ELSEVIER

Comparative Biochemistry and Physiology Part B 132 (2002) 359–365

CBP

www.elsevier.com/locate/cbpc

Antibacterial effect of a magnetic field on *Serratia marcescens* and related virulence to *Hordeum vulgare* and *Rubus fruticosus* callus cells

Elena Piatti[a,*], Maria Cristina Albertini[a], Wally Baffone[b], Daniele Fraternale[c], Barbara Citterio[b], Maria Piera Piacentini[a], Marina Dachà[a], Flavio Vetrano[d], Augusto Accorsi[a]

[a]*Università degli Studi di Urbino, Istituto di Chimica Biologica 'Giorgio Fornaini', Via Saffi 2, 61029 Urbino PU, Italy*
[b]*Università degli Studi di Urbino, Istituto di Scienze Tossicologiche, Igienistiche e Ambientali, Via S. Chiara 27, 61029 Urbino PU, Italy*
[c]*Università degli Studi di Urbino, Istituto di Botanica 'Pierina Scaramella', Via Bramante 28, 61029 Urbino PU, Italy*
[d]*Università degli Studi di Urbino, Istituto di Fisica, Via S. Chiara 27, 61029 Urbino PU, Italy*

Received 5 November 2001; received in revised form 6 February 2002; accepted 7 February 2002

Abstract

The exposure to a static magnetic field of 80 ± 20 Gauss (8 ± 2 mT) resulted in the inhibition of *Serratia marcescens* growth. Callus cell suspensions from *Hordeum vulgare* and *Rubus fruticosus* were also examined and only the former was found to be affected by the magnetic field, which induced a decreased viability. *S. marcescens* was shown to be virulent only toward *H. vulgare* and this virulence was reduced by the presence of the magnetic field. The modification of glutathione peroxidase activity under the different experimental conditions allowed us to speculate on the possibility of an oxidative-stress response of *H. vulgare* both to *S. marcescens* infection and magnetic field exposure. Since the control of microbial growth by physical agents is of interest for agriculture, medicine and food sciences, the investigation presented herein could serve as a starting point for future studies on the efficacy of static magnetic field as low-cost/easy-handling preservative agent.

Keywords: Callous cells; Magnetic field exposure; Antibacterial effect; Food preservative agent

1. Introduction

The control of microbial growth is necessary in many practical situations and significant advantages have been obtained in agriculture, medicine and food science by investigating this area of microbiology. This control usually involves the use of chemical or physical agents, which either kill microorganisms (cidal agents) or prevent their growth (static agents). Many chemical anti-microbial agents are able to kill or inhibit microbial growth (Krämer, 1992). Among them, preservatives are of significant importance in food, where static agents are used to inhibit the growth of microorganisms in that they are non-toxic. Nonetheless, physical agents (e.g. low temperature, freezing, drying and irradiation by short electromagnetic waves) should be preferred, when possible, to avoid the direct addition of substances potentially harmful for public health. On the other hand, if food of plant origin is taken into account,

*Corresponding author. Tel.: +39-722-305-242; fax: +39-722-320-188.
E-mail address: e.piatti@uniurb.it (E. Piatti).

1096-4959/02/$ - see front matter
PII: S1096-4959(02)00065-9

it must be considered that the soil contains a wide variety of life forms (including bacteria, fungi and nematodes) which can interact with plants. The relationships among these organisms living in the soil are complex, some being antagonistic and others being mutually beneficial. Indeed, one approach to the biocontrol of fungal and nematode pathogens is based on the disruption of chitin, which is widely present in the cell walls of fungi and in the outer covering of nematodes, by chitinase produced by certain naturally occuring bacteria and fungi (Nuero, 1995; Yun et al., 1996; Hollis et al., 1997).

