Is there a Role for Glycine Betaine in Cutting Propagation?©

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INTRODUCTION

Glycine betaine (GB) is a compound naturally synthesised by some higher plants in response to abiotic stresses. Its role when produced in these plants is an osmoprotectant, helping protect cells, proteins, and enzymes from stress due to drought, salinity, heat, and freezing temperatures. In addition, GB has been proven to protect the Photosystem II complex in some plants under various abiotic stress situations (Papageorgiou and Murata, 1995; Murata et al., 1992). Glycine betaine is synthesised in the chloroplasts, and research has proven it to be a nontoxic, non-perturbing, very water-soluble, and electrically neutral compound with a molecular weight of 117.15 $g \cdot mol^{-1}$ (Sakamoto and Murata, 2002). A plant's natural ability to synthesise GB isn't defined by its membership in a particular taxonomic group, these plants are spread over a number of plant families. In addition, a small number of plants, including sugar beet, wheat, and spinach, are known to be natural GB accumulators (Bohnert et al., 1996). It is only relatively recently that the chemical pathway for the synthesis of GB in higher plants has been confirmed, but the exact way in which it protects the plant from abiotic stresses is still unknown.

In addition to plants, GB naturally occurs in a wide range of other organisms, including all seaweeds, marine invertebrates, many microorganisms, and all mammals, including humans. Glycine betaine has two roles in human metabolism, one of which is as an osmoprotectant, helping protect the kidneys, liver, and heart. The kidneys can synthesise this compound, but more often it is taken in as part of a diet, as many foods contain glycine betaine. There is also building evidence that GB plays a role in athletic performance (Craig, 2004).

My introduction to GB was in 2005 when I returned to Lincoln University after a break of many years to sit some applied science papers, one of which was plant physiology. Glycine betaine was talked about in some of our lectures, and this prompted my interest in finding out more. Much of what was published that I read on the subject at that time seemed to focus on the possibility of genetically engineering the GB synthesis pathway into plants. The potential for alleviating abiotic stresses on crop plants through the application of GB in a world with increasing water supply problems and large areas of saline and sodic soils had been noted (Flowers and Yeo, 1995; Mäkelä et al., 1996). There were no references I could find at that time directly relating GB to ornamental plants or to their propagation, but as a plant propagator at heart that was where I saw the potential. If drought stress in cuttings could be reduced by applying GB, a natural plant product, then this would be a great extra tool for propagators to have.

Finally, 9 years after first learning about GB, I set about doing three very basic, low-input experiments. Due to the complexity of the factors involved there may not ever be a simple answer to the question posed in the title, but this is my initial attempt to come up with one.

MATERIALS AND METHODS

A lack of relevant information meant that the following had to be decided for these experiments:

- Is GB best applied as a foliar spray, full cutting immersion, or basal end soak?
- What strength solution should be used?
- How long should the application time be?
- Which plants should be used?

The glycine betaine used in these experiments was purchased from Sigma-Aldrich. A product of Finland, it is a by-product of the sugar beet industry, where it is refined from

the sugar beet molasses by chromatographic separation. Glycine betaine in the crystallised form like this has to be kept refrigerated. Solutions of 0.5 M and 1.0 M GB were used in these experiments.

Experiment 1: Examine the Effects of Repeated Foliar Application of 1.0 M Glycine Betaine on *Griselinia littoralis* Cuttings

This plant was chosen because it is an industry standard in New Zealand and a very popular native plant that is able to grow in a wide range of environmental conditions. The leaves are shiny, smooth, and a little leathery, providing a test for whether or not the GB would be effective as a foliar application. Seven days of spraying was possibly excessive, since 1.0 M is a strong solution. However, I was hoping there would be a good visual difference between the two trays at the end of the 3-week trial period.

- Leafy tip cuttings approx. 25 cm long were taken in mid-December. No leaves were removed.
- All were wounded on one side and given a 5 s dip in Liba, 10,000 softwood (1,000 ppm IBA).
- Cuttings were stuck into Jiffy 7 coir pellets. There were two trays, 49 cuttings per tray.
- Trays were placed in an enclosed plastic tent on a 22°C heat pad with intermittent mist.
- Each day for the first 7 days the GB treatment tray was sprayed with a very fine mist of 1.0 M GB solution. The control tray was sprayed with water at this time.
- All cuttings were assessed and the experiment finished at 21 days.

