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Full Length Research Paper

Effect of Millimeter Waves with Low Intensity on Peroxidase Total Activity and isoenzyme Composition in Cells of Wheat Seedling Shoots

Anahit V. Nerkararyan, Mariam A. Shahinyan*, Marieta S. Mikaelyan, Poghos O. Vardevanyan

Yerevan State University, Faculty of Biology, Department of Biophysics, A. Manoogian 1, Yerevan, Armenia, 0025
*Corresponding Author: Phone (+374 10 571061); Fax (+374 10 554641); E-mail: m.shahinyan@ysu.am

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Abstract. In present work the effect of millimeter waves on both peroxidase total activity and isoenzyme spectrum in wheat seedling cells has been investigated. It is followed from obtained data that the influence of millimeter waves with low intensity on germinating seeds and plant extract invokes the changes of peroxidase isoenzyme spectrum of wheat shoots. At multiple effect of external field when seedlings are cultivated by irradiated water isoenzyme composition changes are observed as well. The enhancement of peroxidase activity is accompanied with isoenzyme number increasing which indicates that at external effects other genes controlling peroxidase synthesis are activated that results in changing of isoenzyme spectrum.

Key words: wheat seedling cell, peroxidase total activity, isoenzyme spectrum, millimeter waves, irradiation

1. INTRODUCTION

During evolution process plants produce the mechanisms of adaptation to unfavorable factors of surrounding. The ability of plant cells and organisms to react respectively to external effects is a necessary condition of existence and adjustment to surrounding medium. Among enzymes providing antioxidant protection of plants peroxidase plays an important role being responsible for controlling of H_2O_2 concentration and organic peroxides in cells (Alieva et al., 2010; Lin and Kao, 2001; Allison and Schultz, 2004; Csiszar et al., 2008). Peroxidase is one of marker enzymes and responds to wide spectrum of physiological processes in plants practically firstly activating to stress response (Ye et al., 1990; Zimmerlin et al., 1994).

This enzyme is localized in different organelles in plant cells (Troitskaya et al., 2000; Mensen et al., 1998). This fact supposes differential involvement of its isoenzymes into plant protective systems (Alieva et al., 2010; Siegel, 1993). Peroxidase is an enzyme possessing of pronounced polymorphism. The presence of great number of this enzyme isoforms permit them working in different conditions and realize different functional loading (Duroux and Welinder, 2003; Graskova, 2002). Variety of peroxidase isoenzymes is a result of changes of amino-acid composition of protein part of enzyme molecule, sugar composition of carbohydrate part or aggregation of low-molecular forms (Sadvakasova and Kunaeva, 1987). According to recent data there have been revealed more than 50 (Csiszar et al., 2008), and more than 73 genes (Veitch, 2004; Welinder et al., 2002; Delannoy et al., 2006) encoding peroxidase polypeptide sequence which has 308 amino-acids and comprises the proximal and the distal domens (Veitch, 2004). The changes of peroxidase activity, isoenzyme spectrum as well as thermostability are observed at different biological, physical and chemical effects on plants in different periods of their life cycle (Andreeva et al., 1979; Karpets et al., 2009).

Living organisms are being intensively exposed to electromagnetic irradiation (EMI) effect of extremely high frequencies (EHF) from artificial sources since the investigations of the effect of these waves on biosystems have great importance. It has been revealed that EMI EHF effects on different levels of living material organization (Babayan et al., 2006; Betskii et al., 2004; Devyatkov et al., 1991; Kalantaryan et al., 2010), including molecular level. It has been shown that this effect results in activity changing of many enzymes – lactate-dehydrogenases, alcoholedehydrogenases, peroxidases et al. (Nerkararyan et al., 2011; Vardevanyan et al., 2013). The mechanisms of EMI effect with low intensity on cells and the whole organism hitherto are not revealed.

In the present work the influence of EMI EHF on peroxidase total activity and isoenzyme spectrum in wheat seedling cells has been investigated.

