

## **The possible origins of presumptive Somaclones in barley tissue cultures**

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Over the past few years there has been much interest in the somatic cell culture of cereals. Genetic variation in plants regenerated from somatic cell callus (somaclones) has been reported in barley (1). If there was a high enough percentage of regenerated plants with useful variation then somacloning would be an added 'tool' available to breeders to improve existing varieties in shorter times than are currently feasible using conventional breeding techniques. The first aims of the tissue culture project at the ARI were to assess callus production and subsequent regeneration of plants for a range of barley genotypes. Whilst callus could be induced in 21 varieties, precocious germination of the zygotic embryo and low regeneration rates have led us to investigations of the source of regenerants arising from somaclonal callus.

### **Methods**

Immature barley embryos cultivar 'Mink' 0.5-1.5 mm were dissected from seeds which had been surface sterilized with 0.2% Benzalkonium chloride for 10 mins then rinsed in sterilized distilled water. One hundred and eighty embryos were excised. Ninety were plated directly whilst the remaining 90 were dissected into embryonic axis and scutellum. Each part was cultured individually giving a total of 270 cultures. The cultures were maintained on an initiation media, (modified N6 containing 20 g/L sucrose, 5 mg/L picloram and 8 g/L agar, pH was adjusted to 6.0). After one month they were transferred to regeneration media, (modified N6 without growth regulators). Cultures were assessed at regular intervals and samples were taken for histology at all stages of development.

### **Results and discussion**

Seventy six of the 90 intact cultured embryos survived. Nineteen showed precocious germination within 2 weeks of culturing whilst a further 10 developed shoots by 5 weeks. Of the 90 scutellar parts only 5 have shown any organised shoot growth whilst 39 exhibited root growth. Both scutellar and embryo cultures produced hard nodular opaque callus. In addition, the embryo cultures produced varying amounts of friable loose watery callus. This watery callus was also produced by the embryonic axis cultures. A low frequency of hard nodular callus was observed in the embryonic callus cultures but the position in which this arose suggested that remnants of scutellum on the original explant were responsible for such callus.

Histological examination of the nodular callus revealed meristematic regions below the surface of the epithelium connected to vascular tissue. This vascular tissue and the meristematic regions were fragmented throughout the callus by the growth of large, vacuolated parenchymous cells. These meristematic regions gave rise to the roots observed in high frequency in scutellar callus. In the embryo cultures there were islands of meristematic cells remaining at the site of the embryonic axis even when precocious germination was overtly suppressed. These also became dispersed throughout the callus in older cultures. True somaclones are thought to be derived from embryoid structures arising from somatic cells. Our studies indicate that it is probable that many shoots are derived from a proliferation of cells from the meristematic region of the shoot primordia which is carried over in the callus despite auxin suppression of germination.

This may in part explain the low numbers of regenerants observed in the scutellum cultures and the low number of variants overall.

1. Maddock, S.E. and Semple, J.T. 1986 *J. Exp. Bot.* 37, 1065-78.