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Pulsed electric fields on phenylalanine ammonia lyase activity of tomato cell culture

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ABSTRACT

Pulsed electric field (PEF), among numerous stresses, provides a large potential as an external stimulus to enhance secondary metabolite biosynthesis from plant cell cultures. PEF treatment was combined with the sub-culturing technique to increase phenylalanine ammonia lyase (PAL) activity, the key enzyme for secondary metabolite synthesis from tomato cell culture. In reference to the analysis, maximum accumulation of total phenolic (TP) compounds in the cells was determined as a function of stress reaction time (SRT). Also, PEF application increased PAL activity. PEF resulted in an increase in the porosity of the cell membrane, thus decreasing cell vitality. On the other hand, SRT decreased vitality, while increasing TP content. Sub-culturing enhanced the cell structure and improved the cell membrane permeability. PEF treatment potentially offers an alternative to other methods for increasing PAL activity in plant cell cultures.

1. Introduction

Plant cell cultures have received attention because of their importance as potential renewable sources of invaluable secondary metabolites, such as pharmaceuticals, nutraceuticals and food additives (Cai et al., 2011; Ferri et al., 2011). Valuable medicinals, flavors, essences and colorants which cannot be produced by microbial cells and chemical synthesis can be obtained from plant cell cultures (Dicosmo and Misawa, 1995). Manufacturing these phytochemicals using plant cell cultures is independent of geographical location, climatic conditions, seasonal variations and growth conditions, when compared with extracting them from plant sources (Gueven and Knorr, 2011). In vitro biosynthesis of these phytochemicals by plant tissue cultures allows fast proliferation of the cell mass within a short cultivation time (about 2–4 weeks), and thus leads to a continuous supply of products with uniform quality and yield (Dornenburg and Knorr, 1995; Rao and Ravishankar, 2002). Therefore, plant cell cultures offer a good alternative to traditional agriculture. However, biochemical pathways and production protocols of bioactive secondary metabolites under controlled and reproducible conditions have not been fully studied (Cai et al., 2011; Rao and Ravishankar, 2002).

Secondary metabolites are large molecules with complex chemical structures that are not necessary for the survival of plant cells, but they are invaluable components of the plant defense mechanism. They are known to protect plants from variations in the growth environment such as air pollution, herbicides, heavy metals, wounding, hot and cold stress, UV and pathogens by influencing metabolic activities against adverse conditions (Gaspar et al., 2002). Among numerous enzymes of the phenylpropanoid pathway, phenylalanine ammonia lyase is the signal that stimulates the secondary metabolism in plant tissue cultures (Dixon et al., 2002).

Methods to increase secondary metabolite biosynthesis and hence PAL activity in plant cell cultures involve chemical and physical stresses or their combinations. Chemical stresses involve using chemicals for elicitation, cell immobilization and membrane permeabilization. Physical stresses involve applications of High Hydrostatic Pressure (HHP), cold shock, UV, ultrasound and Pulsed Electric Field (PEF) (Dornenburg and Knorr, 1995; Rao and Ravishankar, 2002).

PEF is a non-thermal technology that involves application of short repeated high voltage pulses to biological cell materials. PEF is a food preservation alternative that causes less degradation in nutritional food components (Angersbach et al., 2000). PEF is

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readily used for the inactivation of microorganisms and enzymes, pasteurization of fluid foods, as well as permeabilization of plant cells to intracellular metabolites (Toepfl, 2006). PEF application causes a stress reaction which results in secondary metabolite biosynthesis. The advantage of PEF application is that it also leads to enhanced membrane permeability resulting in the excretion of intracellular metabolites (Cai et al., 2011). PEF causes irreversible or reversible pore formation that enhances membrane permeability. High energy pulsing (E > 1.0 kV/cm) causes irreversible pore formation that is useful for food preservation and mass transfer in food products (Janositz et al., 2011). Reversible PEF treatment (E = 0.5–1.0 kV/cm) stimulates secondary metabolite release without destroying cell vitality (Guderjan and Knorr, 2005).

In the present study, assessment of PEF in conjunction with the sub-culturing technique was used to determine PAL activity and the accumulation of total polyphenols which stimulate secondary metabolite biosynthesis in the *Lycopersicon esculentum* (LE) cell culture as a model system. First, the growth curve of *L.* esculentum was generated in small batches. Time required for culture development before PEF application was determined according to the sub-cultivation frequency. After PEF treatment, dynamic profiles of cell concentration, cell vitality, pH and medium conductivity were followed to analyze the effect of PEF application on the target plant cell culture, in addition to the analyses of PAL activity and the content of total polyphenols.