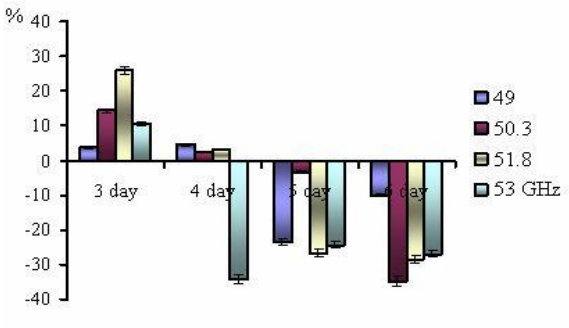

Fig. 1: The change of peroxidase total activity in cells of EMI EHF irradiated wheat seeds by different frequencies compared with control in percents.

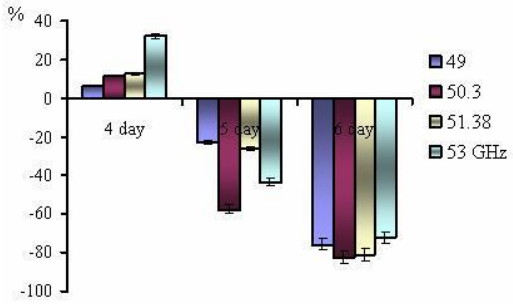

Fig. 2: Peroxidase total activity change in extract of wheat seedlings irradiated by EMI EHF with different frequencies compared with control in percents

2. MATERIALS AND METHODS

The wheat (*Triticum aestivum*) seeds of "Bezostaya" sort were used in experiments. Three approaches were applied: in the first case the early germinated seeds were irradiated – the dry seeds had been moistened by non irradiated water during 12 hours, and then these seeds were germinated and irradiated during germination one-fold, moreover they were cultivated by non irradiated water every day (in vivo irradiation). In the second case the plant extract from seedlings of non irradiated seeds was irradiated (in vitro irradiation). In the third case the seeds were moistened by previously irradiated water and they were cultivated by irradiated water of respective frequency and duration every day during their germination. The non irradiated germinating seeds were taken as control samples. The germination of seeds was realized in thermostat in 25^0C. The irradiation was performed using the generator G4-141 with working interval of 37.50-53.57GHz and power flux density 60μW/cm^2. Frequency signal stability was ±0.05□ and frequency deviation of output signal in persistent regime of generation did not exceed 6MHz. Irradiation with 30min duration was realized at following frequencies: 49GHz, 50.3GHz, 51.8GHz and 53GHz. Plant extract was obtained in 0.15M tris-HCl buffer containing 0.1M DTT (1,4-dithiothroitol), pH 8: 0.2ml buffer was added to 100mg plant material and the shoots of germs were grinded in cold pounder. Extraction was carried out on magnetic mixture during 30 minute in refrigerator. The obtained mass was centrifuged during 15 min with 18000g. The supernatant liquid was used in experiments.

Peroxidase activity was determined in pirogallol (1,2,3-threeoxibenzol) oxidation reaction (Baden and Corbett, 1979) by optic density changing of reaction mixture during 2min, at room temperature and 450nm, by spectrophotometer SF-4A. The reaction started since the moment of injection of 0.15M H_2O_2 solution into reaction mixture. Reaction mixture contains 1.1ml H_2O, 0.8ml 0.06M Na-P (potassium-phosphate)

buffer (pH 6.8), 0.5ml 0.003M pirogallol, 0.2ml plant extract, 0.2ml 0.15M H_2O_2. Instead of extract 0.2ml distillated water was added into control solution. Solution of H_2O_2 was added immediately before optic density measurement. Protein amount was determined by Lowry method (Lowry et al., 1951). Peroxidase total activity was calculated by following formula:

$$A = \frac{\Delta D \cdot f}{c \cdot t}$$

Where A is the total activity of enzyme (optic density/min per 1mg protein), ΔD – optic density change, c – protein amount (mg/ml), f – dilution coefficient, t – optic density measurement duration (min).