The international debate on the problem of the possible biological effects of electromagnetic fields, in which we were involved in the past (Dachà et al., 1993; Piacentini et al., 2001), led us to test the possibility that such fields could be used as low-cost and easy-handling agents with which to inhibit microbial growth on plant tissues. Accordingly, we designed an experimental model in which the bacillus bacteria *Serratia marcescens* was added to callus cells from *Hordeum vulgare* L. (barley) and *Rubus fruticosus* L. (blackberry) in the absence or presence of an 80 ± 20 Gauss (8 ± 2 mT) static magnetic field (MF). The choice of *S. marcescens* was due to the fact that it has been found to be the most widely present member of the Enterobacteriaceae family in fresh horticultural products. *H. vulgare* and *R. fruticosus* were chosen because they represent two completely different plants of agricultural interest (the former is an annual herbaceous plant, while the latter is a perennial shrub). The results of the experiments allowed us to speculate on the possibility of an oxidative-stress response of *H. vulgare* both to *S. marcescens* infection and MF exposure.

2. Materials and methods

2.1. Isolation and growth of bacteria

S. marcescens was isolated from *Yucca elephantipes* (fam. Agavaccae) and identified using an API 20E system (Bio-Merieux, France). Bacterial cells were then grown for 18 h at 27 °C after inoculation in 20 ml of TSB (Triptone Soy Broth, Oxoid, UK). The concentration of cells was adjusted to 10^3 per ml by counting using a Thoma chamber. The *S. marcescens* culture was monitored every 6 h over a 24-h period to estimate the growth time course.

2.2. Plant cell culture and incubation preparations

Fifty caryopses of *H. vulgare* were placed in 200 ml of distilled water plus 1 ml of Tween 20 and stirred for 30 min. The caryopses were then washed with distilled water and sterilized with 90% ethyl alcohol for 30 s and with 2.5% (v/v) sodium hypochlorite under vacuum for 30 min. The caryopses were then rinsed three times with distilled water, dried with sterile filter paper and plated in batches of nine in Petri dishes (9 cm diameter) containing 20 ml of MS (Murashige and Skoag) medium pH 5.8 (the pH was adjusted before adding agar) supplemented with 4.5 mM 2,4-dichlorophenoxacetic acid (2,4-D), 20 g/l sucrose and 0.8% (w/v) agar. All cultures were incubated at 25 ± 2 °C under a light intensity of 50 mmol m^{-2} s^{-1} from cool, white fluorescent lamps under an 8-h photoperiod. After three weeks, calli obtained from caryopses in the micropilar zone were excised and placed in the same fresh medium under the same conditions. Every 4 weeks calli were subcultured and multiplied in the same medium. In a typical experiment, 2.5 g of calli were suspended in 50 ml of MS medium and incubated at 25 °C in an orbital shaker at 115 rpm for 24 and 48 h in the absence or in the presence of a loop of bacteria (10^3 cells/ml) from a 24-h-old culture.

Young stems of *R. fruticosus* were sterilized in 70% ethanol for 3 min, then immersed in 5% sodium hypochlorite for 15 min before being washed 3 times with distilled sterile water. The 5 mm-long explants were plated in Petri dishes on KM (Kao and Michayluk) medium pH 5.6 supplemented with 72 g/l of anhydrous glucose, 2 mg/l of α-naphthalenacetic acid (NAA) and 0.4 mg/l N^6 benzyladenine (BA), as reported by Mezzetti et al. (Mezzetti et al., 1997) and incubated in a control chamber at 25 ± 2 °C in the dark. When calli had completely formed (after 4 weeks) they were excised from explants and plated in the same fresh medium under the same conditions; subcultures were carried out every 30 days. In a typical experiment 2.5 g of calli were suspended in 50 ml of KM medium and incubated at 25 °C in an orbital shaker at 115 rpm for 24 and 48 h in the

