1. Introduction

Seeds are the primary means of propagation in many crops. The use of genetically uniform tissue culture derived plants could be considered advantageous for crops such as coffee, in which vegetative propagation is difficult to practice. Micropropagation technique is greatly constrained due to high cost involved and inadequate standardization of hardening procedure. During transfer of plants from laboratory to field, generally high mortality rates result that further lower the efficacy of results achieved in-vitro (Mathur et al., 1989). For these reasons, efforts have been made to overcome the constraints by encapsulating somatic embryos (Kitto and Janick, 1985; Redenbaugh et al., 1987) in different matrices and to grow them on different media. Studies on somatic embryogenesis and related encapsulation of somatic embryos have been reported by Redenbaugh et al. (1993). The first successful examples of synthetic seed technology were in alfalfa and celery. Fujii et al. (1989) grew plants at low frequency from synthetic seeds of alfalfa, planted directly in the field. Kirin Brewery Co Ltd., Japan and Plant Genetics Inc., USA conducted field trails in Japan with 20,000 F1 synthetic seeds in 1988 (Sanada et al., 1993). Recently, Rao et al. (1998) reviewed the concept, methods and
micropropagation as well as germplasm conservation through preservation of encapsulated zygotic and somatic embryos in liquid nitrogen. In coffee, somatic embryogenesis has been achieved from different tissues (Sondahl and Loh, 1988; Sreenath and Naidu, 1997; Muniswamy and Sreenath, 1997). However, reports on encapsulation and subsequent plant regeneration for producing synthetic seeds in coffee are very much limited. Here we report plant regeneration from encapsulated zygotic and somatic embryos of coffee.

2. Materials and Methods

The somatic embryos were regenerated directly from the cultured zygotic embryos of CxR cultivar on MS medium (Murashige and Skoog (1962), supplemented with 1-Naphthaleneacetic acid (NAA) and 6-Benzylaminopurine (BAP). The zygotic embryos of C. arabica S.4348 and S.2794 were isolated from mature green fruits. These embryos were cultured on MS medium with ABA (1 mg/l) for 30 days for embryo maturation and then used in encapsulation study.

2.1. PREPARATION OF ENCAPSULATION MATRIX AND BEADS:

Different concentrations of sodium alginate and calcium chloride were tested to standardize bead formation. Sodium alginate solution (2-6%) was prepared in MS medium supplemented with 3% sucrose, with or without growth regulators. pH of the solution was adjusted between 5.6 and 5.8 and sterilized at 121°C for 20 min. The embryos were mixed with sodium alginate solution and dropped through a Pasteur pipette into calcium chloride solution (2%) held in a conical flask with continuous stirring on a magnetic stirrer. The resulting calcium alginate beads containing entrapped embryos were left in the calcium chloride solution for 30 minutes to complete complexion. The beads were then removed from the solution by using sterile stainless steel sieve. The beads ranging in diameter between 4-6 mm were placed on different media for germination of embryos.

2.2. CULTURE MEDIA:

Encapsulated embryos were cultured in-vitro on nutrient medium and ex-vitro in potted vermiculite for germination. For in-vitro germination, MS medium supplemented with 0.1, or 0.5 mg/l of Kinetin (Kn) or 0.1 mg/l of BAP with 3% sucrose was used. The pH