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PHOTOAUTOTROPHIC MICROPROPAGATION - A REVIEW

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ABSTRACT

Sugar in a medium is considered to be an essential cause for high production costs of plantlets in a conventional heterotrophic micropropagation. The heterotrophic mode of nutrition and poor mechanism to control water loss render micropropagated plants vulnerable to transplantation shocks. High sucrose and salt containing media often employed for raising cultures and the poor light conditions seem to restrict the photosynthetic efficiency of the leafy shoots. The growth of plantlets *in vitro* is often greater under photoautotrophic conditions then heterotrophic conditions, provided that the *in vitro* environment is properly controlled for promoting photosynthesis.

The multiplication of plants by tissue culture - micropropagation, offer many advantages over conventional method for the rapid production of large number of plants, independent of climatic conditions with conservation of space and time. This approach has considerable potential for the conservation of germplasm for breeding programmes. Also, for the multiplication of rare plants which can be introduced into natural habitat thereby ensuring conservation of ecosystem. Micropropagation has many advantages over conventional vegetative propagation and its commercial use in horticulture, agriculture and forestry is currently expanding worldwide. However its widespread, commercial use for major crops is still restricted as a result of relatively high production cost. The concept of photoautotrophic micropropagation has recently been proposed as a means of reducing production cost and automation - robotization of the micropropagation process.

Photoautotrophic micropropagation

This strategy assumes that autotrophic culture will have persistant leaves that live longer and would be more photosynthetically productive *ex vitro*. (Grout and Millam, 1985). The objective is to modify culture induced phenotype towards autotrophy in culture. To do this, the oxygen concentration can be reduced in culture environment, which depresses the photosynthetic rate (Shimada *et al.*, 1988). Alternatively sugar is reduced or completely eliminated from the medium (Kozai, 1988), while the photosynthetic photon flux (Kozai, 1988, Kozai *et al.*, 1987) and the CO_2 concentration (Desjadins *et al*, 1988) are increased (Debergh, 1988). Increasing light intensity alone can not raise the net photosynthetic rate for cultures at there CO_2 compensation point. Such a photoautotrophic tissue culture system has the added advantage that microbial contamination is less of a problem when sugar is eliminated from the medium (Fujiwara *et al.*, 1988). In this system, a gas permeable, clean plastic film is used as a vessel closure (Kozai, 1988). This plastic film improves gas exchange of the cultures, CO_2 enrichment or O_2 reduction, increases the light penetration to the container contents and decreases the relative humidity to the vessels. In adopting the photoautotrophic tissue culture system, *in vitro* culture has been exchanged for a hydroponic system. This has the advantage that contamination by bacteria and fungi are no longer a problem *in vitro*. Extensive climate control larger vessels and robotization are possible and the *ex vitro* acclimatization

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stage is less of problem (Kozai, 1988). However, one must accept the concurrent loss of many of the advantages of micropropagation, such as growth rates and miniaturization (Debergh, 1988) and assumes the problems of hydroponic system, such as algae control and the requirements of chronic nutrient solution adjustments (Fujiwara *et al.*, 1988).

Reasons for high production cost

Labour costs for multiplication, rooting and acclimatization of plantlets are said to account for approximately 60% of the total production costs in conventational micropropagation. Automation or robotization or both at these culture stages therefore is essential for a drastic reduction of production costs in the future in the micro-propagation industry (Aitken-Christe, 1991, Jan Rowe, 1986). In reality, however manipulation of explants-plantlets at the multiplication and rooting stages is difficult to automate in conventional micropropagation. Other reasons for the high production costs in conventional micropropagation include:

(a) A long time period (often several weeks or more) required for each culture stages.
(b) A low multiplication rate.
(c) A loss of plantlets due to biological contamination and physiologic and morphologic disorders during the multiplication stage.
(d) A relatively high percentage of death of the plantlets due to serious environmental stresses during the acclimatization stage.
(e) Large variation in size, quality and morphology of plantlets.
(f) Significant energy costs for lighting, cooling or air-conditioning, sterilization, washing, etc.
(g) Significant cost for gelling agent, culture vessels, macro and micro nutrient, plant growth regulatory substances, sugar, etc.
(h) An over production or shortage of plantlets at various time of the year due to unpredictable circumstances.
(i) A significant space (or its related costs) required for cultures and their preparation (Kozai, 1990).

