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Comparison of a conventional semisolid medium system with the Plantform™ Bioreactor system: micropropagation of *Cordyline fruticosa* 'Purple' and *Aronia melanocarpa* 'Viking'

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Abstract

Various bioreactors, using temporary immersion systems (TIS) based on different designs and working principles, can have positive effects on development and multiplication of plantlets during micropropagation. Both number and quality of propagules produced in the TIS, when compared to those from a semisolid medium micropropagation system, may vary substantially depending upon both plant species and bioreactor type. This study compared the *in vitro* propagation performance of both *Aronia melanocarpa* 'Viking' and *Cordyline fruticosa* 'Purple' grown in the Plantform™ Bioreactor TIS system and on a semisolid medium system. The number of shoots per explant increased significantly in the bioreactor system compared to the semisolid medium system for both species although some of the shoots of cordyline were not marketable. In the Plantform™ Bioreactor culture, the multiplication rate for cordyline was 8.9 marketable shoots per production cycle vs 2.2 marketable shoots produced on semisolid medium, a 400% increase. For aronia grown in the Plantform™ Bioreactor culture, the multiplication rate was 11.9 shoots per production cycle vs 6.6 shoots produced on semisolid medium, a 180% increase. Additionally, shoots of both species grown in the Plantform™ Bioreactor system demonstrated significant increases in both total shoot fresh and dry weights. Some cordyline shoots also demonstrated various hyperhydricity symptoms and leaf curling and necrosis in the TIS production cycles; further experimentation needs to be conducted to address this issue before the system can be recommended for this crop. The Plantform™ Bioreactor system can be recommended for improved micropropagation of *Aronia melanocarpa* 'Viking' since the numbers of shoots were substantially increased and there were no abnormalities of the shoots or leaves as compared to those produced on a semisolid medium system.

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Introduction

In tissue culture, the more important problems of commercial micropropagation traditionally performed on semisolid medium include low proliferation rate, use of a large number of culture containers, contamination, large shelf area for cultures, high labor costs, energy expenses, and lots of time spent on subcultures. To overcome many of these problems, large-scale propagation techniques with simpler protocols, fewer tools and equipment, and lower labor inputs have to be taken into consideration[1]. Bioreactors are the most promising technique in terms of reducing labor and production costs while providing propagules in very large quantities[2]. Bioreactors using a Temporary Immersion System (TIS), designed to take advantage of the positive effects of the liquid medium on the growth and organogenesis of plant tissues compared to the effects of a semisolid medium on propagation, are based on the principle of alternately contacting plant tissues with a liquid medium and with air[3,4]. The bioreactor with TIS generally contacts the explant with a liquid medium usually 1–5 times a day for 5–10 min, depending on the type of plant, then the liquid medium is removed. Georgiev et al.[5] evaluated TIS bioreactors used by 15 research groups, according to the number of containers used, the mechanism for moving the air and liquid medium, and the version or type of bioreactor technology[6–12].

The Plantform™ Bioreactor system, developed in Sweden, is a highly preferred micropropagation system, with its numerous advantages such as 1) economical cost per unit, 2) high light transmissibility, 3) simplicity of use, 4) reduced space requirements for operation, and 5) possibility of connecting large numbers of bioreactors serially[13]. Individual Plantform™ bioreactors are small volume single culture container systems, the outer walls are made of polycarbonate, and the inner baskets, ventilation vents, legs, and lids are made of polypropylene material – all of these are resistant to high temperature (http://plantform.se/index. html)[14].

Aronia melanocarpa, a North American native plant commonly known as aronia or chokeberry, has gained importance in recent years in Turkey, where it is known to be one of the most antioxidant-rich fruit species[15]. At the same time, this plant can be used as an outdoor ornamental landscape plant. Although it can be propagated both by seeds and vegetatively through cuttings, micropropagation is preferred for producing large numbers of true-to-type and disease-free propagules[16].

The genus *Cordyline* contains numerous ornamental plants of tropical origin. Cultivars of the Ti plant or cordyline,

Cordyline terminalis and *C. fruticosa* are both grown as containerized plants that are prized for their large, often colorful leaves. This plant is propagated both by seed and by traditional vegetative propagation methods such as stem cuttings. While conventional vegetative propagation allows for clonal production, these methods are time-consuming; a low rate of proliferation can result in the many years needed to create a large number of elite clones, with the concomitant requirement for stock plant cultivation. For these reasons, cordyline is among the containerized ornamental plants where classic *in vitro* micropropagation has been applied[17–19].

