Plant Cell Monogr (2)
A. Mujib · J. Šamaj: Somatic Embryogenesis
DOI 10.1007/7089_035/Published online: 2 December 2005

Environmental Design Considerations for Somatic Embryogenesis

Takanori Hoshino (✉) · Joel L. Cuello

Department of Agricultural and Biosystems Engineering, The University of Arizona, 507 Shantz Building, Tucson, AZ 85721-0038, USA
takanori@email.arizona.edu, cuelloj@email.arizona.edu

Abstract In addition to the biomolecular, physiological, and biochemical aspects of somatic embryogenesis, careful design of environmental conditions is necessary to ensure the successful induction and development of somatic embryos for different plant species. A dissolved oxygen concentration, for instance, below 10% generally inhibits the differentiation of somatic embryos, while the same is promoted at 40, 80, or 100%, depending on the plant species. Certain plant species also exhibit inhibition of somatic embryo differentiation at high dissolved oxygen concentration, such as at 80%. Cell density influences somatic embryogenesis by changing the concentrations of conditioning factors released by plant cells and embryos into the culture medium. High initial cell density, in general, results in inhibition of somatic embryo differentiation on account of inhibitory compounds released by cells into the culture medium. Partial medium replacement has been employed to rectify this situation. In terms of the general influence of light, red light promotes and blue light inhibits the induction of somatic embryos. Blue light, however, generally promotes the development of somatic embryos.

1 Introduction

Investigation of the various critical aspects of somatic embryogenesis is necessary in order to establish protocols for the successful induction and development of somatic embryos for different plant species. Recent studies, for instance, have focused on the biomolecular (Takahara et al. 2004), physiological (Godbole et al. 2004; Konradova et al. 2002), and biochemical (Sharma et al. 2004; Ramarosandratana and Stade 2004) aspects of somatic embryogenesis. The environmental factors that interact with and influence somatic embryogenesis constitute another critical aspect that needs careful consideration. This is especially true since it is a given that certain environmental factors need to be controlled and regulated for the important practical applications of somatic embryogenesis, i.e., artificial seed technology and automated plant mass production using bioreactors (Onishi et al. 1994).

Only a handful of studies, however, have addressed the optimization of specific environmental factors for somatic embryogenesis. Examples include those that investigated the effects on somatic embryogenesis of cell density

and conditioning factors (CFs; Bellincampi and Morpurgo 1987, 1989; Vries et al. 1988; Osuga and Komamine 1994; Higashi et al. 1998), dissolved oxygen (DO) concentration (Kessell and Carr 1972; Jay et al. 1992; Archambault et al. 1994; Shimazu and Kurata 1997), medium pH (Hofmann et al. 2004), nutrient and plant hormone composition in the medium (Jimenez 2001), as well as humidity (Meskaoui and Tremblay 1999; Bomal and Tremblay 1999). This chapter underscores the effects on somatic embryogenesis of three critical environmental factors: (1) DO concentration; (2) cell density; and (3) light quality and intensity.

2 Dissolved Oxygen Concentration

The effects of DO concentration on somatic embryogenesis are mainly twofold: influencing the biomass of undifferentiated cells; and influencing the development or differentiation of somatic embryos. Archambault et al. (1994) reported that the biomass (0.7–9.7 g of dry weight per liter) of undifferentiated cells of transformed California poppy (*Eschscholtzia californica*) at high DO (60% of air saturation) exceeded that of the control (0.2–10 g of dry weight per liter) at a DO of 20%. By contrast, a low DO (5–10%) yielded a lower biomass (0.2–3.3 g of dry weight per liter) compared with that of the control. Jay et al. (1992) reported that the stationary phase of the dry-mass curve of the undifferentiated cells of carrot (*Daucus carota* L.) occurred after 10 days of culturing for 100% DO, while that for 10% DO occurred with a 3-day delay. There was no significant difference in the final dry mass, approximately 4.5 g of dry weight per liter, for 100 and 10% DO levels. Jay et al. (1992) concluded that the results had a nutritional basis. They showed that while glucose uptake commenced after 4 days of culturing for 100% DO, glucose uptake started after 6 days of culturing for 10% DO. Also, complete consumption of glucose (defined as less than 2 g L^{-1}) in the medium occurred on day 10 for 100% DO, while it took another 3 days (on day 13) for 10% DO for the glucose to be completely consumed. The foregoing results indicated that high DO concentration generally resulted in higher biomass of undifferentiated cells. It should be noted, however, that inhibitory effects at 40% relative oxygen partial pressure in bioreactors were observed by Hohe et al. (1999) on cell proliferation of florist cyclamen (*Cyclamen persicum*) relative to the effects at 20 and 30%. A reduction of up to two thirds in yield in a packed cell volume and a decrease of more than 50% in growth rate in one genotype were observed.

