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Scanning Electron Microscopy Studies and In Vitro Regeneration of Passiflora edulis Sims var. edulis for Conservation

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Effect of Landfill Leachate on Brassica chinensis Dry Matter Weight and Iron Concentration
Scanning Electron Microscopy Studies and In Vitro Regeneration of Passiflora edulis Sims var. edulis for Conservation

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Abstract—In this study, different combinations of benzylaminopurine (BAP) and naphthalene acetic acid (NAA) were used to establish an efficient in vitro regeneration in two different types of explants: stem and shoot tip of seedlings obtained from in vitro seed germination of Passiflora edulis Sims var. edulis. The results obtained showed that BAP induced shoots while NAA induced roots. Scanning Electron Microscopy (SEM) studies were carried out to observe and differentiate the morphology of in vitro and in vivo grown Passiflora edulis Sims var. edulis. SEM techniques were done to observe the stomata and microscopic characters of both the axial and adaxial surfaces of in vitro and in vivo leaves of Passiflora edulis Sims var. edulis.

Index Terms—In vitro, in vivo, Passiflora edulis Sims var. edulis, regeneration, Scanning Electron Microscopy (SEM).

I. INTRODUCTION

The family Passifloraceae consists of 18 genera and about 430 species, most of which are tendril climbing vines native to warm regions of the world [1]. In America, the family is represented by four genera (Ancestrothysus, Dileka, Mitostemma and Passiflora), of which Passiflora is numerically and economically the most important genus of the family. There are about 500 species of Passiflora worldwide, but only less than 10 species could be found in Malaysia. Passiflora edulis Sims is considered as the most important species of the genus Passiflora, mainly because of its botanical and commercial value, as well as for crop breeding and genomic programs.

Passiflora edulis Sims var. edulis is also known as purple passion fruit and is a vigorous perennial woody climber. It is originated in the Amazon region of Brazil, but has been commercially cultivated in Hawaii, Australia, New Zealand, Fiji, South Africa and Kenya [2]-[4]. In Malaysia, it is widely distributed in the northern, east coast, and central regions. The leaves are green and alternate, turning into 3 lobed leaves when they mature. The bisexual solitary flower is borne at leaf base of new growth. It is about 4 to 5 cm in diameter and very conspicuous, colourful and fragrant. It has five whitish petals and two purplish rows of thread like rays called corona. The pistil consists of an ovary triradiate style, each branch terminating in a sticky stigma. The fruit is round or oval, about 4 to 5 cm long and greenish yellow or purple when ripe and smooth [2]. They grow swiftly about 15 to 20 feet per year [4].

Passiflora edulis Sims var. edulis is widely grown in the tropics for its edible fruits as they are good sources of Vitamins A and C. The fruit has anticarcinogenic effects and the pulp acts as a stimulant and tonic. The flower extract of Passiflora edulis has sedative and hypnotic effects. Glycosides, phenols and alkaloids are the major constituents in Passiflora edulis [3], [4]. The identified constituents in Passiflora edulis includes anthocyanins, carotenoids, γ-lactones, L-ascorbic acid, flavour components, volatile oil constituents, minerals, amino acids, carbohydrates, the cytoplasmic enzyme pyruvate kinase, cycloartenone triterpenes, cyclo passilic acids A-D, and their saponins, cyclopassi-floidosides I-VI [4], [5].

Tissue culture studies of Passiflora started as early as 1966 and since then many reports on tissue culture based methods applied to the genus have been published [6]. Most of the in vitro culture techniques for Passiflora were developed using Passiflora edulis Sims f. flavicarpa Deg. explants and have been used for micropropagation [7], [8], organogenesis [9], somatic hybridization [10], [11], and genetic transformation [9] of the species. Organogenesis-based plant regeneration system is currently being established in passion fruit [6], [11]. However, reports on in vitro plant regeneration and taxonomic studies of Passiflora edulis Sims var. edulis are scanty especially on the micromorphological aspects. Therefore, the objectives of this study were to study the influence of different combinations of BAP and NAA hormones on in vitro regeneration, to observe and differentiate the characteristics of Passiflora edulis Sims var. edulis grown in vivo and in vitro via Scanning Electron Microscopy (SEM), and to increase micromorphological taxonomic information of Passiflora edulis Sims var. edulis.

II. MATERIALS AND METHODS

A. Seed Germination

Seeds of Passiflora edulis Sims var. edulis were obtained from ripe fruits collected from the Institute of Biological Sciences garden in University of Malaya, Kuala Lumpur. Seeds were germinated via in vitro and in vivo techniques. The pulps were removed completely to be cultured in vitro and in vivo using aseptic techniques to obtain explants from seedlings. The seeds were then washed under running tap water for 30 minutes and treated with 70%, 50%, and 20% (v/v) commercial bleach for 10 minutes at each concentration. Next, seeds were rinsed in distilled water for 2 minutes and