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Anna Broszkiewicz[1] , Jerzy Detyna[1]*, Henryk Bujak[2]

[1]Department of Mechanics, Materials Science and Engineering, Faculty of Mechanical Engineering, Wrocław University of Science and Technology, Smoluchowskiego 25, 50-370 Wrocław, Poland; [2]Department of Genetics, Plant Breeding and Seed Production, Wrocław University of Environmental and Life Sciences, Pl. Grunwaldzki 24A, 50-363 Wrocław, Poland;
* Corresponding author:e-mail: jerzy.detyna@pwr.edu.pl

INFLUENCE OF THE MAGNETIC FIELD ON THE GERMINATION PROCESS OF TOSCA BEAN SEEDS (*PHASEOLUS VULGARIS L.*)

ABSTRACT:

The experiment was aimed at determining, whether exposure to static magnetic fields of intensities (1T, 2.5T, and 5T) stimulates the growth of Tosca bean seeds (*Phaseolus vulgaris* L.). Each treatment group in the experiment underwent exposure to the magnetic field for a specific period of time, that is: 900s, 1800s, or 3600s. The measurements of root volumes were taken every 24 hours. The first measurement was taken after 96 hours since the beginning of germination. The collected data was analyzed using statistical methods.

The experiment allowed to determine that the magnetic field had a significant influence on the lowering of bean seeds germination rate. The extent of this influence was dependent on the combination of factors: magnetic field force- time of exposure.

Keywords: biostimulation, germination process, magnetic field, plant, plant growth stimulation

Highlights:

- The magnetic field has a significant impact on the germination rate of Tosca bean seeds.
- The rate of germination depends on the combination of factors (intensity of the magnetic field - exposure time).
- There is a high rate of bean root growth treated with a static magnetic field.

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INTRODUCTION

Although the research on the magnetic field's influence on living organisms has been conducted for almost one hundred years, the exact mechanism behind the observable magnetically induced changes has not been fully explained yet. Numerous hypotheses trying to account for the influence of magnetic field on the changes of biochemical processes in cells have been formulated. Some of them point out, for example, the field's influence on the ions of ferromagnetic elements found in prosthetic groups of enzymes of the electron transport chain (e.g. some cytochromes), or enzymes partaking in the decomposition of H2O2 (catalases, peroxydases). Others suggest alterations in the functioning of entire protein structures, or even tissues. Many hypotheses concern the changes caused by physical phenomena induced by a magnetic field, for instance changes in the properties of liquid crystals (cell membranes exhibit many properties of liquid crystal structures), as well as the Hall, Dorfman, and Ettinghausen effects (Polk and Fellow, 1991; Rosen, 2010; Podleśny and Pietruszewski, 2007; Blanchard, 1996; Aceto *et al.*, 1970). Moreover, water treated with a magnetic field changes its physicochemical properties, which can influence the course of some biochemical reactions (Reina *et al.*, 2001; Es'kov and Darkov, 2003; Moussa, 2011; Grewal and Maheshwari, 2011; Małuszyńska *et al.* 2016). Even such a simplified summary of hypothetical explanations of underlying mechanisms induced by magnetic biostimulation of plant cells indicates what a complex phenomenon it is.

The hitherto conducted research focused mainly on the plants of economic importance (Rcacuciu *et al*., 2008; Marks and Szecówka, 2010; Carbonell *et al.*, 2000; Kornarzyński and Pietruszewski, 1999; Moon and Chung, 2000; Camps-Raga *et al.*, 2009; Nazari *et al.* 2014). The majority of such works led to the conclusion that the magnetic field has a positive influence on the acceleration of the germination and growth of plants, as well as on the yield increase. Responses of organisms to the treatment with a magnetic field varied. The response was dependent not only on the species of plants, but also on the time of exposure, intensity and character of the field used in the experiment (Aladjadjiyan and Ylieva, 2003; Nimmi and Madhu, 2009; Kobayashi *et al.*, 2004; Bujak and Frant, 2010; Aladjadjiyan, 2010; Vashist and Nagarajan, 2010; Soja, *et al.*, 2003; Pietruszewski and Kania, 2010; Balouchi and Modarres Sanavy, 2009; Podleśny and Gendarz, 2008; Balouchi and Modarres Sanavy, 2009; Szumiło and Rachoń, 2006; Bae *et al.* 2015; Jakubowski 2015; Jakubowski 2016). The exposure dose depends on density of magnetic (electric) field and the time of exposure. The density of magnetic field or electric field is determined by the following formula:

$$\rho = \frac{1}{2}\varepsilon_0 E^2 = \frac{1}{2}\mu_0 H^2 = \frac{1}{2\mu_0}B^2$$

where: ε_0 – vacuum permittivity, μ_0 – vacuum permeability, E – electric field intensity, H – magnetic field intensity, B – magnetic induction.

