GENETIC DIVERSITY OF WINTER WHEAT (*TRITICUM AESTIVUM* L.) GROWING NEAR A HIGH VOLTAGE TRANSMISSION LINE

R. EL-BAKATOUSHI[1]

The objective of this study was to determine the effects of an electromagnetic field on the genetic variability on winter wheat plants cultivated underneath high voltage transmission lines (50 Hz, 6 kV/m). The experiment with winter wheat plants was carried out near Kafr El-Dwar power station (Egypt). The effects were investigated on seed germination, pollen grain viability, RAPD profile and protein molecular weight distributions. The percentage of seed germination did not differ significantly between the exposed and unexposed plants. The results indicated that the plants exposed to the electromagnetic field showed a significant increase in the mean percentage of non viable pollen grains. DNA fingerprinting showed significant differences between DNA patterns of exposed and unexposed wheat plants. The UPGMA clustering analysis indicated high genetic distances between individuals under the high voltage transmission line (ranging from 0.33 to 0.84), compared to individuals from fields free from power towers which clustered together with low genetic distances (ranging from 0.10 to 0.29). The evaluation of genetic variability parameters (actual number of allele (na), effective number of alleles (ne), Nei's gene diversity (h), Shannon's information index (I), number of polymorphic loci and percentage of polymorphic loci) for all loci within each population confirmed the results obtained from the dendrogram. The total amount of soluble protein did not differ between the two groups. It was concluded that the genetic diversity of individuals of wheat plants grown under transmission lines increases, although seed germination and the amount of total soluble protein does not change.

Key words: genetic diversity, wheat, Electromagnetic field, RAPD, SDS – PAGE.

INTRODUCTION

Environmental stresses come in many forms and plant species vary in their sensitivity and response to environmental stresses because they have various capabilities for stress perception, signalling and response (Bohnert *et al.*, 1995). Electromagnetic fields from such seemingly innocuous devices as power lines, mobile phones and domestic wiring is one kind of stress, which can affect the plant exposed to it directly or indirectly A wide range of physiological effects, stimulation of the biological processes and nutrient metabolism has been observed in plants as a result of the exposure to a high voltage electric field (Ynikiene and

[1] Biological and Geological Sciences Department, Faculty of Education, University of Alexandria, 21526 El-Shatby, Egypt, E-mail ranyaelbakatoushi@yahoo.com, Fax. +034865671, Tel. 0102417351

Pozeliene, 1998; Zhang *et al*., 1997). Effects on plant growth, the role of electric field effects on cell polarity (Schnepf, 1986), on calcium transport (Hepler and Wayne, 1985), and on auxin transport (Rathore and Goldsworthy, 1985) have been observed. Several researchers have described the effect of such fields on the growth rate of the plants; both significant increases of plant growth and impairment of productivity in response to electromagnetic fields have occurred. Fischer *et al.* (2004) studied the effects of weak $16\frac{2}{3}$ Hz magnetic fields on growth parameters of young sunflower and wheat seedlings and showed that the shoot and the root fresh weights of sunflower plants significantly increased, but wheat dry weight and germination rates remained unaffected. Treated wheat exhibited higher root fresh and dry weights and also higher germination rates. More positive results concerning magnetic or electric field effects on seed germination have been reported by Davies (1996) and Zhang and Hashinaga (1997). However, Rabold *et al.* (1990) showed that extremely low frequency (60 Hz) 360 V/m electric fields can inhibit growth in plant root model cell systems. Electric fields can cause deformation inside wheat grains through compression or tension of particular layers (Sumorek and Pietrzyk, 1999), and inhibited the biological properties of the membrane protein (Laberge, 1998; Walter *et al*., 1997; Macginitie *et al*., 1994). Karcz and Burdach (1995) and Plätzer *et al.*, (1997) observed increased activity of Hþ-ATPases in electric fields, leading, for example, to enhanced lily pollen tube growth. These results show that the electrical field is effective on the single cell or protoplast level and also suggest that under certain conditions, an intact organism may benefit either partly or as a whole. Bai *et al.* (2003) investigated the original mechanism of the biological effects of the electrostatic field (4 kV/m) on barley and (4.5 kV/m) sugar beet seeds for 10 minutes. Their results showed that electrostatic fields with a certain intensity could increase the content of free radicals in seeds.

