Review

Plant response to alternative matrices for *in vitro* root induction

Gaurab Gangopadhyay, Subhash Kanti Roy and Kalyan K. Mukherjee*

Cytogenetics Laboratory, Department of Botany, Bose Institute, 93/1 APC Road, Kolkata – 700009, West Bengal, India.

The quest for alternative matrices for plant tissue culture is a continuing process. This inquiry has two aspects. One being commercial for low cost tissue culture, and the other obviously is the better root induction vis-à-vis higher percentage of survival, particularly with respect to micropropagation. The advantage of alternative matrices raises the concomitant question of plant intelligence in sensing its environment. The present review apart from summarizing the works with alternative matrices, attempts to raise some issues related to plant sensitivity, which remain unanswered due to lack of present understanding in this area of plant biology.

Key words: Alternative matrix, plant tissue culture, root induction.

INTRODUCTION

Plant's association with the soil dates back to the origin of land plants having early rhizoidal roots anchoring the substratum. It was indeed unknown to mankind that roots could perceive gravity (Perrin et al., 2005). Soil may be defined as a thin layer of earth's crust which serves as a natural medium for growth of plants. It is the unconsolidated mineral matter that has been subjected to, and influenced by, genetic and environmental factors-parent material, climate, organisms and topography all acting over a period of time. Soil differs from the parent material in the morphological, physical, chemical and biological properties. Also, soils differ among themselves in some or all the properties, depending on the differences in the genetic and environmental factors. Thus some soils are red, some are black; some are deep and some are shallow; some are coarse textured and some are fine-textured. They serve as a reservoir of nutrients and water for plants, provide mechanical anchorage and favorable tilth. The components of soil are minerals, organic matter, water and air, the proportions of which however, vary and all of them together form a system for plant growth (http://www.krishiworld.com/html/soils1.html).

Roots, the "hidden half" of plants, which remain underground in the soil, serve a multitude of functions. They are responsible for anchorage, supply the plants with water, nutrients, and exchange various growth substances with the shoots. Roots perform the basic functions in most ferns and in all seed plants, whereas additional traits (e.g. formation of storage organs, determination of the depth of the regenerating buds, or aeration of inundated organs) are characteristic of roots of exclusive groups of plants.

The root-soil interface is the site where the most interactions between the plants and their environment occur. Roots constitute a major source of organic material for the soil and thus affect its structure, aeration, and biological activities. While organic chemicals move out of the roots into the soil, inorganic ions move in: some of the entering materials are needed for normal metabolism of the plants and are actively sought. Others are not required but are either neutral or toxic. Insufficient or excessive accumulation of most elements would damage plants, and therefore, their uptake is controlled at the root surface (Waisel et al., 2002).

The search for suitable alternative of soil probably dates back to the time when plants or plant parts were first grown *in vitro*, that is, advent of the techniques of plant tissue culture. Plant tissue culture owes its origin to the ideas of the German scientist, Haberlandt, at the beginning of the 20th century. The early studies led to root cultures, embryo cultures, and the first true callus/tissue cultures. The period between the 1940s and the 1960s was marked by the development of new techniques and

---

*Corresponding author. E-mail: kalyan@bosemain.boseinst.ac.in

the improvement of those that were already in use. The 1990s saw continued expansion in the application of the *in vitro* technologies to an increasing number of plant species, which in recent times have culminated in re-search in plant transgenics (Thorpe, 2007).

The wide and almost universal application of agar powder, a mixture of polysaccharides derived from red algae as the gelling material in culture media for all the aforesaid branches of plant tissue culture is due to its fol-lowing advantage: high clarity, stability (after auto-claving), non toxic nature and resistance to alteration during culture (MacLachlan, 1985; Henderson and Kinnersley, 1988). However, wide spread use of agar in plant tissue culture media has certain limitations too in many plant systems, which in the long run affects plant survival after transfer to soil from culture vessels.

The present review attempts to address the short-comings of conventional agar-gelled media and highlight the use of alternative matrices in plant tissue culture, the trend of which has been initiated in recent times.

