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# GENETIC EFFECT OF MAGNETIC FIELD ON GROWTH AND PROTEIN CONTENTS OF MAIZE, BEAN AND *Bacillus sp IN VITRO*.

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## ABSTRACT

Physical mutagenesis as radiation, electrical field, magnetic field...etc., were inspired to enhance or modify their biological behavior. This work was carried out to detect the change in some properties and chemical constituents of maize (variety S.C.10) and bean (variety Nebraseca ) seeds after exposure to magnetic field such as root weight and length, shoot height, proteins, amino acids and protein banding pattern. The density flux of magnetic field was 0.5 Tesla and exposure times were 15 and 30 min. Seeds were placed at suitable distance between magnetic dipoles. Paper chromatography detected increase in some amino acids as aspartic acid, glutamic acid, and methionine in both maize and bean seeds after exposure to magnetic field. The total protein of maize seedlings decreased by 66% after 30 min of exposure to magnetic field. Protein pattern detected new bands after 30 min of magnetic field exposure in maize and bean. The germination and growth of maize and bean seeds were affected after magnetic field exposure. Root weight of maize was decreased 66% but in bean the decrease of root weight was not significant. The maize shoots were increased 66% and 233% after magnetic field exposure for 15 and 30 min, respectively. The bean shoots increased 27.3% and 63.6% after magnetic field exposure for 15 and 30 min, respectively. The results indicated that the magnetic field can produce plants free of *Bacillus* sp, increases some amino acids contents and also increases maize and bean shoots growth.
**Keywords**: Maize, Bean, Magnetic field, *Bacillus sp*, Protein pattern.

## INTRODUCTION

Chemical and physical mutagens were used widely for producing mutations and/or increasing genetic variability in target materials. It is well known that mutagens could directly induce physiological changes, point mutations and chromosomal aberrations. Magnetic field (MF) can also be used for the same purpose due to their biological effects (Mastude *et al*., 1993). There are a lot of biological effects include carcinogenesis that has been attributed to MF, but there is no clear data about the mutagenic effect of MF (Schreiber *et al*., 2001; Novikov *et al*., 2002). The evaluation of MF effects on the biological systems is difficult, since most of the biological structures are heterogeneous (Goodman *et al*., 1995).

The previous studies indicated that the MF affects on plant growth and germinations, and that frequency of the field is an important factor on germination rates. Maximum germination rates (20% increasing in the treatment higher than the control) were obtained at around 10 Hz. The MF of 10 Hz also produced a statistically significant effect on plant growth, as measured by leaf area. The difference in growth rate between treated and control plants decreased after the field was removed (Namba *et al*., 1995).

The application of high magnetic fields (1 Tesla and above) produced marked effects on germination and growth of plants, especially corn and peas (Chen, 1999). Recently Novak *et al*. (2007) found that the MF decreases the number of yeasts, and also down their growth, which it seems that the MF kills a part of yeasts. They reported that this result was similar to the experiments with bacteria *E. coli*, *S. aureus* and *L. adecarboxylata*.

Certain types of stressful environmental conditions can activate stress genes to produce stress proteins that enable organisms to tolerate such stresses. Biochemical markers have been used to distinguish between homozygous and heterozygous individuals and to estimate the level of genetic variability in plant populations (Mulchinger *et al* 1992). Hussien and Stegemann (1978) reported that sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) of total grain proteins was a rapid method to screen varietal differences.

This work aimed to estimate the effect of magnetic field on germination and growth of maize, bean and *Bacillus sp in Vitro*. Chemical constituents of protein and amino acids contents and protein banding pattern were also evaluated.

## MATERIALS AND METHODS

### Plant materials

Seeds of "Nebrasca" bean variety (*Phaseolus vulgaris* L.) and grains of "Single Cross 10" maize variety (*Zea mays* L.) were kindly supplied from Agriculture Research Center, Giza, Egypt. MS medium (Murashige and Skoog, 1962) was used in maize and bean seeds cultures. Nutrient broth medium, consists of 0.8% nutrient broth and distilled water, was used to grow *Bucillus* sp. wild type strain.

### Magnetic field

The seeds were exposed to MF (Newport Pangell Instrument, Oxford, UK). The MF applied was 10 kg at 0.5 Tesla as field strength or density flux (H) with a current of 0.25 Amps. After MF exposure for two times 15 min and 30 min, seeds were sterilized in 5.25 % sodium hypochlorite for 15 min and washed many times with sterile distilled water, blotted and planted on MS medium and incubated at 23ºC in light for 10 days.

### Amino acids and protein determination

Amino acids were estimated using paper chromatography and Ninhydrin solution for displaying method (Cassidy, 1957). Protein extraction was carried out according to Bollage and Edelstein (1992). Total protein concentration was estimated according to method of Brandford (1976).