Bioreactors have been suggested as a system than could be used to overcome problems of low proliferation rate seen in some micropropagation systems utilizing semisolid medium. However, results are not always superior or consistent. Some researchers, in the case of *Spathiphyllum*, found plants grown on semisolid medium produced more offshoots than plants grown in a TIS bioreactor system[20,21]. However, Takayama & Akita[22] reported a superior proliferation rate using a bioreactor for *Spathiphyllum*. Differences in plant variety can play a significant role here. It also appears that the effects of different types of bioreactors on development and proliferation rate of plantlets may be a significant source of variation in multiplication rates. In terms of shoot proliferation, other species placed in the same bioreactor system showed similar variation. Both *Rubus* and *Echinacea* had superior plantlet production in the Plantform™ Bioreactor system, but *Digitalis* produced fewer shoots in this bioreactor system[23]. In a study conducted with carob (*Ceratonia siliqua*) using the same bioreactor system, it was reported that both the number and length of shoots were greater using the Plantform™ Bioreactor when compared to culture on conventional semisolid media[24]. Clearly, both plant species and bioreactor type impact the success rate of micropropagation using a bioreactor. For many years, vitrification has also been a significant factor to consider when using liquid medium rather than a semisolid medium for *in vitro* culture[25,26] and we encountered some hyperhydricity issues in these trials. This study aims both to investigate the use of the Plantform™ bioreactor system for the micropropagation of two horticultural plants and to compare the results obtained from that system with micropropagation of these plants using a similar semisolid nutrient medium *in vitro*.

Materials and methods

In vitro shoots, 8–9 mm in length of two cultivars (*Cordyline fruticosa* 'Purple', *Aronia melonacarpa* 'Viking') were used as explant sources in both the bioreactor and semisolid medium systems. To reduce impact of existing growth regulators on experimental results, the *in vitro* plants used as explant sources were grown on semisolid MS medium without growth regulators for 30 d before explants were collected. MS + 0.5 mg/l BAP + 0.5 mg/l BAP + 0.1 mg/l NAA nutrient medium was used for cordyline proliferation as this medium has been used previously in our lab for this purpose. For aronia, DKW (Driver-Kuniyuki Walnut medium[27] + 0.5 mg/l BAP + 0.1 mg/l NAA nutrient medium used for cultivation and multiplication. This medium had also been used successfully in the lab. Sucrose at 30 g/l was added to all nutrient media, pH was adjusted to 5.8 with 1.0–0.1 N NaOH and HCl solutions. The media were not different except for the solidifying agent, in both bioreactor and conventional semisolid cultures[28]. Gelling for conventional semisolid media was performed with addition of 6 g/l agar after pH adjustment. Culture vessels for semisolid nutrient medium were 250 ml glass culture containers with transparent lids; 50–60 ml of nutrient medium was poured in liquid form. Plantform™ bioreactors (180 mm × 160 mm × 150 mm) were used as TIS based bioreactors. After the addition of 400 ml of the liquid nutrient medium into bioreactors, the nutrient medium in bioreactors and glass containers were sterilized in an autoclave for 20 min at 121 °C. For setup, 40 *in vitro* shoots were placed in the bioreactors and 15 shoots were inserted in the semisolid medium in each glass container. In the Plantform™ bioreactor, the frequency of immersion was carried out with compressed air for 10 min every 6 h; forced aeration was not applied between immersion periods. A simple timer and aquarium pumps were used for the upward movement of the liquid medium. Bioreactors and culture vessels containing semisolid media were incubated at 25 ± 2 °C, under daylight fluorescent lamps, 35 micromole/m/s light intensity, with 16 h/day photoperiod conditions.