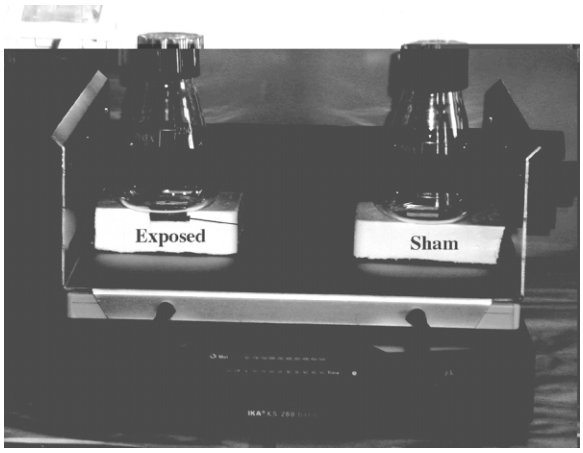

Fig. 1. MF exposure system. The orbital shaker does not interfere with the applied magnetic field.

absence or presence of a loop of bacteria (10^3 cells/ml) from a 24-h-old culture.

2.3. Magnetic field application

MFs were produced by metal magnetic disks of known intensities, placed under the cone-shaped flasks containing each sample (Fig. 1). Field magnitude and homogeneity of 8 ± 2 mT (80 ± 20 G) were checked by means of a gaussmeter (Hall-effect gaussmeter, GM04 Hirst Magnetic Instruments Ltd, UK). The choice of the intensity of the static MF used in our experiments was made according to the report by (Fanelli et al., 1999) in which this intensity was indicated by the authors as the minimum intensity necessary to induce effects on their animal cell model. For this type of exposure, no shielding against the natural variations of terrestrial MFs was required, their values (ranging at approx. 0.075 mT, 0.75 G) being negligible with respect to the MF intensities applied. Simultaneous experiments by omitting the magnet were performed as controls (sham).

2.4. Microscopic observations

Observations were made using a phase-contrast microscope coupled with a Hamamatsu 5985 camera. The images were acquired and visualized by a Macintosh computer. The same microscope magnification ($\times 200$) was used for both plant cell types.

2.5. Analysis of viable cells

Cell viability was assessed by a staining technique involving the addition of 3 ml of a stock solution of fluorescein (5 mg of fluorescein diacetate in 1 ml of acetone) to 1 ml of cell suspension. The samples were left at room temperature for 10 min in the dark and examined at 490 nm.

2.6. Enzyme and protein determinations

Ten ml of cell suspension were spun down and washed twice with 10 mM NaH_2PO_4, 10 mM Na_2HPO_4, pH 7.2 (PBS). The cell pellet was then resuspended in 1 ml (final volume) of 50 mM NaH_2PO_4, 50 mM Na_2HPO_4, 50 mM NaCl, pH 6.5 and lysed by sonic disruption (30 s in a Heat Systems Model 350 sonicator, Plainview, NY). Protein content of the lysate preparation was determined by the method of (Bradford, 1976), using bovine serum albumin as standard. Glutathione peroxidase (GSH-Px) activity was measured spectrophotometrically as reported by (Beutler, 1975). Control experiments were conducted to verify that bacterial contamination did not affect plant cell enzyme and protein determinations (data not shown).

2.7. Chemicals

All chemicals were of analytical grade. Solvents and reagents were from Sigma (USA).

3. Results

3.1. Virulence of S. marcescens toward H. vulgare and R. fruticosus callus cell suspensions

On the basis of *S. marcescens* growth time course (not shown), *H. vulgare* and *R. fruticosus* callus cells were incubated with *S. marcescens* for 24 and 48 h to evaluate bacteria virulence to the plant cells. As shown in Table 1, when *S. marcescens* cells were added to *H. vulgare* cell suspensions the number of bacterial cells in suspension in the medium decreased (Table 1, a vs. g), suggesting the presence of an interaction between the two cell types. The microscopic observation confirmed that an adhesion occurs between the two cell types (Fig. 2a, 1 vs. 2) and it appears to be virulent since a number of dead plant cells were also observed.