SHORTCOMINGS OF AGAR-GELLED MEDIA

As addressed briefly in previous section, agar is by far the best support system to keep plant cultures from being submerged in the medium. From the physical and chemical point of view it is convenient to work with agar since it melts at about 100ºC and solidify at about 45ºC. In addition, agar does not react with media components nor is it digested by plant enzymes.

On the other side, different brands of agar vary in their purity and gelling strength. Concentrations generally range between 0.5 and 1%. Furthermore, agar does not gel well under acidic conditions and thus may not be ap-propriate for situations in which a low pH (<4.5) is re-quired. The concentration of agar may be critical to plant growth in culture. Media that is too soft may produce hyperhydricity in tissue culture raised plants whereas media that is too hard may cause reduced plant growth (http://trc.ucdavis.edu/egsutter/PLB153/Lectures/media.pdf).

These shortcomings, however, become compounded later and manifested in the physiological status of the tissue culture raised plantlets, especially during the stages of rooting and hardening. The survival percentage during transition from test tube to soil is often become very limited since the *in vitro* raised plantlets in agar-gelled media are very seldom adapted readily to *in vivo* condition (Pierik, 1996). A closer look to the morpho-anatomical aspects of the tissue-culture raised plantlets in agar-gelled media in the following paragraphs will pro-bably give some insight about the cause of their poor survival when transplanted directly to soil.

Leaf and shoot system

The *in vitro* propagated plants possess thin, soft, photo-

synthetically hypoactive leaves having less epicuticular wax, trichomes and malfunctioned stomata resulting in excessive transpiration (Gangopadhyay et al., 2002). Water is the most important component of plants and in-efficient management of it may lead to poor rate of hard-ening and transplantation. High relative water content appears to be an important factor for both survival and growth of micropropagated plants after transplantation (Diaz-Perez et al., 1995a,b). Trichomes – their number and distribution also play one of the major roles of this management (Pereira-Netto et al., 1999; Perez-Estrada et al., 2000) in addition to cuticle and stomata. Scanning electron microscopy revealed precariously low abun-dance of trichomes in the adaxial surface of leaves of tissue culture raised plantlets within the glass vessels in both herbaceous as well as woody plant materials (Gangopadhyay et al., 2002). A similar type of work of Bandyopadhyay et al. (2004) in teak, a major timber-yielding tree of Indian sub continent, which is essentially a hardy plant, revealed efficient water economizing mechanism with predominance of diverse types of trichomes in the leaves of *in vivo* condition, the paucity of which in plants growing in agar-gelled media probably resulted in low rate of survival after field transplantation.

Malfunctioning of stomata of micropropagated plants after transplantation has been reported to cause rapid water loss resulting in low survival rate. This malfunc-tioning has primarily been attributed to the inability of sto-mata of such plants to close properly when those plants have been taken out from glass vessels (Santamaria and Kerstiens, 1994). Results of the work of Bandyopadhyay et al. (2004) indicated that the *in vitro* teak plants showed remarkably higher number of stomata than the *in vivo* ones and there was a   significant positive correlation bet-ween number of stomata and relative water content in the leaves of former plants. It was speculated that high rate of water loss from these large number of stomata which did not learn till then how to close at the sense of desic-cation outside the cultural environment led to low survival rate of such plants after transplantation.

The epidermal cell layer of the tender aerial parts of higher plants is covered by a cuticular membrane, which constitutes an effective barrier against desiccation. When plant stomata are closed or absent, gaseous exchanges between the plant and the atmosphere occur across the cuticular barrier. In addition to the loss of water by trans-piration, permeation into the plant of chemicals deposited on its surface is dependent on the transport properties of the cuticle. The cuticle consists of a polymer matrix of long-chain polyesters, the cutin, in which waxes are em-bedded and deposited on the external surface. Cuticular wax composition can be quite complex and varies greatly according to species, age, and environmental conditions (Carreto et al., 2002).

