Synthetic Seeds Production and Regeneration of *Oxalis triangularis* for Mass Propagation and Conservation

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**Abstract**—*Oxalis triangularis* is an attractive ornamental plant propagated by using bulbs and has no natural viable seeds. It is an ideal decorative plant for growing in pots and other containers. The plant is not widely found in Malaysia and mostly obtained from Thailand. Hence, it is necessary to propagate this plant through tissue culture system for large scale production. In the present study, production of synthetic seeds was attempted from this species and the synthetic seeds managed to survive after 7 and 30 days after storage at 4°C. The rate of synthetic seeds conversion to complete plants after 7 days was 96.67% with 4.57 mean shoot numbers, while after 30 days of storage, the conversion rate slightly decreased to 90% with only 3.97 shoots formation per bead. The present work reports and discusses the potential of tissue culture technique as an alternative method for mass propagation and conservation of this interesting and attractive ornamental plant for future uses and exploitation.

**Index Terms**—*In vitro* propagation, BAP, NAA, synthetic seeds.

I. INTRODUCTION

*Oxalis triangularis*, commonly known as “False Shamrock”, is an edible perennial plant belonging to the Oxalidaceae family. It is native to Brazil. *Oxalis triangularis* grow from bulbs, and their propagation is obtained by division of the bulbs. Like other bulbous plants, *Oxalis triangularis* goes through dormancy period on a regular basis. Somatic embryos and synthetic seeds (embryos or micro shoots encapsulated in artificial endosperms) hold great potential for large scale clonal propagation of superior genotypes of heterogeneous plants [1], [2]. They have also been used in commercial plant propagation and for the multiplication of parental genotypes in large scale hybrid seed production [3], [4].

In Malaysia despite popular demand, this species is usually imported from Thailand, hence there is a necessity to mass propagate this species. Nowadays, mass propagation by using tissue culture method is quite common, the current work reports and discusses the production of artificial seeds from micro shoots derived from stem explants of this species. The subsequent *in vitro* regeneration was also achieved at high rates and the regenerated plantlets were successfully acclimatized. The development of encapsulated or synthetic seed technology brings up a new prospect in agriculture and floriculture industry. Production of synthetic seeds is effective and acts as an important alternative method of propagation in commercially important plants, which do not produce seeds. Plants could be produced in large scale with high volumes. Consequently, genetic uniformity and stability of the plants could be maintained. Due to the sterility, it could be easily transported and has potential for long term storage without losing viability. The aims of this paper are to produce artificial seeds and to investigate the ability of the synthetic seeds to regenerate after 7 and 30 days of storage at low temperature (4 ± 1°C).

II. MATERIALS AND METHODS

A. Explant Sources and Encapsulation Matrix

Micro shoots of *Oxalis triangularis* were induced from stem explants cultured on MS medium supplemented with 0.5 mg/l NAA and 0.5 mg/l BAP. The stem cultures were maintained in the culture room at 25 ± 1 °C for 16 hours light and 8 hours dark. Illumination was at 1000 lux and relative humidity was 90 – 100%. The standard method for preparation of capsule matrix was followed [5].

B. Final Stage Preparation of 3% (w/v) Sodium Alginate Solution (NaCa3H-Os)

To prepare 3% (w/v) sodium alginate solution in 100 ml MS basal medium without calcium chloride dehydrate (*CaCl*2·2,H2O), 1g sodium alginate powder was dissolved gradually. Sucrose (3.0 g) and hormones (1.0 mg/l NAA and 1.5 mg/l BAP) were added. Media pH was adjusted to 5.8. This solution was autoclaved for 20 minutes at 121°C and 104kpa.

C. Preparation of Calcium Chloride Dehydrate Solution (*CaCl*2·2,H2O)

Calcium chloride dehydrate solution was used as a complexion agent. To prepare 75 mM (w/v) calcium chloride dehydrate in 100 ml distilled water, 1.47 g *CaCl*2·2,H2O was dissolved gradually. This solution was autoclaved for 20 minutes at 121°C and 104kpa.

D. Encapsulation Techniques and Bead Formation

They were then, being dropped one by one in the *CaCl*2·2,H2O solution. The micro shoots were allowed to get encapsulated (hardened) by allowing them to remain in *CaCl*2·2,H2O solution for 30 minutes. These beads were taken out and transferred into sterile distilled water to wash out the excess *CaCl*2·2,H2O solution and were blotted with sterile tissue paper.