Table 1. Effects of MF on *S. marcescens, H. vulgare* and *R. fruticosus*, and on the virulence of the bacterium to the plant cells

Cell preparations and experimental conditions		Bacterial growth ($\times 10^6$/ml)		Plant cell number (% of values at 0 h)		Cell viability (% of values at 0 h)		Protein concentration (% of values at 0 h)		GS (UI/g of protein	
		24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h	24 l	
a-*S. marcescens*		3.15 ± 0.23	2.62 ± 0.2	–	–	–	–	–	–	–	–
b-*H. vulgare*		–	–	150 ± 2.8	225 ± 3	113 ± 2	132 ± 2	124 ± 7	150 ± 6	0.52 ± 0.08	0.63 ± 0.21
c-*R. fruticosus*		–	–	210 ± 4	230 ± 6	108 ± 3	156 ± 11	157 ± 3	171 ± 5	2.76 ± 0.65	2.30 ± 0.91
d-*S. marcescens*		1.85 ± 0.3^a	1.42 ± 0.1^a	–	–	–	–	–	–	–	–
e-*H. vulgare*	+MF	–	–	110 ± 3^b	158 ± 3^b	89 ± 3^b	75 ± 2^b	75 ± 5^b	63 ± 4^b	0.91 ± 0.07^b	0.76 ± 0.07
f-*R. fruticosus*		–	–	200 ± 4	240 ± 10	105 ± 1	186 ± 10	157 ± 5	164 ± 5	2.58 ± 0.5	2.36 ± 0.21
g-*S. marcescens* + *H. vulgare*		2.01 ± 0.3^a	1.49 ± 0.2^a	105 ± 0.25^b	8 ± 4.8^b	82 ± 1^b	41 ± 5^b	70 ± 2^b	47 ± 2^b	0.69 ± 0.06	1.91 ± 0.05
h-*S. marcescens* + R. fruticosus		3.5 ± 0.4	1.99 ± 0.3	195 ± 7.5	210 ± 10	110 ± 2	125 ± 5	200 ± 12	300 ± 15	2.99 ± 0.06	1.89 ± 0.11
i-*S. marcescens* + *H. vulgare*	MF	$1.1 \pm 0.1^{a,g}$	$0.04 \pm 0.01^{a,g}$	$115 \pm 1^{b,g}$	$50 \pm 3^{b,g}$	$102 \pm 0.25^{b,g}$	68 ± 3	$92 \pm 1^{b,g}$	$58 \pm 4^{b,g}$	0.6 ± 0.05	1.01 ± 0.06
l-*S. marcescens* + *R. fruticosus*		2.6 ± 0.16	2.4 ± 0.16	192 ± 8	200 ± 10	110 ± 3	142 ± 8	184 ± 8	291 ± 15	2.81 ± 0.06	1.78 ± 0.2

Data reported refer to 24 h and 48 h after the beginning of the incubation and are the means ± S.D. of 9 separate experiments. The superscript letter(s) to the values indicate that the datum differs significally ($p \leq 0.05$, paired *t*-test statistical analysis from the *Cell preparation and experimental conditions* denoted by that letter.

The effects of *S. marcescens* infection on plant cell suspensions were further investigated by determining cell number and viability, protein concentration and GSH-Px activity. As shown in Table 1 (b vs. g), in the presence of *S. marcescens*, *H. vulgare* cell number and viability, protein concentration and GSH-Px activity all decreased as compared to control values. None of the effects reported above for *H. vulgare* were observed when *R. fruticosus* cells were incubated with *S. marcescens* (Table 1, a vs. h, c vs. h and Fig. 2b, 1 vs. 2). Note that the microscopic observation performed with the same magnification on both plant cell types allowed a better detection of the presence of shrunken *H. vulgare* cells.

3.2. Effects of magnetic fields

When *S. marcescens* cells were grown in the absence and in the presence of an 80 ± 20 Gauss (8 ± 2 mT) static MF, a decrease in the number of cells was observed at 24 and 48 h of incubation in comparison with control (Table 1, a vs. d). Exposure of *H. vulgare* callus cells to MF induced a decrease in cell number and viability, a reduction in protein concentration and an increase in GSH-Px enzyme activity (Table 1, b vs. e). Microscopic observation revealed that many cells were severely damaged and remnant of cells were observed (Fig. 2a, 1 vs. 3). On the contrary, exposure of *R. fruticosus* callus cells to MF did not induce significant variations in the parameters evaluated (Table 1, c vs. f, and Fig. 2b, 1 vs. 3).