The physical and chemical properties of cuticular wax determine functions vital for plant life. It restricts non-stomatal water loss, protects plants against ultraviolet

radiation and reduces water retention on the surface of the plant thus minimizing deposition of dust, pollen and air pollutants (Kunst and Samuels, 2003). Interestingly enough, scanning electron microscopy revealed low to very low abundance of epicuticular wax in the abaxial surface of the leaves of *in vitro* raised banana plantlets in agar-gelled media, where the *in vivo* banana leaves are conspicuous in the presence of epicuticular wax (Gangopadhyay et al., 2002).

Some other epicuticular structures, unique for certain plants in normal condition were found strikingly lacking in the plantlets raised *in vitro* in agar-gelled media as indicated in the same study made by Gangopadhyay et al. (2002). *Chenopodium album*, a semi cultivated leafy vegetable, which grows throughout India during the winter of tropical climatic condition as a companion weed with the ravi crops (Gangopadhyay et al., 2002a) is such an example. The plantlets of *C. album* in agar-gelled media did not reveal much salt gland after SEM study, which was otherwise a notable feature in the adaxial surfaces of leaves of *in vivo* grown *C. album* (Gangopadhyay et al., 2002).

Hyperhydricity, also known as vitrification, is a morphological, anatomical and physiological malformation that makes the plant tissue water-swollen (Paques and Boxus, 1987), and it is a common observation in the shoot system of many *in vitro* raised plantlets growing in agar-gelled media. The outcome is an undesirable *in vitro* culture microenvironment caused by the accumulation of ethylene and other volatiles in the headspace of the culture vessel, a condition distinctly different from the natural environment (Zobayed et al., 1999).

Root system

It is a general observation that the step of transfer from tissue culture vessels to soil is often very difficult because the *in vitro* produced plants are not well adapted to an *in vivo* climate. Apart from the many adaptation problems of the leaf and shoot systems as discussed previously, the system of root regeneration *in vitro* in agar-gelled media appears to be one of the most vulnerable one. In many cases even negatively gravitropic roots appear in agar-gelled media within glass vessels. Furthermore, the *in vitro* formed roots do not function properly (fewer root hairs) *in vivo*, are rather weak, and often die; in soil, *in vitro* formed roots often have to be replaced by newly formed subterranean roots. As a consequence of the nonfunctional roots, transpiration outside the glass vessels is too high and can result in the loss of many plants. It has been suggested that to promote functional root formation, it is necessary to regenerate roots *in vivo* instead of *in vitro* since rooting of micropropagated shoots *in vivo* is usually more cost effective than rooting *in vitro* (Pierik, 1996).

ALTERNATIVES OF AGAR

The search for alternatives of agar began when the once sophisticated techniques of plant tissue culture confined only in impeccably maintained research laboratories started to be used by common mass in large scale commercial plant propagation on industrial basis. From that point the concept of cost effectiveness emanated and shedding of extra cost started from various angles. The cost of micropropagation is influenced by a number of factors. Though there is difference in opinion whether media costs really contribute a significant portion of the total cost (George, 1996) but refined agar powder generally used in plant tissue culture media, no doubt, is a costly commodity.

