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ALTERNATING MAGNETIC FIELD IRRADIATION EFFECTS ON THREE GENOTYPE MAIZE SEED FIELD PERFORMANCE

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A b s t r a c t. In this study alternating magnetic field treatments at low frequency (60 Hz) with combinations of three magnetic flux densities (20, 60 and 100 mT) and three exposure times (7.5, 15 and 30 minutes) were used as pre-sowing seed treatments in three maize (*Zea mays* L.) genotypes (CL-12 X CL-11, CL-4 X CL-1 and CL-13 x CL-1). In the case of CL-12 X CL-11 genotype these treatments increased significantly the seedling emergence rate, seedling dry weight and emergence in soil. The best treatment was found at magnetic flux density of 100 mT and exposure time of 7.5 min, with significant improvements in seedling emergence rate by 123.2%, field emergence by 110% and seedling dry weight, 21 days after planting, by 30.1%. In the case of CL-4 X CL-1 genotype it was found a negative biostimulation for seedling emergence percentage; finally in the CL-13 x CL-1 genotype there was not any significant effect. These results show that electromagnetic field treatment provide a simple and ecologically well compatible method to improve seed vigour in maize but is necessary to find the optimal irradiation parameters to induce a positive biostimulation in the maize seeds which also depends on the seed genotype.

Keywords: *zea mays*, electromagnetic field, seed vigour, biostimulation

INTRODUCTION

The magnetic field treatment is one of the most investigated physical pre-sowing seed treatments in agriculture (Pietruszewski 1999, Vasilevsky 2003, Martínez *et al*. 2009). Perception mechanisms were attributed originally to ferri-magnetisms. Magnetic effects on plants can be explained in the framework of the ion cyclotron-resonance and the radical pair models, two mechanisms that also play an essential role in the magnetoreception of other organisms (Galland and Pazur 2005).

Several studies have shown that strong magnetic fields change cell membrane characteristics, cell metabolism, cell reproduction and various other cellular functions like mRNA quantity, gene expression, protein biosynthesis and enzyme activities. Also, it has been reported that magnetic fields affect plant growth characteristics at the organ and tissue level in seeds, young seedlings and plants (Pittman *et al*. 1979, Wadas 1992, Atak *et al*. 2003, Pietruszewski 2007, Hernandez *et al*. 2007a). Pre-sowing treatments with an alternating magnetic field showed generally positive effects on seedling growth, seed vigour and crop yield (Pietruszewski 1993). All results showed that the frequency of alternating fields, magnetic flux density, seed exposure times, absolute exposure dose and the polarity (N o S) are important characteristics of a magnetic field treatment to produce positive biostimulation effects.

In Latin-American countries, maize (*Zea mays* L.) is one of the basic crops where seed vigour and in particular the physiological seed quality plays a fundamental role for seedling emergence and crop establishment (Zepeda *et al*. 2009, Zepeda *et al*. 2002, Van de Venter 2000). Quality seed is a factor for sustainable progress (Hampton *et al*. 2002). Therefore, pre-sowing treatments for seed vigour enhancement could play an important role in maize production (Hernandez *et al*. 2007b), this characteristics allow a seedling to have more strength to emerge from the soil (Valadez *et al*. 2007). Biophysics methods as pre-sowing treatments are considered as harmless for the environment; however, these treatments must be applied with suitable parameters in order to improve the seed vigour and ensure better plant development at later stages (Podleśny *et al*. 2005, Hernandez *et al*. 2006).

Since the magnetic field effect on seed vigour don't have a linear response with the field strength and exposure time, it is necessary to explore this effect over a broad range of magnetic field parameters and different maize seed genotypes. Therefore, the objective of the present research is to investigate the effects of low frequency of alternating magnetic field on the three genotype maize seed vigour.

MATERIALS AND METHODS

Maize seeds from cross CL-4 x CL-1 (Genotype 1: G1, produced in 2006), CL-13 x CL-1 (Genotype 2:G2, produced in 2006) and CL-12 x CL-11 (Genotype 3: produced in 2006) has been provided by the Mexican Genetic Quality control assurance institute (IREGEP). These have been used in the present research project. The seed lots were graded for uniformity by using 8 mm and 9 mm screens and the

1000-seed weight averages were 420, 460 and 465 g for 1, 2 and 3 genotypes respectively. Magnetic fields, at 60 Hz, with 20, 60 and 100 mT flux densities were used in three different exposure times: 7.5, 15 and 30 minutes.

