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# Influence of magnetic fields on the activity of enzymes: α- and β-amylase and glutathione S-transferase (GST) in wheat plants

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A b s t r a c t. The paper presents the impact of magnetic fields on enzyme activities in plants. Three species of wheat with different ploidy levels were used in the experiments: *Triticum monococum* (diploid), *T. dicocum* (tetraploid), and *T. aestivum* (hexaploid). Air-dry seed samples, made up of 100 seeds each, were treated with an alternating magnetic field of low frequency (16 Hz) for 2 h. The control samples were not tested with the magnetic field. After the 13th day of magnetic field treatment, measurements were conducted on the following enzymes: α- and β-amylase and glutathione S-transferase. The magnetic field caused a reduction in the activity of alpha- and beta amylases. This can be really important in breeding and seed production and in certain sections of the agricultural and food industry. Plants grown from treated seeds will be more resistant to sprouting in the future. The magnetic field caused a higher activity in the glutathione S-transferase enzyme. It caused that the plants have a higher resistance to pathogen attack, oxidative stress, and heavy-metal toxicity.

K e y w o r d s: magnetic field, germination of wheat, magnetic biostimulation, alpha-amylase, beta-amylase, glutathione S-transferase

## INTRODUCTION

Examination of the impact of magnetic fields on the activity of enzymes is a new concept in science. In accessible literature there are only a few publications on this subject. In previous publications, only the impact of a frequent magnetic field was investigated on the activity of respiratory enzymes: peroxidase, catalase and superoxide dysmutase (Tugulea *et al*., 2000; Xi-G and Fu, 1993) *ie* enzymes connected with chlorophyll synthesis, and esterase (Davies *et al.*, 1969). This publication tries to explain the impact of a ma gnetic field on the activity of enzymes: alpha- and beta-amylase and glutathione S-transferase.

Amylases are very popular in plants. There are two main types of amylases:
– Endoamylases which attack alpha-1,4 bond in polymers in an incidental way;
– Exoamylases which attack alpha-1,4 bond only from the non-reduction ending of the substrate chain (Davies *et al*., 1969).

Alpha- and beta-amylases belong to the hydrolase class and were the earliest enzymes detected. They decomposed hydrolysed starch, glycogen and similar oligo- and polysaccharides (Toczko and Grzelińska, 1997).

Amylases are typical digestive enzymes which are popular in animals, plants and microbes. Particularly a lot of alpha- and beta-amylases are present in the course of germination of cereal grains. The level of alpha- and beta-amylases significantly increases during the germination of cereal grains.The action of alpha-amalyse results in a rapid drop in viscosity of the substrate, a change in the colouring complex with iodine, and a very slow-acting reduction in reaction mixture. Alpha-amylases are activated because of the $Ca^{+2}$, $Zn^{+2}$, $Cl^{-}$ ions (Toczko and Grzelińska, 1997).

An increase in the alpha synthesis can lead to a ripening of cereal grains during sprouting (premature germination) (Dinh and Masojć, 1994).

Beta-amylase acts on substrates (starch, amylose, glycogen) from the non-reduction ending of the substrate chain and that is why beta-amylase is called exoamylase. Beta-amylase causes the hydrolysis of every second alpha-1,4 glycoside, tearing out the rest of the beta-maltose.

Beta-amylose which comes from plants displays an optimal activity in the range of pH 4-5.5, while bacterial beta-amylose acts in the range of pH 6-7.

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Amylases hydrolysing substrates are independent of the chain length and the degree of branching and they cause a break in the glycoside bond between the dioxide glycoside and the oxygen of the glycoside bond (Toczko and Grzelińska, 1997).

The high activity of amylases in seeds proves that they initiate starch wrestling (Davies *et al*., 1969). Alpha-amylase breaks glycoside bonds in the middle of the polysaccharide chain. Their activity creates a mixture of glucose, maltose, isomaltose and oligo-saccharides. Beta-amylase tears off the rest of the maltose from the non-reductive end of the polysaccharide chain. If the substrate is glycogen, the amylopective enzymatic reaction gets stopped at the point of the fork of the polysaccharide chain. Mixtures of maltose and dextrias are created.