In terms of the effects of DO on the development or differentiation of somatic embryos from embryonic callus cells, Kessell and Carr (1972) reported that lower than 16% DO was quite detrimental to the production of carrot somatic embryos. Jay et al. (1992) reported that carrot somatic-embryo pro-

duction was inhibited by approximately 75% at 10% DO compared with that at 100% DO. They also found that the DO level supplied during cell proliferation did not affect cell differentiation. Archambault et al. (1994) reported that cell differentiation of transformed *E. californica* cells was slow at 60% DO and was inhibited at low (5–10%) DO. Feria et al. (2003) also reported that the total number of somatic embryos that were induced from embryogenic cells of coffee (*Coffea arabica* cv.) was greater (71 072 somatic embryos per liter) at 80% DO than that (36 941 somatic embryos per liter) at 50% DO. Meanwhile, Shimazu and Kurata (1999) reported that the total number of somatic embryos that differentiated from carrot embryogenic cells was not affected at 4–40% DO. They also found that the development of carrot somatic embryos into the torpedo-shaped or heart-shaped stage was enhanced at 20–40% DO, while the same was completely inhibited at less than 7% DO. Also, they found that increasing DO from 4 to 7% increased the sugar consumption by the somatic embryos. By contrast, no significant difference in sugar consumption was observed when DO was varied from 20 to40%. Feria et al. (2003) reported that the development of coffee somatic embryos into the torpedo-shaped stage was enhanced at 50% DO, and was inhibited at 80% DO. Thus, different levels of DO were required to enhance torpedo-shaped differentiation.

3
Cell Density

The predominant effects of cell density on somatic embryogenesis appear to be indirect, rather than direct. The adjustment of cell density influences somatic embryogenesis through the following: (1) change in the concentrations of the CFs which plant cells and embryos release into the culture medium; (2) change in the amount of nutrients or gas which individual plant cells or embryos can consume; and (3) physical stress caused by increasing the physical contact among plant cells and embryos when cell density is increased. In studies that investigated the effects of cell density on somatic embryogenesis, it was established that the change in the concentrations of the CFs which plant cells and embryos release into the culture medium was the most significant aspect of manipulating cell density (Osuga and Komamine 1994; Osuga et al. 1993, 1997; Bellincampi and Morpurgo 1987, 1989; Higashi et al. 1998).

Bellincampi and Morpurgo (1987, 1989) investigated the effects of CFs released from plant cells of carrot (*D. carota* L.) into cell suspension culture medium, and determined that at least two different CFs were released from carrot cells into the culture medium. In the first study (Bellincampi and Morpurgo 1987), they concluded that (1) the first CF increased growth by cell division activity, and significantly enhanced the plating efficiency (defined as the ratio of the number of proliferating colonies to the number of initial plat-

ing units) of carrot cells, (2) the CF was physically and chemically very stable, being resistant to boiling and to acid or alkaline pH, and was strongly hydrophilic, and (3) the CF had a low molecular mass of 700 Da. Their results also suggested the species-unspecificity of the CF.

