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Review Article

Somatic embryogenesis induction of *Syzygium cumini*

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Abstract

Somatic embryogenesis serves as an effective alternative system for *in vitro* cultivation of endangered plants (*Syzygium cumini*), as it allows for the propagation of plants under a controlled environment. So produce hundreds of embryos that can be used as artificial seeds. Somatic embryos of *Syzygium cumini*, family *Myrtaceae*, were induced from the calli of a sterile leaf explant in Murashige and Skoog's medium with 6 ppm 2,4-D. After the transfer of the developed calli into liquid media supplemented with the same concentration of growth regulator, all three embryonic stages (globular, heart and torpedo) were observed after 6 weeks. Liquid media with growth regulators appeared to enhance the development to torpedo-stage embryos, especially at six weeks of age cultures. 12 weeks after the transfer of the callus into liquid media, flasks containing predominantly one microscopic stage were pooled and plated on fresh solid media lacking a growth regulator, where the embryogenic calli germinated showing shoots and aerial parts. Embryogenesis production protocol is considered a good tool to save plants from extinction, especially in *Syzygium cumini* which is a very important anti-diabetic drug.

Introduction

The genus *Syzygium*, which contains around 1200 different species, is the biggest in the family *Myrtaceae*. In addition, *Syzygium* is the sixteenth-largest flowering plant genus in the world [1]. *Syzygium cumini* is indigenous to the Indian subcontinent [2]. According to Gajera [3], Vijay Kothari [4] and Arunpandiyan [5]; *Syzygium cumini* is also known as "Jambolan", Eugenia cumini, "Jambul", and "Kala Jamun" in India. *Syzygium cumini* is an endangered plant in Egypt, present only in the Antoniadis Garden in Alexandria, Egypt.

Syzygium cumini is a traditional medicinal plant. Its fruit pulp, seed, bark, and leaves showed the medicinal value and have been used in various pharmaceutical formulations for the treatment of various kinds of diseases due to therapeutic prospects. It reduces metabolic abnormalities [6] and reduces blood sugar levels significantly. An ethanol extract from a plant was found to have a dose-dependent impact on fasting blood glucose [7]. It also has analgesic [8], anti-microbial [9-11], anti-inflammatory [12], anti-oxidant activity [13], hypolipidemic [14], cardioprotective [15], antidiarrheal, antiallergic, antifertility [16], anti-clastogenic, gastroprotective, antidermatophytic, antiviral [17], antianemic [18], carminative, anti-neoplastic, radioprotective, anti-HIV [19], diuretic, antipyretic (reduce fever), anticancer, anorexigenic, antiarthritic, aphrodisiac, antiscorbutic and cytotoxic activities [20-22]. It has protective and disease-preventive health properties and also contributes to the flavor, color, texture, and aroma of the plant [23]. Carotenoids, flavonoids, sterols, phenolics, anthocyanins, and terpenes are the major phytochemicals present in the stem, fruits, bark, seed, and leaves. About 30 different phytochemical compounds have been reported in the pulp. *S. cumini* fruit is rich in anthocyanins, gallic acid, ellagic acid, glucoside, caffeic acid, ascorbic acid, coumaric acid, isoquercetin Maslinic acid, Corosolic acid, Betulinic acid, myricetin and kaempferol [24-

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26]. *S. cumini* fruit and leaves contain various essential oils; 82% of the total essential oils are found in the leaves. It was found that oils induce apoptosis in the HT116 human colon cancer cell by stimulating the Bax protein and the activation of caspases as well as inducing apoptosis by activating the ERK pathway [27].

Somatic embryogenesis is an *in vitro* biological process in which bipolar structures (somatic embryos) can be induced to form from somatic cells and regenerate into whole plants [28]. Plant regeneration through the somatic embryogenesis pathway offers an advantage over the organogenesis approach, avoiding the risk of developing chimeras [29].

Somatic embryogenesis is a technique of *in vitro* plant propagation where somatic cells gain the embryogenic ability to form an entire plant. Typically, somatic embryogenesis consists of two stages: first, somatic cells start dedifferentiation and then redifferentiation to gain the capacity for embryogenesis, and then embryogenic cells form somatic embryos and develop into plants. Plant regeneration by somatic embryogenesis has numerous advantages, including the potential to develop a complete plant from a single cell and the ability to produce embryos automatically and in large quantities in a bioreactor, as well as synthetic seeds that can be planted in the field. The polarity of embryos permits direct growth into plantlets without any need for the rooting stage required in organogenesis for plant regeneration. In addition, a unicellular origin can prevent chimerism, thereby reducing the burdensome screening work required during the later stages of transgenic plant development. Somatic embryogenesis resembles zygotic embryogenesis in many ways. SE is an outstanding experimental model for examining the molecular mechanisms underlying early embryogenesis.