The exposure dose can be described using the formula:

$$D = \rho t$$

where: t – exposure time (Pietruszewski and Kania, 2010).

MATERIALS AND METHODS

The main objective of the conducted research was to determine the influence of high intensity magnetic field stimulation (1 T, 2,5 T, 5 T) on the germination of Tosca bean seeds, at different times of exposures of the seeds (900 s, 1800 s, and 3600 s). The study had a preliminary character. Previous experiments conducted using stimulation with a constant magnetic field of low intensities (2 mT and 4 mT) slowed down the process of Tosca seeds germination. This observation allowed to formulate a preliminary research hypothesis, that the application of a high intensity magnetic field will also have a significant influence on the root growth rate of this plant. The samples comprised of 30 to 37 Tosca bean seeds. The number of seeds that we used in our experiment was determined on the basis of literature and on the tests power calculations. Treatment groups were treated with a magnetic field induced by a PPMS superconducting electromagnet (Quantum Design). The process of germination took place in an environmental chamber in which the day was set at 16 h and the temperature was 293°K. For the purpose of the experiment, symbols of groups were introduced in Tables 1 and 2. All statistical tests were carried out at the significance level α=0.05.

Table 1

Symbols of treatment groups and the corresponding intensities and times of exposure to the magnetic field.

Inductionvalue (T)	1.0			2.5			5.0		
Time (s)	900	1800	3600	900	1800	3600	900	1800	3600
Measurement 1	1T/15/1	1T/30/1	1T/60/1	2,5T/15/1	2,5T/30/1	2,5T/60/1	5T/15/1	5T/30/1	5T/60/1
Measurement 2	1T/15/2	1T/30/2	1T/60/2	2,5T/15/2	2,5T/30/2	2,5T/60/2	5T/15/2	5T/30/2	5T/60/2
Measurement 3	1T/15/3	1T/30/3	1T/60/3	2,5T/15/3	2,5T/30/3	2,5T/60/3	5T/15/3	5T/30/3	5T/60/3

Table 2

Symbols of control groups according to the number of measurement and the corresponding treatment-groups.

For groups of induction (T)	1.0			2.5			5.0		
Time (s)	900	1800	3600	900	1800	3600	900	1800	3600
Measurement 1	K11	K11	K11	K21	K21	K21	K31	K31	K31
Measurement 2	K12	K12	K13	K22	K22	K22	K32	K32	K32
Measurement 3	K13	K13	K13	K23	K23	K23	K33	K33	K33

It was decided that the measure of the germination rate would be the change in the volume of tap roots of the beans. The measurements were taken using an electronic calliper with an accuracy of 0.1 mm. A simplifying assumption was made that a cone will serve as a good model of a tap root. For each group three measurements were taken, one every 24 hours. The first measurement was taken after 96 hours since the beginning of germination.

RESULTS AND DISCUSSION

The observations of the process of seed germination led to the conclusion that both within individual groups, and between separate groups, there is a significant variation in the rate of tap root growth. Therefore, the task of determining if control groups can comprise the right base for the comparison with the treatment groups was very important (Tadeusiewicz, 2015; Tadeusiewicz, 2009).

It was assumed that the only factors varying the rate of tap root growth in control groups were the biological and genetic traits of every seed. Aside from these, the results obtained in each control group should not show any significant statistical variation (Table 3).

Table 3

Selected statistical parameters in control groups

Parameters	Control group 1(T)			Control group (2,5T)			Control group (5T)		
	K11	K12	K13	K21	K22	K23	K31	K32	K33
n		37			36			35	
$\overline{x}$	14.11	35.79	64.07	15.03	34.88	60.08	18.99	40.28	64.33
s_x	9.87	21.14	30.4	6.44	14.1	20.85	13.81	23.58	29.14
$V_x = \frac{s_x}{\overline{x}}$	0.6996	0.59	0.47	0.43	0.4	0.35	0.73	0.59	0.45

The average volumes of roots in control groups (in corresponding measurements) were very similar. However, the high values of the coefficients of variation proved high variability in these samples. The highest variability in root volumes was recorded in the first measurement (in all groups). In the consecutive measurements the values of the coefficient descended. It can be assumed that together with the development of the roots, the environmental factors started to play an increasingly important role, which caused the diminishment of variation in the growth rates.