Glutathione S-transferase (GSTs) belongs to the super-family of most cytosolic enzymes found in almost all organisms. This 'family' fulfils a detoxifying function. The GSTs catalyse the conjugation of tripeptide glutathione (GSH) to endogenous or exogenous electrophilic substrates (Pascal and Scalla, 1999). They are one of the most important systems of defence against carcinogenic compounds. Glutathione transferase enzymes conjugate isothiocyanates, leading to excretion (Lin *et al*., 1998). GST in plants were identified first and have been intensively studied because of their ability to detoxify herbicides, and individual GSTs conferring herbicide tolerance have been characterized from most major crop species. Recently, another GST subclass plant has been implicated in numerous stress responses, including those arising from pathogen attack, oxidative stress and heavy-metal toxicity (Riechers *et al.*, 1997).

Many plant species contain GST activity when assayed with xenobiotic and potential endogenous substrates.

They have recently been studied in maize, where they are responsible for tolerance to triazines, chloroacetamides and thiocarbomate sulfoxides. GSTs would be involved in the protection of important cell components against a toxic reactive form of oxygen (Pascal and Scalla, 1999).

There are at least three groups of glutathione-transferases: theta (θ), tau (τ) and dzeta (ζ). The theta class contains the maize (*Zea mays*), and the dzeta class is limited. Tau glutathione-transferase was characterized from maize too and resembled tau glutathione-transferase from dicotyledonous plants which metabolise herbicides (Cole *et al*., 1998). Glutathione S-transferase from the tau (GSTU) class is unique to plants and plays an important role in stress tolerance and secondary metabolism as well as in catalysing the detoxification of herbicides in crops and weeds (Thom *et al.*, 2002).

For example cruciferous vegetables, especially broccoli which prevents cancer, contain isothiocyanates that induce carcinogen-detoxifying enzymes (Masojć *et al*., 1997).

The investigation described in this paper indicates a strong, positive impact that the magnetic field has on the activity of enzymes: alpha-, beta-amylases and GSTs.

## MATERIALS AND METHODS

Three species of wheat of different ploidy levels: *Triticum monococum* (diploid), *T. dicocum* var Polonicum (tetraploid), *T. aestivum* var. Sigma (hexaploid) were used for the experiments.

Seeds after treatment with magnetic fields were examined with the following laboratory tests:

– the activity of enzymes:

- α- and β-amylase,
- glutathione S-transferase (GST).

Air-dry seeds were treated with an alternating magnetic field of low frequency (16 Hz) for 2 h. Magnetic flux density was 5 mT. The magnetic field generator was constructed by the University of Technology in Wrocław. The generator produces a homogenous magnetic field of a 16 Hz frequency and strictly determines the magnetic flux density. The design of the generator allows the elimination of electric components in the field and enables the treatment of seeds with frequent magnetic fields. To avoid overheating seeds, the equipment is cooled by air stream. The samples were made up of 100 seeds each. The control samples were not tested on magnetic fields. The activity of enzymes was examined after 13 days of magnetic field treatment.

The activity of alpha- and beta-amylases was investigated for two-day seedlings of every wheat species. For each sample, 1.5 g of weighed seedlings which were grown from seeds treated with magnetic fields and controlled ones were tested. Then the plant material was grated in a mortar with 10 ml $H_2O$. The obtained homogenate was twisted for 10 min at 8 500 g (10 000 r.p.m.) in K-24 creamer. Then the solution was filtered through cotton wool into a test-tube and diluted with $H_2O$. The activity of α- and β-amylase was determined with the Bernfeld method (Toczko and Grzelińska, 1997).

The results were calculated as: μmole of beta-amylase per 1 g of wheat seedlings and reactions per minute and mg of starch hydrolysed by alpha-amylase in 1 g of wheat seedlings per 1 min.

The GST activity was also determined in two-day seedlings of each species. The seedlings were cut and placed into a tube. Next, the tissue was macerated at room temperature in a cold extraction buffer (a 100 mg of tissue used up to 100 μl) using a grinder. Then it was centrifuged for 10 min at 14 000 r.p.m. in a cold rotor. The supernatant was transferred into a fresh eppendorf tube. The reaction buffer was added (120 μl per each sample) and the reaction was started by adding 6-12 μl of enzyme extract.

After obtaining the conjugate, the enzyme activity was measured by a spectrometer at 340 nm. The enzyme activity was calculated, knowing that the extinction coefficient for conjugate is 9.6 $mM^{-1}$ $cm^{-1}$, and divided by the time of reaction and the protein concentration in the sample (Jakoby, 1981).