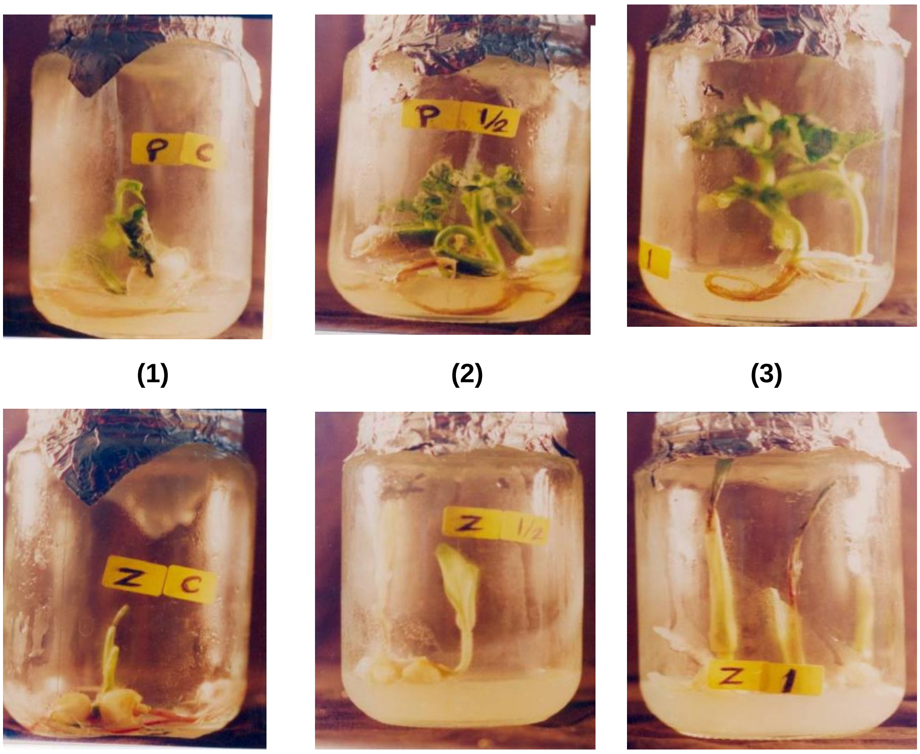
### Protein electrophoresis

Samples of 1g kernel flour from each of the maize and bean seed varieties, that exposed to the MF and unexposed seeds as controls, were used for protein electrophoretic analysis. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analysis was performed (Laemmli, 1970). The sample preparation and extraction of seed water-soluble proteins was performed (Stegemann *et al* 1980). Gel was photographed and scanned by Gel Doc Bio-Rad System (Gel – Pro analyzer V3).

## RESULTS AND DISCUSSION

### Seed germination and growth

The seeds of maize and bean were exposed to MF for 15 and 30 min. The effect of treatments on root and shoot growth is shown in Figure (1) and Table (1). The root weight of maize and bean seedlings decreased with increasing the time of MF exposure comparing to the control. Similar results were obtained for root length after treatment with MF, which the length decreased comparing to the control after 15 and 30 min of exposure. In contrast, the shoot height increased after treatments with MF in both maize and bean plantlets. These results are in agreement with those of Chen (1999) who reported that the application of high magnetic fields (1 Tesla and above) produced marked effects on germination and growth of plants, especially corn and peas. Namba *et al*. (1995) found that maximum germination rates (20% increasing in the treatment higher than the control) were obtained at around 10 Hz. The MF of 10 Hz also produced a statistically significant effect on plant growth.




(1) (2) (3)

(4) (5) (6)

**Fig. 1: Seedling growth on MS medium after 10 days of germination. Bean: (1) without MF exposure, (2) 15 min of MF exposure and (3) 30 min of MF exposure. Maize: (4) without MF exposure, (5) 15 min of MF exposure and (6) 30 min of MF exposure.**

**Table (1): Root length, weight and shoot height of maize and bean seedlings after 10 days of germination.**

| Seeds | MF/time | Root length (cm) | Root weight (g) | Shoot height (cm) |
|---|---|---|---|---|
| Maize | Control | 6.0 | 36.2 | 3.0 |
| | 15 min | 5.5 | 35.7 | 5.0 |
| | 30 min | 5.5 | 12.2 | 10 |
| Bean | Control | 3.0 | 17.2 | 5.5 |
| | 15 min | 2.5 | 14.0 | 7.0 |
| | 30 min | 2.5 | 13.3 | 9.0 |

The seeds of maize and bean were infected with *Bacillus* sp after exposure to MF for 15 and 30 min. The treated and untreated seeds with MF were germinated on MS medium. The results showed that the plantlets of treated seeds did not infect by *Bacillus* sp, while the plantlets of untreated seeds were infected with *Bacillus* sp (Table 2). These results indicated that MF can produce plants free of *Bacillus* sp. Moore (1979) reported that growth of microorganisms could be stimulated or inhibited depending upon the field strength and frequency of the pulsed magnetic field. Novak *et al*. (2007) found that the MF decreases the number of yeasts, and also down their growth, which it seems that the MF kills a part of yeasts. They reported that this result was similar to the experiments with bacteria *E. coli*, *S. aureus* and *L. adecarboxylata*.