In lieu of agar, many plant based alternatives have been proposed since late 1980s. Henderson and Kinnersley (1988) first demonstrated that growth and differentiation of plant cell cultures was increased when media were gelled with corn starch instead of agar. The plant system(s) tested by the said workers, however, was tobacco and carrot, the all time favorite model materials of plant tissue culturists. The plant biotechnologists from Indian Institute of Technology, Kharagpur, India subsequently reported the efficacies of sago (from *Metroxylon sagu* Rottb.) and isubgol (from *Plantago ovata* Forsk.) as gelling agents in culture media of *Chrysanthemum*, a plant of floricultural interest. Isubgol continued to be an alternative of agar since it was also used effectively by Babbar and Jain (1998) in tissue culture media for *in vitro* seed germination, shoot formation and rooting in *Syzygium cuminii* and anther culture in *Datura innoxia*. The same group of workers (Babbar et al., 2005) successfully used guar gum, a galactomannan derived from the endosperms of *Cyamopsis tetragonoloba*, as the gelling agent for plant tissue culture media. Its suitability as a gelling agent was demonstrated by using guar gum-gelled media for *in vitro* seed germination of *Linum usitatissimum* and *Brassica juncea*, *in vitro* axillary shoot proliferation in nodal explants of *Crataeva nurvala*, rooting of regenerated shoots of the same, *in vitro* androgenesis in anther cultures of *Nicotiana tabacum*, and somatic embryo-genesis in callus cultures of *Calliandra tweedii*. Subsequent innovation was again made by the same group of workers (Jain and Babbar, 2006) when xanthan gum, a microbial desiccation-resistant polysaccharide prepared commercially by aerobic submerged fermentation from *Xanthomonas campestris*, was successfully used as a solidifying agent for plant tissue culture media. Its suitability as a substitute to agar was demonstrated for *in vitro* seed germination, caulogenesis and rhizogenesis of *Albizzia lebbeck*, androgenesis in anther cultures of *D. innoxia*, and somatic embryogenesis in callus cultures of *C. tweedii*.

Maintenance of cultures in liquid media is a common practice for many plant systems and has been found to be more advantageous than conventional agar-gelled media. Substrates other than agar, including sorbord plugs (Roberts et al., 1990; 1994), foam (Gebhardt, 1985; McComb and Newton, 1980; Roche et al., 1996), vermiculite (Rugini and Verma, 1982), vermiculite and gelrite

mixture (Jay-Allemand et al., 1992), peat (Gebhardt and Friedrich, 1987) and rockwool (Lin et al., 1995) have been used to overcome the problems of poor rooting in agar and to enhance survival on transfer to the glass-house. Filter paper bridges have been used for woody Australian plants *Grevillea rosemarinifolia* (Ben-Jaacov and Dax 1981) and *Banksia coccinea* (Sedgley 1996). Excellent root growth for *Trifolium subterraneum* has been reported by Barrett-Lennard and Dracup (1988) using porous agar. However, each of these supports has certain shortcomings. For example, roots grown on the filter paper matrix are often problematic to take out with-out imparting injury. Proper rooting space is often not available when glass beads are used. Inert synthetic sup-ports (Roberts et al., 1990; Pierik 1991a, b) though are very much effective but often are not cost effective.

In an excellently worked out and compiled PhD thesis, Newell (2006) presented a strong argument on the basis of example and thorough physiological studies that the suppressed rooting performance using agar-gelled rooting media is due to hypoxia-compromised physiology. Furthermore, the said thesis has elegantly demonstrated that plants rooted in IVS (*in vitro* soil less rooting) media acclimatize normally and the work has envisaged the opportunities to clonally propagate many plants pre-viously deemed to be recalcitrant to conventional plant tissue culture practices.

Coir and *luffa*-sponge as matrix in liquid media

Considering all the merits and shortcomings of the alter-natives of agar, the present group made an endeavor to use certain unconventional matrices derived from ligno-cellulosic fiber from different plant origins (Gangopadhyay et al., 2002). Initially three were tried – paddy straw, jute fiber and coir. Of these three, coir was selected as the most suitable matrix due to its unique moisture retention capacity for prolonged period. Coir belongs to the group of hard structural fibers. It is an important commercial product obtained from the husk (dried sclerenchymatous fiber) of the coconut, *Cocos nucifera*, a fibrous drupe and is composed mainly of lignin and cellulose (Thampan, 1989), consequently it is biodegradable. The unique moisture retention capacity of coir was reflected when not less than ten different plant materials (*Nicotiana tabacum*, *Andrographis paniculata*, hexaploid *C. album*, two diploid cytotypes of *C. album*, *C. murale*, *Beta vulgaris* (both table beet and sugar beet, *Tectona grandis* and *Musa* spp.) were grown on this matrix with very little amount of liquid culture media even for prolonged sub cultural duration (Gangopadhyay et al., 2002; Bandyopadhyay et al., 2004). In the aforesaid studies the approach was never to replace agar during the stage of culture initiation or establishment. Rather, for the later stages, viz. multi-plication and rooting, the use of liquid media with coir as solid support was found to be either comparable or better over agar-gelled media. That liquid media with coir result-