In order to verify the assumption that the average volumes of roots in general populations, which were allotted the corresponding control groups, are not statistically significantly different from each other, the Student's t-test was conducted. The hypotheses system:

$$H_0 : x_{i,j} = x_{i+1,j}$$
$$H_1 : x_{i,j} \neq x_{i+1,j}$$

where:
$x_{i,j}$ - average volume of a bean root in the general population, from which the control sample
number '*i*' was drawn, and measurement '*j*' was taken.

Table 4

Results of Student's t-test for control groups

Compared control groups	Statistical t value	p-value	No basis for rejecting H_0?
K11-K21	-0.471	0.639	yes
K11-K31	-1.716	0.091	yes
K12-K22	0.217	0.829	yes
K12-K32	-0.849	0.399	yes
K13-K23	0.654	0.515	yes
K13-K33	0.038	0.970	yes
K21-K31	-1.542	0.130	yes
K22-K32	-1.168	0.248	yes
K23-K33	-0.705	0.484	yes

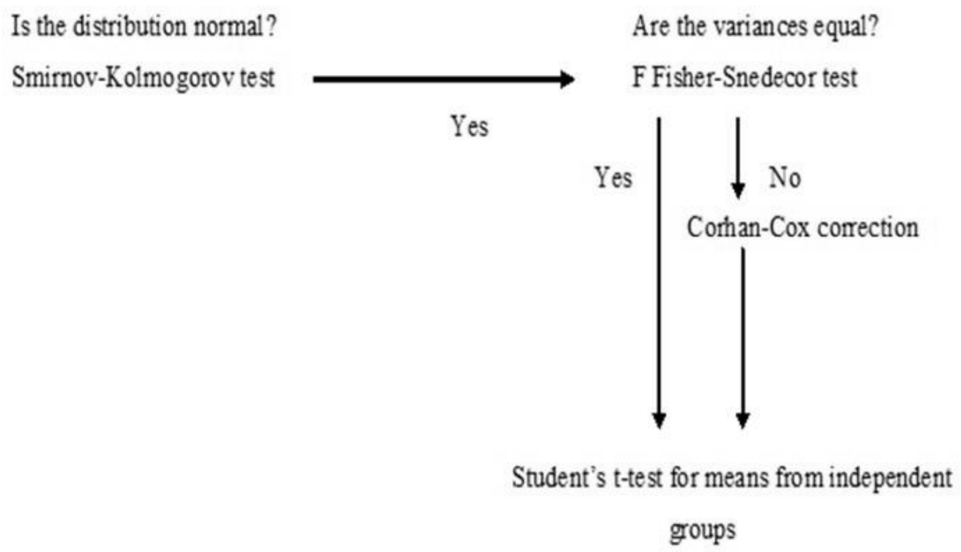

Fig. 1. Scheme of the equality of means hypothesis testing (for independent samples, source: own work)

For calculations, the level of confidence of α = 0.05 was accepted. This testing procedure required the verification of the hypotheses concerning the normal distribution and the equality of variance of the researched groups. The verification procedure was conducted according to the graph in Fig. 1. In the intermediate tests (Smirnov-Kolmogorov and Fisher- Snedecor) the level of confidence was also $\alpha = 0.05$. The results of the conducted verification of the hypotheses about the equality of mean values (Table 4) show that with the set level of confidence of $\alpha = 0.05$ there is no basis for rejecting the hypotheses saying:

- All control groups have a normal distribution (Smirnov-Kolmogorov).
- The mean values of volume in groups do not differ from each other (Student's t-test).

It can be stated that all the control groups (in pairs) are similar to each other and, despite the observed differences in measurements, they constitute the right basis for comparison with the corresponding treatment groups. These groups come from general populations which do not differ in any statistically significant way. The results of the measurements of root volumes were presented using descriptive statistics tools (Fig. 2-4, Tables 5-7).

