Proposed propagation methods:

Pomaderris clivicola and *Bertya pedicellata*

Prepared by University of the Sunshine Coast for

North Burnett Regional Council

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Propagation of cuttings

Shoots from each of the 34 *Pomaderris clivicola* plants and 82 *Bertya pedicellata* plants will be collected and placed in labelled plastic clip-lock bags with a light spray of water, and kept cool in plastic tubs containing ice bricks. Shoots will be transported overnight to the University of the Sunshine Coast propagation facilities and the cuttings will be set into propagation mix on the following day(s).

The shoots will be dissected into cuttings, and the cuttings will be pruned by removing approximately 60% of their leaf length. Each cutting will be dipped 0.5 cm into powder containing 8 g/kg IBA for 1 second and placed 1 cm deep into a 70-mL Hyco propagation tube containing propagation mix. The propagation mix will consist of a 75/25 (v/v) mixture of perlite and shredded pine bark, with 3 kg of 8-9 month of Osmocote™ fertiliser (Scotts International, Heerlen, the Netherlands) and 1 kg gypsum (Queensland Organics, Narangba, QLD) incorporated per m^3.

The propagation trays will be placed under mist irrigation in a translucent white polyethylene chamber at the University of the Sunshine Coast. Misting will be provided for 1 minute approximately every 15 minutes, depending on climatic conditions at the time of setting.

Cuttings will be assessed twice-weekly by removing the translucent insert from the inside of each propagation tube and examining the propagation mix for roots. The cuttings will be moved to an adjacent greenhouse with hourly overhead watering once their roots have reached the bottom of the tube.

Tissue culture

The shoot tips and any discarded nodes from the 34 *Pomaderris clivicola* plants, and as many as possible of the 82 *Bertya pedicellata* plants, will be dissected to approximately 2-3 mm length, removing their macroscopic leaves. The shoots will then be surface sterilized, proliferated, and converted into plantlets (Fig. 1) using the following methods.

The dissected shoots will be washed in 70% ethanol (v/v) for 1 min in 70-mL vials containing one drop of Tween 20. They will then be rinsed in sterile distilled water for 1 min, and transferred into new vials containing 5% sodium hypochlorite with one drop of Tween 20 (note: the sodium hypochlorite concentration will be reduced if bleaching becomes apparent). The vials will be swirled for 30 min on an orbital shaker at 110 rpm and the shoots will then be rinsed three times in sterile distilled water. Shoots will be placed on sterile paper to remove excess liquid between solutions. Shoots will be plated (5 shoots per 90-mm Petri dish) onto shoot induction medium consisting of half-strength MS medium with 30g L^{-1} sucrose, solidified with 8 g L^{-1} agar, and with pH adjusted to 5.8 prior to autoclaving (121ºC, 20 min). The shoot tips will be maintained at 25ºC for 4 weeks under a 16-h photoperiod (approx. 50 µmol m^{-2} s^{-1} with fluorescent tubes). The explant source (shoot tip vs. node) will be recorded and tracked for every shoot.

Uncontaminated shoot tips will be transferred to 375-mL glass jars containing 50 mL of shoot proliferation medium consisting of full-strength MS medium with 30 g L^{-1} sucrose and 1 mg L^{-1} BA, solidified with 8 g L^{-1} agar, and with pH adjusted to 5.8 prior to autoclaving (121ºC, 20 min). Shoots will be proliferated in this medium for 4-week passages at 25ºC under a 16-h photoperiod (approx. 100 µmol m^{-2} s^{-1}) until required for conversion to whole plantlets (i.e. with roots).

When required for conversion to plantlets, shoots of at least 25-mm length will be dissected and transferred to root induction medium consisting of half-strength MS medium with 30 g L^{-1} sucrose and various concentrations of IBA (Fig. 1), solidified with 8 g L^{-1} agar, and with pH adjusted to 5.8 prior to autoclaving (121ºC, 20 min). The shoots will be placed in the dark for

1 week to allow formation of root primordia. The shoots will then be transferred to hormone-free half MS medium with 30 g L^{-1} sucrose, solidified with 8 g L^{-1} agar, and with pH adjusted to 5.8 prior to autoclaving (121ºC, 20 min). These shoots will be placed under a 16-h photoperiod (approx. 100 µmol m^{-2} s^{-1}) at 25ºC for 3 weeks to allow root elongation.