2. Materials and methods

2.1. Plant cell culture

The *L. esculentum* cell culture was obtained from the Federal Research Center for Cultivated Plants (Berlin, Germany) and propagated in AM1 medium (Table 1). Cell suspension cultures were established by transferring cell aggregates into 60 ml of liquid AM1 medium in 250 ml Erlenmeyer flasks. The flasks were continuously agitated at 100 rpm using a rotary shaker. The cell cultures were transferred to fresh medium every 14 days. *L. esculentum* cell cultures were maintained at 25 ± 2 °C with a 24-h photoperiod under fluorescent light.

2.2. Chemicals

Chemicals for the vitality test (TTC- Test, Towill and Mazur, 1974) were 2,3,5-Triphenyl-tetrazoliumchloride (Merck, Darmstadt, Germany), ethanol (Carl Roth, Karlsruhe, Germany), sodium dihydrogenphosphate (Merck, Darmstadt, Germany), potassium hydrogenphosphate (Merck, Darmstadt, Germany).

Chemicals for the determination of total phenolic compounds were methanol (VWR, BDH-Prolabo, Lutterworth, Leicestershire, England), chloroform (Merck, Darmstadt, Germany), Folin–Ciocalteu reagent (Sigma – Aldrich Chemicals GmbH, Deisenhofen, Germany), sodium carbonate (Merck, Darmstadt, Germany), 3,4,5-Trihydrobenzoic acid (Sigma – Aldrich Chemicals GmbH, Deisenhofen, Germany) and ethanol (Carl Roth, Karlsruhe, Germany).

Potassium hydrogenphosphate (Merck, Darmstadt, Germany), boric acid (Merck, Darmstadt, Germany), borate solution (Merck, Darmstadt, Germany), sodiumchloride (J.T. Baker, B.V. – Deventer, Netherlands) and phenylalanine (powdered solid) (Carl Roth, Karlsruhe, Germany) were the other chemicals that were used for phenylalanine ammonia lyase enzyme activity tests.

2.3. Treatments

2.3.1. Characterization of L. esculentum growth

L. esculentum suspension cell cultures inoculated and maintained as given above were incubated for 17 days. Dry weight, cell vitality, total phenolic materials, phenylalanine enzyme activity, pH and conductivity of the cell culture were monitored by making relevant measurements on the 0, 3, 6, 9, 11, 13, 15 and 17th days of incubation during generation of the growth curve of *L. esculentum* cells (Fig. 1).

2.3.2. PEF application

Effects of PEF on cell vitality of the tomato cell culture was investigated using a central composite design (CCD) scheme (Design Expert 7.0®, Stat Ease, Minneapolis, USA) (Table 2). For the response of cell vitality, experimental conditions (coded variables) were PEF as 600 (−1), 900, 1200 (+1) V/cm, pulsed numbers (PN) as 1 (−1), 5, 9 (+1) and stress reaction time (SRT) which is required for the formation of the stress effect as 0 (−1), 4, 96 (+1) hours as 2

Table 1
Chemical composition of AM1 medium (Murashige and Skoog, 1962).

Chemicals	Ingredients	Concentration (g/l)	Final concentration (ml/l)
Macro nutrients	KNO_3	25.0	10
	NH_4NO_3	6.2	
	$CaCl_2 \cdot 2H_2O$	4.4	
	$MgSO_4 \cdot 7H_2O$	3.7	
	KH_2PO_4	4.0	
Micro nutrients	$MnSO_4 \cdot H_2O$	10	100
	$ZnSO_4 \cdot 4H_2O$	8.6	
	H_3BO_3	6.2	
	$NaMoO_4 \cdot 2H_2O$	0.25	
	$CuSO_4 \cdot 5H_2O$	0.025	
	$CaCl \cdot 6H_2O$	0.025	
	KI	0.0075	
Vitamins	Nicotinic acid	0.1	100
	Pyridoxin- HCl	0.1	
	Thiamin-HCl	1.0	
	Myo-inositol	10.0	
Hormones	2.4 Dichlorophenoxy acetic acid	0.4	2.5
	Kinetin	0.5	0.9
	Naphthylacetic acid	1.0	10
Others	Fe-EDTA	36.7	1
	Saccharose	40.0	
	Agar	8	