Division of isoenzymes was carried out in 5.5% polyacrilamide gel by disc-electrophoresis method (Dietz and Lubran, 1967), in glass-tubes (6×100mm). The tubes were filled with gel solution up to 60mm mark, on which the water was carefully stratified for formation of smooth gel surface. Polymerization was carried out under quartz lamp (Q-139) during 2-2.5 hours. Polymerization finishing was fixed by well-defined boundary line between water and gel. Water was moved off, tubes were fixed in cells of electrophoresis apparatus, 0.2ml 40% saccharose solution and plant extract containing 0.6mkg protein were added to gel. Gel solution and electrode buffer were stratified on extract. To form marks 0.7ml 10^{-5}% brome phenol blue solution was added in cathodic buffer. Electrophotetic division was carried out at 7-9^0C during 1.5 hours. During the first 30 min persistent current was given with consideration of 205mA per tube then current strength was increased up to 5mA per tube. Electrophoresis was considered completed the mark reaches to the tube end. After electrophoresis gels were stained by the following method (Safonov and Safonova, 1969). Gels were pulled from tubes, flooded by reaction mixture solution to reveal isoenzymes then put in thermostat. Incubation was carried out during 30min at 37^0C. After incubation gels were washed by water and filled with 0.002% H_2O_2 then were sustained up to 1min. After processing of H_2O_2 gels were fixed by 7% acetic-acid solution. The statistic treatment of obtained data was performed.

3. RESULTS AND DISCUSSIONS

In this work the results of studies on EMI EHF effect on peroxidase total activity and molecular form composition in wheat seedling cells have been represented.

The changes of peroxidase total activity having different direction during growth process of irradiated wheat seedlings (fig. 1) and the increasing of molecular forms of peroxidase were revealed. The certain regularity between peroxidase total activity changes and number of molecular forms was observed. The change of peroxidase total activity of seedlings of wheat irradiated seeds compared with control was represented on fig. 1. Peroxidase 9 isoenzymes were revealed in control variants.

As it is obvious from represented figure, in the third day after irradiation peroxidase activity increases by 3.8%, 14.5%, 25.95% and 10.69% compared with control at irradiation with 49GHz, 50.3GHz, 51.8GHz and 53GHz frequency respectively. In the fourth day peroxidase activity of seedling shoots irradiated by millimeter waves with 49GHz, 50.3GHz, 51.8GHz remains higher than control values by 4.53%, 2.27%, 3.15% respectively, but in seedling shoots irradiated by 53GHz frequency the significant decreasing of enzyme total activity by 34.2% compared with control is observed. In the 5^{th} and 6^{th} days peroxidase activity decreases compared with control in the whole experimental variants.

The following regularity is observed: the higher peroxidase activity change compared with control values the bigger number of isoenzymes in isoenzyme spectrum. At small activity change peroxidase isoenzyme number is equal to that of control. In the 2^{nd} and 3^{rd} days after irradiation the increasing of slow migrating (cathodic) isoenzymes is observed. In the 4-5 days after irradiation in shoot cells peroxidase new molecular forms appear: quickly migrating (anionic) isoenzymes and isoenzymes with average mobility. The magnitude of change of number of fractions in seedlings irradiated by EMI of different frequencies compared with control is different. The maximal number of fractions (12-15) is revealed in seedlings irradiated EMI with 50.3GHz and 51.78GHz frequencies in 3-5 days after irradiation. In the further days, number of fractions decreases. According to several data at stress peroxidase genes characteristic for embryo cells are activated in plant cells by which isoenzyme amount increasing at surrounding medium condition change is explained (Alieva et al., 2010). The effect of EMI EHF on DNA is revealed in other

works as well (Babayan et al., 2006; Betskii et al., 2004; Devyatkov et al., 1991; Kalantaryan et al., 2010), since it may be assumed the mentioned frequencies of EMI effect on DNA structure. It may be also assumed that the decreasing of number of fractions in peroxidase isoenzyme spectrum indicate that organism overcomes stress state.

The effect of irradiation on peroxidase activity at in vitro irradiation was also investigated (fig. 2).

As it is presented from fig. 2, EMI EHF irradiation with different frequencies of extract of seedlings obtained from non irradiated seeds results in peroxidase total activity changing. Moreover the magnitude of change compared with control is higher than in shoots of irradiated seeds. Peroxidase total activity change may be conditioned by enzyme conformational change connected with water structure change under EMI effect with water resonant and near them frequencies.

