INTRODUCTION

Background

Health is an important thing for human life. The use of herbal plant products is increasing along with improved human’s awareness on the concept of a harmonious relationship between human and nature. Moreover, with the development of “back to nature” thinking, many people start to consume food and medicines that are made from natural ingredients. One result is the development of medicinal plant industry.

*Kaempferia parviflora* is a kind of galingale (aromatic ginger), which belongs to Zingiberaceae family. This plant originally came from northern part of Thailand. Most of the Thai use *K. parviflora* as seasoning for their traditional food, traditional medicine, and anti-inflammatory agent. Several research about *Kaempferia parviflora* extract bioactivity showed that it has, among others anti-malarial and anti microbial effect (Yenjai et al., 2004), antioxidant effect on fermented *K. parviflora* rhizomes (Vichitpan et al., 2004), antidepressant effect on aged rats (Wattanathorn et al., 2007), anti inflammatory effect through the inhibition of NO and PGE$_2$ release (Tewtrakul and Subhadirasakul, 2008), and inhibitory effect on *Helicobacter pylori* activity (Chaichanawongsaroj et al., 2010). Although *K. parviflora* has been used to treat inflammatory related diseases, but it hasn’t been commercially cultivated in a large scale.

Indonesia itself has a very large dependence toward imported medicine and conventional medicine ingredients. Development of Indonesian traditional medicine (herbal medicine), which majority made of medicinal plants, has an important role in the development of Indonesian drugs industry and community health services. Approximately 85% of the raw materials for traditional medicine industry were obtained from nature without special cultivation efforts. Besides the uncertain quantity issues, the quality of these materials are also less reliable (Indonesian Ministry of Agriculture, 2007).

The growing medicinal plant industry, requires a sustainable supply of raw materials, so it requires a stable availability of good quality plant materials. To fulfill that demands, medicinal plant cultivation needs to be improved, and to do
that, planting material availability needs to be maintained. A germ free and true-
to-type plant material will be continuously needed in large amount. Conventional
propagation of *K. parviflora* by splitting rhizomes will not sufficient for a large
scale cultivation in the future. A whole year was needed to produce rhizomes for
planting materials (ICS Unido, 2009). Pathogen infestation and dormancy also
posed as potential problem for conventional propagation technique. Another
propagation technique will be needed and plant tissue culture holds promise for
rapid multiplication.

Plant tissue culture is a plant propagation technique by isolating cell,
tissue, or organ of a plant under an aseptic condition until it grows into a perfect
plant. Plant tissue culture offers many advantages over conventional techniques.
The main advantage is that it could offer a rapid multiplication in short time.
Another advantage is with special technique, a germ-free plant could be grown, so
could be used as mother plant. Generally, there are two pathways that were
widely used in tissue culture technique, which are organogenesis and
embryogenesis (Gunawan, 1988).

Organogenesis can be defined as a process of plant cell or tissue forming
various organs. This process provides the basis for asexual plant propagation
largely from non-meristematic somatic tissues. Plant tissues have the ability to
dedifferentiate from their current structural and functional state and to begin a
new developmental path towards other endpoints. *In vitro* plant propagation used
this flexibility as a common approach by regenerating multiple shoot meristems
followed by root meristem induction.

Embryogenesis can be defined as the process of embryo development,
embryo itself is the earliest stage of an organism before it develops any structures
or organs. Usually, in higher plants, embryos are a product of gametic fusion
(zygotes), but as mentioned above, plant cells are unique, thus a morphologically
and functionally correct non-zygotic embryos can also arise from widely disparate
tissues type at different points of plant life cycle. According to Altman
and Loberant (1998), there are two developmental sequences leading to
organogenesis and embryogenesis in tissue culture. They differ in the presence or
absence of a callus stage, but it’s important mainly because it relates to the genetic
stability. A callus stage and meristem cell derived from callus usually lead to genetic aberrations than direct regeneration.

In this tissue culture technique, a part of a plant (explants) was grown on a special basal medium. Those basal medium composed of mineral nutrients (macro and micro), plant growth regulator, vitamins, amino acid, carbohydrate source (sucrose), solidifying agent (agar), distilled water, and additional organic materials. Several researchers have developed mediums for different use, like MS (Murashige and Skog), WPM (Woody Plant Medium) for woody plants, Gamborg B5 medium, and other basal mediums. There are a lot of basal mediums, but Murashige and Skoog medium is the first and most common medium for plant propagation.

The whole medium composition will determine the success rate of plant propagation trough tissue culture, especially mineral nutrients and plant growth regulators (PGR). PGR like auxin and cytokinins are used to regulate plant growth for various needs. PGR is widely used in plant tissue culture technique, but different plant varieties may respond a plant growth regulator in different ways. The effects of PGR are generally specific for explant type and plant species; therefore, a study was needed to determine the best medium composition for rapid multiplication.

**Objectives**

The aims of this research were to study the effect of different MS salt concentration and cytokinin (BAP) concentration to *K. parviflora* growth and shoot multiplication.

**Hypothesis**

1. There is an optimum MS salt concentration for *in vitro* shoot multiplication of *K. parviflora*.
2. There is an optimum BAP concentration for *in vitro* shoot multiplication of *K. parviflora*.
3. There is an interaction between MS salt concentration and BAP concentration, which optimally induce multiple *in vitro* shoot of *K. parviflora*. 