Experiment 2: Examine the Effects of Glycine Betaine on *Lavatera* × *clementii* 'Barnsley' and *Penstemon* 'Alice Hindley' Cuttings Covered Only for the First 3 Days

- Four treatments consisting of:
  - Soak basal end of cuttings for 1 min in 0.5 M GB solution.
  - Soak basal end of cuttings for 1 min in 1.0 M GB solution.
  - Immerse cuttings for 1 min in 0.5 M GB solution.
  - Immerse cuttings for 1 min in 1.0 M GB solution.
  - Plus a control with no GB treatment for the *Penstemon.*
- Tip cuttings of *Penstemon* approximately 12-15 cm long were taken in late January.
- Tip cuttings of *Lavatera* consisting of non-flowering axillary shoots approx. 5-8 cm long were taken in mid-January. These were quite hard to obtain as *L.* 'Barnsley' tends to be in full bud and flower throughout summer. No leaves were removed on any of the cuttings.
- All cuttings were given a 5 s dip in Liba 10,000 softwood (1,000 ppm IBA) after their GB treatment.
- *Lavatera* was stuck into Jiffy 7 coir pellets and *Penstemon* was stuck into Jiffy 7 peat pellets.
- All cuttings were placed in a shallow, slightly opaque plastic storage bin and the lid placed on it. The bin was placed in a well lit room at ambient temperature and no direct sun on it.
- After 3 days the cover was removed, and the cuttings left fully uncovered until the experiment ended. During this time the pellets needed to be gently watered only once.
- All cuttings were assessed and the experiment finished at 21 days.

Experiment 3: **Examine the Effects of Glycine Betaine on Uncovered Cuttings of *Lavatera* × *clementii* 'Barnsley' and *Penstemon* 'Purple Passion'**

- The same treatments were used as in Experiment 2.
- *Penstemon* 'Purple Passion' replaced *P.* 'Alice Hindley' due to a lack of available plant material.
- Cuttings were taken in late February.
- All cuttings were placed in the same shallow, slightly opaque plastic storage bin and the bin placed in the same area as used in Experiment 2, but no lid was placed on it.

- At 14 days all dead cuttings were removed.
- The remaining cuttings were assessed and the experiment finished at 21 days.

RESULTS

Experiment 1

Application of a 1.0 M GB foliar spray on 7 successive days to *Griselinia* cuttings had a negative effect on those cuttings. Leaves on some of the treated cuttings were noticeably starting to yellow by Day 7. Figure 1 shows the cuttings on Day 14, when not only were there yellowed leaves on many cuttings but dark brown patches on a few leaves as well. There were no signs of disease, this appeared to be physiological. The control cuttings in the same environment showed none of these signs, they remained green and healthy. At Day 21, 8 out of 49 treated cuttings had formed roots whereas 22 out of 49 control cuttings had formed roots (Fig. 2). The root mass of the treated cuttings tended to be smaller than those of the controls. All 30 rooted cuttings were potted into 0.75-L pots, and 4 months later all plants were growing well; however, 3 of the 8 treated plants had suffered from tip necrosis as shown in Figure 2 and were shorter plants.

Fig. 1. Experiment 1: The 2 trays of *Griselinia littoralis* cuttings at 14 days. Some treated cuttings in the left tray show signs of deteriorating foliage.

Fig. 2. Experiment 1: Rooted *Griselinia littoralis* cuttings after 21 days. Treated cuttings are on the left (8/49 rooted) and untreated cuttings on the right (22/49 rooted). Note the brown tip on the 3rd from the right treated cutting; there were a number of treated cuttings with similar darkened growth tips, dead at the tip, but none of the controls displayed this.

Experiment 2

On Day 3, when the cover was removed, all cuttings were in good condition (Fig. 3). Unfortunately in my quest to ensure big, leafy cuttings in order to maximise any drought effects, I had made the *P.* 'Alice Hindley' cuttings too tall for the bin, resulting in the tips being bent under the lid. It was too late once I realised my mistake, as the cuttings had all been treated and I had no spare GB to make shorter cuttings. They never recovered from this, and remained bent once the lid was removed. However this did not affect their survival rate. The total *P.* 'Alice Hindley' survival rate was 28 from 30 cuttings, with 2 from the 1.0 M cutting immersion group not surviving the potting up due to very small roots. Twenty-six of the 28 *Lavatera* cuttings formed roots and survived (Figs. 4 and 5). Three months after potting up, all plants were well grown with no visible differences between the treatments.