For data collection and statistical analysis, trials were set up according to a randomized plot design with three replicates of the following: in conventional multiplication (semisolid medium) three vessels, in bioreactor culture, a single bioreactor container was used as a replicate. After the cultures were kept in the conditions described above for eight weeks, the following measurements/calculations were taken in both systems: 1) number of shoots per explant (rate of proliferation), 2) length of shoots, 3) number of leaves per shoot, 4) total fresh and dry weights of shoots formed on an explant, 5) ratio of dry weight to fresh weight of shoots, and 6) appearance and quality of *in vitro* leaves. The dry weights of the shoots were measured after drying at 70 °C for 24 h. The proliferation rate was calculated by dividing the number of shoots formed at the end of the cultural period by the number of explants inoculated at the beginning of culture. The sample averages were compared with a two-tailed independent t-test[23,24,29]. Statistical analysis and SEM calculations were carried out using the SPSS program. The difference between the two micropropagation systems was determined at the significance levels of $p < 0.05$(*) and $p < 0.01$(**). All statistical analyzes were performed separately for each cultivar. This research was conducted in the tissue culture laboratory of Department of Horticulture, Ege University, Izmir, Turkey.

Results

Number of shoots per explant (multiplication rate)

For the number of shoots per explant, there was a significant difference ($p < 0.05$) between semisolid and temporary immersion systems for both cultivars. For *Cordyline fruticosa* 'Purple', the number of marketable shoots produced per explant varied from 8.9 shoots per explant in the Plantform™ bioreactor culture to only 2.2 marketable shoots per explant in the agar medium (Table 1). (Any shoots displaying hyperhydricity symptoms were excluded from this count.) For *Aronia melanocarpa* 'Viking', the number of shoots produced was 11.9 per explant in Plantform™ bioreactor culture, while in the agar medium the number per explant was 6.6 shoots. (Table 2).

Table 1. Comparison of the semisolid medium system and the Plantform™ bioreactor TIS in terms of shoot number, shoot length, leaf number, total shoot fresh-dry weight, dry weight/ fresh weight ratio (dry weight rate), in *Cordyline fruticosa* 'Purple' micropropagation.

Culture type	Shoot number/ explant	Shoot length (cm)	Leaf number/ explant	Shoot fresh weight (mg/explant)	Shoot dry weight (mg/explant)	Dry weight/fresh weight ratio (%)
Semisolid medium	2.2 ±1.0^{b}	3.02 ± 1.52	6.1 ± 2.0^{b}	1066.1 ± 239.0^{b}	110.3 ± 21.6^{b}	9.8 ± 0.2
Plantform™ Bioreactor	8.9 ± 4.7^{a}	3.08 ± 0.77	3.7 ± 0.70^{a}	1923.5 ± 220.1^{a}	184.7 ± 26.3^{a}	9.6 ± 0.4
	*	ns	*	*	*	ns

Different letters within the same column indicate a significant difference at $p < 0.05$ (*).

Table 2. Comparison of the semisolid medium system and the Plantform™ bioreactor TIS in terms of shoot number, shoot length, leaf number, total shoot fresh-dry weight, dry weight/ fresh weight ratio (dry weight rate), in *Aronia melanocarpa* 'Viking' micropropagation.

Culture type	Shoot number/ explant	Shoot length (cm)	Leaf number/ explant	Shoot fresh weight (mg/explant)	Shoot dry weight (mg/explant)	Dry weight/fresh weight ratio (%)
Semisolid medium	6.6 ± 1.7^{b}	3.7 ± 0.8^{b}	12.0 ± 1.6	222.4 ± 23.7^{b}	35.0 ± 3.0^{b}	15.8 ± 0.5^{a}
Plantform™ Bioreactor	11.9 ± 0.6^{a}	4.4 ± 0.6^{a}	11.2 ± 2.0	439.8 ± 26.8^{a}	41.6 ± 2.2^{a}	9.4 ± 0.2^{b}
	*	*	ns	*	*	*

Different letters within the same column indicate a significant difference at $p < 0.05$ (*).

Shoot length (cm)

No statistically significant difference was found between the semisolid medium and the temporary immersion system in cordyline in terms of shoot length (Table 1). The tallest shoots were obtained from aronia (37–44 mm) and the effect of the bioreactor on shoot length was significant in aronia (Table 2).

Number of leaves (leaf number/shoot)

The number of leaves/shoot was between 2.8–3.1 leaves in cordyline and between 11.2–12.0 leaves in aronia (Tables 1, 2). For comparison, no significant differences were found between number of leaves produced in either a TIS or on semisolid media in either agapanthus or spathiphyllum[21,30].