The effect of electromagnetic fields is not limited; it generates some side effects. For example Elansky *et al.* (2001) showed that high voltage transmission lines (HVLs) are able to change significantly the ozone concentration within the atmospheric surface layer over regions where the HVLs is high. Ozone is an air pollutant with significant effects on agriculture (Brimblecombe, 1988). Vaida *et al.* (2008) proved that the ozone concentration near high-voltage lines changed from 10 to 51 ppb. The high voltage electric field has irreversible mutagenic effects on wheat and clover plants (Solman and Hammad, 2002; Magda *et al*., 2006).The cytogenetic effects of the electric field were tested on different plant systems as a valid model for monitoring the hazardous effects of electric field. Electric fields were associated with a significant decrease in mitotic indices, and caused an increase in the percentages of chromosomal aberrations (Mamata *et al*., 1987; Promila *et al*., 1991).

Learning about the molecular mechanisms by which plants tolerate environmental stresses is necessary for genetic engineering approaches to improve crop performance under stress. Different methods are available to investigate the effect of mutagens on plants. Molecular markers allow direct comparisons of the effects on genotypes at the DNA level. A variety of molecular techniques has been developed and is widely used in many fields such as agriculture and biology. Random amplified polymorphic DNA (RAPD) may potentially form the basis of novel biomarker assays for the detection of DNA damage and mutational events (*e.g*. rearrangements, point mutation, small insert or deletions of DNA and ploidy changes) in cells of bacteria, plants, invertebrate and vertebrate animals (Savva, 1996 and 1998; Atienzar *et al*., 2000). RAPD markers are detected by the use of short and long oligonucleotides (10-21 bases) of arbitrary sequence as primers for the amplification of segments of the target genome. The potential value of long primers for generating RAPD polymorphisms was investigated and used in genomic fingerprinting before (ye, 1996; Welsh and McClelland, 1990 and 1991). The assessment of RAPD markers for various purposes has been demonstrated in a number of species such as soyabean (Zhang *et al*., 1996), wheat (Gang and Weber, 1996), rice (Mezencev *et al*., 1997) and barley (Svitashev *et al*., 1998).

Recent proteomic research has provided evidence that environmental stress or stimuli induce the expression of characteristic stress-related proteins in living organisms. Stress proteins are associated with sensing and repairing damage to DNA, helping damage proteins to refold and to regain their conformations and also acting as chaperones for transporting cellular proteins to their destinations in cells (Kultz *et al*., 2005). This opens the possibility of using these proteins, which are involved in an adaptive and protective mechanism of living organisms for the detection of any kind of environmental stimuli.

Uncertainties regarding the mechanisms of how electromagnetic fields are ‘‘perceived’’ by the cells and translated into biological effects, restrained the general use of electromagnetism for plants. This question could be important for field crops grown in the locality of high voltage transmission lines, as in this case large acreages could be influenced adversely.

For crops under high voltage transmission lines, farmers complain about the delay in gathering harvests from these fields and the weakness of these crops comparable to fields free from high voltage transmission lines. Therefore, the aim of this investigation is to evaluate and analyze the impact of high voltage electric fields on the genetic diversity of winter wheat plants compared to fields free from high voltage transmission lines.

MATERIAL AND METHODS

***Experimental sites*.** In this experiment, many parameters were standardised as far as possible, although electromagnetic field strengths will differ between

various parts of fields under power lines. The field used for the experiments was located in a rural environment. Winter wheat plants (*Triticum aestivum* L. cv. Giza 168) were collected from fields near the Kafr El-Dwar power station 310 07' E, 30 0 07' N. The plants were divided into two groups:

Group A – field 100 m or more from the station and completely free from the power line towers.

Group B – field adjacent to the wall of the power station under the high voltage transmission lines (50 Hz, 66 kV/11 m = 6 kV/m) carried by nine power towers.

The two locations were chosen for the uniformity of soil, environmental conditions and the means of irrigation. Five individuals from group A (control) were taken to evaluate the genetic consistency between individuals growing in field free of high voltage transmission lines).

Wheat plants were kindly examined by the Agriculture Research Center, Cairo, Egypt to ensure that all plants from both fields are the same variety (Giza, 168).

Seed germination. Twenty-five seeds were placed on Whatman filter paper in petri dishes. Four petri dishes with 25 seeds were used for two groups. Thus each group was represented by 100 seeds. The seeds were subjected to distilled water. The percentage of germination was recorded on day 2.

