Characteristic of synthetic seeds from two medicinal plants
*(Moringa oleifera and Camellia sinensis)*

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**Abstract.** *Moringa oleifera* and *Camellia sinensis* are medicinal plants and useful for health. Generative propagation of *M. oleifera* plant is quite difficult because the ability of seed germination is low in its survival is low, the growth takes a long time and the lack of vegetative propagation method. *C. sinensis* has difficulty in rooting plantation. One alternative to overcome these obstacles is by planting vegetative propagation through biotechnology approach that is in vitro culture techniques and encapsulation techniques that can produce synthetic seeds. The purpose of this research is to know characteristics synthetic seeds of *Moringa oleifera* and *Camelia sinensis*. Stages done in this research is induction of embryogenic callus in MS medium which has been modified. The somatic embryo of *M. oleifera* and *C. sinensis* obtained will be encapsulated and placed at CaCl₂·2H₂O, when the seed has been hardened rinsed with aquades.

Synthetic seed *C. sinensis* yellowish green and 5.5 to 5.7 mm in diameter and fresh weight of synthetic seed produced ranges from 260-290 mg. Characteristics of synthetic *M. oleifera* produced seeds have clear and yellowish-looking embryo, and are 5.8-6.0 mm in diameter and have a fresh weight of 250-300 mg.

1. Introduction

Medicinal plants are an important element of indigenous medical systems that has persisted in developing countries [1]. Traditional medicine has a long history of serving peoples all over the world [2]. Recently, the use of traditional medicine based on plants has received considerable interest. Different plants produce diverse products like Moringa oleifera and Camelia sinensis.

*Moringa* (*Moringa oleifera* Lam) belongs to the Moringaceae family. *Moringa oleifera* is one of thirteen species most widely cultivated. The leaves contain more vitamin A compared to carrots, more calcium than milk, more iron than spinach, more vitamin C than oranges, and more potassium than bananas, and that the protein quality of *Moringa* leaves rivals that of milk and eggs [3] The extracts of *Moringa* leaves shows antimicrobial and antifungal activity in addition to cancer preventive effect [4][5]. Some of the compounds isolated from *Moringa* preparations which are reported to have hypotensive, anticancer and antibacterial activity include 4-(4'-O-acetyl-a-L-rhamnopyranosyloxy), Benzyl isothiocyanate, 4-(a-L-rhamnopyranosyloxy) benzyl isothiocyanate, niazimicin, pterygospermin, benzyl isothiocyanate, and 4-(a-L-rhamnopyranosyloxy) benzyl glucosinolate [6].

According to ethanobotanical studies its roots are bitter, acrid, thermogenic, digestive, carminative, anthelmintic, constipating, anti-inflammatory, emmenagogue, diuretic, ophthalmic, expectorant and stimulant. They are useful in dyspepsia, anorexia, verminosis, diarrhea, colic, flatulence, paralysis,
inflammations, amenorrhea, dysmenorrheal fever, strangury, vesicle and renal calculi. It is used in cough, asthma, bronchitis, pectoral diseases, splenomegaly, epilepsy and cardiopathy [7]. Due to these medicinal values, this plant have to be conserved and multiplied to reach commercial requirement.

Moringa is now found in all tropical regions around the world ranging from parts of South Asia through West Africa, including Indonesia. Although the spread of Moringa is vast, but in Indonesia these plants become very rare nowadays. The spread of Moringa is also rare for seed germination and viability or survival are low, and there is the lack of vegetative propagation method [5]. Moringa distribution limited is the main reason that drives the need to make efforts so that the necessary conservation plant propagation techniques to produce many seeds of Moringa in a short time.

