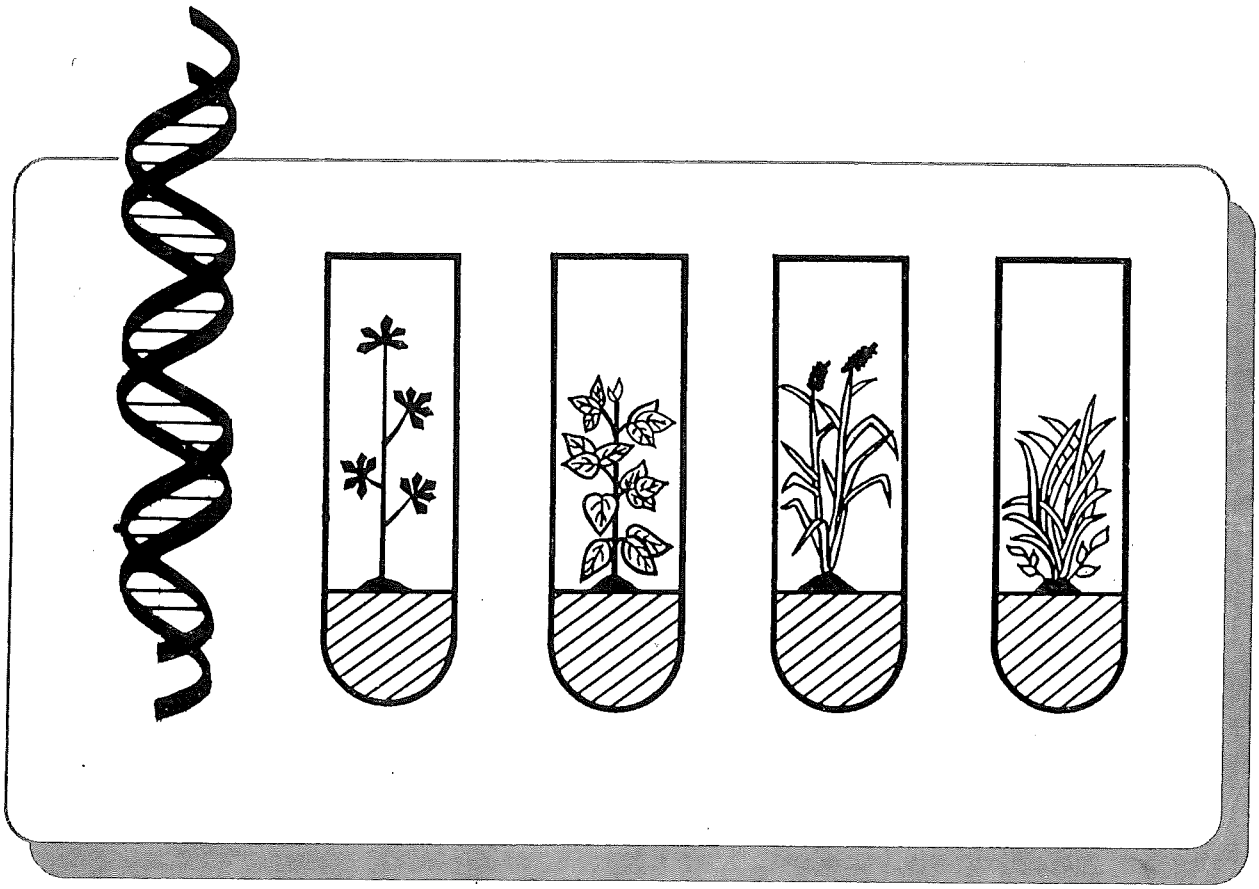
Annual Report

Biotechnology Research Unit 1988-1992

March 1993



CIAT
Centro Internacional de Agricultura Tropical
International Center for Tropical Agriculture

2.4 Isolation and *In Vitro* Culture of Cassava Immature Pollen and Zygotic Embryos
(Collaboration: C. Iglesias, Cassava Program Genetics Section)

Efficient systems for the isolation and in vitro culture of cassava pollen would be a way to avoid the possible detrimental effect of anther wall tissue on microspore development observed in previous cassava anther culture research. In vitro germination of cassava zygotic embryos will be useful in designing protocols for germinating and growing interspecific hybrid embryos as well as for the former to contribute to the construction of a cassava molecular map. The methodology is as follows:

