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REVIEW

Application of photoautotrophic suspension cultures in plant science

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Abstract

Whereas most plant suspension cultures are grown heterotrophically in the presence of sugars, a limited number of photoautotrophic cultures have been established which are able to grow with CO_2 as the sole carbon source. Photoautotrophic cultures are useful to address various aspects of photosynthesis, source-sink regulation, nitrogen metabolism, production of secondary metabolites, and defence responses. The homogenous populations of these cultures provide an ideal and sensitive system to obtain reproducible results. The availability of an increasing number of photoautotrophic cultures from different economically important species provides the basis also for practical applications.

Additional key words: nitrogen metabolism; photosynthesis; plant pathology; secondary metabolites; source-sink transition.

Introduction: Higher plants consist of photosynthetically active source tissues that export sugars to photosynthetically less active or inactive sink tissues. In addition, the different tissues are under control of various endogenous and exogenous stimuli such as phytohormones, nutrients, and abiotic factors. To circumvent the problems associated with the analysis of such a multi-factorial system, cell suspension cultures of a number of plant species and tissues have been established. In such cell cultures single cells or small cell clusters are grown in strictly controlled environmental conditions. Plant suspension cell cultures have proven to be valuable experimental systems to address various aspects of defence response, ion transport, secondary metabolite production, gene regulation, and signal transduction (Ebel and Mithofer 1998). Low concentrations of substances and short incubation times are sufficient to elicit cellular reactions because no barrier (*e.g.* cuticula) is present and no thick cell layers have to be penetrated. Since every single cell is exposed to exogenously applied stimuli within a few seconds, it is possible to analyse even very fast and transient effects such as the post-translational activation of proteins in signal transduction pathways or ion fluxes. Plant suspension cultures are also considered as a source of valuable products such as sweeteners, pharmaceuticals, flavours, fragrance, aromatic compounds, and enzymes (Mühlbach 1998). The industrial production of plant compounds from these cell cultures will save natural resources as many medicinal plants are threatened and endangered due to over exploitation and disparity in their regeneration and propagation. However, most of the suspension cultures are grown in the presence of sugars and their photosynthetic activity is low or they are photosynthetically inactive. Photomixotrophic (PM) cultures are available from some species; they are photosynthetically active but require the presence of exogenous sugars in the culture medium for growth. For a limited number of species photoautotrophic (PA) cultures have been established which grow in the absence of any reduced carbon source and solely utilise photosynthesis to provide energy and carbon for growth. They combine the advantages of plant suspension cultures with carbon autotrophy as the most typical feature of plant cells. Due to some unknown reason it seems to be difficult to establish photoautotrophic

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Abbreviations: Chl – chlorophyll; 2,4-D – 2,4-dichloroacetic acid; Lhc – light-harvesting complex; NAA – naphthylacetic acid; PA – photoautotrophic; PM – photomixotrophic; PS – photosystem; RuBPCO – ribulose-1,5-bisphosphate carboxylase/oxygenase.
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cell cultures and PA cultures are available only from a limited number of plant species (Widholm 1992). In particular, PA cultures from only few agriculturally important species have been established and PA cultures from monocotyledons or established model plants are missing with the exception of photoautotrophic cultures from the tomato species *Lycopersicon esculentum* (Stöcker *et al.* 1993) and *L. peruvianum* (Beimen *et al.* 1992). PA cultures proved to be valuable experimental systems for the analysis of various aspects of plant metabolism, which are in particular related to chloroplasts and photosynthetic activity. Since several enzymes and pathways of secondary metabolite biosynthesis are exclusively located in plastids, photoautotrophic suspension cell cultures offer a new potential for secondary metabolite production. PA cultures were used for the selection and characterisation of herbicide resistant cell lines (Thiemann and Barz 1994a,b) and to investigate circadian oscillation of light-harvesting complex (Lhc) mRNAs (Winter *et al.* 1996) among other studies. In recent years PA cultures have been established as a model system to analyse the regulation of source/sink transition. The photoautotrophic suspension cell cultures of *Chenopodium rubrum* and *L. peruvianum* have been extensively used to study the source/sink transition in response to sugars and stress related stimuli (Krapp and Stitt 1994, Roitsch and Tanner 1994, Godt *et al.* 1995, Roitsch *et al.* 1995, 2000, Ehneß *et al.* 1997, Klein and Stitt 1998, Hofmann *et al.* 1999, Roitsch 1999, Link *et al.* 2002). Effects on photosynthesis and sink metabolism may be analysed within the same experiment and it is possible to relate the regulation of sugar metabolism to the activation of defence responses. Also various aspects of sugar signalling may be addressed without the necessity of sugar depletion. The present review will focus on the growth and maintenance of PA cultures and elucidate their potential as experimental system to address various aspects of primary and secondary metabolism.