The magnetic field intensity changes from the periphery to the centre of the ring electromagnet. The seeds were placed inside a plastic holder on a circular strip of 2 cm width on a volume $V = 0.297 \cdot 10^{-3}$ m^3, where the magnetic field induction is approximately constant. A Gaussmeter (Lakeshore, model 410) was used to measure the magnetic field intensity.

The seed emergence test as a kind of seed vigour test was established at the experimental station of Montecillo, Texcoco, Mexico (treatments 30, including the controls of the three genotypes). A loamy clay soil was used as substrate.

Each plot consisted of 0.75 m rows, 25 seeds per row and spaced 0.025 m. The seeds were sown 4 days after the pre-sowing magnetic field treatment (including the control) by placing the pedicel in the soil, leaving the silk scar exposed. The seeds were covered with 0.06m soil. The seedbed, with dimensions of 4.5 m x 1.8 m x 0.40 m, was watered every day at the same hour. A micro-tunnel, elaborated with metal and semitransparent plastic, covered the seedbed, and placed to inclemency outdoors under environment conditions and natural light cycle. The average temperature was 14.05ºC, ranging from –0.4ºC to 28.8ºC. The seedling emergency began on the 5th day after sowing. From this day, daily counting of emerged seedlings were made during the next 7 days. Finally the total number of emerged seedlings, the seedling emergence rate according to Maguire (1962) and the seedling dry weight at the 16th day after drying at 40ºC during 72 h were used in the statistical analysis.

Vigour test were carried out in a randomised complete block design with 3 replications. The experimental unit included 25 seeds. Variance analyses were based on the ANOVA procedure SAS (SAS, 2008 version). Differences among treatments were tested on significance by the least significant difference (LSD) at 5% probability level (Steel and Torrie 1980). Significant statistical differences were found, since the fourth day after the seedling emergency, in the seedling emergence rate and emergency establishment percentage for CL-4 X CL-1 genotype. In the case of CL-12 X CL-11 genotype significant statistical differences were found at 3rd day, after the initial emergence, on the seedling emergence rate and emergence percentage. The seedling dry weight was evaluated at the 16th day after initial emergence. Only normal seedlings according to the ISTA rules (ISTA, 1993) were evaluated. The accuracy in these measurements was an essential component of the methodology used in this study (TeKrony 2003).

RESULTS

The alternating magnetic field had significant effects on seedling emergence rate and seedling emergence (%) for CL-4 X CL1 genotype. On the CL-13 x CL-

1 genotype there was not significant effect, finally the CL-12 X CL-11 genotype had significant effects in the three parameters evaluated: seedling emergence rate, seedling emergence and seedling dry weight.

The ANOVA results, for the three seed performance parameters, revealed significant ($P<0.05$) differences among treatments (Tab. 1). For CL-4 X CL-1 genotype, the pre-sowing irradiation effects for 30 minutes at 60 mT; it can be seen that were significant increase of the seedling emergence rate 14.0% with respect to seedling from control seeds. On the other hand, the seedling emergence percentage showed for 7.5 minutes an effect of negative biostimulation, decreases 16.2% when compared with control samples. The effects for CL-12 X CL-11 genotype, as a function of the alternating magnetic field exposure time, on seedling emergence rate, seedling emergence and dry weight show the seedling emergence rate and seedling emergence (%) respectively, observing positive biostimulation for magnetic flux densities of 60 and 100 mT for all the exposure times (7.5, 15 and 30 minutes); the highest increment occurs at 7.5 minutes exposure time and corresponds to 123.2% for seedling emergence rate and 110.0% for seedling emergence rate when compared to the control seed.