**Table (2): Effect of MF on seeds of maize and bean infected with *Bucillus sp.***

| Seeds | MF/time | *Bucillus sp* growth |
|---|---|---|
| Maize | Control | ++ |
| | 15 min | - |
| | 30 min | - |
| Bean | Control | ++ |
| | 15 min | - |
| | 30 min | - |

**Protein and amino acid contents**

Total protein quantity was estimated in seedlings of maize and bean before and after exposure to MF for 15 and 30 min (Table 3). The total proteins of maize and bean seedlings after treatments were lower than that in control for both times of exposure (15 and 30 min). The decrease in total protein of bean was more after treatment for 30 min than 15 min.

**Table (3): Percentage of total proteins in seeds of maize and bean.**

| Seeds | MF/time | Total protein (%) |
|---|---|---|
| Maize | Control | 5.7 |
| | 15 min | 4.7 |
| | 30 min | 4.5 |
| Bean | Control | 12.1 |
| | 15 min | 11.9 |
| | 30 min | 4.1 |

The amino acids determination showed increase in the amino acids of aspartic, glutamic, and methionine in both maize and bean seeds after treatments with MF for 15 and 30 min as shown in Table (4), while the amino acids of proline, serin and leucin did not affect by the MF.

**Table (4): Determination of amino acids in seeds of maize and bean after exposure to magnetic field.**

| Seeds | MF/time | Amino acids | | | | | |
|---|---|---|---|---|---|---|---|
| | | Aspartic acid | Glutamic acid | Methionine | Proline | Serin | Leucin |
| Maize | Control | + | + | + | + | + | + |
| | 15 min | +++ | +++ | +++ | + | + | + |
| | 30 min | +++ | +++ | +++ | + | + | + |
| Bean | Control | + | + | + | + | + | + |
| | 15 min | +++ | +++ | +++ | + | + | + |
| | 30 min | +++ | +++ | +++ | + | + | + |

**Electrophoretic patterns**

The electrophoretic patterns of seed water-soluble proteins of two varieties of maize and bean without and after treatments with MF for 15 and 30 min are shown in Figure (2) and Table (5).

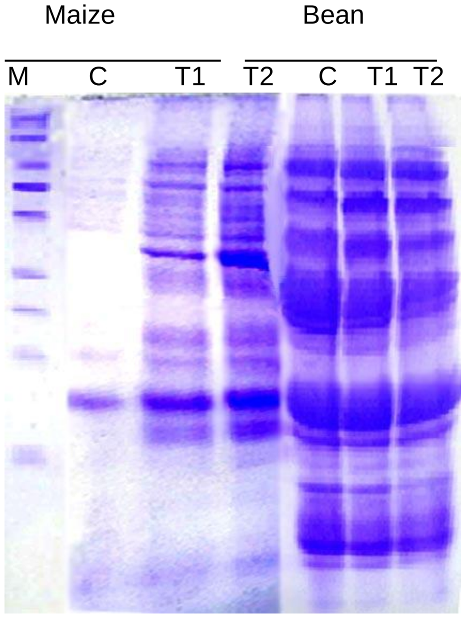



**Fig. 2: Electrophoretic patterns of water-soluble proteins for maize variety "S.C. 10" and bean variety "Nebrasca" (C: control, T1 and T2: treatmrnts with MF for 15 and 30 min, respectively). M: protein markers (212, 120, 97.4, 66.2, 45, 31, 20, 14.4 and 6.5 kDa).**

The protein patterns after treatments with MF showed differences comparing with the control in band numbers and loci. Some bands appeared in the patterns after treatments especially in maize variety where the control showed 11 bands while the treatments gave 16 bands after both 15 and 30 min. On the other hand, in the protein patterns of bean some bands disappeared after treatments especially for 30 min.