Establishing, maintenance, and preservation of PA cell cultures: PA cultures are established from PM cultures. Initially callus cultures are established from explants on sugar-containing solid medium, and photosynthetic active green parts are selected to establish a PM suspension culture line. The sugar content of the medium for growing the PM culture is slowly and gradually decreased while selecting green tissues to finally establish a PA culture that grows in a medium lacking any sugar (Sato *et al.* 1979, Hüsemann 1985). This process may take many months. Chlorophyll (Chl) content, carbon assimilation in light, and ultrastructure of chloroplasts routinely monitor the increasing photosynthetic activity. The development of chloroplasts in cells of PA cultures is limited compared to mesophyll cells of green leaves (Widholm 1992, Horn and Widholm 1994a). PA cultures are characterised by slow growth rates with doubling times of about 1.5 to 3.0 d. Most PA cultures are maintained in constant light, which seems to be important for the synthesis and accumulation of Chl. However, there are certain PA cultures such as from *Euphorbia* which are grown with an 18 h photoperiod (Hardy *et al.* 1987) and from *Asparagus* which require a 16 h photoperiod for optimal growth (Peel 1982).

PA cultures are characterised by a demand for an elevated CO_2 concentration and most of the PA cultures are grown in the presence of at least 1 % CO_2. One widely used method for maintaining PA cultures is shaking them in so-called two-tiered flasks which are essentially two Erlenmeyer flasks that are connected by a glass tube (Fig. 1). The cell suspensions are present in the top part while the lower vessel contains a carbonate buffer system ($K_2CO_3/KHCO_3$) to provide an elevated CO_2 concentration as carbon source (Meyer and Spiteller 1997).

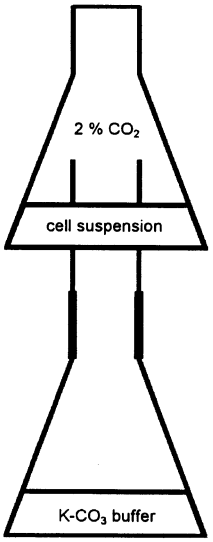

Fig. 1. Two-tiered flask used for maintaining PA cell cultures.

Apart from two-tiered flasks, different research groups have also used Petri dishes, bubble tubes, flasks with controlled environment, and several kinds of fermenter. Whereas growth rates of *C. rubrum* and final cell densities were higher in the two-tiered flasks, the algae flasks were superior when large quantities of PA cells were required and the physiological status was less critical (Fig. 2). However, for most applications the two-tiered flasks are more appropriate since they provide optimal growth conditions, are easy to handle, and a constant value of an elevated CO_2 concentration may be controlled by choosing the appropriate proportion of $K_2CO_3/KHCO_3$ buffer in the lower flask. A bioreactor system has also been designed to cultivate PA cultures of *C. rubrum* in a large volume over a long period of time in a semicontinuous mode (Fischer *et al.* 1994, Fischer and Alfermann 1995). An airlift bioreactor was used with a working

volume of 0.02 m^3, which is currently the largest volume reported for PA cultures of higher plants.

The cell cultures are grown in Murashige and Skoog (1962) or Linsmaier and Skoog (1965) type basal medium containing only macro- and micro-nutrients with no or very little amounts of growth regulator depending upon the specific species. The use of cytokinins is required at least at the initial stages as they stimulate chloroplast development. Since cytokinins induce certain genes involved in sink metabolism (Ehneß and Roitsch 1997b, Roitsch and Ehneß 2000), it is routinely omitted in later stages. The use of auxin also varies in different species. 2,4-D represses photosynthetic rate in one hour and is also detrimental to greening (Hüsemann and Barz 1977, Yamada *et al.* 1978). In most of the PA cultures NAA is used as auxin. Some PA cultures, such as from tobacco (Chandler *et al.* 1972, Ikemeyer and Barz 1989) and potato (LaRosa *et al.* 1984), require 2,4-D, and also IAA is used for specific PA lines. A few PA cultures are maintained in growth regulator free medium, for example *Chenopodium rubrum* (Hüsemann 1981) and *Spinacia oleracea* (Dalton 1980). Certain plant species require thiamine for the growth of corresponding PA cultures. The desirable goal with PA cultures is to eliminate all organic constituents from the medium in order to have absolute photoautotrophic growth.

