

Pathways of vegetative development via embryogenesis in *Solanum tuberosum* L.

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The concept of totipotency, unique to plants and first proposed by Schwann and Schleiden in 1838, whereby each individual plant cell retains the genetic ability to regenerate back into an intact plant has been exploited for many years. In the laboratory, isolated plant tissues can be induced to de-differentiate and re-differentiate using plant growth regulators and this technology underpins many of the methods used for gene transfer in plant species such as potato. Furthermore, the ultimate demonstration of totipotency whereby an isolated individual plant cell (protoplast) can be induced to divide and regenerate has been used in a number of previous projects involving potato and related germplasm at SCRI.

There exist a number of alternative pathways for vegetative regeneration via an embryogenic route, which have not, until recently, been applied to potato. The underlying principles relate to the natural processes of plant cell development and differentiation. This can be demonstrated by the process in seed plants, where sexual reproduction is initiated by pollen transfer from anther to stigma. One of the two sperm cells carried by the pollen grain fertilizes the egg cell in the flower's carpel, giving rise to a fertilized egg cell or zygote. The subsequent developmental transition of the zygote to a multicellular seedling is termed **zygotic embryogenesis**. Zygotic embryos develop through a series of characteristic morphological stages, in dicotyledonous plants, the globular, heart, torpedo, and bent-cotyledon stages progress to the seedling, which then grows

on to an intact plant. Two other forms of embryogenesis, gametic and somatic, analogous to the normal pathway of zygotic development can be induced *in vitro*, and have wide implications and applications in basic and fundamental studies.



Figure 1 High efficiency production of somatic embryos from internodal explant of potato cultivar "Desiree"

Somatic embryogenesis refers to the initiation of embryos from previously differentiated somatic cells. This relies on the reprogramming of gene expression and triggers many structural changes which are similar to those found in normal, zygotic embryos. This system of regeneration has only recently been unequivocally demonstrated in potato¹. Tissue explants of potato cultivar "Desiree" were induced, using a three stage (maintenance – induction – expression) protocol, to form somatic embryos at a high rate of efficiency (Fig. 1). Confirmation of the progression of

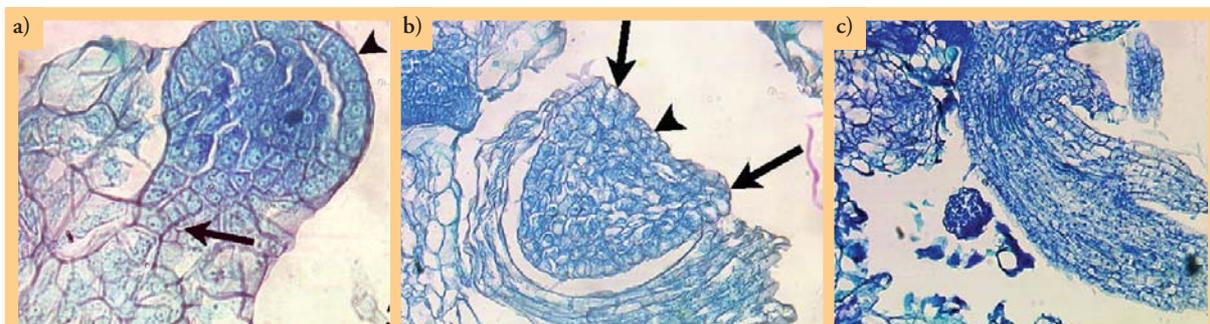


Figure 2 Developmental stages of somatic embryogenesis of potato cultivar "Desiree" : a) globular embryo with visible protoderm (arrowhead) and a suspensor-like-structure (arrow); b) heart shaped somatic embryo containing cotyledonary initials (arrows) and differentiating apical meristem region (arrow head); c) torpedo stage embryo.

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Figure 3 Developing embryos from anthers of potato cultivar Pito.

somatic embryogenesis through each of the characteristic stages (corresponding to the developmental stages observed in zygotic embryogenesis) was performed by a detailed histological analysis (Fig. 2). Specific patterns of gene expression at each developmental stage are currently being investigated.

In **gametic embryogenesis** the development of a gametic cell, which is haploid (n), having half the genetic constitution of the parent plant, is diverted to produce embryos, rather than mature pollen grains or ovules. In the case of microspores (cells destined to become pollen) there is the potential to produce sever-

al hundred plants from a single isolated anther. Following on from this stage, induced or spontaneous doubling of the haploid cells produces doubled haploid ($2n$) plants. These plants are theoretically homozygous, true breeding and are of significance in plant breeding either as parental lines or as finished varieties. Though the techniques work well in several species such as barley and *Brassica napus*, there have only been limited reports of the uptake of this strategy in potato to date. We have recently adapted a method² for the high efficiency regeneration of di-haploid material from anthers/microspores (Fig. 3), of the potato cultivar “Pito” and are expanding this to other cultivars.

In addition to model systems for understanding the processes involved in embryo development, both somatic and gametic embryos may be used as an efficient method for the propagation of large numbers of clonal individuals of valuable lines. We have the capacity to exploit the unique portfolio of vegetative regeneration pathways in potato for a wide range of applications in basic science (gene expression; genetic mapping; haplotyping and other fundamental tools) and for end-user applications in the development and rapid clonal propagation of novel plant material

References

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