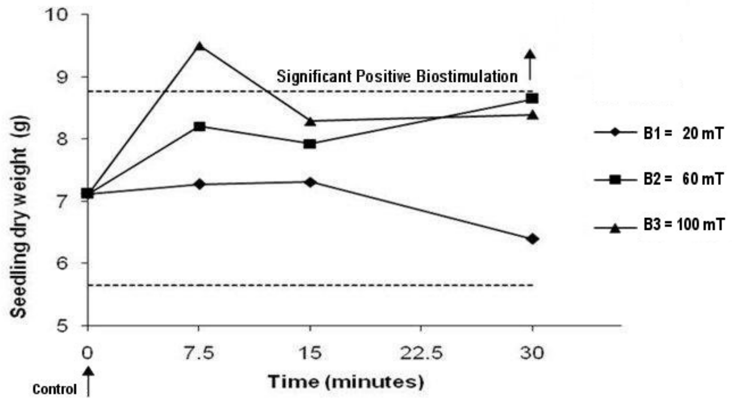
The Figure 1 shows the behaviour of dry weight. In this case only at 7.5 minutes of exposure time and magnetic flux densities of 100 mT, had positive biostimulation; increasing 30.1% when compared to control seeds. Then the combination of exposure time and magnetic flux density with most favourable effects on the evaluated vigour parameters (seedling emergence rate, emergence, and seedling dry weight) were magnetic flux density of 100 mT and irradiation time of 7.5 minutes.

Overall, it was observed that the behaviour of the variables evaluated are modified as a function of the applied treatment, by varying the intensity and exposition time, showing a similar behaviour, for all treatments. First, the evaluated parameters arrive to a maximum value, when comparing with the control, and later they diminish.

The results of this study show that the effects produced by the alternating magnetic field applied pre-sowing depend on genotype and combination of irradiation parameters: magnetic flux density and irradiation time. There is no (CL-13 X CL-1), negative (CL-4 X CL-1) and positive (CL-12 X CL11) bio-stimulations depending on the seed genotype. The negative and positive biostimulations were found with some combinations of time and magnetic flux density, highlighting the importance of finding a combination of parameters to produce favourable effects of biostimulation in different seed genotypes. Variance analysis to 5% level of significance, for CL-11 X CL12 genotype, showed that the best treatment was reached at 100 mT with 7.5 minutes of exposure time since for this treatment the highest seedling emergence rate, seedling emergence, during early stages of growth (third day), and weight dry of seedling aerial parts, at 16th day, were reached.

Table 1. Comparison of the mean of the variables obtained from the vigour test under microtunnel conditions, Montecillo, México

Treat-ments	B(mT)	t (min)	SER	EP	SDW
			Genotype	CL-4 X CL-1 (4th day)	
1	20	7.5	2.91 bdc	88 ba	8.83 ba
2	20	15.0	3.13 bac	94.66 b	8.57 bac
3	20	30.0	2.85 dc	85.33 bac	8.62 bac
4	60	7.5	3.32 ba	93.33 a	9.17 ba
5	60	15.0	2.65 d	76 c	7.52 c
6	60	30.0	3.40 a	94.66 b	8.82 ba
7	100	7.5	3.0 bdac	89.33 ba	8.44 bac
8	100	15.0	3.04 bdac	90.66 ba	7.94 bc
9	100	30.0	2.73 dc	82.66 bc	8.48 bac
10	0	0	2.99 bdac	90.66 ba	9.3 a
			Genotype	CL-13 X CL-1(4th day)	
1	20	7.5	1.91 ba	58.67 a	7.05 a
2	20	15.0	1.92 ba	58.67 a	7.54 a
3	20	30.0	1.43 b	44 a	5.36 b
4	60	7.5	1.40 b	44 a	6.04 ba
5	60	15.0	1.83 ba	56 a	6.46 ba
6	60	30.0	1.82 ba	56 a	7.46 a
7	100	7.5	2.09 ba	62.67 a	6.79 ba
8	100	15.0	1.74 ba	53.33 a	6.41 ba
9	100	30.0	2.15 a	62.67 a	7.05 a
10	0	0	1.72 ba	52 a	6.64 ba
			Genotype	CL-12 X C-L11(3rd day)	
1	20	7.5	1.476 bdac	40 bac	7.276 bc
2	20	15.0	1.307 dc	34.66 bc	7.30 bc
3	20	30.0	1.415 bdc	35.66 bac	6.36 c
4	60	7.5	2.077 ba	56 a	8.2 ba
5	60	15.0	1.95 bac	52 ba	7.92 bac
6	60	30.0	1.90 bac	52 ba	8.64 ba
7	100	7.5	2.12 a	56 a	9.5 a
8	100	15.0	1.97 bac	53.33 a	8.28 ba
9	100	30.0	1.94 bac	49.33 ba	8.39 ba
10	0	0	0.95 d	26.66 c	7.30 bc



The mean values with the same letters are statistically equal (LSD, $\alpha = 0.05$)). LSD, Least significant difference probability level, Treatment 10 = Control without electromagnetic irradiation, SER = Seedling emergence rate, EP = Emergence percentage (%); SDW = Seedling dry weight (g).