Sugar in the medium increases production costs

An essential cause for high production costs is the presence of sugar in the culture medium. This is because

a) Sugar in the medium promotes biological contamination.
b) Small airtight vessels must then be used to reduce the loss of plantlets *in vitro* due to contamination.
c) The relative humidity and ethylene concentration thus tend to be high.
d) The abnormal *in vitro* environment includes physiologic and morphologic disorders such as virtification and growth or development retardation.
e) Plant growth regulating substances are often necessary for promoting differentiation.
f) Acclimatization of micropropagated plantlets is often difficult.
g) Automated micropropagation system using small vessels are thus difficult to develop (Kozai, 1990).

Advantage of photoautotrophic micropropagation

The advantage of photoautotrophic micropropagation are summarized as follows :

a) Growth and development of plantlets are promoted under high CO_2 and high PPF conditions.
b) Physiologic and morphologic disorders can be reduced and relatively uniform growth and development can be expected.
c) Biological contamination can be minimized, therefore loss of plantlets due to contamination can be reduced.
d) A larger culture vessel can be used with a minimum loss of plantlets due to

contamination.

e) Rooting and acclimatization *in vitro* or *ex vitro* or both can be achieved more easily.
f) Environmental control of the culture vessels can be facilited so that the control of growth and development of plantlets can be conducted by environmental control.
g) Automation - robotization or both can be realized by using a larger vessel.
h) The use of plant growth regulating substances, vitamins and other organic substances can be minimized because some of these will be produced endogenously in sufficient quantities by plantlets growing photoautotrophically (Kozai *et al.*, 1991).

The concept of photoautotrophic micropropagation is thus a novel idea for developing an automated micropropagation system to achieve a drastic reduction in the production costs. Photoautotrophic plantlets were obtained on sucrose free rooting medium, under ambient and enriched CO_2 conditions and they performed better *ex vitro* than mixotrophic plantlets grown with sucrose in red raspberry (Deng and Donnely, 1993). Root hairs were more abundant and longer on root tips of photoautotrophic plantlets than on mixotrophic plantlets. The maximum CO_2 uptake rate of plantlet leaves was 52% that of greenhouse control plant leaves. Consecutive new leaves of transplants took up more CO_2 than persistant leaves. The third new leaves of transplants had photosynthetic rates upto 90% that of greenhouse control plant leaves. The results indicate that *in vitro* CO_2 enrichment was beneficial to *in vitro* hardening and that sucrose may be reduced substantially or eleminated from red raspberry rooting medium when CO_2 enrichment is used. The increase in CO_2 concentration could be, therefore considered to increase the net photosynthesis and hence to promote the growth of plantlets *in vitro*, provided that other enviromental factors are controlled favorably for photosynthesis. In fact, the growth promotion of plantlets *in vitro* by CO_2 enrichment by increasing CO_2 concentration outside the vessel and/or by use of gas permeable film as vessel closures under high photosynthetic photon flux (PPF) has been observed for cymbidium (Kozai *et al.*, 1987), carnation (Kozai and Iwanami, 1998),. potato (Kozai *et al.*, 1988), cauliflower (Grout and Donkin, 1987), chinese mustard (Kozai *et al.*, 1991 b), jackfruit (Rahman and Blake, 1988), and strawberry (Fujiwara *et al.*, 1988). Photoautotrophically grown C_3 plants lose upto 50% of photosynthetically fixed carbon due to photorespiration in a normal atmospheric concentration of O_2 (21%) and CO_2 (345 μ mol. mol^{-1}). However, photorespiration is repressed with decreasing O_2 concentration and is almost completely repressed at 2% O_2. The net photosynthetic rate was measured at O_2 concentration of 1, 10 and 21% of Primula malacoides micropropagation for 20 days of half strength MS medium with 30 g l^{-1} sucrose at a PPF of 110 m mol. $m^2.s^{-1}$ (Shimada *et al.*, 1988). The rates at 1 and 10% O_2 were, respectively, nearly 3 times and 1.5 times greater than at 21% O_2 when the CO_2 concentration was approximately 200 μ mol. mol^{-1}. A similar result has been obtained for Chrysanthemum morifolium (Tanaka *et al.*, 1990). The increase in net photosynthesis by reducing O_2 concentration may be of practical value in commercial micropropagation because it is relatively easy to keep the concentration in a vessel at a lower level. Photoautotrophic tobacco cells cultures were gradually adapted to grow in media containing the normally inhibitory concentration of 20g.l^{-1} NaCl (Chang *et al.*, 1997). Photoautotrophic cells have well developed and physiologically active chloroplasts in contrast to heterotrophic cells (Rodgers *et al.*, 1987). Explants of strawberry plantlets were cultured for 21days on half

