production and much of the development work focuses on ways of reducing the labour input by either improving the multiplication rate that it is possible to achieve *in vitro*, or exploring areas such as mechanisation or automation.

PITFALLS IN MICROPROPAGATION AND HOW TO AVOID THEM

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Micropropagation has established a market niche largely in the higher value sector and value-added areas of introduction of new cultivars and virus-free stock. High production costs have limited its market share but the latter is likely to increase with the introduction of automation (18).

The aim of this article is to attempt a state-of-the-art appraisal of micropropagation strategies so that the purchaser of microplants can be reasonably assured that they are likely to be fit for the purpose intended.

Micropropagation pathway analysis. The micropropagation procedure involves critical decision and monitoring steps as outlined in Figure 1. The nursery operator should appreciate the significance of these decisions and make sure that the micropropagator has adopted the appropriate strategy for any given cultivar. These steps are discussed below.

Genetic selection. Genetic selection, allied to the cloning pathway chosen is of critical importance to the production of true-to-type progeny. Many cultivars are inherently unstable in micropropagation because of their genetic construction. Cultivars to avoid, or to accept for micropropagation only after consideration, are chimeras—usually, but not always recognisable visually, e.g. *Pelargonium* × *hortorum* 'Mme. Salleron', 'Mr. Wren', 'Skelly's Pride'; beneficially-infected cultivars, e.g. *Abutilon sellovianum* 'Marmoratum' and those with unstable loci, e.g. *P.* × *domesticum* 'Grand Slam' (1). Only the breeder or grower may be adequately familiar with a cultivar or its antecedents to recognise its instability, but mutation-bred cultivars and those which tend to sport would be included. If these are to be micropropagated, significant levels of variation should be anticipated and the level of acceptability decided.

Guidelines for genetic selection, aside from the exclusions listed above, have been published by Johansen *et al.* (12) for potato,

