Biotechnology for Tropical Plant Breeding in SEAMEO BIOTROP

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Introduction

Tropical biodiversity is the world's very valuable asset. Tropical plant biodiversity provides food, feed, clothing and shelter for a large portion of the world's population. The sustainability of biodiversity, therefore, is very important for life sustainability. Considering environmental degradation and the subsequent increasing number of endangered endemic plant species, strengthening our efforts toward saving biodiversity is indeed essential. The environmental degradation that is mostly caused by anthropogenic activities is derived from the ever-growing population, namely; deforestation, mining, expanding tourism, settlement and many others. The declining environmental condition affects the sustainability of biodiversity. Thus, collecting and assessment of the richness of tropical genetic resources are very critical for conservation and sustainable utilization. Biotechnology and plant breeding, through the production of superior plant seedlings with high productivity, must augment conservation efforts to sustain biodiversity.

Nowadays, agriculture production is pushed further toward marginal land as more fertile land is occupied by settlement. This situation juxtaposed the requirements for new agricultural varieties and clones which are adaptable to harsh environments. However, the seeds market often consists of traditional non-certified seeds with uncertain availability. The very limited availability of certified seeds with new adaptive potential has resulted in their exorbitant prices. The increasing need for certified new seeds often calls for a breakthrough technology supported by genomic breeding techniques, discovered through research on the nursery-raised selected superior clones [1]. Plant breeding programs include the development of clone banks for genetic conservation, clone seed gardens and propagation of important plants resulting from controlled gene crosses. The superior clones are then raised to develop seed gardens as seed sources to produce high-quality plant varieties. The first phase is to identify the parent trees as genetic resources. The second phase is to select superior seeds to achieve high-quality plants.

New Molecular Techniques for Plant Breeding

Selection is the first important technique during the plant domestication process leading to a successful plant breeding program. Selection is a choosing process of plants with the most desirable properties in accordance with the objectives of the plant breeding program from a set of existing plant populations. The primary purpose of the selection is to improve the plant's genetic traits by increasing the targeted gene pools in a population. Nowadays, selection could be done either *in-vivo* or *in-vitro*. *In-vitro* selection is often preferred due to its

Figure 1. Utilization of biotechnology: a). *In-vitro* selection; b). *In-vitro* micrografting; c).
Note: Results of breeding by using mutation technology in *Acacia mangium*.

several advantages such as requiring only a smaller area and having more homogenous controlled conditions compared to field conditions, which make selection highly effective. *In-vitro* selection is usually done to study the relationship between phenotypes and genotypes as well as to implement non-controversial biotechnology approaches for obtaining superior plant cultivars, i.e., by genetic engineering. *In-vitro* plant breeding includes micropropagation techniques for obtaining large quantities of seeds, induction of somaclonal diversity, *in-vitro* tubing and obtaining secondary metabolites [2].

Plants produce various types of secondary metabolites in their tissues, however, their concentrations are very low and their formation depends on the stage of plant development. Extraction of plant secondary metabolites often faces obstacles due to the limited amount of plant supply and the high cost of purification. *In-vitro* culture is an efficient alternative for producing secondary metabolites with various advantages, including independence from varied environmental conditions (geographic location, climate and season), careful selection of multiplied cells that contain certain secondary metabolites, observable cell growth and observable cell metabolic processes [3, 4]. The metabolic processes can be rationally regulated to form bio-active compounds under controlled conditions in a relatively shorter period in a free-microbial-contaminated environment. One example is the production of agarwood employing the *in-vitro* dual culture interaction technique, in which several selected potential fungi are applied to cultured agarwood plant tissue to induce the formation of agarwood compounds as secondary metabolites (Fig. 1a). *In-vitro* micrografting is a vegetative propagation technique carried out in an aseptic environment using *in-vitro* culture techniques aimed at combining the superior characteristics of rootstock and scion (Fig. 1b).

The advantages of using the *in-vitro* micrografting technique include rejuvenating tissue from old plants, allowing a year-round seed production, shortening the production time in providing grafted seeds and disease-free plants, increasing studies on the compatibility and correlation between rootstock and scion, making desired specific combination among genotypes, reducing environmental impacts, shortening the breeding cycle, increasing resistance to diseases and parasites originating from the soil, increasing nutrient uptake, increasing plant's vigor, providing multiple production periods with uniform quality, increasing production and having knowledge of the compatible and incompatible micrografting techniques. In a compatible micrografting, the reciprocal relationship between rootstock and scion occurs normally, which will affect the variability in nutrients distribution patterns, the nutrients movement across the joint junction and the regulation of hormone transport.

Another in vitro breeding technique is the use of mutation technology to bring out new characters. The mutation was induced in *Acacia mangium* tissue culture having high cellulose content, which otherwise is limited in nature. The micropropagation of *Acacia mangium* generation M1 is obtained by mutation breeding technique (Fig. 1c).