Tea (Camellia sinensis L.) is one of the most important plantation crops in the world. The medicinal effects of tea have a history dating back almost 5000 years. Tea is the oldest non-alcoholic caffeine-containing beverage crop in the world and health benefits attributed to tea consumption are well proven. Tea is the chemical components of green tea chiefly include polyphenols, caffeine and amino acids. Tea also contains flavonoids, compounds reported to have anti-oxidant properties having many beneficial effects. Tea flavonoids reduce inflammation, have antimicrobial effects and prevent tooth decay. The pleasing astringent taste and refreshing boost it provides is so deep-pervasive that its potential health benefits and medicinal properties are often overlooked. Ongoing scientific exploration points that the certain potential health benefits derived from tea have important implications on human health. The American Medical Association shows that green tea can lower cholesterol levels, high blood pressure, and reduce the risk of strokes (especially in men). The National Cancer Institute reports that because of the highly effective anti-oxidants in green tea, it can ward off various types of cancer. There are many therapeutic values in green tea, including, aiding digestion, blood purification, ensuring regularity, lowering body temperature, strengthening teeth and bones, boost immune system, enhance heart function, suppress aging, deter food poisoning, fights virus, and lowers blood sugar levels [8].

The chemical components of tea leaves include polyphenols (catechins and flavonoids), alkaloids (caffeine, theobromine, theophylline, etc.), volatile oils, polysaccharides, amino acids, lipids, vitamins (e.g., vitamin C), inorganic elements (e.g., aluminium, fluorine and manganese), etc. However, the polyphenols are primarily responsible for the beneficial healthful properties of tea. The flavonoids have antioxidant, anti-inflammatory, antiallergic and anti microbial effects. Green tea contains six primary catechin compounds namely catechin, gallocatechin, epicatechin, epigallocatechin, epicatechin gallate and epigallocatechin gallate (EGCG), the later being the most active component [8]. Single leaf-node cuttings conventionally propagate cultivated tea. This method of production becomes limiting when large numbers are required from new clones of which only very few stock plants are available. Moreover, the process in establishing tea plants from cutting is lengthy and labour-intensive, rendering it ineffective for rapid dissemination of new clones for large-scale commercial plantings [9]. Conventional tea breeding is well established, though time-consuming and labors intensive due to its perennial nature and long gestation period (4-5 years). additionally, tea breeding has been slowed by lack of reliable selection criteria. Vegetative propagation is standard, yet limited by slow multiplication rate, poor survivability of some clones, and need for copious initial planting material [9].

One alternative techniques to get the plants in large quantities and quickly as conservation effort is using biotechnological approaches such as synthetic seed technology through in vitro culture. Encapsulation technique is a prospective technology developed for seed multiplication and conservation. Encapsulation technique is a technique of wrapping explants (callus, somatic embryos or meristem or shoot tips) with a wrapper specially made explant is not easily damaged and can grow. Packaging plays a role as the endosperm-containing carbon source, nutrients, and plant growth regulator (PGR) synthetic seed The technology can provide advantages such as long-lasting storage, simplify distribution or dissemination, as well as to protect the seed from disease [10].

In recent years, alginate encapsulation technique used in the in vitro alternative to synthetic seed production using somatic embryos. The definition of the seed synthetic that somatic embryos,
seedlings or meristematic tissue other encapsulated artificially and can develop into a plant in conditions in vitro or in vivo encapsulation provide physical protection in somatic embryos, carrying nutrients, growth regulators, antibiotics, fungicides needed during germination and protector of the danger of pathogens [11][12]. Synthetic seed technology can provide benefits, among others, can be stored in the long term, facilitate the distribution or dissemination, as well as to protect the seed from disease [10]. Synthetic seed is a plant biotechnology applications in vitro which offers many benefits, especially M. oleifera and C. sinensis. The purpose of this research is to know characteristics synthetic seeds from somaticembryos of Moringa oleifera and Camillia sinensis.

2. Material and methods

2.1. Plant material
Leaf of Moringa oleifera and C. sinensis were used as explants for callus induction and its plant regeneration. These were collected from Surabaya and Malang East Java, Indonesia.