Fig. 1. Alternating magnetic field treatment effects on seedling emergence rate on CL-11 X CL-12 genotype

DISCUSSION

The improvement, in the three seed vigour parameters, by alternating magnetic field irradiation resulted in favourable biostimulation for CL-11 X CL12, it can be associated with the increment in seed vigour because there were increases in: 1) seedling emergence rate (rapid emergence), 2). in dry weight (rapid development) and 3) emergence (higher potential) when compared with control seeds (Artola *et al.*, 2003). The three vigour parameters were improved and comply with the definition of seed vigour given by AOSA (1983), i.e. "those seed properties, which determine the potential for rapid, uniform emergence and development of normal seedling under a wide range of field conditions".

This positive influence of magnetic field has been also reported by some authors, for example Aladjadiyan (2002) found that corn seed exposed to a 150 mT magnetic field stimulated the shoot development and led an increase in the maize germination. Also magnetic treatment generated by magnet rings with B values of 125 mT and 250 mT for different exposure times showed an increase in the germination rate of maize (Florez *et al.*, 2007). Kato (1988) showed that a magnetic field of 500 mT stimulates root growth of *Zea mays*.

Alexander and Doijode (1995) reported an increase in emergence (127.3%) and germination (36.6%), when compared with seed control in onion, in the case of rice there was a significant increment (161.48%) by the electromagnetic treatment. Seed magnetic field treatment in the range of 72 mT to 128 mT with exposure times between 13 and 27 minutes enhanced yield in soybean, cotton and wheat (Phirke *et al*. 1996).

Dayal and Singh (1986), reported that magnetic fields varying from 15 to 155 mT, applied at different times of exposure, improve the weight and number of primary branches when compared with control samples from tomato seeds. Martinez *et al*. (2009) indicate that germination of tomato seeds was affected by the magnetic treatment, in general, the germination rate of treated seeds was higher than the untreated seeds.

On the other hand, Gusta *et al*. (1978), studied the effects of magnetic fields on dry cereal seeds. They applied different magnetic field intensities to these cereal seeds and they do not found effects on seedling growth and germination. One of these treatments, applied to wheat seeds, showed differences, however after 110 hours of imbibition there was no statistical difference, in the weight dry variable, between the treated and control seeds.

Magnetic fields produce biochemical, physical and physiologic changes in cell structures (Pietruszewski 2007). In this sense, the present research project assessed the effects of three maize seed genotypes (CL-11 X CL-12, CL-4 x CL-1 and CL-13 X CL1), when they are submitted to alternating magnetic field treatments. Three different magnetic flux densities (20, 60, and 100 mT) were applied to the seeds during three different exposure times (7.5, 15 and 30 minutes). It was found, for CL-11 X CL-12 genotype, significant statistical differences ($p \leq 0.05$), three days after starting the emergence, for the emergence rate and seedling establishment percentage parameters. Also, the dry weight, after 16 days, presented this significant difference proving that, in the case of the best treatment, the effects remain over time. In the case of CL-4 x CL-1 genotype only presented significant statistical differences for seedling emergence rate and seedling emergence percentage however significant statistical differences in dry weight were not found. In the case of CL-13 X CL1 genotype was found no significant differences in any of studied vigour variables, in the time range and magnetic flux density applied, resulting in a null impact the alternating magnetic treatment. These results highlight the importance of seed genotype to produce favourable biostimulation effects and also suggest that is necessary to research other magnetic flux densities and exposition times in seed genotypes, as CL-13 X CL1, in order to find positive biostimulation.

Mechanism associated with this phenomenon could be activated by auxin (Boe and Salunkhe 1963), with some combination of irradiation parameters. Auxins regulate essential aspects of plant growth and development since auxin is a hormone that operates through its effects on cell division and elongation. It is transported through files of cells by a process that is thought to depend on the asymmetric distribution of auxin "efflux carriers acting at a cell's plasma membrane" (Estelle, 2001). Mitrov *et al*. (1988) reported an increase in auxin content in maize seeds exposed to magnetic field of 160 G during 10 minutes.