- **Isolation and in vitro culture of pollen**. Mature and immature male inflorescences from four cassava var. (HMCI, CM91-3, CM523-7 and CM507-37) were used. Flower buds 0.8 to 2.5 mm. in length, corresponding to tetrad to late uninucleate microspore stages (Fig. 1) were gently macerated in 5% sucrose solution sterilized by filtration. According to pollen size (40-100 μm), the slurry was passed through 2 filters of 750 and 150 μm to eliminate somatic tissue. The slurry suspension was collected in a centrifuge tube. The filtrate was allowed to sediment and the supernatant discarded, followed by 3 washes (re-suspensions) with sucrose solution and finally with culture medium. The contents of 50 anthers in a vol of 5 ml sucrose solution resulted in a density of 10,000 microspores ml^{-1} (as determined by hemocytometer count), cultured in 15-mm petri dishes, in a hanging drop system, at 26°C, dark and high humidity conditions (Fig. 2).

- **Zygotic embryo isolation, culture and growth.** Immature seeds obtained from 3 var. (M Col 122, M Cub 18 and M Cub 62) were collected in the field at different stages of fruit development. Under aseptic conditions the seeds were split along the raphe, with the aid of forceps and scalpels to remove embryogenic axes. Immature embryo axes (with their cotyledons separated) were cultured between 25 to 45 days after pollination (torpedo to cotyledonary stage).

  We have developed a very efficient system for the rapid isolation and in vitro culture of large quantities of mature and immature cassava pollen in a very short time, starting with male flower buds. About 50 flower buds processed with 5 ml of sucrose solution will permit the manipulation of thousands of pollen grains at a time. Isolation and in vitro culture of immature cassava pollen has allowed us to obtain cell proliferation from microspores cultured at the tetrad stage. Induced microcallus was obtained by direct pretreatment of the isolated microspores with high osmoticum (Fig. 3).

  We have also developed a technique for growing cassava immature zygotic embryos in sterile, *in vitro*, conditions. The technique has been used to recover plants from inter-specific crosses used in the molecular mapping project, as well as for difficult-to-germinate *M. esculenta* and wild *Manihot* spp. (Fig. 4).

