Technical improvement of a new bioreactor for large scale micropropagation of several *Vaccinium* cultivars

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Abstract

The aim of this study was to evaluate the new Plantform bioreactor for large scale micropropagation of several *Vaccinium* cultivars. Micropropagation in the bioreactors was very cost effective by elimination of agar, more plants per unit, less handling due to no positioning of explants in the bioreactor and rooting in the same unit. Ex vitro was facilitated with no agar in the medium and due to aeration of the bioreactor the plant quality was improved.

Keywords: plantform bioreactor, temporary immersion system (TIS), large scale micropropagation, *Vaccinium*

INTRODUCTION

Conventional micropropagation is expensive and labour intensive. Until now, few laboratories have been truly profitable. However with development of a new methodology using liquid culture systems in bioreactors it is now possible micropropagation to become cost-effective. One of the first described bioreactors (RITA) using temporary immersion system (TIS) was by Alvard et al. (1993). This bioreactor consisted of one unit. Another system based on the Twin Flasks system was described by Escalona et al. (1999, 2003). In this system the explants are placed in one vessel and the nutrients in another. We used the RITA system for apples (Zhu et al., 2005) and the Twin flask system for several horticultural crops (Welander et al., 2007). However we found problems with existing equipment. The RITA system was too small for our purposes and the vessels also changed colour after being autoclaved a few times. The 5-L glass vessels used for the twin system were very heavy especially since both vessels had to be transferred together to the laminar flow hood. The glass vessels had large height making it difficult to transfer explants with no contamination and the small bottom area made it difficult for the plants to expand. For these reasons we developed a new bioreactor called Plantform which was described by Welander et al. (2014). This bioreactor is easy to work with because it is composed of one unit with low weight, is easy to fill and change the medium, and easy to connect to pumps timers and electrical valves. The aim of this research was to evaluate the Plantform bioreactor for different *Vaccinum* cultivars.

MATERIALS AND METHODS

Plant material

Plant material used for micropropagation in the plantform bioreactors were *Vaccinium corymbosum* 'Oskar', *V. angustifolium* 'Emil', and 'Putte', and *V. corymbosum* × *V. angustifolium* 'Northblue'.

Assembly and function of the bioreactors

Figure 1 shows the assembly of the bioreactors, and Figure 2 describes the movement of the nutrients and aeration.

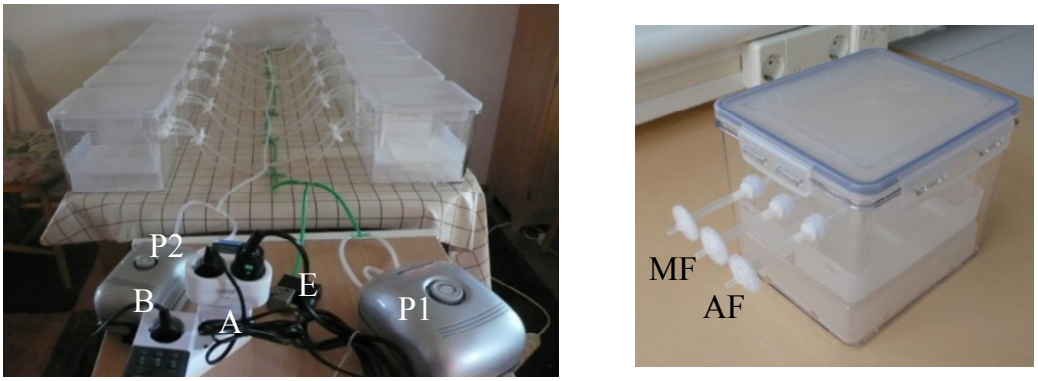

Figure 1. Assembly of 12 bioreactors. The green tubes are connected to the middle filter (MF) on the bioreactors. One end is connected to P1, the electric valve (E) and timer A, while at the other end there is a stopper. The white tubes are connected to one of the outer filters (AF). One end is connected to P2 and timer B and the other end to a stopper.

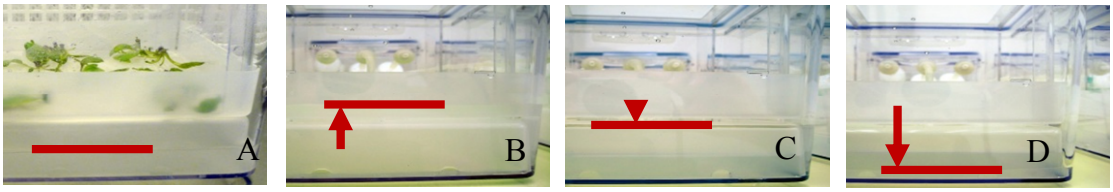

Figure 2. Movement of the nutrient in the bioreactor and aeration. A: When P1 and P2 are off, the medium is at the bottom of the bioreactor and the electric valve is open. B: When P1 is on, the medium floods the basket and the electric valve is closed. C: When P1 is off and P2 is on, the electric valve opens and the medium is drained back. D: As long as P2 is on, the headspace is ventilated.