Pazur *et al*. (2006), grown barley seedlings under static magnetic and electromagnetic fields, noted influence of the applied MF and EMF, affecting Ca^{2+} levels, via mechanisms of ion-cyclotron resonance. Phytocrome A activity and circadian regulation are driven by Ca^{2+} oscillations (Dodd *et al*., 2005). The phytochromes are chromoproteins that are capable of regulating almost all phases of plant development (Winkel 1998, Smith 2000; Hernandez *et al*. 2008a, Hernandez *et al*. 2008b). The interaction of magnetic field with seeds produces multiple reactions in seeds; these reactions were found in the present research project through the seed emergence test, and a suitable combination of irradiation parameters conduce to favourable effects of biostimulation. We also found that effects of alternating magnetic field depend on the seed genotype. Pietruszewski (2007) pointed out that the fundamental role of the research is a selection of biological material, and these are extremely complex system. In this research can be seen that each genotype have effects different to same irradiation parameters.

The increment of maize production in the world is very important due to the fact that this cereal is used for human and animal consumption as well as to produce energy, making it increasingly important to develop new technologies to increase its production. Since a critical problem in the seed industry is the production of seeds with low vigour, the development of ecological and low cost technologies to improve the seed vigour, which can be implemented as a pre-treatment sowing, could be very useful. For this reason it is important to determine the optimal parameters of different irradiation sources in a broader germplasm for future investigations. Alternating magnetic methods can be considered as a physiological treatment that could improve significantly the vigour of maize seeds under optimal irradiation parameters.

CONCLUSIONS

1. In the present research project it has been found that the effects of alternating magnetic field treatments, in maize seeds, depend on the seed genotype. Then it was found zero, negative and positive biostimulation effects for CL-13 X CL-1, CL-4 X CL-1 and CL-11 X CL-12 genotypes respectively.
2. Positive effects are dependent on the irradiation parameters (intensity and exposure time) used in the pre-sowing seed treatment. The best result was obtained with CL-12 X CL-11 maize genotype when irradiated with magnetic flux density of 100 mT, applied during 7.5 minutes. In this treatment it was observed a significantly increment in the studied vigour parameters and hence the physiological quality of seeds.

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WPŁYW ZMIENNEGO POLA MAGNETYCZNEGO NA WSCHODY NASION TRZECH GENOTYPÓW KUKURYDZY W WARUNKACH POLOWYCH

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S t r e s z c z e n i e. W prezentowanych badaniach zastosowano zmienne pole magnetyczne o niskiej częstotliwości (60 Hz) w kombinacji z trzema poziomami indukcji magnetycznej (20, 60 i 100 mT) oraz trzema czasami ekspozycji (7.5, 15 i 30 minut) do przedsiewnego traktowania nasion kukurydzy (*Zea mays* L.) trzech genotypów (CL-12 X CL-11, CL-4 X CL-1 oraz CL-13 x CL-1). W przypadku genotypu CL-12 X CL-11, traktowanie polem magnetycznym spowodowało istotne zwiększenie liczby wschodów siewek, suchej masy siewek, oraz wschodów w glebie. Najlepszą kombinacją doświadczenia okazało się traktowanie nasion polem o indukcji 100 mT przy czasie ekspozycji 7,5 min, które spowodowało istotną poprawę liczby wschodów siewek (o 123,2%), liczby wschodów polowych (o 110 %) oraz suchej masy siewek, 21 dni po wysadzeniu do gleby (o 30,1 %). W przypadku genotypu CL-4 X CL-1 stwierdzono negatywną biostimulację w zakresie procentowego wskaźnika wschodów. W przypadku ostatniego genotypu, CL-13 x CL-1, nie stwierdzono żadnego istotnego wpływu stymulacji nasion polem magnetycznym. Te wyniki pokazują, że stymulacja polem elektromagnetycznym stanowi dobrą i ekologiczną metodę poprawy wigoru nasion kukurydzy, ale konieczne jest znalezienie optymalnych parametrów dla wywołania dodatniej biostymulacji nasion kukurydzy, co uzależnione jest także od genotypu tych nasion.

S ł o w a k l u c z o w e: kukurydza (*Zea mays* L.), pole elektromagnetyczne, wigor nasion, biostymulacja