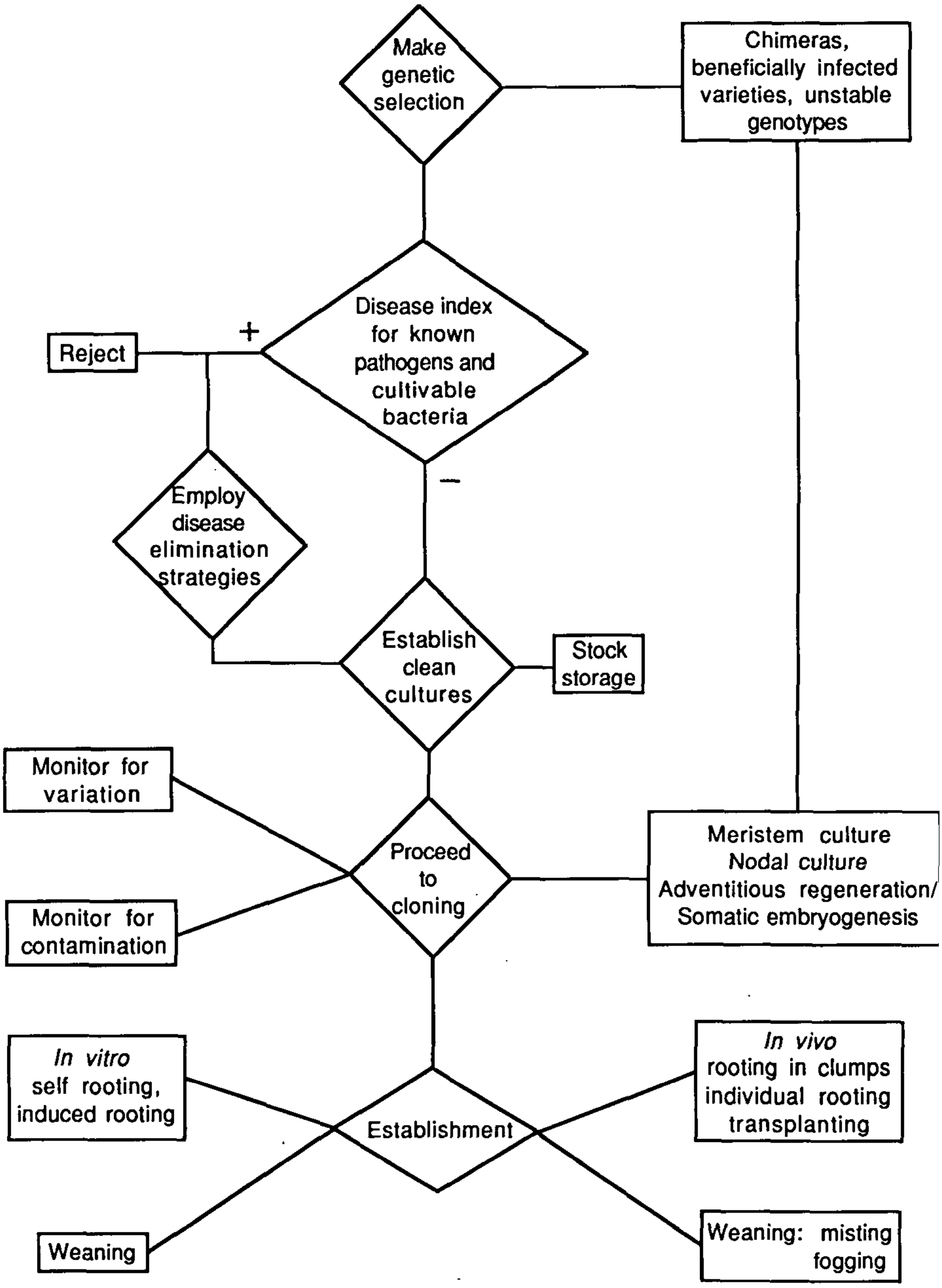

Figure 1. Factors influencing the production of good quality microplants.

viz, that not less than 10 vigorous uniform individuals be used to initiate clonal propagation. In addition, it is recommended here that the client/breeder and micropropagator should discuss the question of inherent stability for each cultivar entered for micropropagation. If a suspect cultivar is entered, special consideration should be given to the cloning strategy to be used (see below).

Disease indexing and contamination monitoring. To avoid clonal disease transmission and losses *in vitro* and on establishment, it is essential to clone clean ('axenic') cultures. To achieve this, rigorous screening procedures should be employed. Pragmatically, one should look for known pathogens of the crop—viruses, bacteria, and vascular fungi (12). One should also screen for cultivable bacteria, which include common endophytes, e.g. *Erwinia, Pseudomonas, Corynebacterium,* and *Xanthomonas,* and be aware that occasional exotic bacteria may also be found (4).

Screening techniques should be sensitive and non-strain specific for the known pathogens of the crop, e.g. ELISA and DNA probes. Culture indexing may be employed for the cultivable bacteria. Screening is an aspect fraught with potential problems and can only be covered by a "best endeavours" approach.

Management of clean cultures. Once clean, or preferably axenic, cultures have been obtained they are at risk of contamination in the laboratory. Sources of contamination are transferred from contaminated cultures or directly from the micropropagator. *Bacillus* and other heat-resistant, spore-forming bacteria are commonly encountered.

Two management approaches should be used. Firstly, sample cultures should be regularly culture-indexed during subculture for cultivable bacterial contaminants. Secondly, stock handling should be minimised by storage under slow growth conditions (16). Deep cold storage should be avoided for cultivars that are unstable in adventitious regeneration.

The cloning pathway. Plants may, at least in principle, be cloned by a number of pathways (Figure 1) that are grouped into two fundamentally different types—bud culture and adventitious regeneration. In the former, which includes bud tip, meristem and nodal culture, the structural organisation of the somatic layers is theoretically maintained. In the latter, buds arise *de novo* from single cells or groups of cells in one or more somatic layers (6).

Nodal culture, and meristem culture, if *via* precocious axillary bud proliferation and not *via* intervening callus, can be used to propagate "normal" and chimeral cultivars, giving true-to-type progeny with the *caveat* that in chimeras one genotype may be selected for preferentially, under *in vitro* pressures, resulting in increased instability. Meristem culture but not nodal culture may result in the elimination of beneficial infections (1).