**Reagents:**

Extraction buffer:
100 mM Tris-HCl, pH 6.5
1 mM EDTA

Reaction buffer:
100 mM Tris-HCl, pH 6.5
1 mM glutathione
1 mM 1-chloro-2,4-dinitrobenzene (CDNB) – soluble in DMF.

## RESULTS AND DISCUSSION

### Impact of the alternating magnetic field on the activity of enzymes

The activity of α- and β-amylase in wheat seedlings grown from seeds treated with frequent magnetic fields and untreated (control) is shown in Table 1. The function of these enzymes is hydrolysis of the reserved polysaccharides of seeds, specifically starch, mono- and oligosaccharides. The amylases put the starch supplies in motion and then prepare the seeds for germination.

As seen from the obtained results, seedlings grown from seeds treated with magnetic fields are characterized by a lower activity of enzymes than untreated seeds.

The activity of α-amylase was lower by 21.7 for *Triticum dicocum* to 40.5% for Triticum aestivum than in control seeds. Differences of β-amylase activity were lower by 8.7 to 3% in comparison to the control.

The same tendency – a decrease of α-amylase activity – was also noticed by Pittman *et al*. (1979) and Pietruszewski (1996), but in their experiments the effect of constant and alternating (50 Hz) magnetic fields was lower.

Amylolytic activity is connected with the hormonal metabolism of seeds. ABA decreased the activity of α-amylase in germinating seeds similarly to the frequent magnetic field (Masojć *et al*., 1997). It could indicate one more possible explanation of the magnetic field influence – the influence of a hormonal metabolism of germinated seeds and seedlings. The sprouting seeds are connected with an increase in activity of amylolytic enzymes. The sprouting of seeds is really unprofitable for agriculture. Sprouted seeds can not be used – not as seed material, but also not for consumption or pasturage. They lose their seed quality. The magnetic field causes a reduction in the activity of enzymes and it can be really important in breeding and seed production, and in various sections of the agriculture and food industry.

Plants grown from treated seeds will be more resistant to sprouting in the future, which is very important for their quality and possibility of practical usage (for example – storage).

The activity of glutathione S-transferase (GST) is presented in Table 1. As seen from the obtained results, higher GST activity was characteristic of seedlings grown from seeds treated with magnetic fields than of untreated ones (control) – by 11.3% in *Triticum monococum* and 54.2% in T*riticum dicocum*. Only in hexaploid *Triticum aestivum* the activity was lower.

Glutathione S-transferase belongs to multigene families common to all plants. GST is the most important system defence cell against unfavourable or harmful activity of chemical substances. GSTs in plants were the first to be identified and have been intensively studied because of their ability to detoxify herbicides, and individual GSTs conferring herbicide tolerance have been characterized from most major crop species. Recently, another plant GST subclass has been implicated in numerous stress responses, including those arising from pathogen attack, oxidative stress and heavy-metal toxicity.

**T a b l e 1.** The activity of β-amylase (μmol/1g seedlings/1min), α-amylase (mg starch /1g seedlings/1 min), glutathione S-transferase (u/μg protein)

| Treatment | *Triticum monococum* | *Triticum dicocum* var. Polonicum | *Triticum aestivum* var. Sigma |
|---|---|---|---|
| | β-amylase | | |
| Control | 1104.60 | 1124.09 | 662.76 |
| Seeds treated with a magnetic field | 1072.12 | 1026.62 | 617.27 |
| | α-amylase | | |
| Control | 1214.47 | 896.68 | 778.77 |
| Seeds treated with a magnetic field | 843.34 | 702.46 | 463.35 |
| | glutathione S-transferase | | |
| Control | 0.173 | 0.110 | 0.250 |
| Seeds treated with a magnetic field | 0.195 | 0.240 | 0.130 |

## CONCLUSIONS

1. A higher activity of GSTs was characterized in seedlings grown from treated seeds, except for *T. aestivum*.

2. The obtained results indicate that a magnetic field causes a higher activity in enzymes. It causes the plant to have more resistance from pathogen attacks, oxidative stress and heavy-metal toxicity. A higher GST activity causes a vital prolongation in the seeds and it permits a longer storage time so seeds do not lose their vigour.

3. The different behaviour of *T. aestivum* is caused by its complicated genetic structure compared to that of the diploid and tetraploid species.

4. The observed favourable effects of frequent magnetic fields could be connected with changes in enzyme activity. It can be used in agricultural practice.

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