leaf biomass, oil concentration, and oil yield. In this case, a new progeny trial representing successive advanced generation breeding of *M. cajuputi* should be established. The leaf biomass assessment should be done together with oil concentration and oil yield.

### 4. Conclusion

Genetic improvement in the previous breeding generation of *M. cajuputi* by applying selection on 1.8-cineole content and oil concentration yielded a high and positive genetic gain on the respective trait concerned and total oil yield. However, continuing such a selection strategy by increasing IS or in advanced generation breeding indicated a small and even negative correlation between the correlated gains of leaf biomass and then oil yield. Therefore, incorporating oil concentration and leaf biomass into an index selection is necessary to maintain simultaneous correlated gains of oil yield, minimizing the adverse effects of sole selecting on a single trait. Economic considerations for both traits can be approached using other parameters and gains achieved during previous breeding generations. It suggests that excess weight on leaf biomass in compiling the selection index should be prioritized at least four times higher than the oil concentration.

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