The use of adventitious regeneration in complex explants or

callus cultures will result in chimeral breakdown and may result in high levels of genetic instability—somaclonal variation (14). The variation found may depend on the specific genotype being cloned, particularly in ornamental plants where, as in pelargoniums, polyploid and aneuploid genotypes may exist side by side in different cultivars (8).

Cultivars containing unstable loci may mutate at very high frequency *in vitro* and these should be handled with special consideration (5).

Production monitoring. Production should be regularly monitored for bacterial contaminants as discussed above. It is important in this regard to recognise that media constituents, e.g. salts, may inhibit bacterial growth and consequently visual examination may not be adequate. Consequently, losses of cultures to contamination may occur on transfer to reduced strength rooting media.

Monitoring production for variation must always be carried out for each new genotype entered into micropropagation to avoid risks. Two types of variation may be encountered in micropropagated plants—random and non-random (or directed) changes. Random variation may be anticipated in adventitious regenerants at relatively low frequency at around 1 to 10%, while directed change may occur at high frequency, occasionally up to 100 per cent, e.g. change in leaf shape in *Saintpaulia ionantha* 'Rose' (6).

The above are examples of changes in the genome which may or may not be heritable. Epigenetic (non-heritable) changes may also be expressed in the phenotype, the latter induced by the microenvironment and/or media factors and by the presence of contaminants in the cultures. The gaseous environment: O_2, CO_2, C_2H_4, and H_2O, interacting with the hormone concentration in the medium, may induce vitrification and/or apical necrosis (13). Both conditions affect multiplication rates and quality of growth. Apical necrosis may result in break of lower buds and uneven cultures and progeny.

Problems resulting from the microclimate may be controlled by provision of appropriate light intensity and quality and by control of the gaseous environment by the use, for example, of differentially permeable membranes as covers (3). The latter may also facilitate weaning (see below).

Finally, it should be recognised that there may be residual effects of the hormones on the performance of the established progeny (15).

Rooting and establishment. A number of strategies are used for rooting and to achieve the establishment of microplants (see Figure 1). Where induced rooting is employed, care should be taken to avoid influencing the root/shoot ratio in such a way as to alter the plant habit. In the case of plants to be used as stock for cuttings,

manipulation of the root/shoot ratio to achieve reduced apical dominance may result in more productive stock (11). This is an area which merits further research.

Self-rooting, or rooting in clumps, is also frequently used to reduce costs. The latter may result in irregular progeny requiring grading by the grower.

The issue of rooting aside, the establishment of microplants may be difficult because of poorly adapted photosynthetic apparatus and softness (subliminal vitrification) (9,10). The latter problem can be addressed by the provision of misting or fogging facilities (17), or by hardening the material *in vitro* by manipulation of the micro-environment, for example by the use of differentially permeable double-membraned containers (3) (Figure 2).

CONCLUSIONS

Mass clonal propagation via micropropagation depends on careful genotype (cultivar) selection. Inherently unstable genotypes may, on grounds of rarity etc., merit consideration but for these it is imperative that the appropriate cloning pathway be adopted and that the prospect of variation in the progeny be accepted. The breeder or grower has an important role in advising the micropropagator of potential risks due to instability. The micropropagator for his or her part should avoid unstable genotypes or issue a disclaimer.

The micropropagator has responsibility, unless exempted, to ensure the clean (axenic) status of mother cultures and to monitor production for contamination. Further, the micropropagator, should monitor production for clonal stability and should provide the appropriate microclimate and media to ensure quality growth on establishment.

Finally, if micropropagation is to increase its market share, production costs must be reduced. It is likely that this may be achieved eventually, for example by exploiting adventitious pathways of regeneration to produce artificial seed *via* somatic embryogenesis. It has been stressed here that this pathway carries the greatest inherent risks of variation in the progeny. Nursery operators should be alert during the "learning phase" of the risks associated with adventitious regeneration.