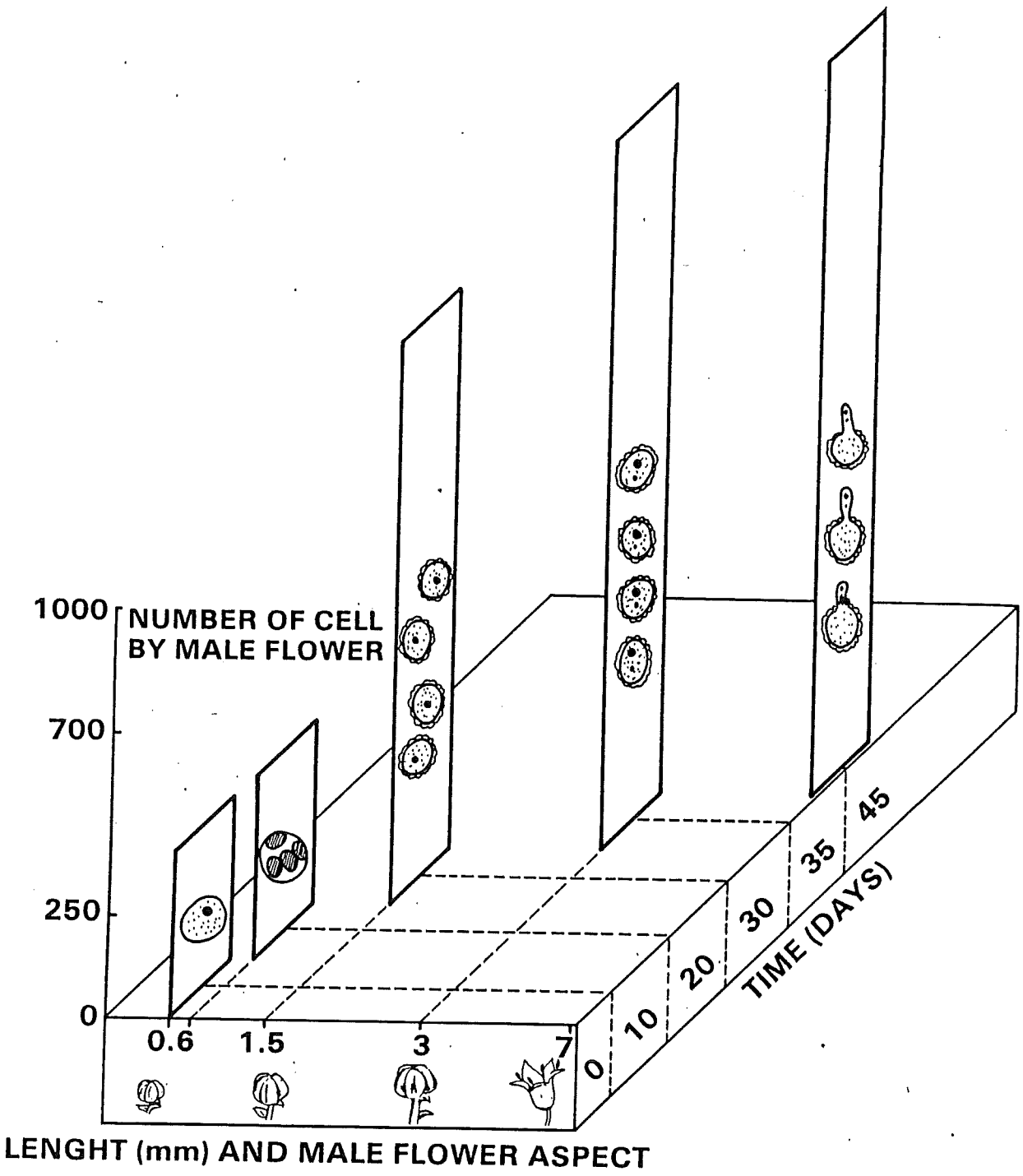


Figure 1. Developmental stages of cassava pollen grains (microsporogenesis): relationships of microspore-pollen stage to the morphology of flowers.