In the second study (Bellincampi and Morpurgo 1989), evidence was provided for the presence of a second growth-stimulating CF. But while the plating efficiency as influenced by the first CF was completely dependent on the initial cell density, the plating efficiencies as influenced by the second CF after 20 days of growth remained very similar for different initial cell densities. They suggested that the second CF had relatively low hydrophilicity and, thus, diffused slowly, and might have also been unstable.

Sung and Okimoto (1981) explored the relationship between cell density and embryo differentiation of carrot (*D. carota* L.). In their study, they found that a globular embryo was induced even under low concentrations of exogenous auxin (in this case 2,4-dichlorophenoxyacetic acid) at low cell density (2×10^4 cells mL^{-1}). Differentiation into torpedo-shaped embryos, however, was completely inhibited under that condition. By contrast, the differentiation of somatic embryos was strongly inhibited at high initial cell density (4×10^6 cells mL^{-1}). This fact indicated that an inhibitory CF was released from carrot cells during cell proliferation, and differentiation of somatic embryos was repressed when a high concentration of inhibitory CF was brought about by high cell density. This result was also supported by the results obtained by Fridborg and Eriksson (1975). They found that the differentiation of carrot somatic embryos was stimulated by the addition of activated charcoal, and that the differentiation was observed even in the presence of 1 mg L^{-1} α-naphthalene acetic acid, which would typically inhibit differentiation. They suggested that inhibitory compounds were removed by the activated charcoal.

Osuga et al. (1993, 1994) reported that cell density did not affect the development of carrot embryogenic cell clusters into globular or heart-shaped embryos. They also found that the total numbers of somatic embryos obtained at different initial cell densities were statistically similar when initial cell densities ranged from 0.5 to 2.0×10^3 cell clusters per milliliter. No torpedo-shaped embryo formation, however, was observed when the cell density exceeded 1.0×10^3 cell clusters per milliliter. Previous studies reported that the rate of somatic embryo development was enhanced when cell density was high (Halperin 1967; Hari 1980). Osuga (1993, 1994) concluded in his study, however, that such enhancement at high cell density was caused by stimulation of growth of single cells (or very small cell clusters) into embryogenic cell clusters by cell division. This conclusion agreed with the results obtained by Bellincampi and Morpurgo (1987, 1989).

Osuga et al. (1997) also found an enhancement in the development of embryogenic carrot cell clusters into globular embryos at high cell density with partial replacement of the medium. They also confirmed that this was not caused by either physical stress or the enrichment of nutrients by replacement

of the medium. They found that a greater number of globular embryos was obtained with partial medium replacement compared with entire medium replacement. Thus, they concluded that both inhibitory CF and promotive CF were released during cell proliferation. The results of Higashi et al. (1998) supported the inhibitory effects of high cell density (defined as greater than 1.0-mL packed cell volume per liter of medium). They found that the inhibitory effects caused by physical stress and by the change in the amount of available nutrients were not as critical as the negative effects of the inhibitory CF, which was released during cell proliferation and had a molecular mass of less than 3500 Da. Interestingly, Osuga et al. (1993, 1994) also reported that when globular embryos were cultured at different embryo densities, their results showed that the rate of torpedo-shaped embryo formation decreased linearly as embryo density increased from approximately 100 embryos per milliliter to 500 embryos per milliliter.

4
Light Quality and Intensity

That light affects somatic embryogenesis has been known for over 30 years through the pioneering studies by Ammirato and Steward (1971) on the effects of light on the growth of somatic embryos of hemlock water-parsnip (*Sium suave*) cells and by Halperin (1966) and Ammirato and Steward (1971) on the effects of light on the morphological characteristics of carrot somatic embryos. Of the critical environmental factors, however, light is the one whose effects on somatic embryogenesis have been the least investigated. Indeed, there is a paucity of published literature on the subject. What is more, three major issues make it difficult to analyze the specific effects of light quality and intensity on somatic embryogenesis in existing literature. These include (1) the different definitions of light quality used in the available studies, (2) the problematic spectral noises generated by the conventional experimental lighting systems, consisting of fluorescent tubes and light filters, used in such studies, and (3) the different light intensities applied to embryogenesis, which makes difficult the isolation of the morphological effects from the photosynthetic effects. Further studies are clearly needed to analyze and determine the specific effects of light environments on somatic embryogenesis.

