

EFFECT OF MICROWAVE ON THE DYNAMICS OF SOME OXIDOREDUCTASE ENZYMES IN *BRASSICA NAPUS* GERMINATION SEEDS

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Abstract: This paper presents the effect of microwaves on the catalase and peroxidase activity in rape germinating seeds. Two experimental lots were projected: one in which the non germinated seeds were exposed 1 hour, respectively, 5 hours, to microwave rays, and the second in which the germinated seeds (after 24 hours) were exposed to microwave action for the same interval. We used a microwave generator with a horn antenna, at the 10.75 GHz and a power density under 1 mWcm⁻¹. For a period of 8 days, the dynamics of the catalase and peroxidase activity were analyzed at 2 days, 4 days, 6 days and 8 days. The results showed that the microwaves determined variations of catalase and peroxidase activities depending on the age of the plants, time of exposure to microwave action and state of seeds (germinated and non germinated) exposed to microwave.

INTRODUCTION

Some areas are affected by high frequency microwaves affecting germination, plant growth and enzymatic activity which determined the decreased productivity of crops. Plants and stress presents a great importance lately as a field of study and research. Among the various types of stress can remember: temperature, drought, salinity, microwaves and from these the last have a major importance. Microwaves are at the heart of these studies because of the drastic effect that they have not only the body but also on animals, plants and microorganisms. It was found that microwaves affect plant growth and the prolonged exposure to microwave affects seed germination. The microwaves weak intensity does not affect plant growth but the dose increased influence by the meaning of slowing the germination.

The effect of electromagnetic microwave irradiation on agricultural crops has scarcely been studied yet. However, some studies regarding the effect of electromagnetic microwave irradiation on wheat detected increased of the germination (Bhaskara et al. 1998). Also, Bhaskara et al. 1995 used successfully electromagnetic irradiation of the radio-frequency range and of the microwave range on seeds of charlock, wheat, soybeans, peas and rice with the aim of pre-storage destroying of the microorganisms. Yoshida et al. 2000, treated soybean seeds with microwave rays for 6 to 12 minute for improving the triglycerides distribution in the seed coat. Yi-Ping Chen, 2006 revealed in their results that microwave pretreatment of *Isatis indigotica* seeds enhanced UV-B stress resistance in the seedlings, increasing the activities of catalase, peroxidase, and superoxide dismutase. All these results suggest that microwave radiation enhances plant metabolism. The effect of microwave treatment on the seeds and their characteristics of vitality has not been fully explained (Aladjadjiyan A., 2002). Some authors have conducted studies regarding the effect of microwaves on seed rape to enhance oil production (Valentová et al. 2000, Novotná et al., 1999, Oberndorfer et Wolfgang, 1999). The used of microwave on the germinations seeds may have other utilizations, such as for example it was demonstrate that microwave induced stimulation of L-DOPA, phenolics and antioxidant activity in fava bean (*Vicia faba*) for Parkinson's diet (Reena et Kalidas, 2004).

This paper presents the influence of microwaves (various periods of time) on the activity of catalase and peroxidase during seeds germination of *Brassica napus*, one of the main plants used as biofuel.

MATERIALS AND METHODS

Biochemical researches have been conducted in the laboratory of Molecular Biology and Experimental and exposure to microwaves of the two lots was done in the Applied Physics Laboratory. The research was conducted on seeds of *Brassica napus*, which were exposed to different time on microwave action. For this we used a microwave generator with a horn antenna, at the 10.75 GHz and a power density under 1 mWcm⁻¹. Rape seed analyzed were divided into two groups: germinated and ungerminated. The seeds of the first batch were made in advance germinated 24 hours before the experiment. First seeds have been disinfected with peroxide 3%, then rinsed with distilled water, after which they were transferred into sterile Petri plates, containing 2 layers with paper Whatman 1, steeped in 10 ml distilled water. In each Petri plate were put every 1000 seeds and were carried out three rehearsals for each variant. Seeds were kept at room temperature under controlled conditions and a wet day for 8 days. Each batch of seeds (germinated and ungerminated) was exposed to microwaves action different periods of time, an hour and 5 hours, too. At the same it was release two controls were not exposed to microwaves action. Biochemical investigations were carried out in different phases of seedling development samples and the sample were collected at an interval of 48 hours, correlated with the growth and development of vegetative for 8 days (Figure 1).