Pollen viability. Stainability of the pollen grains of wheat plants in aceto-carmine stain for the two treatments were performed as an index of determining pollen viability and statistically analyzed using t-tests.

DNA analysis. Fresh leaves of plants were collected and total genomic DNA was extracted using Wizard genomic DNA extraction kit promega (USA). 10-mer to 21-mer arbitrary primers were used for RAPD analysis. Six primers were screened for their amplification (Table 1). PCR amplification was performed in total volume of 25 µl containing 10× reaction buffer, 2,5 mM dNTPs, 5 mM MgCl2, 10 pmol/reaction primer, 100 ng of genomic DNA and (0.5 U μl^{-1}) of Taq polymerase (promega, Germany) in Thermocycler Gene Amp 9700, (Applied Biosystems (ABI), (USA). After a denaturation step for 5 min at 95 °C, the amplification reactions were carried out for 40 cycles. Each cycle comprised of 1min at 95 °C, 1min of annealing temperature ranged from 28 °C to 30 °C in the primers used and 1min at 72 °C. The final elongation step was extended to 10 min. Amplification products were separated on agarose gel electrophoresis using 1.5% (w/v) agarose in 0.5× TBE buffer and stained with ethidium bromide and photographed by using gel documentation system. Amplification products were compared with molecular weight marker IX (100–1500 bp).

Table 1

Sequences of six primers used in this study

No. of primers	Sequences of primers (5` → 3`)
1 Primer (B2)	CGC TGT CGC C
2 Primer (G2)	GAG GCC AGT GTC TGT TTG
3 Primer (RAPD2)	ATG CCC CTG T
4 Primer (VO)	GGG AAA ACG ACA ATT GC
5 Primer (A9b7)	GGT GAC GCA GGG GTA ACG CC
6 Primer (BAIF)	GTG GGG GTA GGA TGA GAT GAT

The obviously reproducible RAPDs were scored and entered as binary characters (1 for presence and 0 for absence). The percentage of polymorphism was estimated as the proportion of polymorphic bands with the total number of bands. Also polymorphism in RAPD profiles included disappearance of normal band and appearance of new band in comparison to all data of group A as a control.

Genetic similarity was calculated on the basis of genetic distances coefficients using the NTSYS-pc program (Rohlf, 2002). The similarity matrix was subjected to cluster analysis by the Unweighted Pair Group Method with Arithmetic averages (UPGMA) (Sneath and Sokal, 1973).

Genetic variation for all loci within each group was calculated using Popgene32 (Yeh *et al*., 1999).

***Total seed protein electrophoresis*.** Soluble proteins were determined according to the method described by Bradford (1976). The identification and characterization of different protein fractions were obtained using one-dimensional Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS – PAGE). Polyacrylamide slab gel (12.5) was carried out using the discontinuous buffer system as described by Laemmli (1970).

The banding pattern, molecular weight and band percentage were analysed using TotalLab version 1.11 software (Nonlinear Dynamics Ltd., Durham, USA) in the presence of protein molecular weight marker. The obvious protein bands were scored and entered as binary characters (1 for presence and 0 for absence). Genetic similarity was calculated on the basis of genetic distances coefficients using the NTSYS-pc program (Rohlf, 2002). The similarity matrix was subjected to cluster analysis by the Unweighted Pair Group Method with Arithmetic averages (UPGMA) (Sneath and Sokal, 1973).

RESULTS

***Seed* germination.** The two groups did not differ significantly in the percentage of germination (P = 0.258).

***Pollen grains*.** A highly significant increase in the percentages of abnormal pollen was observed in plants exposed to electromagnetic fields (Table 2).

Table 2

Percentages of non-staining pollen grains in *Triticum aestivum* L. flower buds, in groups

	No. of examined pollen grains	Percentage of non-staining pollen grains / plant						Mean % of non viable pollen grains ± SE
Group A	4857	0.191	0.23	0.11	0.22	0.16	0.38	0.22 ± 0.09
Group B	5033	8.73	4.27	1.83	3.43	2.74	3.10	4.02** ± 2.44

** Significant from control at 0.01 level (t-test)

Table 3

Changes of total bands in control, and of polymorphic bands and varied bands in *Triticum aestivum* group A

