### 1 Introduction

The process of androgenesis is unique, in that haploid embryos arise from single gametophytic cells. Clear evidence for the unicellular origin of somatic embryos is lacking for most regeneration systems (Fransz and Schell 1991); therefore, totipotent microspores offer a potentially useful subject for both basic and applied studies. Although anther culture can be an efficient method of producing doubled haploid lines for breeding applications, culture of isolated microspores provides a more useful experimental system, allowing direct manipulation and observation during all stages of development.

Recent progress in the ab initio isolated microspore culture of maize is summarized in this chapter.

### 2 In Vivo Microspore Development

In normal in vivo development, following meiosis, uninucleate haploid microspores are released from the quartet and subsequently undergo two mitotic divisions yielding a mature trinucleate pollen grain (Fig. 1; Pescitelli and Petolino 1988). The early uninucleate stage is characterized by a relatively large nucleus which occupies one-third to one-half of the cell volume. During the mid-uninucleate stage, the microspore enlarges in size and the nucleus becomes smaller and more densely staining. With the formation of a large central vacuole, the nucleus is pushed to the side opposite the germ pore. During this stage (late uninucleate) the nucleus becomes enlarged and undergoes the first pollen grain mitosis. In the early binucleate stage, the nuclei are initially similar in size but soon differentiate. The vegetative cell, distinguished by its larger size and more diffuse staining, then migrates across to the opposite side (mid-binucleate) and comes to rest near the germ pore (late binucleate). The generative cell then migrates towards the germ pore where the second pollen mitosis results in the formation of a trinucleate pollen grain.
Fig. 1. Diagram of the developmental stages of normal in vivo microspore development in maize, g Generative nucleus; v vegetative nucleus; s sperm nuclei (provided by Gary Pace)

3 In Vitro Microspore Development

The preferred stage of microspore development for maize anther culture is mid- to late uninucleate (Genovesi 1990). In isolated microspore culture, there appears to be a bias towards the late uninucleate to early binucleate stage (Coumans et al. 1989; Pescitelli et al. 1989). Statistical analysis of the developmental stage effects supports this observation (Gaillard et al. 1991). The prominence of generative cell divisions in isolated microspore culture (see below) indicates the prevalence of binucleate structures. (Technically, these binucleate structures should be referred to as pollen; however, for consistency, the term microspore culture will be used herein.) Pescitelli et al. (1989) observed that development continues during cold pretreatment with the stage progressing from early/mid-uninucleate to late uninucleate/early binucleate. It is possible that the enhanced response found from microspores exposed to an extended pretreatment (Gaillard et al. 1991) was due to the higher percentage of binucleate microspores present at the onset of the culture period.

Following isolation, during the first few hours of culture, a large percentage of microspores become swollen and stain brightly with fluorescein diacetate (Fig. 2). This phenomenon was reported by Coumans et al. (1989), who found that after 2–3 days, 10 to 40% of the microspores were still swollen, while most of the others had shriveled and degenerated. Typically, mortality is very high during the initial stages of isolated microspore culture. Under optimum conditions, viability after 1 day was reported to be in the range of 27 to 36% with only 10%