The effect of MF on the virulence of *S. marcescens* toward the plant cell suspensions was also evaluated. As far as bacterial growth is concerned, we still observed a decreased growth in the presence of MF, but *H. vulgare* cells, which responded to MF with a decrease in cell number and viability, and protein concentration, showed a recovery of these parameters upon the addition of *S. marcescens*. Only GSH-Px activity remained unaffected (Table 1, g vs. i). Microscopic observation showed that *H. vulgare* cells undergoing simultaneous exposure to MF and *S. marcescens*, appeared to be healthier than cells which were subjected to separate exposures to the two agents (Fig. 2a, 2 vs. 4; Table 1, i vs. g, Cell number and Cell viability columns).

As expected, *R. fruticosus* cells which were not infected by *S. marcescens* did not change their behavior even when exposure to MF was superimposed (Fig. 2b, 2 vs. 4).

Pretreatment with MF for 24 and 48 h affected neither the subsequent growth of *S. marcescens* nor its virulence vs. *H. vulgare* callus cells (data not shown).

4. Discussion

While continuing our investigations on the problem of the possible biological effects of electromagnetic fields (Dachà et al., 1993; Piacentini et

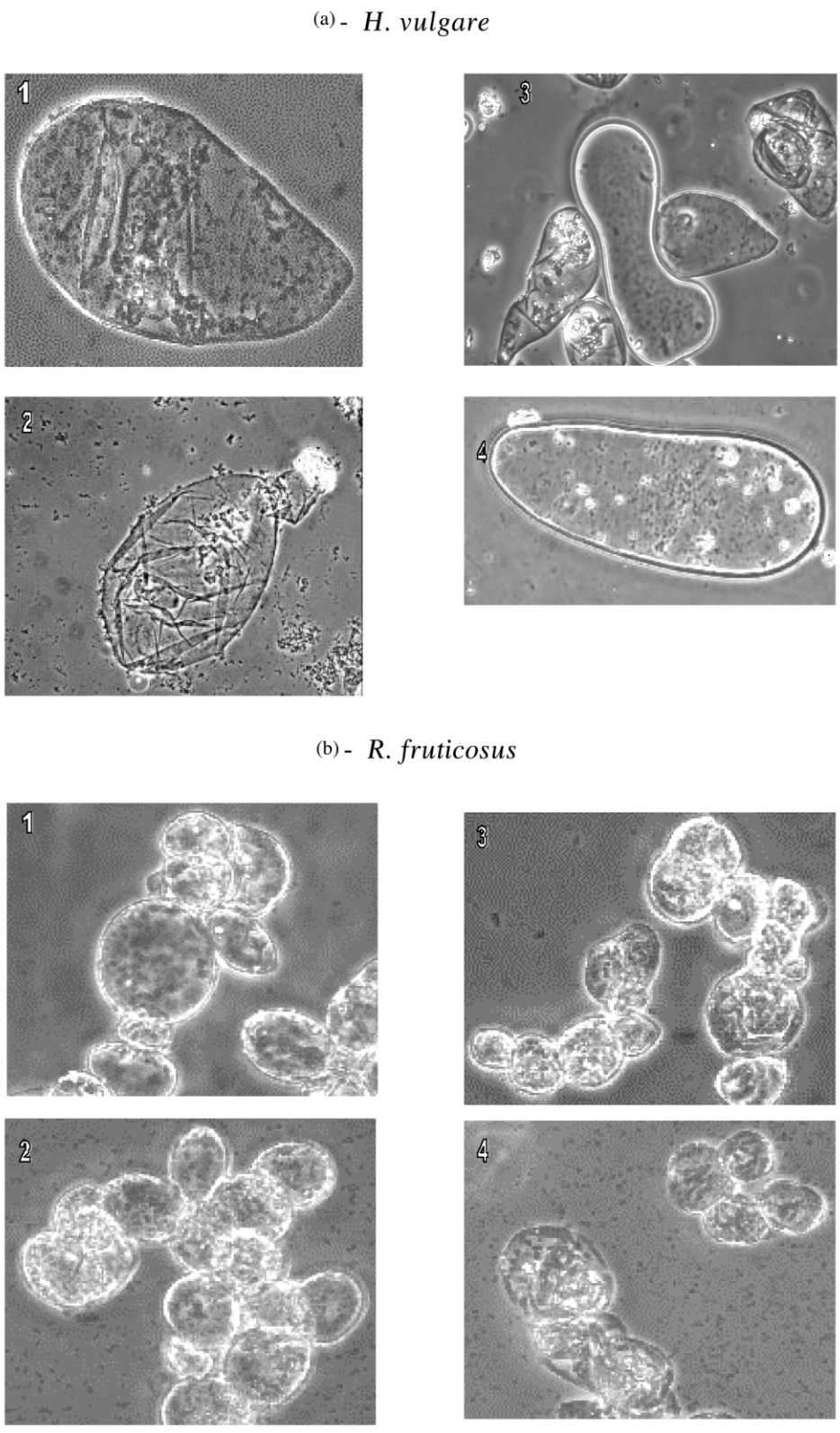

Fig. 2. Optical microscopy of *H. vulgare* (a) and *R. fruticosus* (b) callus cell suspensions. (1) Controls. (2) Infected with *S. marcescens*. (3) Exposed to magnetic field. (4) Infected with *S. marcescens* and exposed to magnetic field. Cells were photographed at the same magnification (×200).

al., 2001), in the present study we examined the effects of 80 ± 20 Gauss (8 ± 2 mT) static MFs on *S. marcescens* growth and the possible interaction of this bacterium with both *H. vulgare* and *R. fruticosus* callus cell suspensions.