Fig. 3. Experiment 2: All cuttings are in good condition at 3 days, immediately after lid removal.

Fig. 4. Experiment 2: Rooted *Lavatera* cuttings after 21 days. Cuttings immersed in 1.0 M GB are on the left and 1.0 M GB basal-soaked cuttings on the right.

Fig. 5. Experiment 2: Rooted *Lavatera* cuttings after 21 days. Cuttings immersed in 0.5 M GB are on the left and 0.5 M GB basal-soaked cuttings on the right.

Experiment 3

The *P.* 'Purple Passion' cuttings took several days to show any noticeable deterioration but then their demise was rapid, and at Day 14 they were all dead. There were no signs of disease on them. The *Lavatera* struggled too, and at 14 days all cuttings from both the 0.5 M and 1.0 M immersion treatments had died. These were removed and the other *Lavatera* were left for another 7 days before the experiment finished and they were assessed. At Day 21 the *Lavatera* cuttings still alive included all seven controls, six of the 0.5-M base-soaked cuttings, and five of the 1.0-M base-soaked cuttings. At that stage only two had formed roots; a control and a 1.0-M base-soaked cutting. I had decided to end all three experiments after 21 days to allow for continuity between the experiments, but since these few surviving *Lavatera* were in a place where they could be left for longer, that is what I did. The container was left in its original position but each afternoon the sun started to directly hit the plants through a glass window. Leaves on the control plants wilted slightly each afternoon the sun shone on them, whereas the remaining *Lavatera* from both treatments did not wilt as much. This can just be observed in Figure 6, with the control plants on the left hand side of the picture. Thirty-five days after the experiment started I potted all the remaining *Lavatera* with roots into 0.75-L pots. There were two controls, four 0.5-M GB base-soaked rooted cuttings, two of which have since died, and three 1.0-M GB base-soaked rooted cuttings.

Fig. 6. Experiment 3: At 14 days all the *Lavatera* cuttings from both the 0.5 M and 1.0 M immersion treatments had died. The photo shows the surviving cuttings from the control and basal-soaked treatments.

DISCUSSION

The 1.0 M solution applied in Experiment 1 is many times stronger than the foliar spray concentrations of 0.05, 0.1, and 0.14 M used by Mäkelä et al. (1998) on field tomatoes, and another trial using 0.1 M GB foliar spray on glasshouse-grown summer turnip rape, soybean, pea, tomato, and wheat (Mäkelä et al., 1996). Without the benefit of laboratory equipment to test for minute traces of GB in leaves and other plant parts for proof of its uptake, I assumed Experiment 1 would have a big effect on the cuttings, and this effect observed and noted so that it could then be used as a standard for making future comparisons. Results from Mäkelä et al.'s glasshouse trials in 1996 showed that GB translocation through the sprayed plants started very soon after spraying, with the GB moving to the roots first and then to the other plant parts. Overall results from these trials using HPLC and autoradiography showed that GB was xylem-phloem mobile but the translocation itself depended on light and humidity conditions. Surfactants were used in the trials, and they noted that the physical structure of the leaves also played a role in the success of GB uptake (Mäkelä et al., 1996). There were a couple of differences here; the application rate they used was 1/10 or less rate that I used, and it was applied only once to entire young plants with a full and functional root system. However despite these differences it has provided me with future plans for more experiments.

In Experiment 2, I wrongly assumed many of the cuttings would die over the days immediately following the lid removal on Day 3. The control *P.* 'Alice Hindley' survived just as well as the treated *P.* 'Alice Hindley', so there did not appear to be any drought relief needed from the GB treatment. Unfortunately I did not have a control line of *Lavatera*, but all four different GB treatments had good survival rates for the cuttings. The results from Experiment 2 show that *P.* 'Alice Hindley' and *Lavatera* can be propagated successfully using the method outlined above. I liked using the Jiffy pellets in these experiments, as they provided a good WHC and good porosity, so vital for root formation. In addition, in Experiment 3 I could remove the dead cuttings simply by lifting out those pellets.

Future plans include applying GB to cuttings at rates similar to those used by Mäkelä et al., both by immersing the cuttings and soaking the bases, and placing the cuttings in a range of drought stress-inducing conditions. Larger numbers of cuttings will be propagated so that results can be analysed and presented rather than just reporting trends.

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