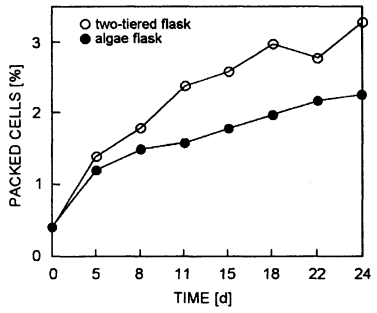

Fig. 2. A comparison of the growth of PA cultures of *C. rubrum* in a two-tiered flask (*open symbols*) and an algae flask (*closed symbols*). Equal volumes of cells were taken and percentage of packed cells was calculated after centrifugation. From a representative experiment of C. Wöhrle (Diploma thesis, University of Regensburg 1991).

Table 1. List of available photoautotrophic suspension cell cultures from different plant species.

Plant species	Author
Amaranthus cruentus and *A. powellii*	Xu *et al.* (1988)
Arachis hypogaea	Kumar (1974)
Aspargus officinalis	Peel (1982)
Catharanthus roseus	Tyler *et al.* (1986)
Chenopodium rubrum	Hüsemann and Barz (1977)
Cicer arietinum	Orthen *et al.* (2000)
Cytisus scoparius	Yamada and Sato (1978)
Datura stramonium and *D. innoxia*	Yasuda *et al.* (1980)
Daucus carota	Bender *et al.* (1985)
Dianthus caryophyllus	Rebeille *et al.* (1988)
Digitalis purpurea	Hagimori (1982)
Euphorbia characias	Hardy *et al.* (1987)
Glycine max	Horn *et al.* (1983)
Gossypium hirsutum	Blair *et al.* (1988)
Hyoscyamus niger	Yasuda *et al.* (1980)
Lycopersicon esculentum	Stöcker *et al.* (1993)
Lycopersicon peruvianum	Beimen *et al.* (1992)
Marchantia paleacea var. *diptera*	Taya *et al.* (1995)
Mesembryanthemum crystallinum	Willenbrink and Hüsemann (1995)
Morinda lucida	Igbavboa *et al.* (1985)
Nicotiana tabacum and *N. plumbaginifolia*	Bergmann (1967)
Peganum harmala	Barz *et al.* (1980)
Ruta graveolens	Corduan (1970)
Solanum tuberosum	LaRosa *et al.* (1984)
Spinacia oleracea	Dalton and Street (1976)

Most of the PA cultures were established from C_3 plants (Table 1). There is only one report of PA cultures from a C_4 species, *Amaranthus* cell lines APO-P and ACR-P (Xu *et al.* 1988). From CAM species, a PM culture has been established of *Kalanchoë blossfeldiana* which grows in the presence of 3 % sugar (Mricha *et al.* 1990). Willenbrink and Hüsemann (1995) established a PA culture of *Mesembryanthemum crystallinum*, a C_3 species that under stress behaves as CAM species. So far there are no reports of PA culture from any cereals. The

general problem, as stated by Widholm (1992), may be due to the lack of uniform greening in cereal cells.

Several reports describe techniques for preservation of PA cell cultures. Luo and Widholm (1997) suggested cryopreservation of PA cell cultures. PM cultures of soybean were stored in liquid nitrogen after a two step freezing method. These cultures were then gradually thawed and within 40-47 d the Chl content of the cells was recovered. Malik (1996) described that different cultures of *Chloroflexus* can be maintained for more than 18 months of storage under dim irradiance (100-200 lx) at 37-40 °C as slow growing liquid cultures. During this storage no shaking, regular subculture, or transfer to fresh medium was required. Malik suggests that this method is superior to cryopreservation as the maintained cell suspensions can provide a continuous source of inocula from the same batch of cell suspension for routine work. In another method, the PM cell suspensions of different species were first placed under heterotrophic conditions where the Chl content declined to near zero. Once these cultures were transferred back into photomixotrophic or photoautotrophic conditions, rapid re-greening and continuous growth was observed (Lozovaya *et al.* 1996a,b).

Cell viability of several PA cultures was determined using the red exclusion dye phenosafranin (Blair *et al.* 1988, Roeske *et al.* 1989). Other exclusion dyes that were established for mesophyll cells and which proved to be useful for PA cultures are Evans Blue (Cosio *et al.* 1983) and fluorescein diacetate (Widholm 1972). Thiemann *et al.* (1989) measured the viability of PA cultures of *C. rubrum* by their ability to generate reducing equivalents to reduce nitroblue tetrazolium chloride, which was easily quantified in the ethylacetate phase by spectrophotometry. This method is similar to the triphenyltetrazolium chloride reduction assay, in which the reduced formazan dye can be extracted into ethanol to be measured by absorption (Towill and Mazur 1975).