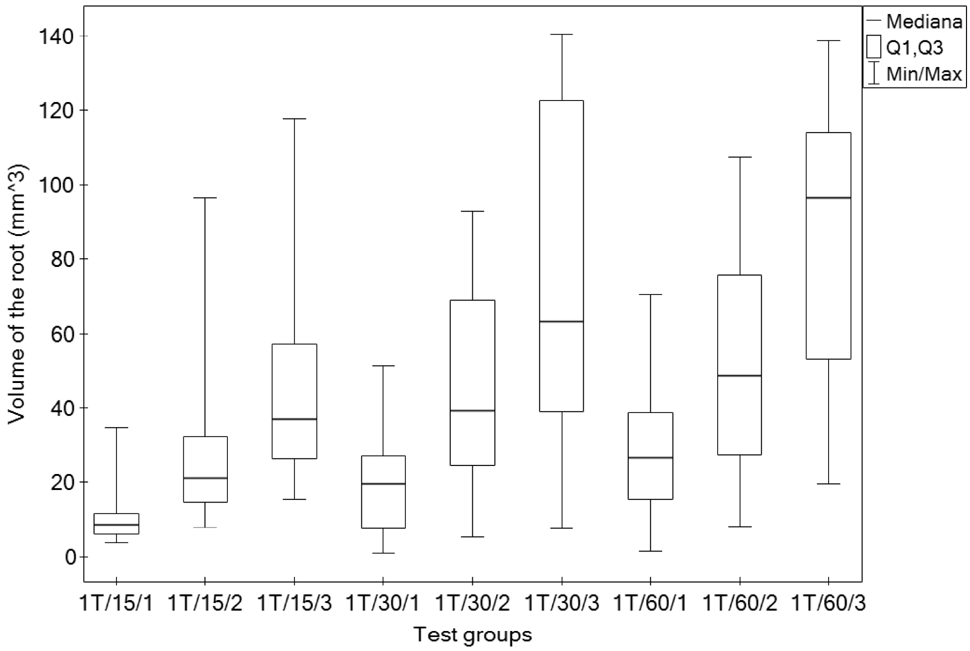

Fig 2. Median, Q1, Q3, min and max volume of roots in treatment groups treated with a magnetic field of 1T intensity

Table 5

Volume of the root in treatment groups treated with a magnetic field of 1T intensity – selected statistical parameter

	Test groups 1(T)								
Parameters	1T/15/1	1T/15/2	1T/15/3	1T/30/1	1T/30/2	1T/30/3	1T/60/1	1T/60/2	1T/60/3
n	30	30	30	30	30	30	30	30	30
$\overline{x}$	10.65	28.22	45.76	20.32	45.11	75.43	28.27	51.41	87.65
s_x	7.31	17.23	25.19	13.83	26.56	43.26	17.34	28.94	36.66
$V_x = \frac{s_x}{\overline{x}}$	0.69	0.61	0.55	0.68	0.59	0.57	0.61	0.56	0.42

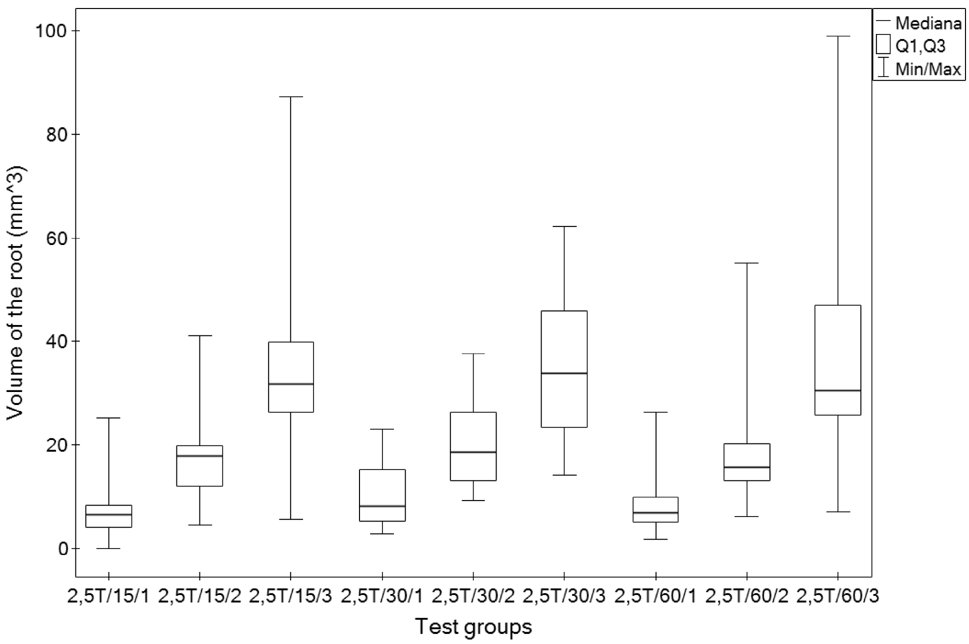

Fig.3. Median, Q1, Q3, min and max volume of roots in treatment groups treated with a magnetic field of 2.5T intensity.