Somatic embryogenesis has a number of benefits, including reduced material requirements, high genetic stability, reduced mutation rates, and greater regeneration rates. It is frequently used for mass propagation in vitro, cell mutation induction, cell fusion, hybrid zygotic embryo rescue, germplasm resource conservation, synthetic seed preparation, and molecularly-assisted breeding. In recent years, other characteristics of this strategy have also become popular research topics [30].

This study describes the conditions required to induce somatic embryogenesis in *Syzygium cumini* from a callus initiated from one piece of leaf, which produces hundreds of embryos, which are considered "artificial seeds."

Materials and methods

Aseptic plant

Syzygium cumini leaves (Figure 1) (obtained from Antoniadis Garden, Alexandria, Egypt) were placed in approximately 10 ml of tap water with 0.3 ml of 95% ethanol for 30 minutes to ensure wetting. The leaf is dissected into many parts. The leaf parts were then removed and sterilized in a 2.5% sodium hypochlorite solution as an effective bactericide and fungicide for 5 minutes before being rinsed with sterile distillate water [31].

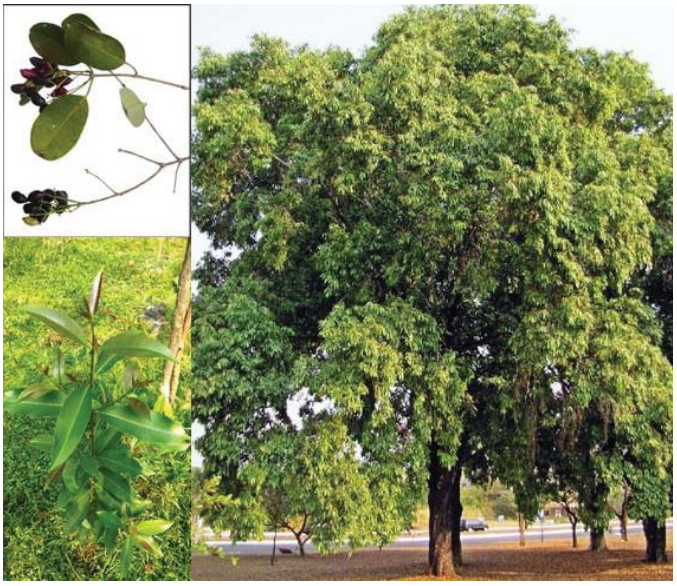

Figure 1: *Syzygium cumini* tree and leaves.

Culture media

The culture medium containing Murashige & Skoog's (MS) medium (1962) was augmented with 3% (w/v) sucrose, 1% (w/v) agar, and standardized pH (6) was maintained before autoclaving at 121 °C for 18 min. Media (static culture) is dispensed in Petri dishes. The basal medium was supplied with various (1, 2, 3, 4, 5, or 6 ppm) concentrations of 2, 4-Dichlorophenoxyacetic acid (2, 4-D); or was used without growth regulators (MS). Incubation of explants was done in Petri dishes containing 10 ml of the medium under aseptic conditions and inoculated cultures were maintained for 16 hours light (33000 Lux) and 8 hours dark at 28 °C and 22 °C, respectively. The liquid culture was prepared with the same content without agar. Liquid cultures were grown in 50 ml of medium in 250 ml Erlenmeyer flasks under continuous light (8000 Lox) at 25 °C on a shaker incubator set at 78 - 80 rpm.

Callus initiation

The aseptic explant (part of the leaf) was transferred to a 2,4-D static medium. After 6 weeks of incubation, only well-developed yellow-white friable (YWF) and green friable (GF) regenerating calli (3 mm pieces) (Figure 2) from the optimal concentration of 2,4-D (6 ppm) were sub-cultured to MS basal media supplied with 2,4-D (6 ppm) to increase biomass.

Embryo development

The tissues were then subcultured into liquid media of the same composition for 6 weeks and then transferred for 6 more weeks into the liquid media to promote embryo growth and development. Culture flasks containing embryos were examined microscopically after 12 weeks of growth in liquid media and were categorized into flasks containing principally globular stage embryos, heart stage embryos, or torpedo stage embryos. Each embryo category was pooled, the excess medium was decanted, and the embryos were divided into three 250 ml Erlenmeyer flasks. The tissues were then subcultured onto

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solid media containing no hormones (hormone free) for 4 weeks to promote embryo growth and development (Figure 3). Calli germinated in approximately 30 days to a plantlet with a shoot and root system when grown in 16 hours light (33 000 Lux) and 8 hours dark at 28 °C and 22 °C, respectively.