2.2. Sterilization and inoculation of explant
Sterilization was carried out by washing the leaves with distilled water for 10 minutes, and then soaked in surfactant solution for 10 minutes, followed by three times of rinses with distilled water for 5 minutes. The explant were soaked in antifungal for 10 minutes, followed in NaOCl solution for 2 minutes. The explant were further surface sterilized using 96% alcohol for 3 minutes, then rinsed with sterile distilled water. The explants were surface dried on sterile filter paper and cultured on MS medium supplemented with different levels of plant growth regulators for callus induction and somatic embryogenesis

2.3. Medium composition and culture condition
MS (Murasige and Skoog) medium consisting of MS basal medium with vitamins, and 30% of sucrose. The pH was adjusted to 5.6-5.8 with NaOH atau HCl before autoclaving at 121°C for 20 minutes. The growth regulators were added to the culture media before autoclaving. Cultures were incubated in a growth chamber at 24°C, 70% of relative humidity and 16/8 h our (light/dark) photoperiod with light supplied by cool white fluorescent lamps at an intensity 60 µmol m^{-2}s^{-1}

2.4. Medium of induction and maturation of embryonic calluses
The M. oleifera explants were placed on MS medium supplemented with 1 mg L^{-1} NAA and 1 mg L^{-1} kinetin for the induction of embryonic callus formation. Embryonic calluses were induced for 4 weeks. The C. sinensis explant was cultured in MS medium supplemented BAP medium 1mg / l and NAA 0.1 mg / l.

Somatic embryos were induced within 4 weeks on MS media supplemented with 3 mg L^{-1} BAP. The C. sinensis explant were placed on MS + BAP 1mg/l + NAA 0.1 mg/l for maturation of embryonic embryos. The greenish- yellow, globular pro- embryos were observed and these were sub-cultured on the same medium for maturation. Matured somatic embryos (heart- and torpedo shaped) were sub-culturing every 2 weeks.

2.5. Encapsulation technique
The embryoosomatic of M. oleifera and C. sinensis were encapsulated by the same method. 4% sodium alginate was added with 0.3 mg L^{-1} NAA and 3 mg L^{-1} BAP in a calcium free liquid MS medium containing 30% sucrose. Encapsulation was done by mixing embryo on Na- alginate gel and MS. The somatic embryos was introduced into a mixture of sodium alginate and liquid MS and then taken with a disposable sterile plastic pipette with the position of all parts of the embryo covered by sodium alginate. The explant was release drop by drop into 75 mM CaCl2 solution for 15 to 20 minutes, then the synthetic seeds were washing in aquadest to remove residue. The observed parameters of synthetic seed characteristics were diameter of synthetic seed, color and texture.
3. Result and discussion
The embryogenic callus formed is transferred to the maturation medium. Maturation is the maturation stage of embryogenic callus (Oetami, 2015). The maturation stage of the embryogenic callus is the developmental stage of the globular structure to form cotyledons. The first stage of embryosomatic is the formation of globular phase. The embryoid growth begins with a globular phase with the formation of a rounded bulge, usually from the callus. The next stage is the heart phase, marked by the formation of the gap so that there is a hollow between two bulges and looks like a heart shape, and greenish-white. The heart phase embryo is characterized by a gap in which SAM (Shoot Apical Meristem) is formed. At the torpedo stage, it looks that the cell is growing longer, and is whiteish-green.

3.1. Synthetic seeds of Moringa oleifera
Production and application of synthetic seeds has been widely practiced in the field of plant biotechnology such as massive clonal propagation and in large quantities, germplasm conservation, facilitate storage and transport of seeds [13]. Seed technology is a highly perspective technique to be developed in seed propagation and conservation.

The maturation medium used was MS with 3 ppm BAP, according to the [5], the right hormone for maturation was BAP concentration of 3 ppm. BAP is a cytokinin that is often used in tissue culture techniques. Cytokinins are derivatives of adenine. Cytokines are very important in the regulation of cell division and morphogenesis. The effect of cytokinin in plant tissue culture is related to the process of cell division, root growth inhibition, and micro root induction.