	Primers Group A individuals														
	1				2			3			4			5		
	C.b	T.N.B.	F.P.B.	%P	T.N.B.	F.P.B.	%P	T.N.B.	F.P.B.	%P	T.N.B.	F.P.B.	%P	T.N.B.	F.P.B.	%P
Primer 1	1	5	0	33.33	5	0	33.33	6	1	41.66	5	0	33.33	7	1	50.00
Primer 2	0	6	0	40.00	3	0	20.00	7	0	46.66	7	0	46.66	7	0	46.66
Primer 3	0	4	0	36.36	3	0	27.27	2	0	18.18	4	0	36.36	4	0	36.36
Primer 4	1	6	0	35.71	3	0	14.28	7	0	42.85	6	0	35.71	6	0	35.71
Primer 5	0	9	0	42.85	9	0	42.85	10	0	47.61	5	0	23.80	4	0	19.04
Primer6	0	2	0	13.33	2	0	13.33	2	0	13.33	2	0	13.33	2	0	13.33
Mean				33.59			25.17			35.04			31.53			33.51
Total		32	0		25	0		34	1		29	0		30	1	

Table 4

Changes of total bands in control, and of polymorphic bands and varied bands in *Triticum aestivum* group B

Primers	Group B individuals																							
	6				7				8				9				10				11				12			
	C..B.	T.N.B.	F.P.B.	%P	C..B.	T.N.B.	F.P.B.	%P	C..B.	T.N.B.	F.P.B.	%P	C..B.	T.N.B.	F.P.B.	%P	C..B.	T.N.B.	F.P.B.	%P	C..B.	T.N.B.	F.P.B.	%P	C..B.	T.N.B.	F.P.B.	%P
Primer 1	1	9	1	66.66	1	6	0	41.66	1	4	0	25.00	1	5	0	33.33	1	6	0	41.66	1	2	0	8.33	1	7	0	50.00
Primer 2	0	8	0	53.33	0	5	1	33.33	0	6	1	40.00	0	5	0	33.33	0	5	0	33.33	0	2	0	13.33	0	4	1	26.66
Primer 3	0	7	3	63.63	0	4	0	36.36	0	1	0	9.09	0	5	0	45.45	0	5	0	45.45	0	2	0	18.18	0	1	0	9.09
Primer 4	1	8	0	50.00	1	7	1	42.85	1	5	1	28.57	1	8	2	50.00	1	6	1	35.71	1	4	0	21.42	1	6	1	35.71
Primer 5	0	11	2	52.38	0	7	0	28.57	0	6	0	23.80	0	10	0	47.61	0	3	0	14.28	0	12	1	57.14	0	6	0	28.57
Primer 6	0	5	1	33.33	0	2	0	13.33	0	1	0	6.66	0	2	0	13.33	0	8	6	53.33	0	6	0	40.00	0	6	0	40.00
Mean				53.22				32.68				22.18				37.17				32.79				26.4				31.67
Total		48	7			31	2			23	2			35	2			33	7			28	1			30	2	

RAPD analysis. Six primers were used to analyze PCR products and they generated 88 RAPDs which ranged in size from 100–1500 bp (Fig. 1). The total number of fingerprinting bands and the percentage of polymorphism for each species are summarized in Tables 3 and 4.

The total number of RAPD bands in group A (control) ranged from a minimum of 25 bands in individual 2 to a maximum of 34 bands in individual 3 with an average percentage of polymorphism 25.17 and 35.04 respectively. On the contrary, the minimum total number of bands recorded for Group B was 23 bands in individual 8 with 22.18 % as an average of polymorphism and the maximum total number of bands was 48 bands in individual 6 with 53.22 % as an average of polymorphism. The fingerprinting bands nearly disappeared in group A compared with group B. In Group A the maximum estimated with the primer 6 (6 bands), followed by the primer 3 in individual 6 (3 bands), pursued by the primer 4 in individual 9 and primer 5 in individual 6 (2 bands). Only a few common bands were detected between Group A and Group B (one band in primer 1 and primer 4) (Tables 3 and 4). The principal differences observed between group A and group B individuals were a variation in band intensity, loss of normal bands and appearance of new bands. The appearance of new bands reached a maximum of 15 bands in group B individuals 6, 10, and the disappearance of bands reached a maximum of 29 bands in group B individual 8 (Table 5).