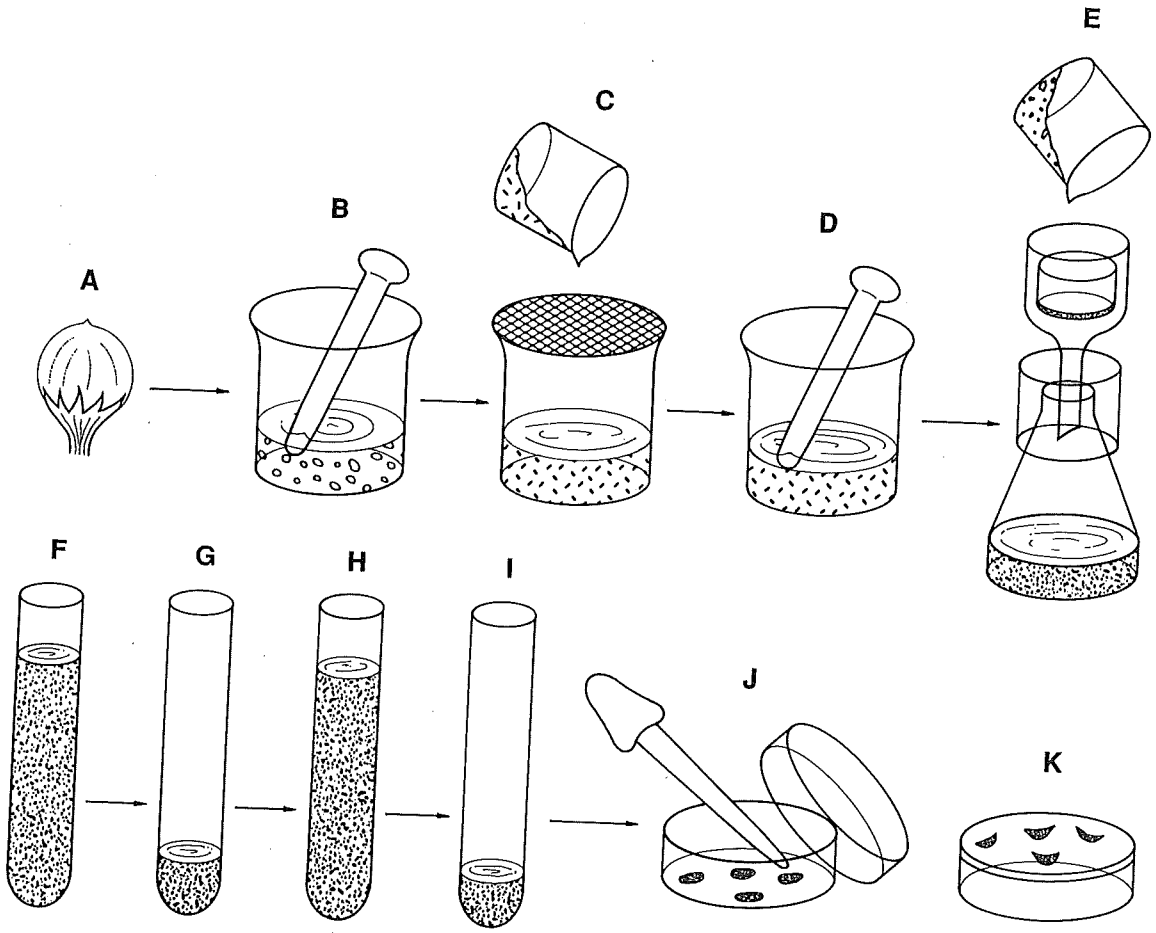


Figure 2. Steps A-K followed to isolate large numbers of cassava microspores at the tetrad stage.

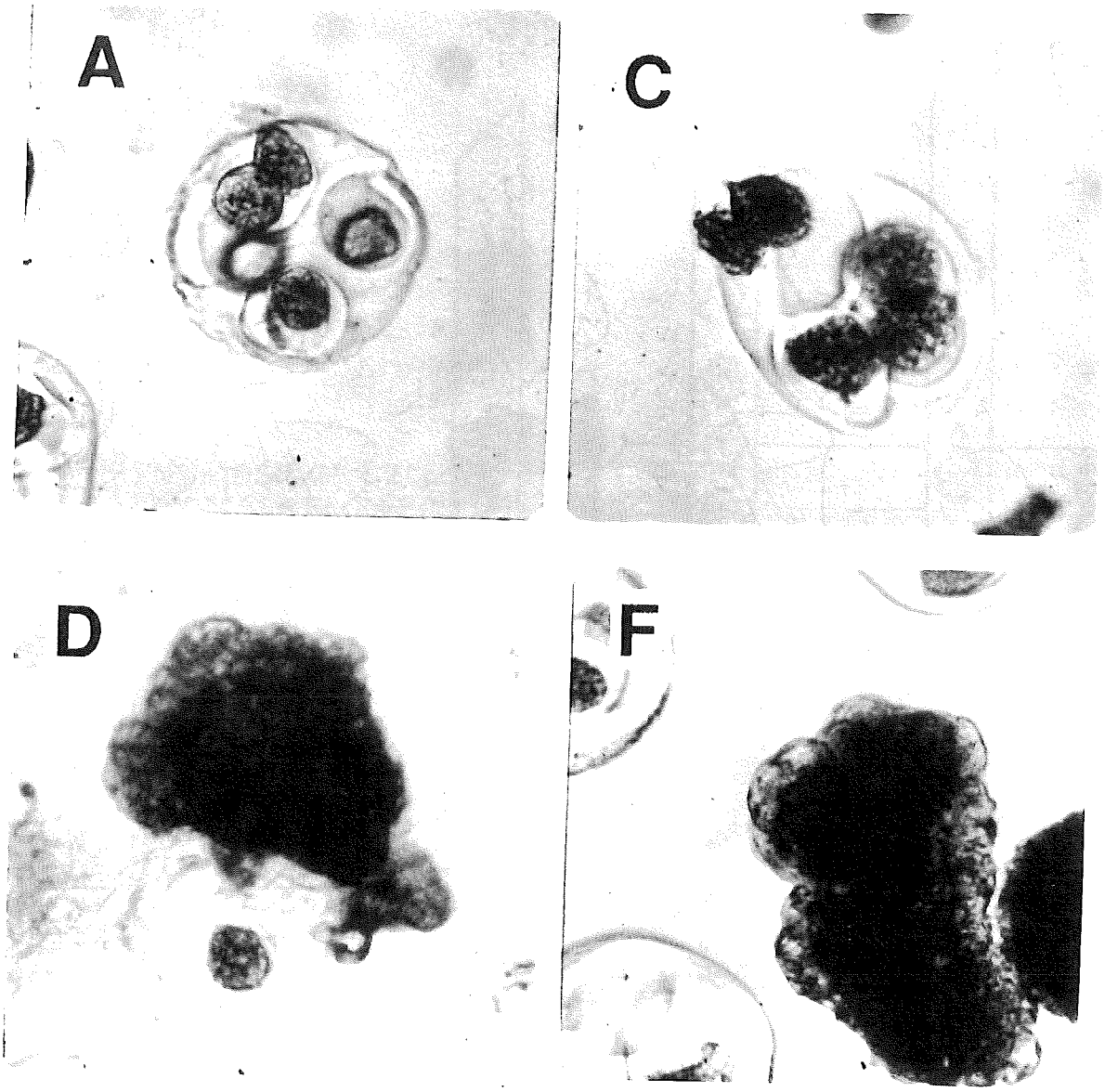


Figure 3. Induction of mitosis and cell proliferation from isolated cassava microspores at the tetrad stage: A. isolated tetrad-stage microspore; C. first mitosis of tetrad cells; D. and F. micro-calli grown from microspores.