The results of our experiments indicated that when the cells were separately submitted to MF, *S. marcescens* growth was inhibited and *H. vulgare* cell viability was diminished, while *R. fruticosus* was unaffected. It should be underlined that in *H. vulgare* the GSH-Px activity was significantly enhanced after 24 h of MF exposure, an effect observed by (Churin et al., 1999) in biotically and environmentally stressed *H. vulgare* leaves.

When *Serratia* was added to plant cells only *H. vulgare* was subsequently found to be infected. This result likely depends on the presence of lectins on the *H. vulgare* cell surface (Partridge et al., 1976; Cammue et al., 1985) which seem to be more specific for *Serratia* polysaccharides than are *R. fruticosus* lectins (Lienart et al., 1991). These proteins can bind *N*-acetylglucosamine-containing carbohydrates on the surface of *S. marcescens*, as supported by our microscopic observations. On the other hand, the addition of 500 μM *N*-acetylglucosamine to the medium hindered the adhesion between the two cell types (data not shown). In *H. vulgare*, cell number and viability as well as protein concentration, strongly decreased while GSH-Px activity was enhanced, confirming that the plant was under attack by the pathogen.

The exposure of *H. vulgare* cells to MF during *S. marcescens* infection produced a recovery of all the parameters evaluated in comparison with the parameters measured after MF exposure or bacterial infection alone. A partial explanation of the above results may be found by considering that an increase in reactive oxygen species (ROS) has been reported to be an early defense strategy used by plants to combat infection by pathogens (Wojtaszek, 1997) and that the increase in GSH-Px activity is a cellular response to protect itself from oxidative damage. On the other hand, Reiter (1997) suggested that EMF may prolong ROS half-life thus increasing their toxicity. Thus, the partial protection of *S. marcescens*-infected *H. vulgare* cells by MF exposure may be explained by a greater utilization of ROS which interfere with the bacterial growth (bacteriostatic effect) and a decrease in the negative effect on the growth and multiplication of plant cells.