Photosynthetic characteristics and ageing of PA cultures: A comparison of PA cultures with green leaves is one way of assessing the photosynthetic properties of these cultures. In most of the cases the Chl content of PA cultures is much lower than that of green leaves. The Chl content per chloroplast is the same but chloroplast number per cell varies and may account for the lower Chl content of PA cultures. In PA cultures of *Nicotiana tabacum,* the number of chloroplasts per cell was 90 as compared to leaf cells which contained 200 chloroplasts per cell (Takeda *et al.* 1989). Electron micrographs revealed the same structure of chloroplasts in PA cultures as in green leaves (Hüsemann *et al.* 1984, Horn and Widholm 1994a). The maximum activities of photosystem (PS) 1 and 2 measured during the different developmental stages and on the basis of Chl content were half of those in leaves of *N. tabacum* (Takeda *et al.* 1989). Treatment of PA cultures of protonema of the moss *Physcomitrella patens* and *L. esculentum* with β-lactam antibiotics showed that these antibiotics specifically inhibit plastid division in the moss. This inhibition can not be compensated by exogenous application of cytokinin and removal of the antibiotic resulted in the division of macrochloroplast (Kasten and Reski 1997). The analysis of PA and PM cultured tobacco cells revealed that the contents of certain polypeptides in the thylakoid such as α and β subunit of coupling factor I (CFI), of polypeptides of the reaction centres of PS1 and PS2, and also of the Chl binding polypeptides of the Lhc of PS1 and PS2 were reduced in the cultured green cells compared to green leaves and that the effect was in particular pronounced in the PM culture (Takeda *et al.* 1989). The characterisation of eight PA cultures of *C. rubrum* resistant to certain photosynthesis inhibiting herbicides revealed different mutations in the D1 protein of PS2 which did not result in a reduced electron transport in PS2 (Thiemann and Barz 1994a,b). A PA culture of *Euphorbia characias* adapted to a low irradiance of 70 μmol m^{-2} s^{-1} was highly susceptible to photoinhibition (Bladier *et al.* 1994). In PA cultures of *C. rubrum* higher rates of synthesis and degradation of D1 proteins were observed when the cells were grown at higher irradiances compared to those grown at lower irradiances (Schmid and Schäfer 1992).

The activity of one of the most important enzymes in Calvin cycle, ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBPCO), was also lower in PA cultures than in leaves (Rogers *et al.* 1987, Blair *et al.* 1988, Rey *et al.* 1990b, Widholm 1992). Many PA cultures are characterised by increased content of phosphoenolpyruvate carboxylase compared to RuBPCO during the exponential growth phase and this pattern is reversed during the stationary phase (Hüsemann 1981, Hüsemann *et al.* 1984, Rogers *et al.* 1987, Chagvardieff *et al.* 1990). Activities of NAD- and NADP-dependent malate dehydrogenase and malic enzyme of a PA culture of *C. rubrum* were in the mitochondrial and chloroplast fractions, respectively (Amino 1992). In PA cultures of soybean grown under elevated CO_2 concentration, malate was the dominant fixation product thereby strongly suggesting that phosphoenolpyruvate carboxylase is the primary enzyme involved in carbon fixation in these cells under their normal growth conditions (Horn and Widholm 1994a).

PA cultures are also capable of photorespiration. Photosynthesis was inhibited in the presence of high concentration of O_2 and this effect was inhibited by increasing the concentration of CO_2 (Berlyn *et al.* 1978, Nishida *et al.* 1980, Rebeille 1988, Roeske *et al.* 1989, Rey *et al.* 1990*b*, Bockers *et al.* 1997). Schmid and Schäfer (1994) found a marked reduction in photochemical efficiency, Chl content, and Chl *a/b* ratio when PA cultures of *C. rubrum* were exposed to high irradiance for seven consecutive light periods. The CO_2 compensation concentra-

tion of the PA cells in most cases was 2 to 4 fold higher than in leaves (Rogers *et al.* 1987, Chagvardieff *et al.* 1990). Sinha and Roitsch (2002) showed inhibition of oxygen evolution and electron transport rates of PA cultures of *L. peruvianum* upon treatment with the metabolisable sugars, sucrose and glucose, but not by the non-metabolisable sucrose analogues palatinose and turanose.

In a PA culture of *C. rubrum*, Meyer and Spiteller (1997) showed that the content of oxidised phytosterols increases with the ageing of cell cultures. Extensive turnover of the cell wall pectin and hemicellulose fraction and starch was found during the normal growth phase (Lozovaya *et al.* 1996a) in PA cultures of soybean. The rate of incorporation of ^{14}C was higher in the early growth phase (3-d-old cultures) compared to later stages (10- and 16-d-old cultures). PA cultures of *C. rubrum* can be maintained for approximately 100 d under a 16 h photoperiod (Peters *et al.* 2000). These long living cultures showed an initial cell division phase for 3-4 weeks followed by a stationary phase for the next 4 weeks and finally ageing and cell death within the next 3-4 weeks. These data were based on photosynthetic performance, dark respiration, contents of phytohormones, and the capacity for cell division.