The success of plant breeding depends on several factors, namely genetic

diversity, expected inheritance and potential genetic advantages. So far, morphological identification is used to distinguish new varieties from the existing ones. However, morphological

identification can provide biased results due to similar parental characteristics from which the varieties are derived, especially for plants having narrow genetic diversity. Morphological evaluation is subjective. The appearance of plant morphology is controlled by genetic traits that are influenced by environmental factors. Stronger environmental factors can cause variations in plant morphology and responses to a certain condition, such as soil fertility, pest and disease attacks. Molecular techniques could help in distinguishing plant accessions.

Molecular markers have a very broad potential to shorten the breeding duration and increase the efficiency of breeding programs. Their development allows plant breeders to assess genes almost directly, providing ways to select genes responsible for the desired traits, such as pest and disease-resistant genes in plants. PCR-based markers, such as RAPD, microsatellites or SSR, PCR-RFLP and AFLP, which are relatively easy and cheap to develop have been used frequently to tag genes involved in economically important traits using QTL mapping.

Microsatellite markers were utilized in studying the Sengon (*Falcataria moluccana*) resistance to gall rust disease [5]. Previous RAPD markers could not differentiate accessions showing different resistance and susceptibility against gall rust disease [6]. SSR markers which usually give higher polymorphisms than RAPD and more stable results could be used to evaluate the germplasm diversity. Through the SSR markers a high level of heterozygosity was found in a Sengon population (Fig. 2). The SSR markers were able to genetically differentiate some resistant accessions of Sengon from some susceptible ones. However, some other accessions remained clustered together forming separate groups with intermediate reactions toward the gall rust disease (Fig. 3).

Further investigation on the resistance and susceptibility of Sengon accessions was done using phytochemical screening, which is a technique to determine secondary metabolite presence in the accessions. Different secondary metabolites which are resistant and susceptible to the gall rust disease were found on substances extracted from sengon trees. The susceptible Sengon trunk contained flavonoid, saponin, phenolic, hydroquinone, tannin, triterpenoid and steroid, but did not contain alkaloids. On the contrary, the trunk of Sengon trees which are resistant to the gall rust disease contained flavonoids, saponin, triterpenoid and stronger ssteroids compared to the susceptible ones [7].

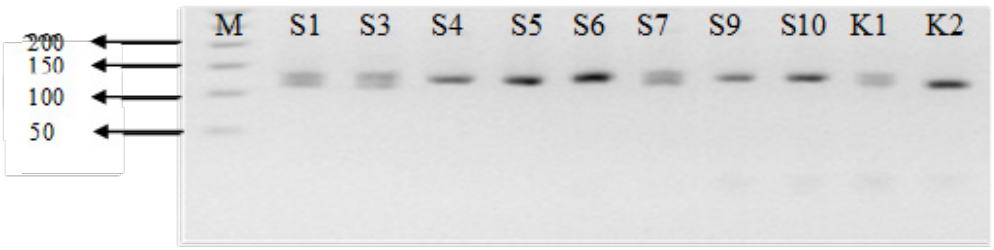

Figure 2. The use of microsatellite molecular markers on *Falcataria moluccana* which is resistant and susceptible to gall rust disease
Notes: M = Marker, bp); S1-S10 (Sengon is resistant against Kediri), K1-K2 (Sengon is susceptible to Kediri).

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The generation of previous molecular markers is often inadequate to differentiate accessions clearly as explained above. The coming of the next-generation sequencing era opens the possibility of generating more powerful markers based on genomic or transcriptomic sequences from relatively cheap sequencing processes. Transcriptomic sequences from Sengon having resistance and susceptibility against stem borer pest and gall rust disease have been published recently [8, 9]. From the available sequences, it is easy to detect single nucleotide polymorphisms (SNPs) among the sequenced accessions. Now SNPs are becoming the choice markers to tackle complicated traits with complicated inheritance patterns. Genome-wide association study (GWAS) is often performed to dissect complicated traits using the association between phenotypes influenced by environmental factors with markers scattered throughout the genome. The use of SNPs and GWAS would accelerate the breeding program of tropical plant species with more precision.

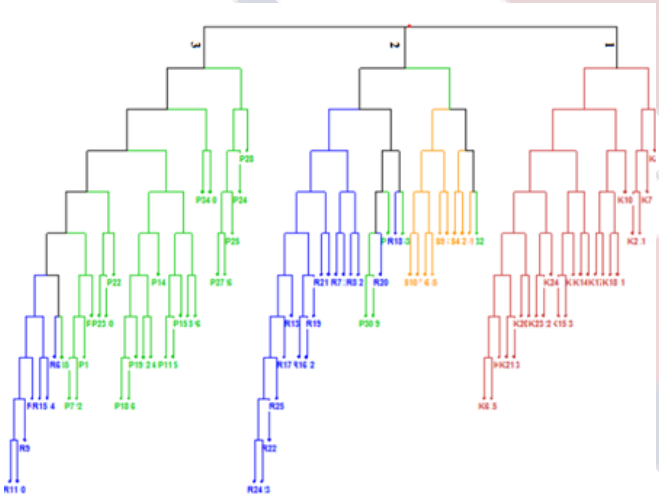

Figure 3. Dendrogram showing clustering of Sengon accessions from two populations of Kediri and Sukabumi into three main groups with different resistance and susceptibility against gall rust disease

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