Results and discussion

Callus induction

In the callus induction step the visual appearance of calluses formed on 3% sucrose and 2,4-D is the most important step. Plant cell, tissue, or organ culture normally requires sucrose as a carbon source for cell proliferation and development [32,33]. The availability of sucrose in the culture medium has been found to affect somatic embryogenesis in many woody plant species [34,35]. The total number of somatic embryos was highest at 3% sucrose in media. Media with 2,4-D concentrations 1,2,3,4 and 5 ppm could not induce calli; meanwhile, media with a concentration of 6 ppm was able to induce them as it was reported that at high concentrations of synthetic auxins such as 2, 4-dicholorophenoxyacetic acid (2,4-D) induce callus [36], yet it needed much longer time to produce calluses from explants, so the optimum concentration for calli induction in each plant varies with the species of plant and type of culture [37]. In addition, it is also related to the age and size of the explant's needs [38].

Induction of somatic embryos

The calli examined microscopically after 6 weeks found globular masses of compact cells at the periphery that were approximately 0.23 mm in diameter. These peripheral cells were smaller, contained a dense cytoplasm, and were more polygonal in shape than the unorganized, highly vacuolated cells within the calli.

Calli subcultured into liquid media [39] rapidly developed into globular, heart, and torpedo embryos, particularly in a medium supplemented with 6 ppm 2,4-D. Globular embryos were approximately 0.4 mm in diameter and contained a multicellular suspensor at the point of attachment to the callus (Figure 4), heart-stage embryos were approximately 0.4 mm in length and 0.3mm in width (Figure 5) and torpedo-stage embryos were approximately 1.8mm length and 0.6 mm in width (Figure 6). Callus transferred from liquid media to solid plain media as it is necessary to remove plant growth regulators at the somatic embryo development and maturation steps. 2,4-D often hampers embryo development and their subsequent conversion into plants at these steps [40] after 30 days developed a shoot and root system (Figure 7).

Conclusion

Calli initiated with MS media containing 6 ppm 2,4-D and then propagated by subculture on solid MS media to increase biomass. Callus is transferred to liquid media forming a suspension culture to accelerate growth to get an embryogenic callus. Microscopical examination showed 3 embryogenic stages (globular, heart, and torpedo). Embryogenic callus was then plated on solid plain MS media that displayed germination with

Figure 2: Yellowish-white and green calli scale bars represent 0.5 cm.

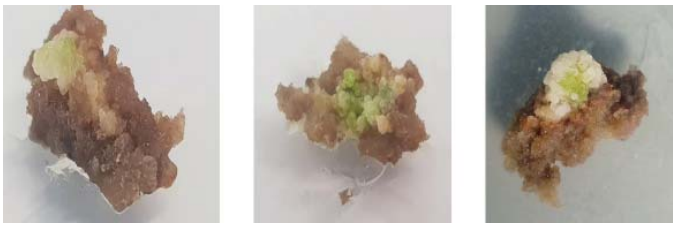

Figure 3: Calli on solid hormone-free media scale bars represent 1 cm.

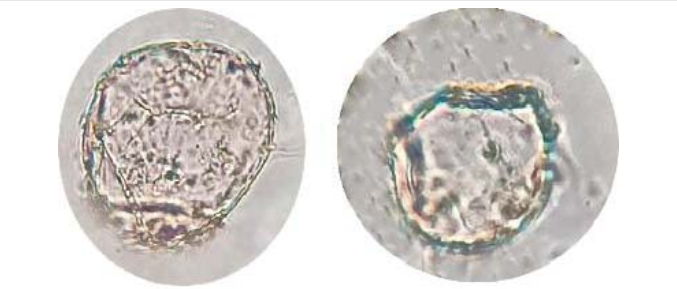

Figure 4: Globular stage the scale bar represents 0.1 mm.

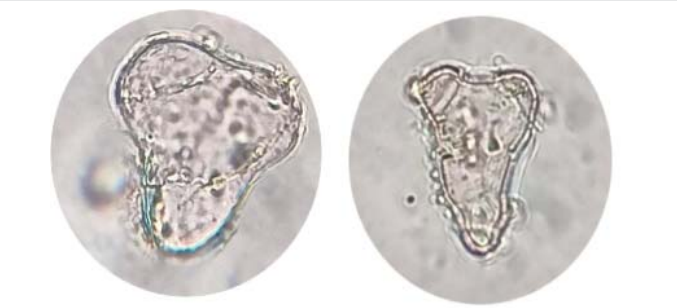

Figure 5: Heart stage the scale bar represents 0.1 mm.

Figure 6: Torpedo stage the scale bar represents 0.4 mm.

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Figure 7: Aerial part and root development the scale bar represents 1 cm.

a germination percentage reaching nearly 25%. Since *Syzygium cumini* is considered an endangered plant, then embryogenesis production protocol could be adopted or mass clonal plant propagation under controlled environmental conditions.

Accordingly, it is highly recommended to try this protocol together with optimizing environmental conditions (temperature, pH, photoperiod, etc...) as well as culture conditions (explant selection, age of the explant, type of the explant, source of the explant, etc...) to get a higher rate and percentage of embryo germination.

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