ed in better rate of multiplication of the propagules than agar-gelled media was evident in *Chenopodium*, *Beta* spp. and *Musa* spp, while the other plant materials studied showed comparable rate of multiplication. The scenario was totally different for the stage of rooting as 100% root induction was attained in liquid media with coir as solid support in each and every plant species tried. Moreover, the roots were normal, positively gravitropic, stout and most importantly with adequate root hair zones. This is of definite advantage than roots induced in agar-gelled media, which often do not function properly due to fewer root hairs (Pierik, 1996). The major drawbacks in the use of agar-gelled media in many plant species due to poor rate of transplantation have been addressed earlier in this review. Those shortcomings were definitely overcome in the plants grown in liquid media with coir. Scanning electron microscopic studies revealed ade-quate hairs (in *Nicotiana*), trichomes (in *Tectona*), surface structures like salt glands (in *Chenopodium*), epicuticular wax (in *Musa*) and well developed stomata on leaves of plants grown in liquid media with coir in comparison to those grown in agar-gelled media. The plants grown in liquid media with coir were nearly identical to *in vivo* ones in terms of development of epicuticular structures, which plausibly helped the plants during hardening and resulted to enhanced rate of successful transplantation. The use of coir as the matrix in liquid culture media was later suc-cessfully extended in *Pandanus amaryllifolius* (Gango-padhyay et al., 2004a) and also in *Gladiolus*, where enhancement of *in vitro* micro corm production was obtained (Roy et al., 2006), which seems to have definite commercial advantage for the propagation of this important floricultural crop.

Apart from coir, *Luffa* sponge, derived from dried fruits of *Luffa egyptica*, was yet another matrix envisaged to be perfectly suitable for this purpose since almost identical X- ray diffraction image of these two matrices were recorded, both of which are of lignocellulosic in nature (unpublished data). To test the efficacy of *Luffa* sponge, the response of aroid *Philodendron*, an exquisite indoor-ornamental with its beautiful leaves and tangled aerial roots, was studied *in vitro*. Philodendrons, like most of the aroids, are adored worldwide for beautifully decorated leaves. The clones produced thereof hence need to maintain their absolute genetic stability so that no unwanted variation in leaf morphology can creep in; and this was attained successfully using *Luffa* sponge as the matrix in liquid culture media (Gangopadhyay et al., 2004). The suita-bility of this unique system was later extended in micropropagation of pineapple, an immensely important horticultural plant, where almost 90% survival rate of the transplanted micropropagated plants was achieved using *Luffa* sponge as the alternative matrix (Gangopadhyay et al., 2005).

Conclusion

The extent to which micropropagation can be practiced

commercially is often being limited by production costs. The cost of micropropagation is influenced by a number of factors. Media costs, however, form only a small proportion of the total cost (George, 1996). Through the use of coir and *Luffa* sponge as the alternative matrices in liquid culture media it has been possible to further cut down certain load of media cost, at least at Stage III of micropropagation. The use of coir or *Luffa* sponge instead of agar during the stages of multiplication, rooting and hardening, not only saves the cost of adding costly gelling agents but also reduces the cost of washing and cleaning. Moreover, 'non-productive cultures' (George, 1996) can be maintained in minimal media with a support of coir or *Luffa* sponge for long period and this has the potentiality to save recurring expenditure. Chances of contamination are also reduced during maintenance of such type of cultures as subculturing takes place only in the form of addition of sterile liquid media. One important factor influencing the cost of micropropagation is how quickly plant numbers can be increased on demand. Although multiplication rates of productive cultures differ markedly from one kind of plant to other as has been observed by Gangopadhyay et al. (2002), but the overall rates of multiplication in coir or *Luffa* sponge with liquid media were either comparable or better than those grown in agar-gelled media. As the rooted plants with coir or *Luffa* sponge were transplanted by holding the matrices without touching the root (the coir or *Luffa* sponge being biodegradable, get mixed with soil in course of time), the rate of survival after transplantation reached almost 100%. High percentage of survival was due to concomitant hardening along with rooting and no damage to the root system. Finally, it can be concluded that the *in vitro* plants grown in aseptic liquid media with alternative matrices have following distinct advantages over the plants grown in agar-gelled media: Large number of positively gravitropic roots with profuse root hairs and non vitrified near normal leaves with well developed epicuticular structures/wax resembling the leaves of *in vivo* plants resulting in high rate of successful transplanttation. These factors probably indicate the potentialities of using coir or *Luffa* sponge as the alternative matrix along with liquid media for economizing micropropagation.