Regulation of source and sink metabolism: PA cultures are valuable to study the regulation of source-sink transition in response to endogenous and exogenous signals such as sugars, hormones, stress related stimuli, and pathogens (Schäfer *et al.* 1992, Krapp and Stitt 1994, Roitsch *et al.* 1995, Ehneß *et al.* 1997, Godt and Roitsch 1997, Klein and Stitt 1998, Goetz *et al.* 2000, Link *et al.* 2002, Sinha *et al.* 2002). Since the cells are photosynthetically active, the effect of specific stimuli on both source and sink specific activities may be analysed within the same experiment. In addition, the response to sugars as metabolic stimuli can be analysed without depleting sugar prior to the start of experiment; this would result in a reduced energy status and thus complicate any conclusions.

PA cultures were used to study the effect of exogenous sugars and certain other stimuli on photosynthesis. Rebeille *et al.* (1988) found that during the log growth phase the rate of photosynthesis was highest which corresponds to the low concentration of sucrose and fructose in PA carnation cultures. Adding of 20 mM sucrose resulted in a rapid increase in sugar content and decline in photosynthetic rate by 75 % within 24 h. Neumann *et al.* (1989) found that PA peanut cells incorporated less $^{14}CO_2$ into C_3 pathway compounds when sucrose was added to the culture medium, although C_4 fixation was not changed. Feeding glucose to a PA culture of *C. rubrum* resulted in a massive accumulation of sugars followed by a reduction in the amount of total Chl (Schäfer *et al.* 1992). In the same study the photosynthetic efficiency as determined by Chl fluorescence was slightly decreased and the rate of oxygen evolution and the activity of RuBPCO decreased considerably when calculated per fresh mass. Krapp and Stitt (1994) showed that feeding glucose to the PA cultures of *C. rubrum* resulted both in increased sugar content and decreased activity of plastid starch phosphorylase and phosphoglucomutase but had no effect on the activities of the corresponding cytosolic enzymes. The effect of glucose and of the glucose analogue 2-deoxyglucose was investigated on the mRNA content of the small subunit of RuBPCO (RbcS) in PA cultures of *C. rubrum* (Klein and Stitt 1998). The transcript level of RbcS was substantially repressed by glucose after 10 h and even low concentration of 2-deoxyglucose resulted in a decrease of the RbcS transcript already after 6 h and also in a substantial depletion of phosphorylated intermediates, ATP and UDP-glucose, and an inhibition of photosynthesis. PA cultures of *L. peruvianum* were used to study the effect of sugars on the rate of oxygen evolution, Chl fluorescence, and delayed fluorescence. Glucose and sucrose reduced the rate of oxygen evolution to 50 % after 48 h when expressed per fresh mass and also the fluorescence yield and photochemical quenching showed a considerable decline (Sinha and Roitsch 2002). The effect of glucose on delayed fluorescence in a PA culture of *L. peruvianum* was measured using an indigenous developed instrument which has been extensively used for identifying and estimating different population of phytoplankton (Gerhardt and Bodemer 1998). Fig. 3 demonstrates a faster decay of delayed fluorescence in the glucose treated sample compared to the PA control culture.

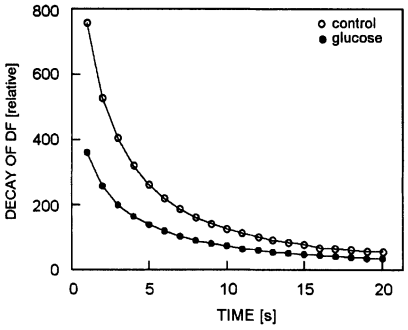

Fig. 3. Effect of addition of glucose on delayed fluorescence (DF) of a PA culture of *L. peruvianum*. DF was measured using an indigenously developed delayed fluorescence spectrometer (Gerhardt and Bodemer 1998). The cells were treated with 50 mM glucose and further incubated for 48 h (*closed symbols*) and compared with a corresponding control culture (*open symbols*). The experiment was repeated three times and a representative curve is presented.