Appearance and quality of *in vitro* shoot-leaves

In the bioreactor culture of cordyline, most axillary shoots formed normally as expected, but the main shoot development and elongation ceased and the leaves on the main shoot displayed light-colored, small, vitrified, necrotic spots. Neither necrotic spots nor vitrification was observed in cordyline grown on semisolid medium. Comparatively, it was determined that growth of aronia in the TIS medium appeared healthy and the form of the shoots and the color of the leaves were similar or better than leaves and shoots produced on semisolid medium. For aronia, development and growth were much faster in the bioreactor, and after only a few weeks in TIS, the shoots filled the container (Fig. 1a, b).

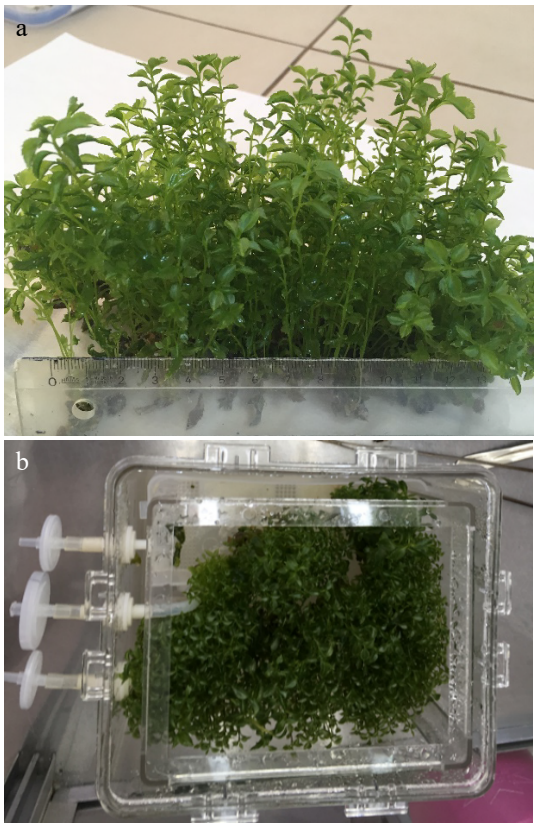

Fig. 1 (a) High quality shoots of *A. melanocarpa* 'Viking' visible in Plantform™ bioreactor. (b) Quantity of shoots of *A. melanocarpa* within Plantform™ bioreactor.

Discussion

Number of shoots per explant (multiplication rate)

Previous studies have differed in terms of the number of shoots per explant produced. In *Spathiphyllum*, it has been reported that more shoots were obtained from the semisolid medium than from the TIS[20,21]. Superior propagation results for a TIS bioreactor culture compared to propagation with semisolid medium was reported for *Fragaria*[31,32], for *Ananas comosus*[33,34], for *Eucalyptus* and *Betula*[29] and for *Musa* 'Dwarf Cavendish'[35]. For *Cordyline*, the multiplication rate of marketable shoots produced with the Plantform™ bioreactor system compared favorably with multiplication rates for these other plants although some hyperhydricity issues need to be addressed. For *Aronia*, although the number of shoots per explant was high, the bioreactor/semisolid medium shoots ratio was not as great as the proliferation rate of *Ananas*, *Agapanthus*, and *Eucalyptus*[33,36]. In other studies, comparing the Plantform™ bioreactor system to semisolid medium system, Welander et al.[23] stated that the total number of shoots of *Rubus, Digitalis,* and *Echineacea* produced in the TIS was similar to or better than those produced on solid medium. Umarusman & Kaçar[24] reported the superiority of the Plantform™ bioreactor system in terms of the number of shoots in the three clones of *Ceratonia siliqua*. In our study, the multiplication rates for both cordyline[17–19,37] and aronia[16] grown on

semisolid media are less than what has been reported in other studies. This variability in *Cordyline* and *Aronia* may be due to the differences in the medium and the plant growth regulators (PGRs) used or to the cultivars used in these trials, so considering that higher proliferation rates of these two species can be achieved, different hormones and different hormone concentrations should be tested in both the bioreactor and semisolid media culture of *Cordyline* and *Aronia*. Using different concentrations of hormones in the bioreactor media might also reduce hyperhydricity issues with cordyline.