However, it remains to be seen that the use of coir or *Luffa* sponge along with the liquid media, which induces root induction with simultaneous hardening is a purely physical mechanism through which roots grow freely with the alternative matrix or there exists a physiological signal transduction chain operating in the presence of these alternative matrices in the liquid medium or both physical and physiological phenomenon are involved in this process.

The main constrain of studying root compared to other plant parts is obvious. Although extensive work for understanding root development to root genetics is being carried out by a number of scientists (Waisel et al., 2002) the present review admittedly is focused on a point that a root can sense its environment and thereby make its own development. Recent work with root indicates that it can sense humidity gradients and thus also construct a three-dimensional environmental perspective (Takahasi and Scott, 1993); similar ability in environmental perception has been shown by Drew et al. (1973). Bose (1924) used continuous recording to report that the behavior of petioles, roots, styles and leaflets of *Mimosa* to thermal, mechanical and light stimuli often oscillated in their approach to a new state of growth. The response of plants to environmental stimuli is termed as tropism (Gilroy, 2008). Response due to touch is known as thigmotropism. Root on growing in the soil, alternative matrices like coir, *Luffa* sponge or agar gel are in touch with the matrices and sense by the plant receptors, which leads to transduction of primary signal and results into conesquent integrated physiological responses. The primary receptor to touch is not yet identified (Braam, 2004), but J. C. Bose worked extensively using *Mimosa pudica* on this line during early 1900. It is now known that intracellular Ca^{2+}, the second most important messenger along with K^+ and Cl^- play most important role in signal transduction in plants and it is their dynamic redistribution that gives rise to action potential or variation potential traveling over large cellular distance from root to leaf and up the stem (Grams et al., 2007) to evoke growth response. Undoubtedly we need much more information before any conclusive statement but our experimental observation is very tempting to conclude that roots can perceive matrix, which perhaps is in the line of thought of a number of scientists working in the area of studying plant intelligence and has been beautifully reviewed by Trewavas (2003).

REFERENCES

Babbar SB, Jain N (1998). Isubgol as an alternative gelling agent in plant tissue culture media. Plant Cell Rep., 17: 318-322.

Babbar SB, Jain R, Walia N (2005). Guar gum as a gelling agent for plant tissue culture media. In Vitro Cellular Developmental Biol. Plant, 41: 258-261.

Bandyopadhyay T, Gangopadhyay G, Poddar R, Mukherjee KK (2004). Trichomes: their diversity, distribution and density in acclimatization of teak (*Tectona grandis* L.) plants grown *in vitro*. Plant Cell Tissue Organ Cult., 78: 113-121.

Barrett-Lennard EG, Dracup M (1988). A porous agar medium for improving the growth of plants under sterile conditions. Plant Soil 108: 204-298.

Ben-Jaacov J, Dax E (1981). *In vitro* propagation of *Grevillea rosmarinifolia*. Hort. Sci. 16: 309-310.

Bose JC (1924). Plant response as a means of physiological investigation. London: Longmans

Braam J (2004). In touch: Plant response to mechanical stimuli. New Phytol. 165: 373-389.