PA cultures of *C. rubrum* and *L. esculentum* were analysed for the induction of sink metabolism and sup-

pression of source activities by different sugars, hormones, stress, and pathogens (Roitsch *et al.* 1995, Ehneß *et al.* 1997, Sinha *et al.* 2002). The mRNA for RbcS was suppressed, while the mRNAs for an extracellular invertase (CIN1) and phenylalanine-ammonia-lyase (PAL), involved in defence response, were coordinately induced in *C. rubrum* cultures by glucose and stress related stimuli such as fungal elicitor chitosan, phosphatase inhibitor, and benzoic acid (Ehneß *et al.* 1997).

A differential effect of the sucrose analogues turanose and palatinose and metabolisable sugars on the transcript levels of extracellular invertase and RbcS and on the activation of MAPK was shown in PA cultures of *L. peruvianum*. These results indicate different sensing mechanisms for metabolisable sugars and those naturally not occurring in plants and that the sucrose isomers are sensed as stress related stimuli (Sinha *et al.* 2002).

PA cultures of C. *rubrum* and *L. peruvianum* were used in our laboratory to study the function and regulation of extracellular invertases (Roitsch *et al.* 2000). In particular the cell wall bound, extra-cellular invertase isoenzyme was shown to be a central modulator of sugar partitioning and defence responses (Roitsch and Tanner 1996, Roitsch and Weber 2000, Roitsch *et al.* 2000, Goetz *et al.* 2001). A full-length cDNA encoding extracellular invertase, Cin1, has been cloned from *C. rubrum*. The sequence analysis of tryptic peptide after biochemical purification of the protein from a PA culture has proven its identity (Ehneß and Roitsch 1997a). In addition to Cin1, a second putative extra-cellular invertase Cin3 with unusual biochemical properties has been cloned and characterised from *C. rubrum* (Ehneß and Roitsch 1997*a*). From a PA culture of *L. esculentum* a family of four extracellular enzyme designated as Lin5, Lin6, Lin7, and Lin8 have been cloned using a PCR based approach and characterised by a highly differential expression and regulation pattern (Godt and Roitsch 1997). The regulation of extracellular invertases by different phytohormones has been identified in PA culture. Application of ethylene to PA cultures of *C. rubrum* repressed the mRNA level of Cin1 (Linden *et al.* 1996). The same culture was used to demonstrate that extracellular invertase and hexose transporter are not only functionally linked to supply sugars to sink tissues but are coordinately induced by cytokinins (Roitsch and Tanner 1994, Ehneß and Roitsch 1997a). Goetz *et al.* (2000) showed that addition of brassinosteroids to tomato cell suspension cultures specifically elevates the activities of cell wall bound invertase, whereas the intracellular invertase activities were not affected. In *C. rubrum* cultures protons were not a second messenger for the regulation of extracellular invertase and PAL genes in response to elicitors (Hofmann *et al.* 1999). Preparations of invertases from PA cultures of *C. rubrum* and heterologous expressed invertases were used to show that the different pH optima and substrate specificities of extracellular and vacuolar invertases are determined by single amino acid substitution (Goetz and Roitsch 1999).

Production of secondary metabolites: PA cultures have a unique potential for the production of secondary metabolites for practical applications in biotechnology. Wink and Hartmann (1980) demonstrated the biosynthesis of quinolisidine alkaloid lupanine from the PM cultures of *Lupinus polyphyllus*. Production of lipoquinones such as phylloquinone, α-tocopherol, plastoquinone, and ubiquinone were reported from a PA culture of *Morinda lucida* (Igbavboa *et al.* 1985). A PA cell culture of *Nicotiana tabacum* produced trigonelline (N-methyl nicotinic acid) which is not found in the heterotrophic culture of the same species (Ikemeyer and Barz 1989). Reil and Berger (1996) demonstrated the elicitation of volatile aromatic compounds only from the PM cultures of *Petroselinum crispum*. The accumulation of ononitol, a major cyclitol of the polyol fraction, was shown in PA cultures of *Cicer arietinum* under salt stress (Orthen *et al.* 2000), though the accumulation of ononitol was higher in PM and heterotrophic cultures under the same conditions.

