To: Marlborough Research Centre Trust

Research Project at UC:

Tissue culture research on Eucalyptus bosistoana selected by NZ Dryland Forests Initiative

Second Report on 30 May, 2021

From: David Leung

Objective 1: to investigate an effective means for sterilisation of micro shoot cuttings

Start date: 20/7/2020

End date: 30/11/2020

Milestones or deliverables:

- at least 80% cultured shoot cuttings free of visible microbial contamination, and
- stay clean for at least 2 rounds of subculture

Status: completed

The above milestones have been achieved. The cuttings of three different lines (3600, 490 and 3245) were successfully surface-disinfected using one protocol as shown in the previous report. The utility of this protocol has been validated with 2 additional lines (1052 and 2948) in late April, 2021.

Objective 2: to investigate the composition of tissue culture medium

Start date: 20/7/2020

End date: 30/10/2021

Milestones or deliverables:

- know the basic nutrients (salts, vitamins and sugars) required for bud break and growth of new shoots from at least 50% of the cultured shoot cuttings
- know the concentrations of two hormones (a cytokinin and an auxin) required for at least 50% of bud break and growth of new shoots

Status: continuing

In the previous report, there were early promising results:

- It was possible to induce bud break on one medium (two lines, 3600 and 490, were used) in the winter (July, 2020).

- There seem to be genotype effect on bud break trialed on one medium using three lines.

Progress made since the previous report,

Bud break has been achieved in all three lines (3600, 490 and 3245) using a particular protocol.

It is important to note the continued direction of this research is to find a common protocol that suits as many clones as possible rather than to have different protocols optimised for different clones. This will simplify commercial propagating operation in the future and make it practical for the industry to mass clone different breeding lines.

Bud break for two additional lines ((1052 and 2948) is underway.

Objective 3: to investigate rooting requirements

Start date: 1/11/2020

End date: 30/12/2021

Milestones or deliverables:

- know the requirements of auxins added to the culture medium for rooting from the micropropagated shoots from Objective 2
- have methodology for isolation and characterisation of endophytes from EB
- know the potential of the isolated endophytes for improving rooting in EB

Status: continuing

Progress since last report:

The new shoots from the bud breaks **(Objective 2)** in lines 3600, 490 and 3245 have to be elongated and separated from each other before rooting trials can begin. More research is underway in this important direction.

It is important to note that increased propagation efficiency here will be achieved even if only a few of the shoots from each line can be elongated after bud break because each elongated shoot can provide more micro-cuttings. In this repeated, cyclic fashion, "*in vitro* stool beds" are established for each line and from which clonal cuttings are continuously supplied, without season limitation, and taken to root and go out to potting mix.

Isolation of endophytes from EB will begin from early June.