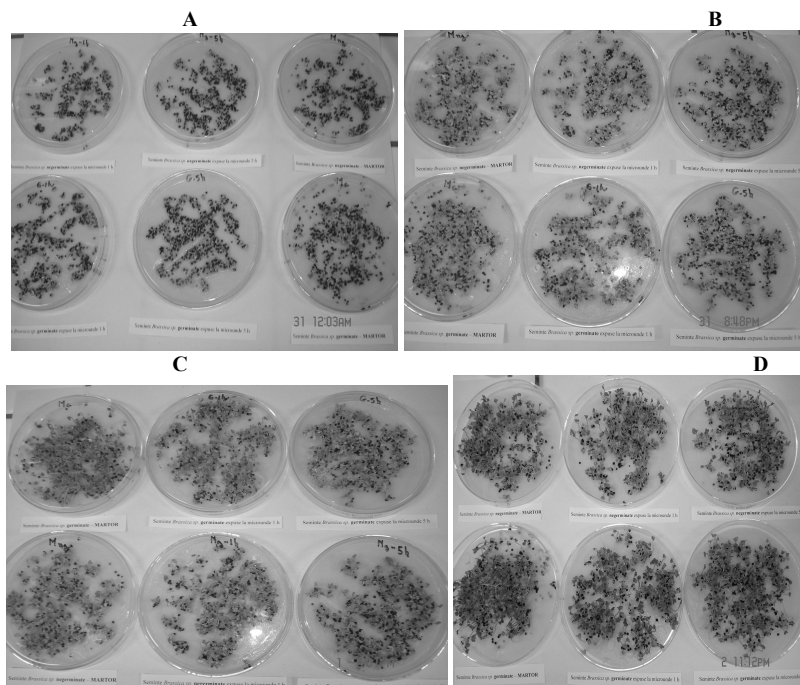


Fig. 1. Aspects of rape seed (germinated and ungerminated) to 2 days (A), 4 days (B), 6 days (C) and 8 days (D) from exposure to microwave

Determination of catalase activity was done by titration method with sodium thiosulphate, while peroxidase activity was determined by spectrophotometric method with o-dianisidine, according to standard method (Artenie et al., 2008).

RESULTS AND DISCUSSIONS

Plants, as most aerobics bodies have developed complex enzymatic and non-enzymatic mechanisms protection to eliminate or reduce the reactive oxygen intermediates (ROI) (Beak and Skinner, 2003). In normal growth conditions, training ROI is achieved with a reduced rate and has an important role in the signaling processes which indicates stress (biotic or abiotic, apoptosis etc). ROI are trained in plant cells that bioproducts of metabolic reactions are toxic products resulting from the reduction of oxygen and formed continuously in cells during metabolism or stress (Allen, 1995, Asada and Takahashi, 1987, Mittler, 2000). Under normal circumstances, the production and removal of ROI is well regulated in metabolic cells (Mittler, 2000). When a plant faces harsh conditions, producing ROI will overcome the elimination and oxidative stress is triggered. The antioxidant enzyme is one of the protective mechanisms which include superoxide dismutase to transform superoxide ions in H_2O_2 and O_2 . The catalase (CAT, EC 1.11.1.6) and peroxidase (PER, EC 1.11.1.7), two other antioxidant enzymes have role in converting H_2O_2 in H_2O and O_2 (Scandalios, 1993, Gara et al, 2003).

Catalase and peroxidase enzymes are involve in various physiological processes by protecting the bodies of free radicals but in conditions of stress is found modifying of their activities. While catalase acting on the large quantities of H_2O_2 present in tissues (vegetal or

animal) that occurs as a result of metabolic changes, peroxidase catabolized small quantities of H_2O_2 remaining undecomposed by catalase.

Exposure 1 hour to the microwaves action the **ungerminated rape seed** stimulates the catalase activity in all intervals chosen for measurement (Fig. 2). Thus, the enzyme activity shows a downward trend as developing seedling up, maximum being at 2 days age (1578.16 UC/g) and less at 8 days (1081.08 UC/g). In case of exposure 5h to microwaves it remark a relatively constant catalase activity comparable to control at seedling in 2 days age, reaching a maximum after 4 day (1568.62 UC/g) and decreases gradually to 8th day (464.67 UC / g).

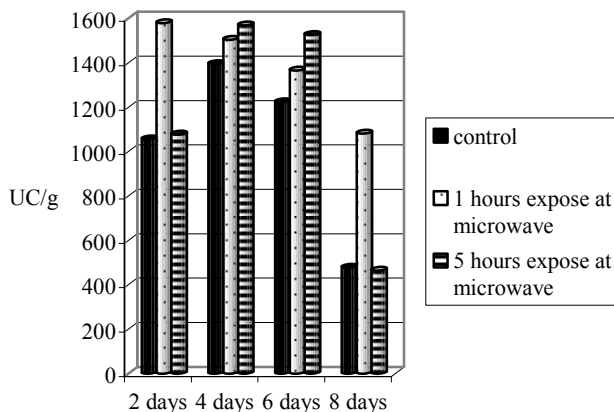


Fig. 2. Dynamics of catalase activity in **ungerminate rape seed** expose to microwaves

Results on catalase activity in the **germinated rape seed** expose to microwaves for 1 hour indicates that this physical factor determines the stimulation of enzyme activity (in the first 6 days), this range between 1695.47 UC/g and 532.68 UC/g (Fig. 3). Unlike seed ungerminate where catalase activity diminishes as seedling development, the situation is altered in the case of germinated seeds when the enzyme activity presents a rising trend until the 6 day when is maximum. The enzymatic activity in the 8 day of collection is lower in relation with control (532.68 UC/g compared with 586.18 UC/g).