Plantlets will be transplanted into punnets containing a sterile potting mix. The potting mix will be composed of a 75/25 (v/v) mixture of shredded pine bark and perlite with 3 kg of 8-9 month slow-release Native Osmocote™ Plus fertiliser (Scotts International, Heerlen, the Netherlands), 3 kg lime (Unimin, Lilydale, VIC), 1 kg Micromax^{R} micronutrients (Scotts Australia, Baulkham Hills, NSW), 1 kg Hydroflow^{TM} wetting agent (Scotts Australia, Baulkham Hills, NSW) and 1 kg of gypsum incorporated per m^3. The punnets, each containing fifteen 12-mL tubes, will be placed in sterile 1-L plastic take-away food containers. The containers will be watered with sterile distilled water containing Fongarid^{TM} and Mancozeb^{TM}, and the container lid will be replaced with another container and sealed with plastic film to create a volume of 2 L.

The sealed punnets will be exposed to increasing light intensities over 3 weeks to gradually prepare the plants for the nursery environment. The punnets will be moved to a glasshouse and receive watering for 30 min at 4 times per day. The lid will be removed from the punnets after 3-4 days to commence hardening-off.

The plants will be re-potted after 3 weeks into 70-mL Hyco tubes containing potting mix. The potting mix will be composed (as above) of a 75/25 (v/v) mixture of shredded pine bark and perlite with 3 kg of 8-9 month slow-release Native Osmocote™ Plus fertiliser (Scotts International, Heerlen, the Netherlands), 3 kg lime (Unimin, Lilydale, VIC), 1 kg MicromaxR micronutrients (Scotts Australia, Baulkham Hills, NSW), 1 kg Hydroflow^{TM} wetting agent (Scotts Australia, Baulkham Hills, NSW) and 1 kg of gypsum per m^3.

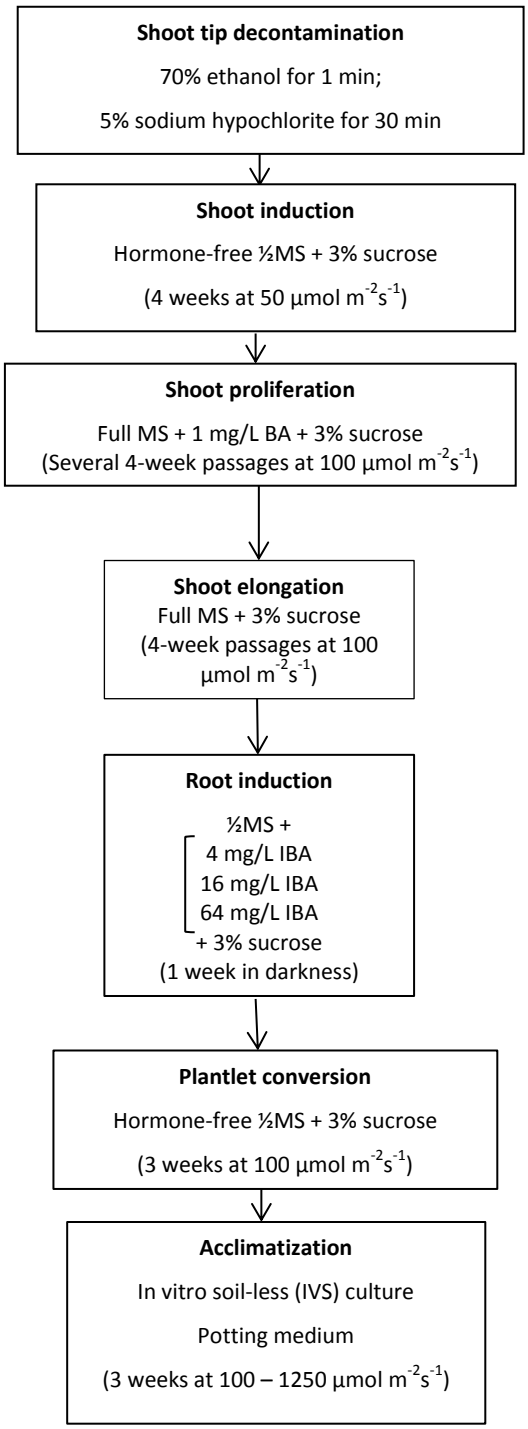

Figure 1 Summary of the tissue culture methods for *Pomaderris clivicola* and *Bertya pedicellata*