

Improvement of shoot proliferation by liquid culture in temporary immersion

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The conventional tissue culture technique for mass propagation of plants uses semi-solid culture medium, solidified by gelling agents. This not only increases the production cost of the micropropagated plants, but also has some weak points which limit the proliferation rate. The major disadvantage is the limited absorbance of the nutrient medium by the basal part of the microshoots. On the other hand, liquid culture systems based on bioreactors, which were hopefully presented as an efficient alternative to semi-solid media, have never been totally satisfactory, being highly suitable for bacteriological cultures, but only few plant species. Indeed, explants permanently immersed in liquid medium frequently show the symptoms of asphyxia, tissue hyperhydricity, and mechanical damages. The temporary immersion system (TIS), based on limiting the contact of the explants with the liquid medium by alternating cycles of immersion and dry periods, combines the advantages of both the conventional semi-solid medium and liquid culture systems. Its advantages are (I) a more uniform contact between the culture medium and the shoots in comparison to the conventional culture in gelled medium, (II) the reduction of asphyxia and hyperhydricity, (III) the dilution of toxic compounds (phenols) released by the shoots, producing culture oxidation and browning, (IV) the periodic replacement of the atmosphere within the culture container, which limits gas accumulation (mainly CO₂ and ethylene), a typical event which occurs when working with the traditional gas-tight glass jars, (V) the possibility to extend consistently the subculture time when using large containers, (VI) the possibility to reduce markedly hand labour and cost of production, as a careful positioning of shoots in the gelled medium is not necessary and the refill of the fresh liquid medium is done easily, and (VII) tissue division, stimulated during bubbling, results with increased proliferation rates and overall shoot quality. In general, the modification of the frequency and duration of medium immersion enables a better control of plant morphogenesis. Since the first report of a liquid culture in TIS of about 30 years ago, numerous prototypes have been developed. Among these, Plantform, a recently-developed TIS device, allowing periodic medium immersion and independent ventilation of containers, has been the focus of the present study. Its performance with ornamental (*Carex oshimensis* ‘Evergreen’ and *Chrysanthemum morifolium*) and fruit species (*Ficus carica* and *Ribes rubrum*) is critically analyzed. Our results confirmed the convenience of the Plantform-based TIS for proliferation of high-quality shoot clusters; moreover, with some species, the system stimulated the contemporary proliferation and rooting of shoots, which can be maintained in proliferation or directly transferred to the acclimatization phase.

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