# WHAT IS SOMATIC EMBRYOGENESIS IN A CONIFER?

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## INTRODUCTION

Somatic embryogenesis has been reported in many plant species, with the earliest in carrot cell cultures over 30 years ago (3). There has been significant progress and many reviews in recent years (5). Woody species often require more complex cultural manipulations than herbaceous species. Reported successes include conifers (e.g. 1,2,4). Somatic embryogenesis has considerable potential as a basic technique for propagation of conifers. The ability to regenerate large quantities of plantlets or even artificial seeds from somatic embryos from superior trees with desirable traits, such as faster growth, better quality wood, and disease resistance would improve existing reafforestation programmes, and be a major asset to the forest industry.

Somatic embryogenesis is the formation of embryos similar to zygotic (sexual) embryos formed in nature, but initiated from somatic cells rather than zygotic cells. Somatic cells are from the plant body, zygotic cells are from the recently fertilised egg. The more traditional tissue culture process, organogenesis, is the initiation *de-novo* of organs, usually shoots and roots, from cells or tissues. Organs may form on the surface of explants or upon an intervening callus phase. Both the organogenesis and embryogenesis techniques have merit. Organogenesis is often the easier technique for propagating a wide range of plant species and the resultant plants are usually true-to-type. Somatic embryogenesis is a more difficult procedure, with cultural requirements often more precise and the appearance of irregularities more frequent. But it also has several advantages that have great commercial appeal. The plants produced are always juvenile, shoot and root axes generally are formed at a similar time and potentially very high numbers of embryos can be formed. For example, in *Pinus radiata* there are at least 10,000 embryo initials per gram fresh weight of embryogenic tissue. The technique could substantially reduce the high labour input compared with that required for multiplication and transfers of shoots and plantlets produced via organogenesis.

Dr. Dale Smith and his research team at the Forest Research Institute (FRI) have been researching embryogenesis in *Pinus radiata* for the past 7 years. Since 1988 this work has been in collaboration with NZFP Forests Ltd. There is still refinement

of conditions required to improve maturation, encapsulation, and liquid culture procedures but results are very encouraging with plants having been established in the nursery. This paper describes some of the techniques and results from this research.

## SOMATIC EMBRYOGENESIS IN *PINUS RADIATA*

**Explant Source:** Immature cones are collected late November through to December (early summer). Over this period of time natural embryos become multicellular and this is the optimum stage of development to initiate somatic embryogenic tissue *in vitro*. The timing is crucial and usually cones are destructively sampled over this period to identify the correct stage of development. This varies among families of trees as well as season.

**Methods:** Immature seeds are easily extracted from the cones, as the cone is still soft and non-lignified. Once seeds are extracted they are sterilized in a hydrogen peroxide solution with a surfactant, and then rinsed in sterile water. Using aseptic techniques the gametophyte (this is food reserve containing the embryo inside the seed coat, and it looks like a grain of rice) is extracted from the seed coat and placed on an embryogenesis medium containing activated charcoal and sucrose in petri dishes. The gametophytes are incubated in low light conditions at 24 ° C. After 2 to 6 weeks embryogenic tissue emerges from the nucellar (sharp) end of the gametophyte. The embryogenic tissue is a mass of elongated cells (suspensors) bearing small embryo initials. If the timing of initiation from the cone into culture is accurate up to 70% of all the gametophytes can produce embryogenic tissue.

Once embryonic tissue masses are 3mm or greater in diameter they are transferred to a maintenance medium. Growth is vigorous on this maintenance medium, with tissues often doubling in weight every 10 to 14 days, and tissues are transferred at two week intervals. The embryo initials remain small and rapidly dividing. When embryo maturation is required, tissue is suspended in liquid medium and put onto an embryo development medium. Tissue regenerates from the suspension over a 4 week period and forms small embryos visible under the stereo microscope. It is then transferred to a medium that contains abscisic acid (ABA). Here, the tissue forms large white embryos (Figure 1). These appear similar to normal zygotic embryos. At this stage the most mature embryos are picked off and placed on maturation medium where they mature further and germinate. They are taken from *in vitro* to *ex vitro* conditions and planted in potting mix. They quickly acclimatise to glasshouse conditions, with little obvious water stress or difficulty compared with many exflasked tissue culture plantlets raised via organogenesis.