Plant pathological studies: Use of PA cultures in plant pathology is still in its initial stages as compared to the numerous studies carried out with heterotrophic cultures (Mühlbach 1998). A detailed analysis of the effect of an elicitor preparation of the fungal pathogen *Fusarium oxysporum lycopersici* on PA cultures of *L. peruvianum* was described by Beimen *et al.* (1992). In the absence of an elicitor the content of phenolics was higher in heterotrophic cultures compared to PM and PA cultures but the maximum contents of phenolics were reached much faster in PA cultures after elicitor treatment. In addition the pattern of elicitor induced phenolic compounds differed in heterotrophic and PA cultures. In another study nine phenolic compounds were isolated which accumulated in the medium of *Solanum khasianum* after treatment with an elicitor preparation from yeast (Mühlenbeck *et al.* 1996). The reaction of heterotrophic and PA cultures in response to potato spindle tuber viroid (PSTVd) infection was investigated in different studies. The PSTVd infected and uninfected heterotrophic cultures of *L. esculentum* showed no difference with respect to growth characteristic and morphology (Mühlbach and Sänger 1981) whereas the PSTVd infected PA cultures of *L. esculentum* differed greatly from the uninfected cells (Stöcker *et al.* 1993). These studies demonstrate the importance of using PA cultures. Co-ordinated regulation of source/sink relations and defence responses was shown in PA cultures of *C. rubrum* (Ehneß *et al.* 1997) and *L. esculentum* (Sinha *et al.* 2002). A fast and transient induction of mitogen activated protein (MAP) kinases as important components of stress signal transduction pathways that link environ-

mental stimuli to cellular responses has been demonstrated in PA cultures. Application of the fungal elicitors, chitosan and E-FOL, resulted in the activation of MAP kinases in PA culture of *C. rubrum* (Ehneß *et al.* 1997) and *L. peruvianum* (Hofmann and Roitsch 2000), respectively. Biochemical evidence for the simultaneous activation of distinct subset of MAP kinase by voltage and defence related stimuli was shown for PA cell cultures of *L. peruvianum* (Link *et al.* 2002).

Analysis of herbicide effects and selection of herbicide resistant lines: Effects of herbicides were compared in PA, PM, and heterotrophic cultures to address the contribution of photosynthesis for growth of the corresponding cultures. Horn *et al.* (1983) showed that 0.5 µM DCMU inhibited the photosynthesis completely in PA culture of *G. max* while the PM and heterotrophic cultures of *G. max* were inhibited up to 70 and 9 %, respectively. In *C. rubrum* cultures, Ashton and Ziegler (1987) showed that both DCMU and atrazine inhibited photosynthesis as well as growth stronger in PA cultures compared to PM cultures grown with 1 or 2 % sucrose. Sato *et al.* (1987) compared the effect of 12 different herbicides on PA, PM, heterotrophic cultures, and seedlings of *N. tabacum* and found that PA cultures were most sensitive to photosynthetic herbicides. Thiemann *et al.* (1989) grew 1.5 cm^3 cell suspension of PA cultures of *C. rubrum* in 24 well-microtiter plates on a shaker. This experimental set-up enables several physiological measurements simultaneously determining lethal concentrations of different herbicides. DCMU resistant colonies after mutagenesis with ethylnitrosoureas were selected from *N. plumbaginifolia* protoplasts grown with limiting amounts of sugars (Rey *et al.* 1989, 1990a). Atrazine resistant PA culture of *N. tabacum* were selected from PM cultures of *N. tabacum* after a 10 months selection period with transfers every 28 d (Sato *et al.* 1988). Different PA cultures of *C. ru-brum* selected as resistant to triazine showed cross-resistance to atrazine and DCMU (Thiemann and Barz 1994b). Alfonso *et al.* (1996) identified a mutant line of *Glycine max* (STR7) PA cultures resistant to atrazine and DCMU. Sequencing of the *psb*A gene coding the D1 polypeptide of PS2 revealed a single change of serine-268 to proline. This particular PA mutant line of *Glycine max* is highly resistant to heat stress (Alfonso *et al.* 2001).

Nitrogen metabolism has been studied extensively with PA cultures of *C. rubrum*. The cultures showed preferential uptake of NH_4^+ in the first week of batch cultivation followed by both NH_4^+ and NO_3^- uptake within the next week till NH_4^+ was exhausted (Campbell *et al.* 1984). Beck and Renner (1989) showed that addition of NH_4^+ to PA cultures grown in medium with NO_3^- as sole N source increased the nitrate reductase activity by mobilising vacuolar NO_3^- and by stimulating NO_3^- uptake. Nitrogen fluxes during lag phase, cell division, and transition to stationary and stationary phase were characterised in PA cultures of *C. rubrum* (Beck and Renner 1990). Peters *et al.* (1995) showed increase in nitrate reductase activity by the application of naturally occurring cytokinins on PA cultures of *C. rubrum*. In a PA culture of soybean, asparagine, glutamine, alanine, and serine were identified as the dominating amino acids which was in agreement with the composition of the phloem sap of a soybean plant (Horn and Widholm 1994b). In the same work there was a positive correlation between the initial nitrogen content of the medium and cellular Chl content.