Table 6

Volume of the root in treatment groups treated with a magnetic field of 2.5T intensity- selected statistical parameters.

	Test groups 2.5(T)								
Parameters	2,5T/15/1	2,5T/15/2	2,5T/15/3	2,5T/30/1	2,5/30/2	2,5T/30/3	2,5T/60/1	2,5T/60/2	2,5T/60/3
n	31	31	31	33	33	33	30	30	30
$\overline{x}$	7.24	17.53	33.04	9.79	20.76	34.43	8.57	18.11	37.37
s_x	5.24	9.23	17.22	5.74	8.81	13.1	5.84	9.62	19.44
$V_x = \frac{s_x}{\overline{x}}$	0.72	0.53	0.52	0.59	0.42	0.38	0.68	0.53	0.52

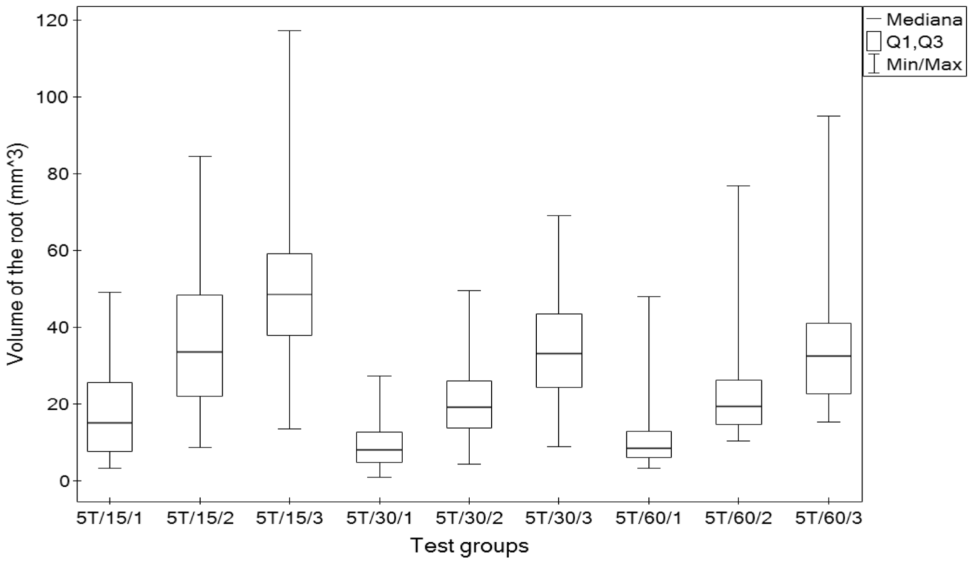

Fig.4. Median, Q1, Q3, min and max volume of roots in treatment groups treated with a magnetic field of 5T intensity.

Table 7

Volume of the root in treatment groups treated with a magnetic field of 5T intensity– selected statistical parameters

	Test groups(5T)								
Parameters	5T/15/1	5T/15/2	5T/15/3	5T/30/1	5T/30/2	5T/30/3	5T/60/1	5T/60/2	5T/60/3
n	31.00	31.00	31.00	30.00	30.00	30.00	32.00	32.00	32.00
$\overline{x}$	18.83	36.93	53.72	9.60	21.31	35.43	10.40	22.53	34.69
s_x	13.19	19.22	26.74	6.63	11.49	15.39	8.02	12.49	15.54
$V_x = \frac{s_x}{\overline{x}}$	0.70	0.52	0.50	0.69	0.54	0.43	0.77	0.55	0.45

The analysis of statistical parameters lead to the following conclusions:

1. In the majority of the groups high and very high dispersion of bean root volumes was observed. High values of the coefficient of variation can be a proof of that. Only in a few groups the observed dispersion was average.
2. The volumes of roots were characterized by the highest variation in the first measurement (V_x usually reached the value of 0.6-0.7).
3. In the consecutive measurements, in all treatment groups, the variation of root volumes decreased at a varied pace. In the third measurement the value of the coefficient of variation was usually between 0.4 and 0.5.
4. During the process of germination, in the majority of the treatment groups, only insignificant changes in the structure of the measured volumes of roots occurred. Medians only slightly “shifted their position” in relation to Q1 and Q3 (box-plots). Only in two groups the change of skewness was observed.
5. Throughout the process of germination the range between Q1 and Q3 rose consistently, however, the dynamics of growth of these two values was proportional in every group. This provided for a relative stability of dispersion and did not lead to significant changes in its structure.
6. It can be assumed that the changes in the subgroup of roots with small volumes (<Q1) and roots with large volumes (>Q3) were responsible for the observed decrease of dispersion in all of the groups. Changes in the minimum and maximum values of root volumes can be considered a faint signal of this influence.
7. The high variance in the values describing central tendencies (mean and median) between separate treatment groups, as well as between control and treatment groups, is worth noting. The meticulous identification of this phenomenon had a great relevance for the accomplishment of the purpose of the experiment, therefore, it was statistically analyzed in more detail.

The following question had to be asked - are the differences in the character of distribution, observed between the control and the treatment groups, significant enough to classify the beans as coming from populations of different properties? The occurrence of these differences should be attributed to the influence of the magnetic field on seeds. In order to verify this assumption a hypotheses system was formulated:

- H_0: median in control group (measurement i) = median in treatment group (measurement *i*).
- H_1: median in control group (measurement i) ≠ median in treatment group (measurement *i*).

where: *i*- number of measurements taken in a group.

In the process of verification of the hypotheses (for any further analysis the level of confidence equaled $\alpha = 0.05$) the nonparametric Mann-Whitney test was used (Tables 8-10).

Table 8

Results of the Mann-Whitney test for the treatment group treated with a 1T magnetic field.

Control group	Test group (*- p-value<α)					
	K11		K12		K13	
	p-value	U	p-value	U	p-value	U
1T/15/1	0.249	1.154				
1T/15/2			0.092	1.683		
1T/15/3					0.006	2.742
1T/30/1	0.053	1.935				
1T/30/2			0.183	1.33		
1T/30/3					0.31	1.015
1T/60/1	0.0003*	3.599*				
1T/60/2			0.027	2.213		
1T/60/3					0.0051*	2.799*

Table 9

Results of the Mann-Whitney test for the treatment group treated with a 2.5T magnetic field.

Control group	Test group(*- p-value<α)					
	K11		K12		K13	
	p-value	U	p-value	U	p-value	U
1T/15/1	0.249	1.154				
1T/15/2			0.092	1.683		
1T/15/3					0.006	2.742
1T/30/1	0.053	1.935				
1T/30/2			0.183	1.33		
1T/30/3					0.31	1.015
1T/60/1	0.0003*	3.599*				
1T/60/2			0.027	2.213		
1T/60/3					0.0051*	2.799*

In the light of the presented parameters it can be concluded that there is no basis for rejecting the H_0 hypothesis in the case of groups where the following field intensities and times of bean exposure were applied: 1.0T- 900s; 1.0T-1800s; 5T -900s.

The H_0 hypothesis should be rejected, and the alternative hypothesis should be accepted as true, in the cases of the groups where the following field intensities and times of bean exposure were applied:

It can be stated that in these groups the distributions (as well as medians) are significantly different from those in their corresponding control groups. Therefore, it can be said that the magnetic field had a significant influence on the process of germination of bean seeds in these groups. It should also be noted that this influence could be observed only in particular combinations of field intensity and time of exposure.

Table 10

Results of the Mann-Whitney test for the treatment group treated with a 5T magnetic field.