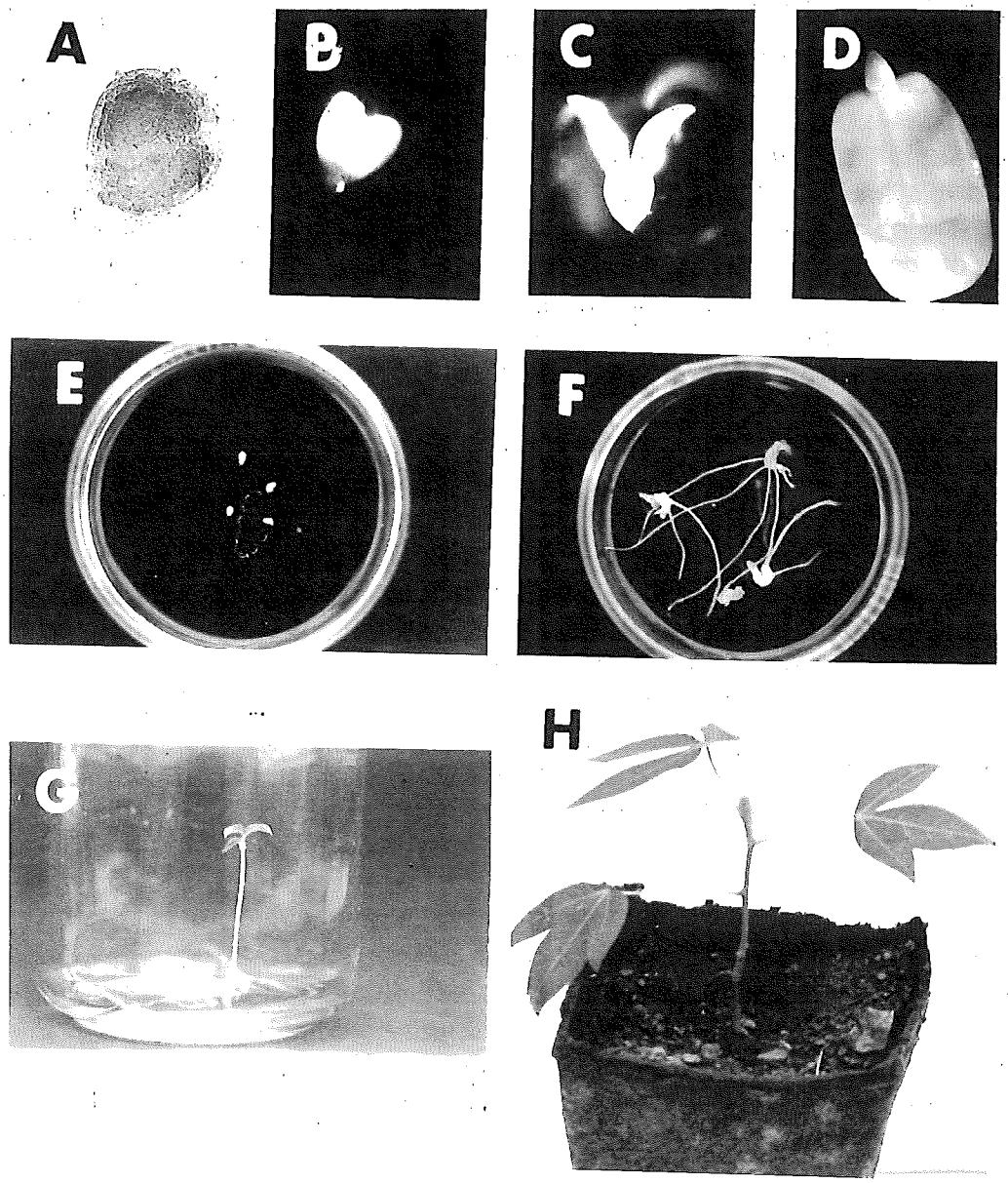


Figure 4. Isolation, culture and growth of cassava immature zygotic embryos: (A & B) heart-shaped embryos isolated from fertilized ovaries; (C & D) torpedo and cotyledonary-shaped embryos; (F-G) growth of embryos in vitro; (H) seedling transplanted into soil from a zygotic embryo.