**Table (5): Densitometric analysis of water-soluble protein bands for two varieties of maize and bean with three treatments of MF, representing band number and molecular weight (MW).**

| Band No | MW (kDa) | Maize variety | | | Bean variety | | |
|---|---|---|---|---|---|---|---|
| | | C | T1 | T2 | C | T1 | T2 |
| 1 | 107.2 | - | - | - | + | + | + |
| 2 | 102.7 | - | + | + | + | - | - |
| 3 | 97.4 | + | + | + | - | - | - |
| 4 | 90.7 | - | - | - | + | + | + |
| 5 | 87.4 | - | + | + | - | - | - |
| 6 | 67.5 | + | + | + | - | - | - |
| 7 | 59.8 | - | - | - | + | + | + |
| 8 | 57.0 | + | + | + | - | - | - |
| 9 | 48.3 | - | - | - | + | + | + |
| 10 | 44.5 | + | + | + | - | - | - |
| 11 | 42.1 | + | + | + | - | - | - |
| 12 | 38.9 | + | + | + | - | + | + |
| 13 | 36.7 | - | + | + | - | - | - |
| 14 | 35.8 | - | - | - | + | + | + |
| 15 | 34.3 | - | - | - | + | + | + |
| 16 | 32.6 | - | - | - | + | + | + |
| 17 | 30.5 | + | + | + | - | - | - |
| 18 | 25.5 | - | - | - | + | + | + |
| 19 | 18.6 | - | - | - | + | + | + |
| 20 | 15.6 | + | + | + | + | + | - |
| 21 | 14.4 | + | + | + | + | + | - |
| 22 | 12.7 | - | - | - | + | + | + |
| 23 | 11.8 | + | + | + | - | - | - |
| 24 | 10.0 | - | - | - | + | + | + |
| 25 | 8.8 | - | + | + | + | + | + |
| 26 | 6.7 | - | + | + | + | + | + |
| 27 | 4.5 | - | - | - | + | + | + |
| 28 | 3.6 | - | - | - | + | + | - |
| 29 | 3.0 | - | - | - | + | + | + |
| 30 | 2.6 | + | + | + | + | + | + |
| 31 | 1.8 | - | - | - | + | + | + |
| Total bands | | 11 | 16 | 16 | 21 | 21 | 18 |

**+: Presence of band.** **-: Absence of band** **C: Control**
**T1: Treatment for 15 min** **T2: Treatment for 30 min**

In maize, five bands with molecular weights (M.W.) of 102.7, 87.4, 36.7, 8.8 and 6.7 kDa were found in the protein band patterns of both treatments for 15 and 30 min. In bean grains, three bands disappeared after treatment for 30 min with MW 15.6, 14.4 and 3.6 kDa, while one band with MW 102.7 kDa disappeared after treatments for 15 and 30 min. Only one band with MW 38.9 kDa was detected in the lanes of bean protein patterns after treatments, where did not found in the control. The number of bands in the pattern of treatment for 30 min was 18 bands which decreased 3 bands comparing with the control (21 bands).

These results indicated that maize can resist the MF stress which produced five new protein bands to overcome this stress. Meanwhile, bean proteins did not affect seriously after treatment for 15 min, but were affected after exposure for 30 min where four protein bands disappeared. These results coincided with those of protein quantity in bean where the quantity decreased after treatment for 30 min much more than that after treatment for 15 min (Table 3).

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## التاثيرالوراثي للمجال المغناطيسي علي النمو ومحتوي البروتينات في حبوب الذرة والفاصوليا و بكتريا الباسيلاس في المعمل

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تم تعريض حبوب الذرة الشامية ( هجين فردي ١٠ ) وبذور الفاصوليا ( نبراسكا ) لفترتين زمنيتين من المجال المغناطيسي (١٥ ، ٣٠ دقيقة ) وتم زراعة البذور المعاملة والغير معاملة (كنترول) علي بيئة موراشيج وسكوج (١٩٦٢) . أظهرت النتائج أن نمو الجذور قل في كلا من الذرة الشامية والفاصوليا بعد التعريض للمجال المغناطيسي ، بينما زاد إرتفاع الساق بدرجة ملحوظة . تعريض البذور للمجال المغناطيسي بعد عمل عدوى ببكتريا الباسيلس أنتج بادرات خالية من النمو البكتيري . أعطى التحليل الكروماتوجرافي للأحماض الأمينية زيادة كمية لبعض الأحماض مثل الجلوتاميك والمثيونين والأسبارتك في كل من الذرة الشامية والفاصوليا بعد التعريض للمجال المغناطيسي وظهر أن التقديرالكمي للبروتين في حبوب الذرة الشامية قل بدرجة ملحوظة بعد التعريض للمجال المغناطيسي خاصة في بذور الفاصوليا بعد التعريض لمدة ٣٠ دقيقة . ظهرت حزم بروتينية جديدة في نماذج التفريد الكهربي لبذور الذرة الشامية بعد التعريض للمجال المغناطيسي ، في المقابل لم تتأثر بذور الفاصوليا بعد التعريض لمدة ١٥ دقيقة وأختفت بعض الحزم بعد التعريض لمدة ٣٠ دقيقة .

**قام بتحكيم البحث**

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