

UNIVERSITI TEKNOLOGI MARA

**PROTOCOL OF SOMATIC EMBRYO
TOWARDS *IN VITRO* ARTIFICIAL
INOCULATION ON *Aquilaria
malaccensis* Lamk. (GAHARU)**

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ABSTRACT

Aquilaria malaccensis Lamk. belongs to the Thymelaeaceae family and known as agarwood in the world and Gaharu in Malaysia, that is commercially identified as a potential aromatic plant. Inoculation for resinous compounds in *A. malaccensis* naturally is difficult. Hence, an artificial inoculation via *in vitro* inoculation method was developed. Stem explants of *A. malaccensis* were cultured on MS media supplemented with 1.0 mg/L BAP and 0.1 mg/L 2,4-D for the callus induction. Embryogenic callus was successfully initiated on MS media supplemented with 1.0 mg/L and 0.1 mg/L 2,4-D added with 500 mg/L casein hydrolysate by producing globular, heart-shaped and torpedo stages. Addition of additive including casein hydrolysate added with 10.0 mg/L abscisic acid (ABA) give a significance effect on the development and maturation of the embryogenic callus. Therefore, induction of a high frequency of somatic embryogenesis in stem explants on MS media is possible. Optimum callus formation from MS media added with 500 mg/L casein hydrolysate and 10.0 mg/L ABA which was used to induce resin of *A. malaccensis* through *in vitro* artificial inoculation. The inoculant known as 4 Moon Booster which was inoculated into MS media then the interaction of the inoculant to embryogenic callus and media was successfully obtained. The result revealed that, embryogenic callus turned into dark brown from creamy white color and give an odour. In addition, the inoculated media were also turned to brown and produced a sense of sensory when the container cap is opened. So, the inoculant gives significance artificial inoculation effect on the embryogenic callus and to the media. A reliable protocol useful for *A. malaccensis* through somatic embryogenesis was successfully established for artificial inoculation purposes.

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CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF STUDY

Aquilaria malaccensis Lamk. is a woody plant of Thymelaeaceae family from the order Myrtales and commonly known as agarwood, eaglewood or gaharu. In a world market, agarwood had been known as the most expensive wood. In a fifteen genus *A. malaccensis*, there are eight species were known to produce of agarwood. (Saikia *et al.* (2012) and Akter *et al.* (2013). There are three species recently can produce agarwood which are *A. malaccensis*, *A. agallocha* and *A. secundaria* but in theory, agarwood can be produced by all members (Selina *et al.*, 2013).

A. malaccensis is a well-known species for the production of good quality agarwood. Gaharu is highly economic importance in Asia due to its uses for the production of incense, perfume and traditional medicine. *A. malaccensis* is a relatively slow-growing plant compared with other *Aquilaria* species. The production of incense perfume and traditional medicine was contributed from the infected part of agarwood as a raw material. The resin of agarwood oil was obtained from the infected agarwood trunk through distillation process known in the east as “agar attar”. This resin has a unique fragrance and also has high export value in the world market (Saikia *et al.*, 2012) and (Akter *et al.*, 2013).

In a case of distillation process for agarwood oil, the agar oil traders have to sacrifice the whole tree as its heart wood serves as the raw materials. The exploitation of the forest area has destroyed the habitat of the agarwood. Consequently, agarwood is included in IUCN red data list of the year 2011 as vulnerable and at the verge of extinction from the natural forest (Saikia *et al.*, 2012). In addition, *A. malaccensis* also have restricted the period of seed viability (Ahmed and Gogoi, 2000). So, modern technologies should be applied for the conservation, existence, and preservation of germplasm of this species.

This seeds of *A. malaccensis* only can be obtained once a year. So, it is difficult to germinate this tree every month. The germination of the random seed is