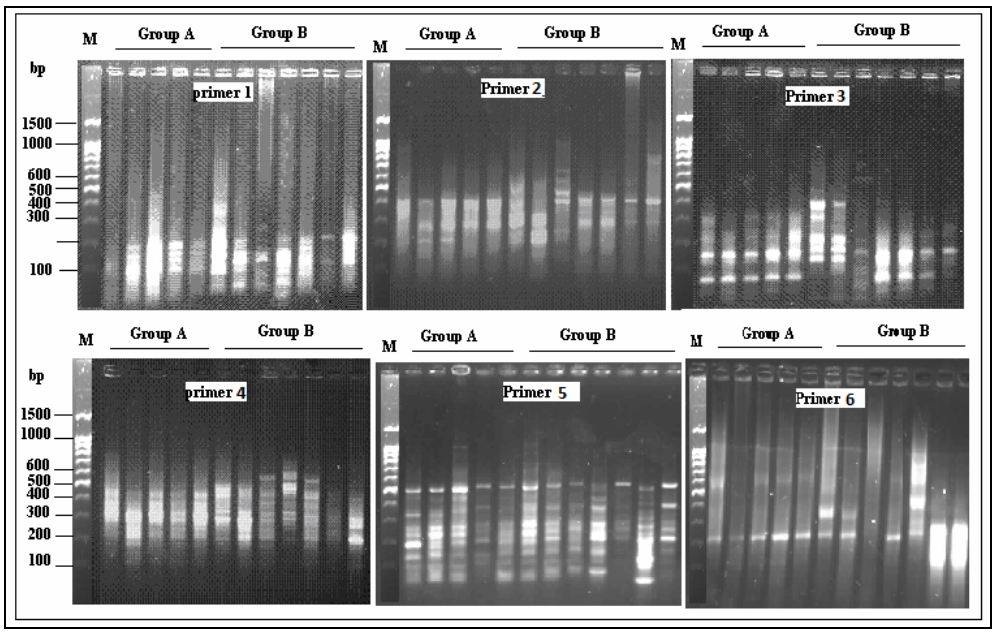

Fig. 1. RAPD profiles of genomic DNA from leaves of *Triticum aestivum* groups.

***Genetic distances between groups*.** A distance matrix using genetic distances coefficients for all individuals based on RAPD results was used for constructing the dendrogram. The UPGMA tree clustered the individuals into two main groups, group I and group II at a similarity distance of 0.48. Group I comprised three individuals from group B plants and group II was divided into subgroups IIa and IIb at a similarity distance of 0.69. The subgroup IIa included other two individuals of group B plants (4, 5). The second subgroup IIb further subdivided into two subgoups IIba and subgroup IIbb at a similarity distance of 0.57. The sub group IIba comprised two individuals of group B plants, while subgroup IIbb contained all individuals of group A plants (Fig. 2).

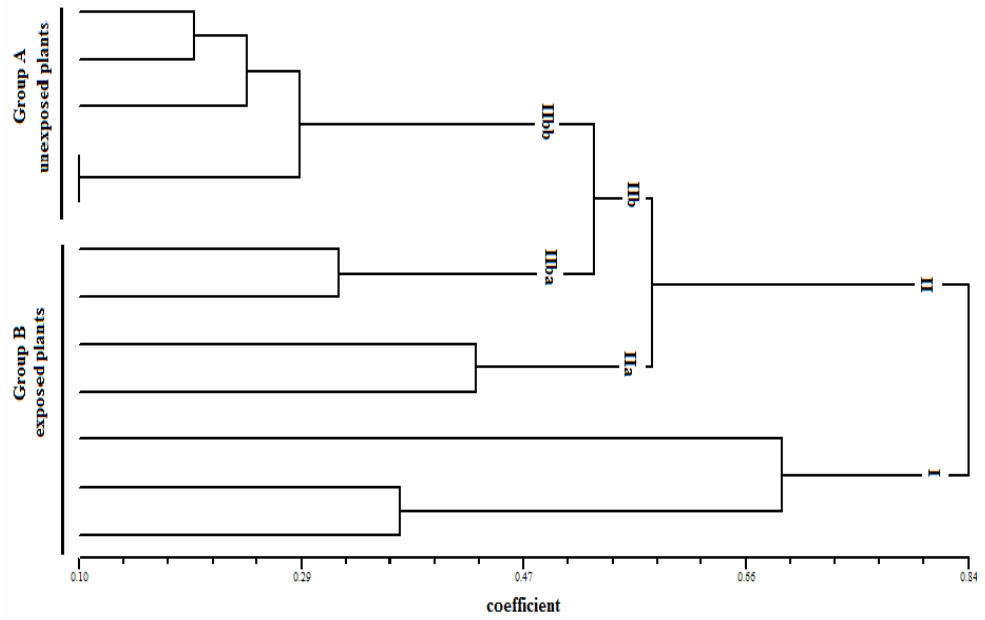

Fig. 2. Dendrogram constructed by UPGMA based on the genetic distances among RAPD profiles of *Triticum aestivum* groups.