Micheler and Lineberger (1987) explored the effects of light quality on carrot somatic embryos by examining the effects of four blue light (480 ± 100 nm), green light (540 ± 50 nm), red light (660 ± 70 nm), and white light, with light intensities ranging from 5 to 50 $\mu mol\, m^{-2}\, s^{-1}$. When cell cultures were exposed under red or green light, a similar number of somatic embryos, approximately 9000 embryos per milliliter, was obtained after 14 days of culturing. By contrast, significant inhibitions were observed under blue

light, resulting in approximately 3000 embryos per milliliter, and especially under white light, fewer than 2000 embryos per milliliter. Also, they showed that the effects of red light and green light did not change with different light intensities, while the negative effects of blue light and white light increased as the light intensity rose from 5 to 30 μmol m^{-2} s^{-1}. Indeed, even low blue light intensity resulted in 25% fewer embryos than in the dark control. Blue light, however, was observed to enhance the differentiation of globular embryos into torpedo-shaped embryos. After 16 days of culturing, 76% of somatic embryos developed into torpedo-shaped embryos under blue light, while only 6 and 18% did in the dark control and red light treatment, respectively. All somatic embryos induced under various light treatments, however, showed significant morphological changes with respect to the somatic embryos grown in the dark. These include the following: leafy cotyledons that were not observed in the dark control, but were observed in more than 80% of somatic embryos in all the light treatments; abnormal somatic embryos with multiple cotyledons under red light treatment and in the dark control (more than 7% in red light and in the dark, while less than 5% in other treatments); orange-pigmented radicles under red light (71% in red light and 0% in other light), while branched radicles were produced under white and blue light (67 and 49% in white light and blue light, respectively, and 0% in other light); and elongated hypocotyls under blue light (88% in blue light, while less than 10% in other light). Similar morphological changes, such as enhanced development of leaves, cotyledons and roots, under light treatments were reported by Ammirato and Steward (1971).

D'Onofrio et al. (1998) investigated the effects of blue light (450 ± 60 nm), red light (670 ± 50 nm), far-red light (> 700 nm), white light, and various combinations of these light qualities on somatic embryogenesis of quince (*Sidonia* sp.) leaves. They reported positive and negative effects of red light and blue light, respectively, on the differentiation of somatic embryos, with more than 0.4 embryos per leaf observed under red light, and fewer than 0.1 embryos per leaf observed in the dark or under blue light. They further correlated the rate of somatic embryo differentiation with photoequilibrium. Photoequilibrium, which is the fraction of physiologically active phytochrome to the total phytochrome, was calculated based on the theory suggested by Mancinelli (1995). The results showed that the ratio of the leaves with embryos was increased exponentially from 0% to approximately 30% as photoequilibrium increased from 0 to 1. Thus, phytocrome activation for somatic embryo induction was suggested. In addition, the blue light treatment resulted in less than half the number of embryo-producing leaves than those exposed to red light plus far-red light even though both treatments had the same photoequilibrium value of 0.43. Since the inhibition occurred at a low photoequilibrium, it implied that less phytochrome was activated. Thus, an interactive mechanism involving phytochrome and a blue-absorbing photoreceptor that caused negative effects on somatic em-

bryo induction was suggested. Similar promotive and inhibitory effects due to the amount of activated phytochrome by red light and far-red light were reported for cruel plant (*Araujia sericifera* L.) somatic embryos by Torne et al. (2001).