Carreto L, Almeida AR, Fernandes AC, Vaz WLC (2002). Thermotropic Mesomorphism of a Model System for the Plant Epicuticular Wax Layer. Biophysical J. 82: 530-540.

Diaz Perez JC, Shackel KA, Sutter EG (1995a). Relative water content and water potential of tissue-cultured apple shoots under water deficits. J. Exp. Bot. 46: 111-118.

Diaz Perez JC, Sutter EG, Shackel KA (1995b). Acclimatization and subsequent gas exchange, water relations, survival and growth of micro cultured apple plantlets after transplanting them in soil. Physiol. Plant. 95: 225-232.

Drew MC, Saker LR, Ashley TW (1973). Nutrient supply and growth of seminal root system. J. Exp. Bot. 24: 1189-1202.

Gangopadhyay G, Das S, Mitra SK, Poddar R, Modak BK, Mukherjee KK (2002). Enhanced rate of multiplication and rooting through the use of coir in aseptic liquid culture media. Plant Cell Tissue Organ Cult. 68: 301-310.

Gangopadhyay G, Das S, Mukherjee KK (2002a). Speciation in *Chenopodium* in West Bengal, India. Genet. Res. Crop Evol., 49: 503-510.

Gangopadhyay G, Bandyopadhyay T, Basu Gangopadhyay S, Mukherjee KK (2004). *Luffa* sponge – a unique matrix for tissue culture of *Philodendron*. Curr. Sci. 86 (2): 315-319.

Gangopadhyay G, Bandyopadhyay T, Modak BK, Wongpornchai S, Mukherjee K (2004a). Micropropagation of Indian pandan (*Pandanus amaryllifolius* Roxb.), a rich source of 2-acetyl-1-pyrroline. Curr. Sci. 87 (11): 1589-1592.

Gangopadhyay G, Bandyopadhyay T, Poddar R, Basu Gangopadhyay S, Mukherjee KK (2005). Encapsulation of pineapple micro shoots in alginate beads for temporary storage. Curr. Sci., 88 (6): 972-977.

Gebhardt K (1985). Development of a sterile cultivation system for rooting of shoot tip cultures (Red Rasberries) in duroplast foam. Plant Sci. 39: 141-148.

Gebhardt K, Friedrich M (1987). Micropropagation of *Calluna vulgaris* cv., Beale HE. Plant Cell Tissue Organ Cult. 9: 137-145.

George EF (1996). In: Plant Propagation by Tissue Culture part 2, In practice Exegetics Limited. pp. 761-786.

Gilroy S (2008). Plant tropism. Curr. Biol. 18: 275-277.

Grams TE, Koziolek C, Lautner S, Matyssak R, Fromm J (2007). Distinct role of elastic and hydraulic signals on the reaction of leaf gas exchange upon re irrigation of *Zea mays*. Plant Cell Environ. 30: 79-84.

Henderson WE, Kinnersley AM (1988). Corn starch as an alternative gelling agent for plant tissue culture. Plant Cell Tissue Organ Cult., 15: 17-22.

Jain R, Babbar SB (2006). Xanthan gum: An economical substitute for agar in plant tissue culture media. Plant Cell Rep., 25: 81-84.

Jay-Allemand C, Capelli P, Cornu D (1992). Root development of in vitro hybrid walnut microcuttings in a vermiculite-containing gelrite medium. Sci. Hort. 51: 335-342.

Kunst L, Samuels AL (2003). Biosynthesis and secretion of plant cuticular wax. Progr. Lipid Res. 42: 51-80.

Lin X, Bergann BA, Stomp A (1995). Effect of medium physical support, shoot length and genotype on in vitro rooting and plantlet morphology of Sweetgum. J. Environ. Hort. 13: 117-121.

MacLachlan J (1985). Macroalgae (Seaweeds): Industrial sources and their utilizations. Plant Soil, 89: 137-157.

McComb JA, Newton S (1980). Propagation of Kangaroo Paw using tissue culture. J. Horticult. Sci. 56: 181-183.

Newell CJ (2006). In vitro soil-less (IVS) rooting medium. Ph.D. thesis, Murdoch University, Australia.