strength MS medium with 0 to 20g/litre sucrose under CO_2 enriched or non riched conditions (Kozai *et al.*, 1991). The best growth occurred without sucrose with CO_2 enrichment, for which the per cent residual inorganic ions in the medium on Day 21 was 3% for PO_4^{3-}, 41% for Mg^{2+} and 53% for NO^-_3. For each ion, this amount was greater in other treatments. Thus better nutrient composition with a higher concentration of PO^{3-}_4 were obtained for photoautotrophic micropropagation than those commonly used for heterotrophic culture.

Disadvantages of photoautotrophic micropropagation

Disadvantages of photoautotrophic micropropagation are often said to be increase in cost for CO_2 enrichment (high CO_2) and lighting (high PPF) for better photoautotrophic growth of plantlets. However, in reality, the cost of CO_2 enrichment will not significant. There are several methods of increasing CO_2 in the vessel at a reasonable cost (Kozai, 1990). The method include.

a) Use of gas permeable film as enclosures.
b) Use of the film in a CO_2 enriched culture room.
c) Use of large vessels with forced ventilation or a CO_2 supply system and
d) Use of chemicals to produce CO_2 inside the vessels.

The methodology to elevate vessel CO_2 concentration as described by Deng and Donnely (1993) are as follows.

The experiment was carried out in specially designed sterile plexiglass incubation chambers (55x30x15cm) with tightly fitting plexiglass lids secured with elastic bands wrapped arround paired hooks. The premixed and analysed gas mixture of either ambient or enriched CO_2 from compressed cylinders were supplied continuously to the chambers at a flow rate of 15 ml. min^{-1} through a flowmeter and scrubbed with a series of filters before they were humidified in a 4.5 litre Erlenmeyer flask and equilibrated (buffered) in small (250 ml) Erlenmeyer flask that preceded the incubation chambers. Each chambers had two air inlets and two air outlets on the opposite side and three sensor probe ports: two for temperature and one for humidity. The chambers and tubing system were surface sterilised with 10% bleach and 70% ethanol, respectively. The temperature inside the chambers were monitored at intervals with a telethermocouple inserted through probe ports in the plexiglass chambers.

There are also several methods of reducing the cost of high PPF and cooling in the culture room. The method include the use of optical fibre, reflective materials and novel light source and the lateral lighting system. The increase in costs for high PPF could be minimized by improving the light source, lighting method and vessel structure.

CONCLUSION

A reduction of production costs by 90% or more is essential for worldwide commercialization of micropropagated plants in agriculture, forestry and horticulture in future. Automation-robotization in combination with environmental control would be a key technology for realizing this drastic reduction in costs and thus for solving worldwide food shortage and reforestation problem.

The use of this approach, therefore, should facilitate the successful multiplication and transfer to soil of important germplasm, thereby assisting the conservation of biodiversity.

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