Shoot length (cm)

Results similar to ours have been reported[21,23,30,32,34] where researchers compared the temporary immersion system with a semisolid medium micropropagation system for *Fragaria, Agapanthus, Ananas, Ceratonia, and Spathiphyllum*. For *Aronia*, when we compare our semisolid medium system results with the values obtained for the semisolid media from other studies, the value of 28–37 mm average shoot length reported by Litwińczuk[38] for semisolid medium micropropagation of *Aronia*, agreed with our research results. However, our findings were not similar to those of Almokar & Pırlak[16], who reported shorter average shoot length for *Aronia* grown in semisolid medium *in vitro*. In our study, the average length of the shoots (30 mm) on semisolid medium for *Cordyline* was less than the mean shoot length obtained by Khan et al.[17].

Fresh and dry weight (mg/explant)

The fresh and dry weight values of the shoots detected per explant are given in Tables 1 & 2. When we examine the average fresh and dry weight values of the two varieties in our trials, it was determined that the Plantform™ bioreactor system significantly increased the fresh and dry weight of shoots compared to the weights of shoots grown on semisolid medium. For the dry weight ratios, no significant differences were found between shoots from either the bioreactor culture or the semisolid medium culture for cordyline. However, the ratio of fresh/dry weight of shoots in the semisolid medium compared to TIS system was statistically different in aronia. For comparison, while there were no differences in shoot fresh weights between TIS and solid medium in agapanthus, the TIS system gave significantly higher values for dry weights[30]. Shoots produced in the TIS system yielded an increase in shoot fresh weights compared to shoots produced on semisolid media for both *Eucalyptus* and *Betula pendula*, but the same was not observed for *Betula pubescens*[29]. The fresh and dry weights of *Ananas* shoots were both higher in the TIS system[34]. The increases in fresh and dry weight mass produced in the bioreactor cultures are generally due to the increases in the number of shoots produced in the TIS compared to shoots on the semisolid medium. This is certainly true for both *Cordyline* and *Aronia* in our study. Notably, the increase in shoot length observed in *Aronia* contributed to this increase in both fresh and dry weights. Paek et al.[39] reported that in a bioreactor system, higher nutrient availability and better growth resulted in more fresh and dry mass accumulation in shoots Certainly, fresh and dry weight gain may vary depending on the species and culture conditions[24].

Appearance and quality of *in vitro* shoot-leaves

Compared to our varied results, Welander et al.[23] reported that in the Plantform™ bioreactor, *Digitalis* and *Echinacea* cultures developed normally, but for *Rubus*, the tips and edges of the leaves were curled due to hyperhydricity. Businge et al.[29] stated that there was no difference in the appearance of *Eucalyptus* and *B. pubescens* shoots and leaves on plantlets produced by either culture method, but there were folds in *B. pendula* leaves produced in a TIS. In another study comparing plants propagated with either a Plantform™ bioreactor TIS or on a semisolid medium system, the quality of citrus rootstocks was found to be higher in the TIS culture[40]. It has been suggested that these physiological disorders, seen as damaged shoots and necrotic folded leaves, were caused by the effect of immersion duration and frequency of the liquid medium that did not fully match up with the demands of the plant species[41,42]. Conversely, the vitrification observed in a TIS system may be less than vitrification observed in either standard liquid or semisolid culture[43].

Conclusions

The Plantform™ bioreactor TIS is preferred in the proliferation phase of micropropagation of *Aronia melanocarpa* 'Viking', due to the better quality of shoots produced and the higher rate of shoot proliferation compared to shoots propagated in a semisolid medium containing identical nutrients and hormones. In contrast, in this study, some *Cordyline fruitcosa* 'Purple' shoots grown in the Plantform™ Bioreactor TIS displayed hyperhydricity and necrotic spots on the leaves and shoots, and the main shoot ceased to grow; we did not see these responses on shoots grown on semisolid medium. These micropropagation complications for cordyline in the TIS may be related to the immersion intervals of the liquid medium, the components of the liquid medium, or they may be responses to some ventilation factors[1,42,43]. Additional trials should be conducted to determine if changes in medium components such as plant hormones as well as changes in frequency of immersion might improve micropropagation of cordyline in a TIS, since micropropagation in a Plantform™ Bioreactor TIS did produce considerably greater numbers of marketable shoots.

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Conflict of interest

The authors declare that they have no conflict of interest.

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