Bach and Krol (2001) reported the effects of various light qualities on Hyacinth (*Hyacinthus orientalis* L.) somatic embryogenesis, focusing on callus proliferation and development of somatic embryos. Greater callus proliferation, expressed as "medium rate or strong reaction of proliferation", was obtained under both red light (647–770 nm, 20 μmol $m^{-2}s^{-1}$) and dark similarly. By contrast, strong inhibition was observed, expressed as "medium or low rate of, or no proliferation", under blue light (450–492 nm, 60 μmol $m^{-2}s^{-1}$) and especially white light (390–770 nm, 60 μmol $m^{-2}s^{-1}$). At the same time, however, greater numbers of developed somatic embryos were observed under blue light. Moreover, when 5.0 μM BAP (6-benzylaminopurine) and 0.5 μM NAA (α-naphtalene acetic acid) was added to the culture medium, the greatest number of somatic embryos, 6–10 embryos per one callus clump, was obtained, compared to only 1–2 embryos per one callus clump was obtained in other treatments. A change in chlorophyll content during both cell proliferation and somatic embryo development was observed under blue and white lights. Indeed, the total amounts of chlorophyll under blue (20.62 mg per 100 g embryo) and white light (18.90 mg per 100 g embryo) treatments exceeded by 3 and 40 times those under red light (6.12 mg per 100 g embryo) and darkness (0.48 mg per 100 g embryo), respectively, when 5.0 μM BAP and 0.5 μM NAA was added to the culture medium.

Latkowska et al. (2000) investigated the effects on somatic embryogenesis of three different genotypes of Norway spruce of red light (670 ± 50 nm) and blue light (450 ± 60 nm) supplied at 30 μmol m^{-2} s^{-1} for 18 h per day. The cell growth of one genotype was inhibited under red light (38% of control) and especially under blue light (10% of control). Such effects, however, were moderated (85 and 65% of control under red light and blue light, respectively) in the case of a second genotype, and were not observed at all with the third genotype. The results indicated that the effects of light quality vary significantly depending on the species or cultivars. Kvaalen and Appelgren (1999) reported higher sensitivity to various light qualities of somatic embryos and seedlings derived from somatic embryos of Norway spruce (*Picea abies* L.) compared with that for seedlings derived from natural seeds. Germination was promoted (98%) and inhibited (50%) when somatic embryos were exposed under red light (670 ± 50 nm) and blue light (450 ± 80 nm), respectively. By contrast, no effect on germination was observed when natural seeds were exposed under various light qualities.

Addressing the previously mentioned three major issues that make it challenging to analyze the specific effects of light quality and intensity on somatic embryogenesis, Takanori and Cuello (2005) determined and optimized the effects of radiation quality and intensity on the induction and development

of somatic embryos from carrot (*D. carota*) embryogenic calli using light-emitting diodes (LEDs), which emit precise narrow-waveband radiation. The specific objectives of their study were as follows: (1) to determine the effects of red light and blue light up to 20 $\mu mol\, m^{-2}\, s^{-1}$ emitted from LEDs on the induction of somatic embryos from carrot embryogenic calli and on the resulting distribution of the embryos among the globular, heart-shaped, torpedo-shaped and cotyledonary stages; and (2) to determine the effects of red light and blue light up to 20 $\mu mol\, m^{-2}\, s^{-1}$ on the development of somatic embryos from carrot embryogenic calli by calculating the developmental coefficients of the somatic embryos.

Their results after 14 days of exposure pertaining to somatic embryo induction showed that (1) red radiation at 10 $\mu mol\, m^{-2}\, s^{-1}$ significantly increased the density of total somatic embryos induced from carrot embryogenic calli, (2) lower and higher intensities of red radiation (1–5 and 20 $\mu mol\, m^{-2}\, s^{-1}$) did not significantly influence the density of induced total somatic embryos, and (3) increasing the intensity of blue radiation (up to 20 $\mu mol\, m^{-2}\, s^{-1}$) appeared to have reduced the density of induced total somatic embryos. In regard to somatic embryo development, the results showed that (1) red radiation (up to 20 $\mu mol\, m^{-2}\, s^{-1}$) had virtually no effect on the development of the carrot somatic embryos, and (2) blue radiation (10 or 20 $\mu mol\, m^{-2}\, s^{-1}$) exerted positive effects on the development of the carrot somatic embryos, especially in the globular and heart-shaped stages.