Control group	Test group (*- p-value<α)					
	K31		K32		K33	
	p-value	U	p-value	U	p-value	U
5T/15/1	1.0000	0.9999				
5T/15/2			0.748	0.321		
5T/15/3					1.619	0.1055
5T/30/1	0.0012*	3.250				
5T/30/2			0.00004*	4.112		
5T/30/3					0.00015*	4.336
5T/60/1	0.00118*	3.245				
5T/60/2			0.00006*	4.023		
5T/60/3					0.00002*	4.726

In order to verify the claim about the presence of statistically significant differences between the populations of seeds treated with the magnetic field and the control groups, the Student's t-test was conducted (for independent samples) to check the equality of means. The following system of research hypotheses was formulated:

- H_0: mean value in treatment group= mean value in control group
- H_1: mean value in treatment group≠ mean value in control group

For this verification the procedure presented in Fig. 1 was used. The results of the nonparametric Smirnov-Kolmogorov test corroborated that with the level of confidence α=0.05 there is no basis for rejecting the claim that the distribution of treatment groups (for all of the measurements and intensities of the field) differs from a normal distribution.

In the case of the Fisher-Snedecor test (checking the equality of variance) the level of confidence α= 0.05 was set as well. When it comes to the groups where the values of variances differed significantly from each other, the Cochran-Cox test was applied to the Student's t-test.

The results of the test (Table 11) undoubtedly indicate that only in the case of the group treated with a 5.0T field for 900s it can be claimed that there is no basis for rejecting the H_0 hypothesis. A similar observation, although not as definite, can be formulated for the groups exposed to the 1.0T magnetic field for 900s and 1800s respectively.

Table 11

Results of the Student's t-test for means (independent samples)

Magnetic field intensity [T]	Compared groups	t-value	p-value	No basis for rejecting H_0?
1.0	K11 - 1T/15/1	1.599	0.115	yes
1.0	K12 – 1T/15/2	1.579	0.119	yes
1.0	K13 - 1T/15/3	2.643	0.010	no
1.0	K11 – 1T/30/1	-2.139	0.036	no
1.0	K12 – 1T/30/2	-1.599	0.115	yes
1.0	K13 – 1T/30/3	-1.206	0.233	yes
1.0	K11 – 1T/60/1	-3.979	0.002	no
1.0	K12 - 1T/60/2	-2.549	0.013	no
1.0	K13 – 1T/60/3	-2.878	0.005	no
2.5	K21 – 2.5T/15/1	5.378	0.0(4)1	no
2.5	K22 – 2.5T/15/2	6.032	<0.0(5)1	no
2.5	K23 – 2.5T/15/3	5.729	<0.0(5)1	no
2.5	K21 – 2.5T/30/1	3.553	0.0007	no
2.5	K22 – 2.5T/30/2	5.029	0.0(4)5	no
2.5	K23 – 2.5T/30/3	6.172	0.0(4)1	no
2.5	K21 – 2.5T/60/1	4.232	0.00008	no
2.5	K22 – 2.5T/60/2	5.715	<0.0(5)1	no
2.5	K23 – 2.5T/30/3	4.541	0.00003	no
5.0	K31- 5T/15/1	0.048	0.961	yes
5.0	K32 - 5T/15/2	0.627	0.533	yes
5.0	K33 - 5T/15/3	1.534	0.129	yes
5.0	K31- 5T/ 30/1	3.573	0.0008	no
5.0	K32 – 5T/30/2	4.214	0.0001	no
5.0	K33 – 5T/30/3	5.098	0.0(4)5	no
5.0	K31 – 5T/60/1	3.144	0.003	no
5.0	K32 – 5T/60/2	3.896	0.0003	no
5.0	K33 – 5T/60/3	5.256	0.0(4)3	no

DISCUSSION

Both the results of the Mann-Whitney and the Student's t-tests led to the conclusion that the stimulation with the magnetic field significantly influenced the rate of germination of bean seeds. This, in turn, created significant differences in both central tendencies and in the character of the distribution of the measured values, which were identified, also in relation to the general population, thanks to the use of statistical tests. The observed differences cannot be explained only by the genetic traits of the plants, nor the environmental conditions. It can be inferred based on the fact that the tests conducted on control groups showed that in their case the process of germination did not lead to the creation of such significant differences in root volumes. In this research only the static magnetic field of three values of intensity and three times of exposure was used, thus, the obtained results should be treated only as preliminary findings. However, they create an interesting incentive for further tests, since they allow to better understand the mechanism of the magnetic field influence on the germination of seeds.

CONCLUSIONS

1. The magnetic field had a significant influence on the germination rate of Tosca bean seeds.
2. A high diversity of the tap roots growth rates was observed among beans treated with a static magnetic field.
3. The rate of germination varied depending on the combination of stimulating factors (field intensity- time of exposure to the field).

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