The resulting somatic embryo will be encapsulated using alginate with a concentration of 4%. Alginate is one of the most appropriate hydrogels in the manufacture of synthetic seeds because it is enriched with nutrients, growth regulators, has low toxicity, low cost, not too sticky, rapidly agglomerates and has biocompatibility properties [14]. Alginate with a concentration of 4% is the most appropriate concentration for the germination of synthetic seeds. Alginate that has too high concentration will cause the seeds produced too hard, thus inhibiting the ability of the embryo to germinate. In accordance with the statement [15] that the conditions of very dense seeds affect the life of the embryo in the seed because the condition does not support the growth and development of the next embryo. High hardness in the seeds is expected to cause the environment to become anaerobic, will further inhibit respiration, and delay the rate of respiration will inhibit the process of seed germination. The process of making synthetic seed begins with 4% alginate solution using dropper drops, then bulb on the pipette is pressed slowly. When the alginate solution comes out of the tip of the pipette immediately the somatic embryo callus portion to be encapsulated is inserted into the droplets of alginate at the tip of the pipette using tweezers rapidly and precisely until all the explants are wrapped. Then the eyeball bullet is pressed back slowly until the alginate on the tip of the pipette trickles and falls on a solution of 75mM CaCl2. Furthermore, the synthetic seeds that have been subjected to hardening are removed from the 75 mM CaCl2 solution and soaked into the aquades. The hardening process is caused by the exchange of Na + ions from Na-alginate with Ca + when dropped into CaCl2 solution to form Ca alginate. The amount of Na + ions exchanging with Ca + ions determines the level of hardness of the capsule. The capsule is expected to be solid enough to prevent leakage [16].

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2\text{Na Alg}_{(aq)} + \text{Ca}^{2+}_{(aq)} \rightarrow \text{Ca (Alg)2}_2(s) + 2\text{Na}^+_{(aq)}
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Characteristics of the resulting synthetic seeds are seeds have a diameter ranging from 5.2 to 5.7 mm with a clear color and yellowish white embryo. The synthetic seeds that have been measured in diameter are then weighed to determine the initial fresh weight of the seed and then the seeds are grown on germination medium with ZPT composition and different concentrations of sucrose. The initial fresh weight of the seed ranges from 250 to 300 mg.
3.2. Synthetic seeds of Camellia sinensis

Synthetic seed incubated for 30 days. After observation, the texture of synthetic seeds solid but not too hard, still vulnerable to damage if not careful when moving. All the synthetic seeds that are formed have the same texture, and the embryo appears to have developed into a cotyledon phase and penetrates the alginate. Synthetic seeds are yellowish green with a diameter of 5.5 to 5.7 mm and a wet weight of 260-290 mg. The synthetic seed forming process is carried out using alginate because it is not toxic to the embryo or the material to be encapsulated, its density can protect the fragile embryo, and serves as a reservoir of nutrients that the embryo uses to survive and even accelerate its growth [17].

Synthetic seeds are formed in greenish white, but the embryo has a compact structure. The color changes that occur in the embryo due to pigment and are influenced by nutrients and environmental factors such as light. Greenish embryo caused by chlorophyll content, due to interaction of NAA and BAP, especially BAP (cytokines) that play a role in the formation of chlorophyll in callus and environmental factors ie exposure to light. The change of color embryo to white-green, because embryo cells have started to form chloroplasts so that embryos become more complex and further differentiate into specific organs.

4. Conclusion

Characteristics of synthetic seeds produced is a soft texture and clear. All the synthetic seeds that are formed have the same texture, Synthetic seed C. sinensis yellowish green and 5.5 to 5.7 mm in
diameter and fresh weight of synthetic seed produced ranges from 260-290 mg. Characteristics of synthetic M. oleifera produced seeds have clear and yellowish-looking embryo, and are 5.8-6.0 mm in diameter and have a fresh weight of 250-300 mg.

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