The foregoing underscores that critical environmental factors, including DO concentration, cell density, and light quality and intensity significantly influence both the production (or induction) and the development (or differentiation) of somatic embryogenesis. Thus, designing for the practical applications of somatic embryogenesis, i.e., artificial seed technology and automated plant mass production using bioreactors, necessitates careful design of their environmental conditions.

References

Ammirato PV, Steward FC (1971) Some effects of environment of the development of embryos from cultured free cells. Bot Gaz 132(2):149–158

Archambault J, Williams RD, Lavoile L, Pepin MF, Chavarie C (1994) Production of somatic embryos in a helical ribbon impeller bioreactor. Biotech Bioeng 44:930–943

Bach A, Krol A (2001) Effect of light quality on somatic embryogenesis in *Hyacinthus orientalis* L. "Delft's Blue". Biol Bull Poznan 38(1):103–107

Bellincampi D , Morpurgo G (1987) Conditioning factor affecting growth in plant cells in culture. Plant Sci 51:83–91

Bellincampi D, Morpurgo G (1989) Evidence for the presence of a second conditioning factor in plant cell cultures. Plant Sci 65:125–130

Bomal C, Tremblay FM (1999) Effect of desiccation to low moisture content on germination synchronization of root emergence, and plantlet regeneration of black spruce somatic embryos. Plant Cell Tissue Organ Cult 56:193–200

D'Onofrio C, Morini S, Bellocchi (1998) Effect of light quality on somatic embryogenesis of quince leaves. Plant Cell Tissue Organ Cult 53:91–98

Feria M, Jimenez E, Barbon R, Capote A, Chavez M, Quiala E (2003) Effect of dissolved oxygen concentration on differentiation of somatic embryo of *Coffea arabica* cv. catimor 9722. Plant Cell Tissue Organ Cult 72:1–6

Fridborg G, Eriksson T (1975) Effects of activated charcoal on growth and morphogenesis in cell cultures. Physiol Plant 34:306–308

Godbole S, Sood A, Sharma M, Nagar PK, Ahuja PS (2004) Starch deposition and amylase accumulation during somatic embryogenesis in bamboo (*Dendrocalamus hamiltonii*). J Plant Phisiol 161(2):245–248

Halperin W (1966) Alternative morphogenetic events in cell suspensions. Am J Bot 53(5):443–453

Halperin W (1967) Population density effects on embryogenesis in carrot-cell cultures. Exp Cell Res 48:170–173

Hari V (1980) Effect of cell density changes and conditioned media on carrot cell embryogenesis. Z Pflanzenphysiol 96:227–231

Higashi K, Daita M, Kobayashi T, Sasaki K, Harada H, Kamada H (1998) Inhibitory conditioning for carrot somatic embryogenesis in high-cell-density cultures. Plant Cell Rep 18:2–6

Hofmann N, Nelson RL, Korban SS (2004) Influence of media components and pH on somatic embryo induction in three genotypes of soybean. Plant Cell Tissue Organ Cult 77(2):157–163

Hohe A, Winkelmann T, Schwenkel HG (1999) The effect of oxygen partial pressure in bioreactors on cell proliferation and subsequent differentiation of somatic embryos of *Cyclamen persicum*. Plant Cell Tissue Organ Cult 59:39–45

Jay V, Genestier S, Courduroux JC (1992) Bioreactor studies on the effect of dissolved oxygen concentrations on growth and differentiation of carrot (*Daucus carota* L.) cell cultures. Plant Cell Rep 11:605–608

Jimenez VM (2001) Regulation of in vitro somatic embryogenesis with emphasis on the role of endogenous hormones. R Bras Fisiol Veg 13(2):196–223

Kessekk RHJ, Carr AH (1972) The effect of dissolved oxygen concentration on growth and differentiation of carrot (*Daucus carota*) tissue. J Exp Bot 23(77):996–1007