With regard to our original goal of testing the possibility of using MFs to inhibit microbial growth on plant tissues, the results presented in this study show that this can be done only when the sole pathogen is MF-sensitive and the plant is not. Our observation that MF affected only *H. vulgare* and not *R. fruticosus* may reflect a profound difference between the metabolisms of the two plant species. As a result, it will be necessary to continue investigations on pathogen/plant pairs of agricultural and nutritional interest and to test the efficacy of these low-cost/easy-handling preservative agents.

References

Beutler, E., 1975. Red cell metabolism. A manual of biochemical methods. Grune and Stratton, New York.

Bradford, M.N., 1976. A rapid and sensitive method for the quantitation of microgram quantity of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254.

Cammue, B., Stinissen, H.M., Peumans, W.J., 1985. Lectin in vegetative tissues of adult barley plants grown under field conditions. Plant Physiol. 78, 384–387.

Churin, Y., Schilling, S., Börner, T., 1999. A gene family encoding glutathione peroxidase homologues in *Hordeum vulgare* (barley). FEBS Lett. 459, 33–38.

Dachà, M., Accorsi, A., Pierotti, C., Vetrano, F., Mantovani, R., Guidi, G., Conti, R., Nicolini, P., 1993. Studies on the possible biological effects of 50 Hz electric and/or magnetic fields. Evaluation of some glycolytic enzymes, glycolytic flux, energy and oxidoreductive potentials in human erythrocytes exposed in vitro to power frequency fields. Bioelectromagnetics 14, 383–391.

Fanelli, C., Coppola, S., Barone, R., Colussi, C., Gualandi, G., Volpe, P., Ghibelli, L., 1999. Magnetic fields increase cell survival by inhibiting apoptosis via modulation of Ca^{2+} influx. FASEB J. 13, 95–102.

Hollis, T., Honda, Y., Fukamizo, T., Marcotte, E., Day, P.J., Robertus, J.D., 1997. Kinetic analysis of barley chitinase. Arch. Biochem. Biophys. 344, 335–342.

Krämer, J., 1992. Lebensmittel-Mikrobiologie. 2nd ed. E. Ulmer GmbH and Co, Stuttgart.

Lienart, Y., Gautier, C., Domard, A., 1991. Isolation from *Rubus* cell-suspension cultures of a lectin specific for glucosamine oligomers. Planta 184, 8–13.

Mezzetti, B., Savini, G., Carnevali, F., Motti, D., 1997. Plant genotype and growth regulator interaction affecting in vitro morphogenesis of blackberry (*Rubus fruticosus*) and raspberry (*Rubus idaeus*). Biol. Plant. 39, 139–150.

Nuero, O.M., 1995. Production of chitinase by Fusarium species. Curr. Microbiol. 30, 287–289.

Partridge, J., Shannon, L., Gumpf, D., 1976. A barley lectin that binds free amino sugars. I. Purification and characterization. Biochim. Biophys. Acta 451, 470–483.

Piacentini, M.P., Fraternale, D., Piatti, E., Ricci, D., Vetrano, F., Dachà, M., Accorsi, A., 2001. Senescence delay and change of antioxidant enzyme levels in *Cucumis sativus* L. etiolated seedlings by ELF magnetic fields. Plant Sci. 161, 45–53.

Reiter, R.J., 1997. Melatonin aspects of exposure to low frequency electric and magnetic fields. In: Lin, J.C. (Ed.), Advances in electromagnetic fields in living systems, Vol. 2. Plenum Press, New York, pp. 1–27.

Wojtaszek, P., 1997. Oxidative burst: an early plant response to pathogen infection. Biochem. J. 322, 681–692.

Yun, D., Paino D′Urzo, M., Abad, L., Takeda, S., Salzman, R., Chen, Z., Lee, H., Hasegawa, P.M., Bressan, R.A., 1996. Novel osmotically induced anti-fungal chitinases and bacterial expression of an active recombinant isoform. Plant Physiol. 111, 1219–1225.