The investigation of effect of EMI EHF irradiated water on germinating seeds was carried out as well. Peroxidase total activity change in cells of seedlings cultivated EMI irradiated water with different frequencies is represented on fig. 3.

Daily cultivation of seedlings by irradiated water shows that the effect of external physical factor appears earlier and peroxidase total activity change magnitude is more than at one-time irradiation of wheat germinating seeds. The enhancement of peroxidase total activity is accompanied by increasing of number of isoenzymes: the number of isoenzymes at first sharply increases – in 2nd day is becomes equal to 17, 17, 17, 15 in EMI irradiated variants with 49GHz, 50.3GHz, 51.8GHz and 53GHz frequencies respectively which indicates that organism is in stress state (Ye et al., 1990; Zimmerlin et al., 1994). In the following days, apparently, the processes develop in organism directed to decreasing of external physical field effect. Number of isoenzyme fractions and peroxidase total activity gradually decrease meanwhile the stress overcoming takes place more slowly than in seedlings exposed to one-time irradiation.

EMI EHF irradiation with low intensity changes water structure in surface layer of irradiated object which results in changing of water properties. The latter may initiate change of physica-chemical properties of cellular membranes that induces the chain of sequential processes in result of which biosystem response to external physical field effect is formed. Peroxidase total activity increasing (Nerkararyan et al., 2011; Vardevanyan et al., 2013) induced by irradiation of seedlings may be conditioned by either activation of genes controlling peroxidase synthesis or changes on epigenetic level, particularly disturbance of enzyme conformation induced by change of water properties.

This may result in changing of quantitative ratio of enzyme molecular forms conditioned by different sensitivity of isoenzymes to changes of solvent properties. This fact corresponds to data of investigations devoted to EMI EHF effect on DNA structure (Babayan et al., 2006; Kalantaryan et al., 2010).

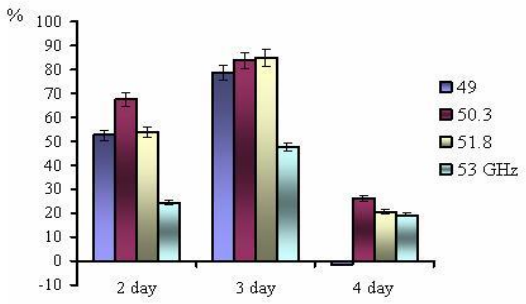

Fig. 3: Peroxidase total activity change in cells of wheat seedlings cultivated by EMI EHF irradiated water with different frequencies compared with control in percents

4. CONCLUSION

Therefore from obtained data it may be concluded that EMI EHF effect with low intensity on germinating seeds and plant extract invokes peroxidase isoenzyme spectrum changes of wheat shoots. Moreover irradiation of seedlings in organism triggers the chain of sequential processes that results in lowering of external physical factor effect while in extract such processes are not observed. At multiple effect of external field when seedlings are cultivated by irradiated water isoenzyme composition changes are observed as well. Since peroxidase activity increasing is accompanied by increasing of number of

isoenzymes, in all appearances, at external effect other genes controlling peroxidase synthesis that results in isoenzyme spectrum changing.

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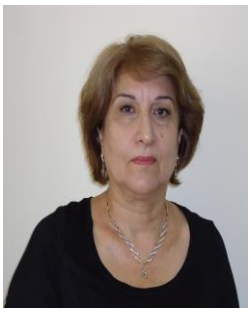

Anahit V. Nerkararyan, Associate Professor at Department of Biophysics, Yerevan State University, Faculty of Biology, Candidate of Biological Sciences, Armenia.

Mariam A. Shahinyan, Junior Scientific Research at Department Biophysics, Yerevan State University, Faculty of Biology, Candidate of Biological Sciences, Armenia.

Marieta S. Mikaelyan, Senior Laboratory Assistant at Department Biophysics, Yerevan State University, Faculty of Biology, Armenia.

Poghos O. Vardevanyan, Head of Department Biophysics, Yerevan State University, Faculty of Biology, Doctor of Biological Sciences, Professor, Armenia.