Konradova H, Lipavska H, Albrechtova J, Vreugdenhil D (2002) Sucrose metabolism during somatic and zygotic embryogeneses in Norway spruce: content of soluble saccharides and localization of key enzyme activities. J Plant Physiol 159:387–396

Kvaalen H, Appelgren M (1999) Light quality influences germination, root growth and hypocotyl elongation in somatic embryos but not in seedlings of Norway spruce. In Vitro Cell Dev Biol Plant 35:437–441

Latkowska MJ, Kvaalen H, Appelgren M (2000) Genotype dependent blue and red light inhibition of the proliferation of the embryogenic tissue of Norway spruce. In Vitro Cell Dev Biol Plant 36:57–60

Mancinelli AL (1994) The physiology of phytochrome action. In: Kendrick RE, Kronenberg HM (eds) Photomorphogenesis in plants, 2nd edn. Kluwer, Netherlands pp 211–269

Meskaoui AE, Tremblay FM (1999) Effects of sealed and vented gaseous microenvironments on the maturation of somatic embryos of black spruce with a special emphasis on ethylene. Plant Cell Tissue Organ Cult 56(3):201–209

Micheler CH, Lineberger RD (1987) Effects of light on somatic embryo development and abscisic levels in carrot suspension cultures. Plant Cell Tissue Organ Cult 11:189–207

Onishi N, Sakamoto Y, Hirosawa T (1994) Synthetic seeds as an application of mass production of somatic embryos. Plant Cell Tissue Organ Cult 39:137–145

Osuga K, Komamine A (1994) Synchronization of somatic embryogenesis from carrot cells at high frequency as a basis for the mass production of embryos. Plant Cell Tissue Organ Cult 39:125–135

Osuga K, Kamada H, Komamine A (1993) Cell density is an important factor for synchronization of the late stage of somatic embryogenesis at high frequency. Plant Tissue Cult Lett 10(2):180–183

Osuga K, Kamada H, Komamine A (1997) Frequency improvement of somatic embryogenesis at high embryo density by partial replacement of medium in carrot suspension cultures. J Ferment Bioeng 84(3):275–278

Rmarosandratana AV, Staden JV (2004) Effects of auxins and 2,3,5-Triiodobenzoic acid on somatic embryo initiation from Norway spruce zygotic embryos. Plant Cell Tissue Organ Cult 79(1):105–107

Sharma P, Pandey S, Bhattacharya A, Nagar PK, Ahuja PS (2004) ABA associated biochemical changes during somatic embryo development in *Cammelia sinensis* (L.) O. Kuntze. J Plant Phisiol 161(11):1269–1276

Shimazu T, Kurata K (1999) Relationship between production of carrot somatic embryos and dissolved oxygen concentration in liquid culture. Plant Cell Tissue Organ Cult 57:29–38

Sung ZR, Okimoto R (1981) Embryonic proteins in somatic embryos of carrot. Proc Natl Acad Sci 78(6):3683–3687

Takahara K, Takeuchi M, Fujita M, Azuma J, Kamada H, Sato F (2004) Isolation of putative glycoprotein gene from early somatic embryo of carrot and its possible involvement in somatic embryo development. Plant Cell Phisiol 45(11):1165–1668

Takanori T, Cuello J (2005) Regulating radiation quality and intensity using narrow-band LEDs for optimization of somatic embryogenesis. In: Proceedings of the 2005 Annual Meeting of the American Society of Agricultural Engineers.

Torne JM, Moysset L, Santos M, Simon E (2001) Effects of light quality on somatic embryogenesis in *Araujia sericifera*. Physiologia Plantarrun 111:405–411

Vries SC, Booji H, Janssens R, Vogels R, Saris L, LoSchiavo F, Terzi M, Kammen A (1988) Carrot somatic embryogenesis depends on the phytohormone-controlled presence of correctly glycosylated extracellular proteins. Genes Dev 2:462–476