Exposure the germinated seed rape a longer period (5 hours) at the microwaves determine generally the same trend of catalase activity as in the case of ungerminate seed, but the magnitude is different probably explained by the fact that the seeds germinated metabolic activity presents a more pronounced compared the ungerminate. Enzyme activity ranged from 387.09 UC/g to 1755.63 UC/g, the minimum is observed when the seedling are 8 days old, while the maximum in a 4th-day of harvest.

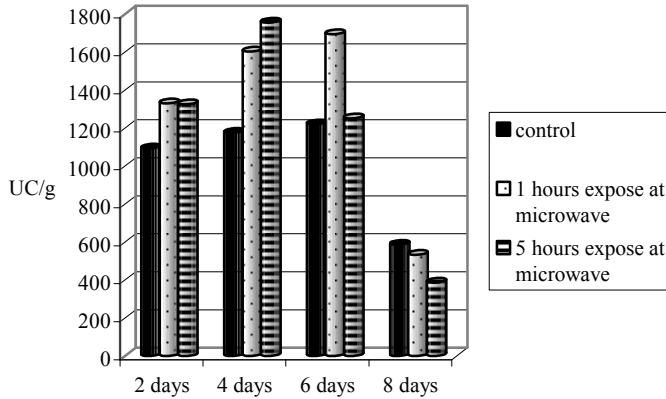


Fig. 3. Dynamics of catalase activity in **germinate rape seed** expose to microwaves

Analyzing the results regarding the exposure of **ungerminate rape seed** to microwave (1 hour and 5 hours) it is observed a stimulation of the peroxidase activity at seedling harvested in the second day, the rest of variants present an inhibitory effect (Fig. 4). Exposure for 1 hour to the microwave causes a slightly higher comparatively with control on the second day of the harvest seedling (0.53 UP/g vs. 0.59 UP/g), after which diminishes 1.46 time at 4 days, 1.07 time at 6 days and 1.8 times at 8 days of harvest. Peroxidase activity is not uniforme, oscillate between 0.59 UP/g and 1.41 UP/g reduced in the first 4 days and higher in the last day of the harvest.

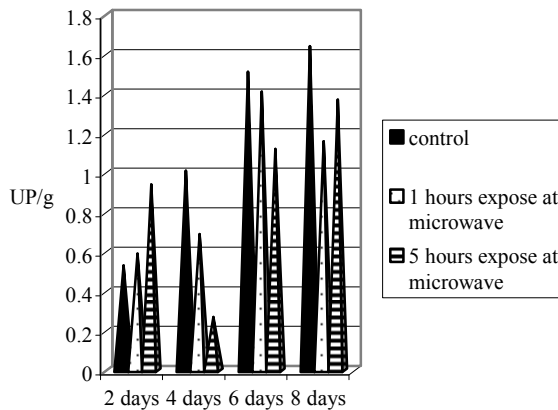


Fig. 4. Dynamics of peroxidase activity in **ungerminate rape seed** expose to microwaves

The peroxidase activity of ungerminate seed rape exposed 5 h to microwaves presents different swing to the control ranging from 0.27 UP/g and 1.37UP/g. All experimental variants excepting those harvested in 4 days presents peroxidase activity an upward trend as developing seedling up.

In the case of **germinated rape seeds** exposed 1 hour to the microwaves peroxidase activity is stimulate only to the seedling harvested on the 6 day. Enzyme activity varies between 0.49 UP/g and 1.71 UP/g intensify as development of the seedling. Analysis of the results obtained as a result of exposure 5h to microwave shows that the peroxidase activity range between 0,7 UP/g and 1,36 UP/g. The exposure prolonged at this physical factor causes at all variants analyzed an inhibition of peroxidase activity in relation to the control, more pronounced in particular on the day of the 6th.

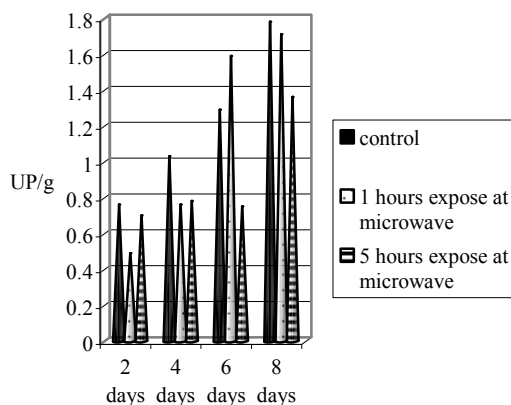


Fig. 5. Dynamics of catalase activity in **germinate rape seed** expose to microwaves

CONCLUSIONS

The results showed that the microwaves determined variations of catalase and peroxidase activities depending on the age of the plants, time of exposure to microwave action and state of seeds (germinated and non germinated) exposed to microwave.

In the second and fourth days after effect of microwaves on the germinated rape seed it is remark the stimulated of catalase activity, while the peroxidase activity is inhibited. The following intervals studied, present a similar behavior with regard to the work of the two enzymes.

Application of physical treatment microwave on the ungerminate rape seed causes stimulating of catalase activity and inhibition of peroxidase activity (except for variations harvested at 2 days).

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