Micropropagation of blueberry

1. Explant establishment in vitro.

Stem sections from rapidly growing shoots from certified plants grown in the greenhouse were used as explants. Cuttings about 6 cm long with 2 nodes were soaked in soap water for 3 min, then pre-sterilised in 70% ethanol for 1 min followed by 7% calcium hypochlorite plus 0.1% Tween for 20 min and the rinsed three times with sterile water. The nodal segments were then placed in test tubes with 3 mL liquid medium consisting of WPM salts (Duchefa, Haarlem, The Netherlands) 2 mg L^{-1} zeatin, 3% sucrose and pH 5.2 until new buds had emerged (3-4 weeks). Only sterile buds were used for further multiplication. Surviving clean shoots were then transferred to the same medium but with addition of 3% sucrose, and 7 g L^{-1} agar.

2. Shoot multiplication in the bioreactors.

Elongated shoots from the agar medium were used for multiplication in the bioreactors. The basal part was cut off with a pair of scissors and the rest was cut into nodal segments. Around 50-100 segments were used per bioreactor (Figure 3). The bioreactor was filled with 400 mL of culture medium consisting of WPM salts (Duchefa, Haarlem, The Netherlands), 3% sucrose, and pH adjusted to 5.2. Zeatin at 0.5 mg L^{-1} was added after autoclaving. The explants were immersed 2 times day^{-1} (8.00 and 16.00) for six min and aerated every hour for two min (8.00-20.00). All cultures were maintained in a growth chamber with 16/8 h photoperiod at 33 µmol m^{-2} s^{-2} and a temperature of 23/18°C (day/night).

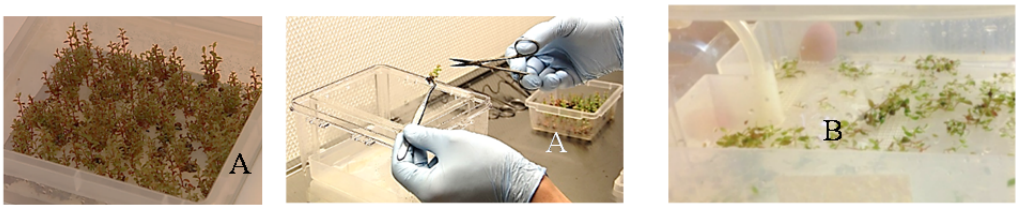

Figure 3. Shoots from agar medium (A) were cut into nodal segments and placed in the bioreactor (B)

3. Rooting in the bioreactors and establishment ex vitro.

The rooting medium consisted of WPM salts, 3% sucrose and 1 mg L^{-1} IBA. The shoots were exposed to this medium for one week. Pre-rooted shoots were then planted in plugs containing fertilized block peat (Hasselfors Garden, Sweden) with 4-7-15 fertilizer incorporated.

RESULTS

Establishment of blueberry shoots

After two months, elongated shoots (Figure 3) were used to start multiplication in the bioreactors.

Shoot multiplication of blueberry in the bioreactors

After five to six weeks, the bioreactor was filled with 300-400 shoots depending on the plant material. The shoots in the bioreactor could then either be used for further shoot multiplication or for rooting. For further shoot multiplication, shoot clusters from the bioreactor were cut into nodal segments. A sterile paper cone was filled with around 100 nodal explants and then thrown into a new bioreactor (Figure 4). From one bioreactor around ten new ones could be loaded.

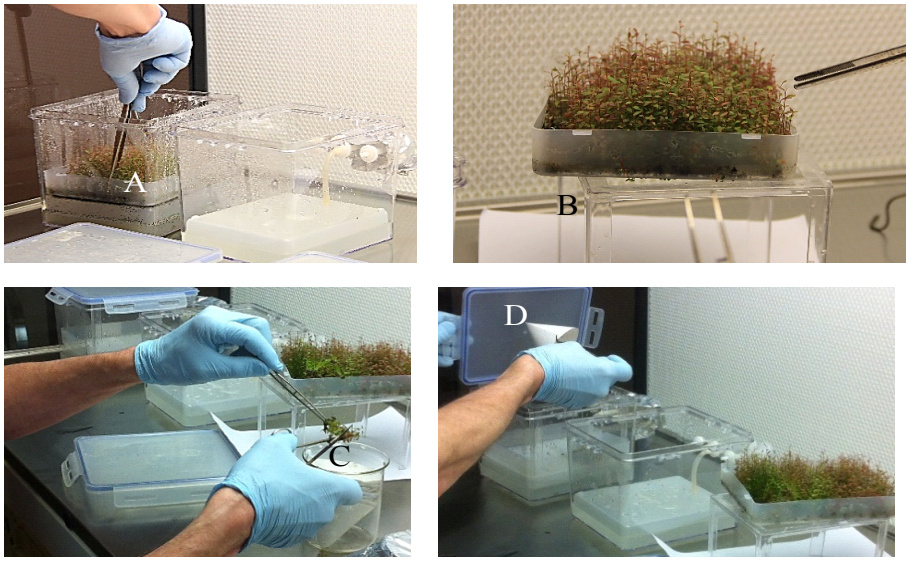

Figure 4. Shoots transferred from one bioreactor to another. The basket (A) with the shoots is lifted from the bioreactor and placed on the legs (B). Shoot clusters (C) are cut with a pair of scissors in nodal segments into a sterile paper cone (D) and then thrown into a new bioreactor.