Studies on diurnal and circadian oscillations: Renner and Beck (1988) showed diurnal fluctuation in nitrate reductase activities in PA cultures of *C. rubrum* grown under a 16-h photoperiod; this activity was cycloheximide sensitive. Winter *et al.* (1996) showed that out of fourteen genes encoding proteins of the Lhc expressed in PA cultures of *L. peruvianum*, *Lhc*a2 (cab7) and *Lhc*b2*1 (cab4), showed a rhythmic oscillation of transcript accumulation. This oscillation in the transcript contents was observed both in the 16-h photoperiod and also under constant darkness, indicating that a circadian clock in PA cultures of tomato controls gene expression. These circadian oscillations of *Lhc* were present even after the application of the inhibitor 2,2′-dipyridyl. Rockel *et al.* (1998) showed day-night oscillations of the mRNA for V-ATPase, subunit c in PA cultures of *M. crystallinum* which was also shown in the leaves of the same species.

Other studies with PA cultures: PA cultures were also used for analysis of plastid DNA content and activities of DNA helicase and DNA polymerase. Richter *et al.* (1987) and Kaldenhoff and Richter (1990) have identified different genes that were induced in *C. rubrum* cultures by light. Cannon *et al.* (1985, 1986) determined the plastid DNA amount in several cultures of *N. tabacum* and *G. max*. They showed that the plastid DNA was doubled compared with leaves of the same species. Activities of DNA helicase (Cannon and Heinhorst 1990) and DNA polymerase (Heinhorst *et al.* 1990) were partially purified from the PM cultures of *G. max*. Anderson (1988) found that addition of 3-30 µM jasmonic acid to PA cultures of *G. max* induced a 30-kDa polypeptide which was not produced in response to abscisic acid, benzyladenine, or gibberellic acid. cDNAs encoding different proteins involved in cell cycle regulation, a D-type cyclin, a mitotic cyclin, and a CDK34-protein kinase were cloned from a PA cultures of *C. rubrum* (Renz *et al.* 1997a,b,c).

Concluding remarks and future perspective: Suspension cell cultures grown in controlled environmental conditions and in sugar and plant growth regulator free me-

dium are an appropriate experimental system for specific areas of both basic and applied plant science research. Since PA cultures have similar properties as green leaves but are not identical to them, the observations and findings in PA cultures have to be verified in leaves of whole plants.

Although PA cultures of several plant species are available to date, it will be required to establish more PA cultures of economically important crop plants. For some unknown reasons, it seems to be difficult to establish photoautotrophic cultures from agricultural important species including monocots or established model plants. The most extensively studied PA cultures are from *C. rubrum, N. tabacum, L. esculentum, L. peruvianum, D. innoxia, Euphorbia, A. powellii, S. oleracea, S. tuberosum, G. max, G. hirsutum,* and carnation. In particular a photoautrophic culture of *C. rubrum* (Hüsemann and Barz 1977) proved to be useful to address various aspects of source/sink regulation in response to sugars and has been used to study various other aspects. PA cultures from *L. esculentum* (Stöcker *et al.* 1993) and *L. peruvianum* (Beimen *et al.* 1992) are promising experimental systems since the transformation of this economical important species is established. Tomatoes have been extensively characterised both by classical genetic analysis as well as in numerous molecular and physiological studies, and an increasing number of EST sequences are becoming available.

PA cultures share all advantages of heterotrophic suspension culture cells because they consist of uniform populations of cells, and are immediately accessible to exogenously applied stimuli, easy to transfer, grown in readily controlled conditions to give reproducible results, free from microorganisms, and can be grown in different vessels including fermenters. They are in particular suitable for plant signal transduction research and may help in the elucidation of complicated stress, pathogen, hormones, and sugar signalling pathways and the interaction of the naturally occurring regulatory networks.

So far nuclear transformation of PA cultures has not been reported and thus need to be established. This will be important to verify correlative data by functional approaches, in particular in the field of signal transduction research. Chloroplast transformation with several advantages over nuclear transformation (see review of Daniell *et al.* 2002) has also not yet been reported for PA cultures and would be other future goal to achieve.

The PA cultures could also be very useful to elucidate the yet not completely understood deoxyxylulose phosphate pathway for terpenoid synthesis that is exclusively located in chloroplasts (Lichtenthaler 1999, Lange *et al.* 2000). This deoxyxylulose phosphate pathway does not occur in humans and animals, making it ideal target for the development of novel antibiotics and antimalaria agents. Moreover, manipulation of the deoxyxylulose pathway in PA cultures might be the basis for the development of new herbicides (Eisenreich *et al.* 2001) and the selective production of pharmaceutically relevant secondary metabolites. The latter aspect is also important to save natural resources and saves endangered plant species from extinction.

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