***Genetic variation statistics for all loci within each population*.** The highest variation values were detected in group B individuals. Nei's gene diversity (h), Shannon's information index (I), number of polymorphic loci, percentage of polymorphic loci and the actual number of alleles (na) were higher than group A (Table 6).

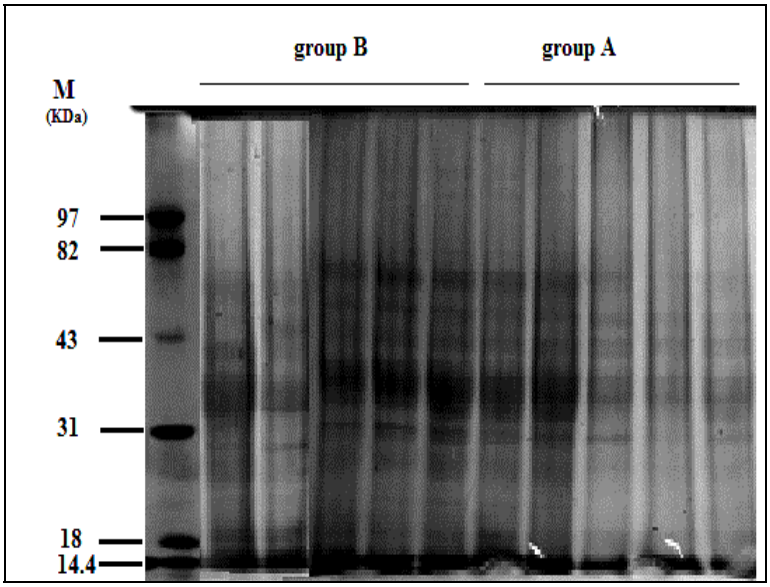

Fig. 3. Electrophotograph produced by SDS-PAGE analysis of soluble protein patterns of *Triticum aestivum* groups.

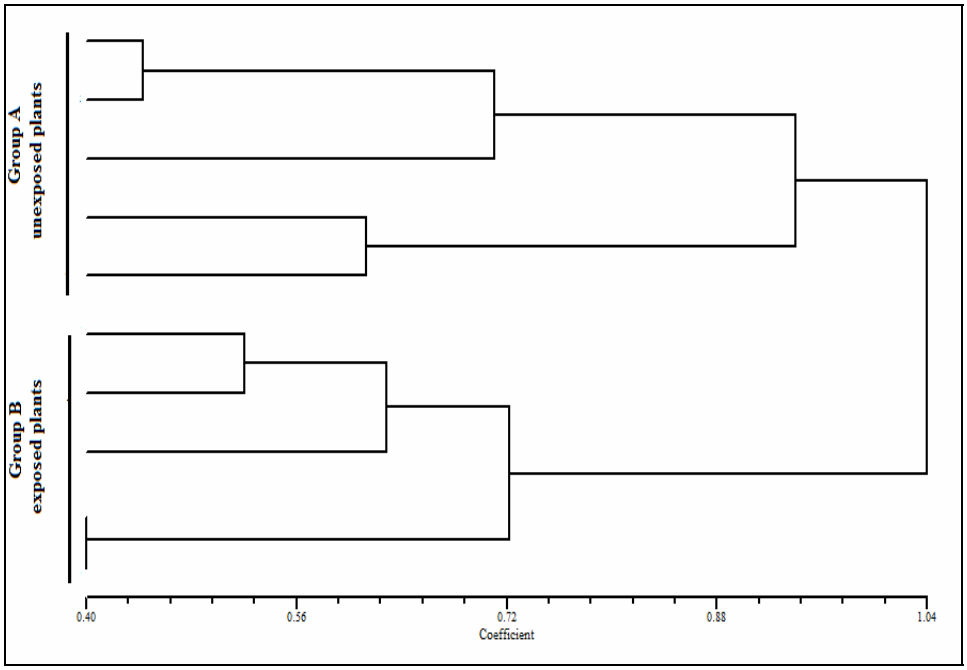

Fig. 4. Dendrogram resulted from UPGMA cluster analysis based on protein electrophoresis of *Triticum aestivum.*