Rooting in the bioreactor and establishment ex vitro

Figure 5 shows how the shoot production medium in the bioreactor is replaced by the rooting medium. The shoots are exposed to this medium for one week and then planted ex vitro.

Figure 5. The shoot production medium (S) in the bioreactor is replaced by the rooting medium (R) witout moving the shoots.

Figure 6 shows pre-rooted shoots planted in commercial plugs containing fertilized block peat and blueberry plants after 3 month outdoors.

Figure 6. Pre-rooted shoots planted in plugs containing fertilized block peat and blueberry plants after 4 month outdoors.

Micropropagation of *Vaccinium*

Figure 7 shows different *Vaccinium* species in the bioreactors during the multiplication stage.

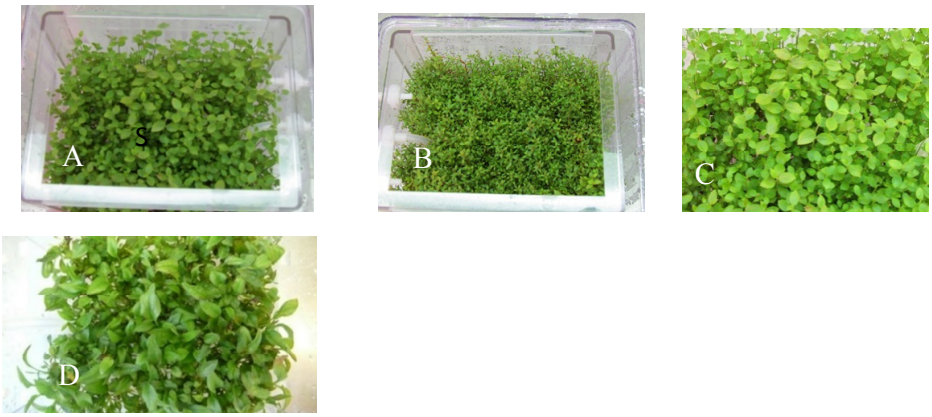

Figure 7. A: *Vaccinium* 'Emil'; B: *Vaccinium* 'Putte'; C: *Vaccinium* 'Oskar'; D: *Vaccinium* 'Northblue'.

CONCLUSIONS

The newly developed plantform bioreactor functions well for both large scale micropropagation and basic research. It is convenient to work with due to one unit with low weight. The medium is easy to fill and replace and filters/tubes are quickly applied. No shoot positioning is needed, no agar and more plants on less space save production cost. The pumps, timers and electric valve are easy to connect. The bioreactors are environmental friendly due to a long shelf life.

Literature cited

Alvard, D., Cote, F., and Teisson, C. (1993). Comparison of methods of liquid medium culture for banana micropropagation – effects of temporary immersion of explants. Plant Cell Tissue Organ Cult. *32* (*1*), 55–60 http://dx.doi.org/10.1007/BF00040116.

Escalona, M., Lorenzo, J.C., González, B., Daquinta, M., González, J.L., and Desjardins, Y. (1999). Pineapple (*Ananas comosus* L. Merr) micropropagation in temporary immersion systems. Plant Cell Rep. *18*, 743–748 http://dx.doi.org/10.1007/s002990050653.

Escalona, M., Samson, G., Borroto, C., and Desjardins, Y. (2003). Physiology of effects of temporary immersion bioreactors on micropropagated pineapple plantlets. In Vitro Cell. Dev. Biol. Plant *39* (*6*), 651–656 http://dx.doi.org/10.1079/IVP2003473.

Welander, M., Zhu, L.H., and Li, X.Y. (2007). Factors influencing conventional and semi-automated micropropagation. Propag. Ornam. Plants *7*, 103–111.

Welander, M., Persson, J., Asp, H., and Zhu, L.H. (2014). Evaluation of a new vessel system based on temporary immersion system for micropropagation. Sci. Hortic. (Amsterdam) *179*, 227–232 http://dx.doi.org/10.1016/j.scienta.2014.09.035.

Zhu, L.H., Li, X.Y., and Welander, M. (2005). Optimization of growing conditions for the apple rootstock M26 grown in RITA containers using temporary immersion principle. Plant Cell Tissue Organ Cult. *81* (*3*), 313–318 http://dx.doi.org/10.1007/s11240-004-6659-9.