Paques M, Boxus P (1987). Vitrification: Review of literature. Acta Hort. 212: 155-166.

Pereira-Netto ABD, Gabriele AC, Pinto HS (1999). Aspects of leaf anatomy of Kudzu (Pueraria lobata, Leguminosae Faboideae) related to water and energy balance. Pesq Agropec Bras, Brasĺlia 34: 1361-1365.

Perez-Estrada LB, Cano-Santana Z, Oyama K (2000). Variation in leaf trichomes of Wigandia urens: environmental factors and physiological consequences. Tree Physiol. 20: 629-632.

Perrin RM, Young LS, Murthy UMN, Harrison BR, Wang Y, Will JL, Masson PH (2005). Gravity signal transduction in primary roots. Ann. Bot. 96: 737-743.

Pierik RLM (1991a). Micropropagation of commercial plants. Acta. Hortic. 289: 45-53.

Pierik RLM (1991b). Commercial aspects of micropropagation. In: Prakash J & Pierik RLM (eds.) New Technologies and Applications Kluwer Academic Publishers, Dordrecht, The Netherlands. Horticult. pp. 141-153.

Pierik RLM (1996). Micropropagation: Technology and Opportunities. In: Prakash J & Pierik RLM (eds) Plant Biotechnology Commercial Prospects and problems, Oxford IBH New Delhi Bombay Calcutta India. pp. 9-22.

Roberts AV, Smith EF, Mottley J (1990). The preparation of micropropagated plantlets for transfer to soil without acclimatization. (Eds Pollard JW, Walker JM). The Humana Press, New Jersey, In: Methods in Molecular Biology, Vol. 6, Plant Cell Tissue Culture, pp. 314-322.

Roberts AV, Smith EF, Horan I, Walker S, Matthews D, Mottley J (1994). Stage III techniques for improving water relations and autotrophy in micropropagated plants. In Physiology, Growth and Development of Plants in Culture. (Eds Lumsden PJ, Nicholas JR, Davies WJ) Klewer Academic Publishers, Netherlands. pp. 314-322.

Roche TD, Long RD, Sayegh AJ, Hennerty MJ (1996). Commercial scale photoautotrophic micropropagation applications in Irish agriculture, horticulture and forestry. Acta Hortic. 440: 515-520.

Roy SK, Gangopadhyay G, Bandyopadhyay T, Modak BK, Datta S, Mukherjee KK (2006). Enhancement of in vitro micro corm production in Gladiolus using alternative matrix. African J. Biotech. 5 (12): 1204-1209.

Rugini E, Verma DC (1982). Micropropagation of difficult to propagate almond (Prunus amygdalus Batsch) cultivar. Plant Sc. Lett. 28: 273-281.

Santamaria JM, Kerstiens G (1994). The lack of control of water loss in micropropagated plants is not related to poor cuticle development. Physiol. Plant 91: 191-195.

Sedgley M (1996). *Banksia*, Family Proteaceae. In Native Australian Plants Horticulture and Uses. (Eds Johnson KJ, Burchetts M) University of New South Wales Press, Australia. pp. 18-35.

Takahasi H, Scott TK (1993). Intensity of hydro stimulation for the induction of root hydrotropism and its sensing by the root cap. Plant, Cell Environ.16: 99-103.

Thampan PK (1989). Handbook on coconut palm Oxford IBH New Delhi Bombay Calcutta India. pp. 1-311.

Thorpe TA (2007). History of plant tissue culture. Mol. Biotechnol. 37: 169-180.

Trewavas A (2003). Aspects of plant intelligence. Ann. Bot. 92: 1-20.

Waisel Y, Eshel A, Kafkafi U (2002). Plant Roots. The Hidden Half (3rd Edition). Marcel, Dekker, Inc. New York Basel.

Zobayed SMA, Armstrong J, Armstrong W (1999). Cauliflower shoot culture: effect of different types of ventilation on growth and physiology. Plant Sci. 141: 209-217.