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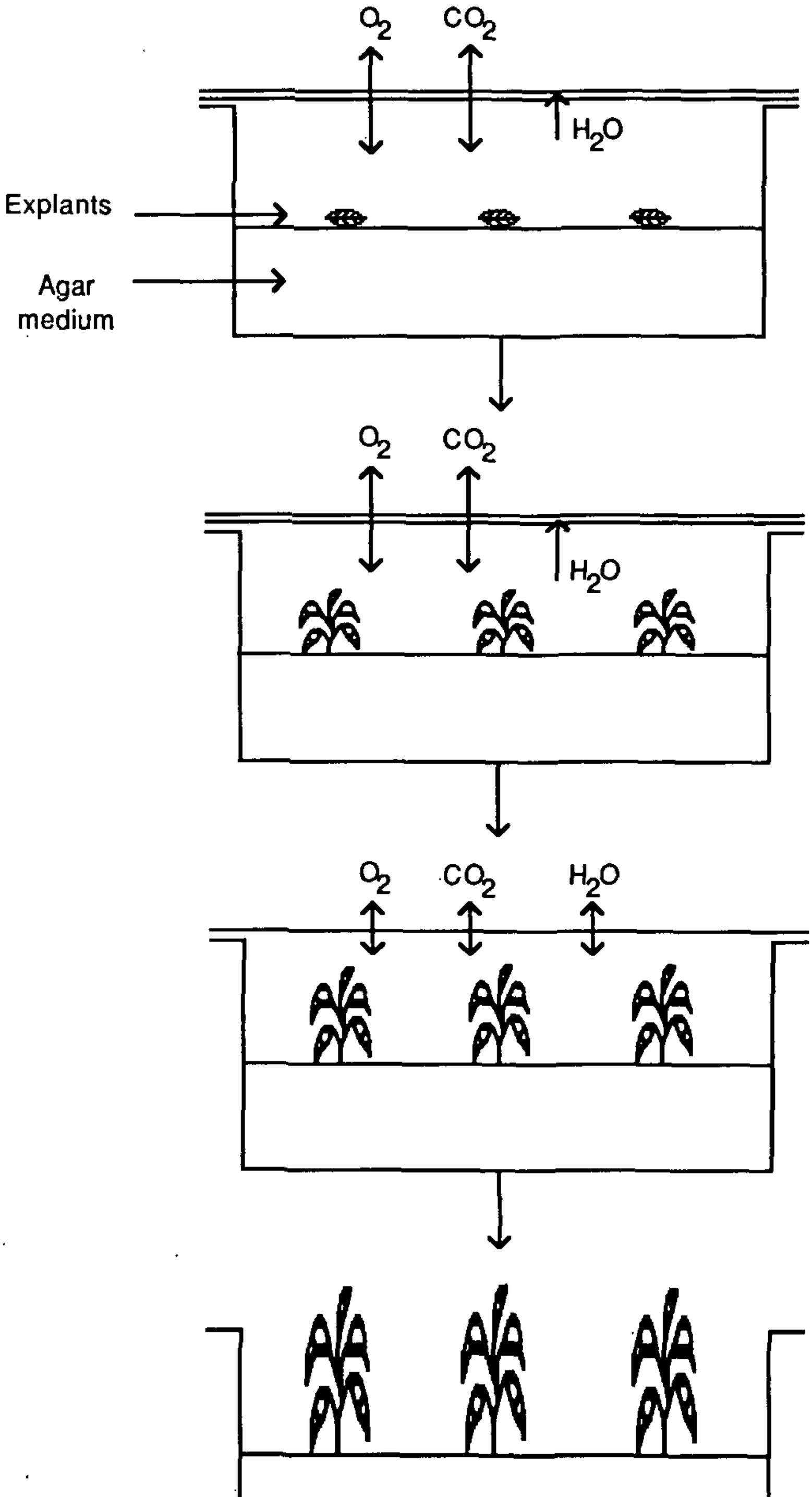

1. Implantation: free diffusion of O_2, CO_2, H_2O loss controlled.

2. Growth: diffusion as above.

3. Weaning: removal o outer membrane, increased H_2O diffusion.

4. Growing on

Figure 2. A double membrane patented system that allows control of the gaseous culture environment during microplant development and weaning (3).

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