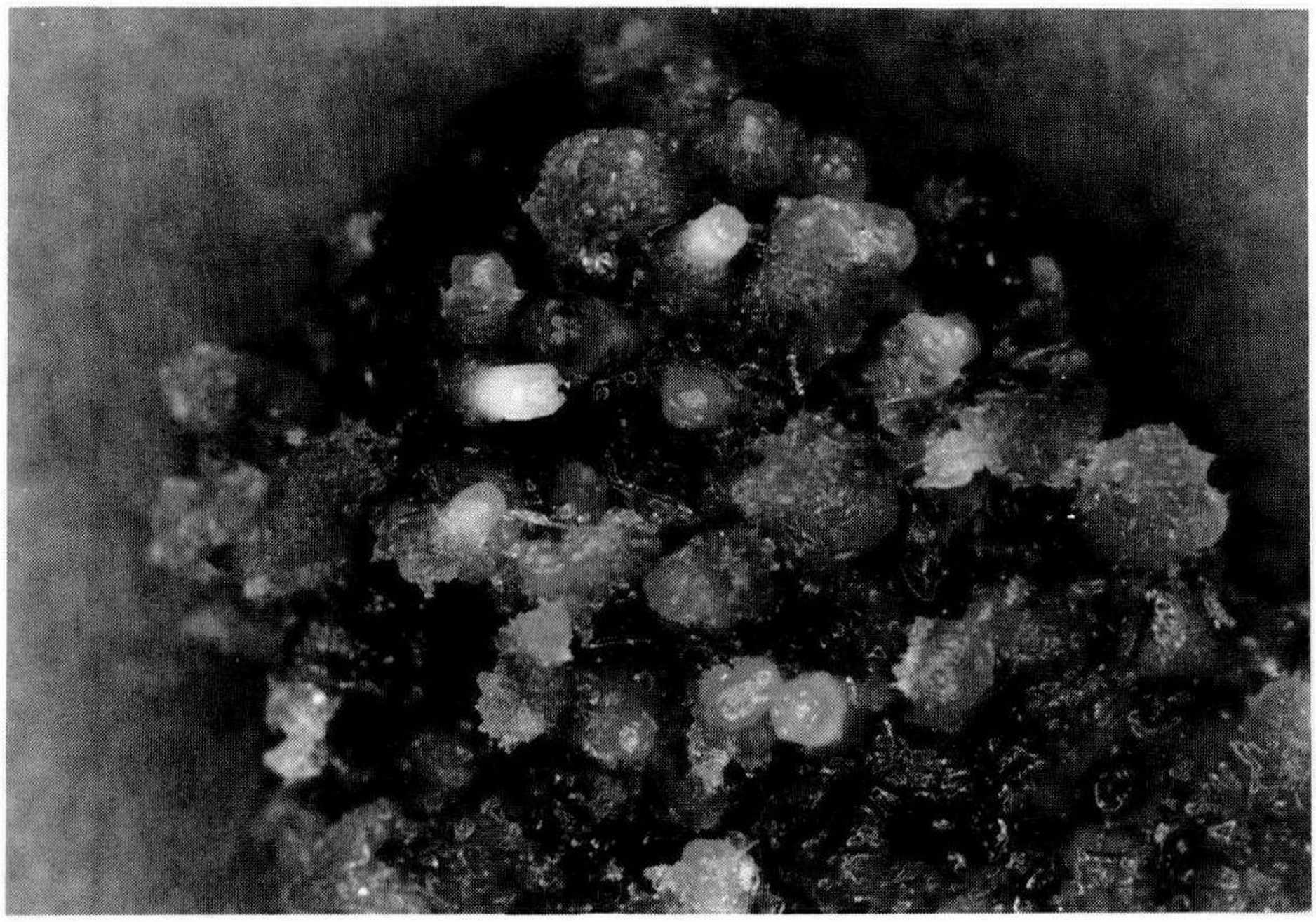

**Figure 1.** Somatic embryos of *Pinus radiata* forming on embryogenic tissue.

**Future Prospects:** In order to realise the potential of this technique further medium modifications (to satisfy physiological and biochemical requirements) are necessary to enable more of the thousands of small embryos present per gram of tissue to mature and form seedlings. Currently only a small fraction reach a plantable size, with many being lost as each developmental stage progresses. The Forest Research Institute is also investigating the use of bioreactors (automated vessels containing liquid media and embryogenic cell suspensions) for cultural phases after initiation of clones on solid medium. It is hoped that encapsulation of the somatic embryos from the bioreactor and sowing them directly into nursery beds will be possible. Early results are promising. Embryogenic suspensions have been multiplied in a bioreactor, and embryo development observed. Mature zygotic embryos have been used to test the concept of artificial seed coats, and the process is clearly feasible.

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