Table 5

Appearance of new bands (a), disappearance of normal bands (b), decrease in band intensities (c) and polymorphic bands (a+b) in *Triticum aestivum* group B

Primers	Group B individuals															
T..B.G.A.	6		7		8		9		10		11		12			
a b	a	b	a	b	a	b	a	b	a	b	a	b	a	b		
Primer 1	8	3	1	2	4	2	6	1	3	1	2	0	5	2	2	
Primer 2	9	2	3	3	7	3	6	1	5	1	4	0	7	1	6	
Primer 3	4	6	3	3	3	1	4	3	2	3	2	1	3	1	4	
Primer 4	9	0	1	0	2	1	5	2	3	1	4	0	5	0	3	
Primer 5	11	1	1	1	5	2	7	3	4	1	9	6	4	2	7	
Primer 6	2	3	0	1	0	0	1	0	0	8	1	6	1	6	1	
Total a +b	43	15	9	10	21	9	29	10	17	15	22	13	25			
	24		31		38		27		37		38					

Table 6

Mean±SE of actual number of allele (na), effective number of alleles (ne), Nei's gene diversity (h), Shannon's information index (I), number of polymorphic loci and percentage of polymorphic loci in *Triticum aestivum* groups

Groups	na	ne	h	I	NPL	%PL
Group A	1.32±0.46	1.21±0.32	0.12±0.18	0.18±0.27	28	31.82
Group B	1.90±0.30	1.53±0.31	0.32±0.15	0.48±0.19	79	89.77

SDS-PAGE. The electrophoretic patterns (SDS-PAGE) for water soluble proteins of the two groups of wheat are illustrated in Fig. 3 and Table 7. A total number of 42 protein bands, with molecular weight ranging between 128.76 to13.80 kDa were recorded from the electrophenograms of the plants studied. The electrophoretic patterns of exposed plants revealed the presence of fourteen peptides ranged from 120.82 kDa to 13.80 kDa. The same number of peptides was recorded for unexposed plants but with different molecular weights ranged from 128.76 kDa to 15.66 kDa. The difference between the percentage of polymorphic bands between exposed and unexposed plants was not significant. Peptides with a molecular mass 60.52 kDa occurred in all individuals of exposed plants but was not found in unexposed individuals. Sixteen peptides with different molecular masses disappeared in exposed plants (Table 7). The amount of the total soluble protein did not differ significantly between both groups (T = 0.52, P = 0.616). The UPGMA tree separated the exposed plants from unexposed at a dissimilarity distance of 1.04 (Fig. 4).

Table 7

Relative concentration (band %), rate of flow and molecular weight (M.wt)
of protein bands of Triticum aestivum groups

Band no.	Rf	M.wt. (KDa)	Band %									
			Triticum aestivum									
			Unexposed plants (Group A)					Exposed plants (group B)				
			1	2	3	4	5	1	2	3	4	5
1	0.09	128.76	7.49*									
2	0.09	127.88	8.53*									
3	0.01	122.56				6.46*						
4	0.12	120.82					5.60*					
5	0.13	119.06						4.70				14.73
6	0.16	111.12	11.61		4.10							
7	0.28	83.45							12.77*			
8	0.29	80/67								8.85*		
9	0.33	76.13										
10	0.34	74.72	6.77	15.95	14.67	8.92	6.91	3.88	7.67		6.58	
11	0.36	73.60						6.74	6.85	9.89		6.89
12	0.38	72.13					6.48				9.19	
13	0.39	71.62				7.65*						
14	0.40	71.59										14.89
15	0.42	70.34		14.80	9.64			7.55	8.27	6.02		
16	0.44	69.62				6.78	6.41					
17	0.48	66.381							8.27	11.51	11.71	
18	0.49	65.59	10.14	11.84	7.68							
19	0.50	64.72					7.25	7.59			5.31	2.40
20	0.51	63.78	4.63			6.49						
21	0.53	60.52						1.80	3.13	3.34	2.93	6.95
22	0.56	54.06	4.99			4.44	4.31		10.65	11.26		2.52
23	0.58	51.25		4.97	6.15			9.79				
24	0.59	49.11					4.22				8.22	
25	0.60	46.22								7.80	2.42	4.78
26	0.61	44.07		15.22					7.79			

Table 7

(continued)

27	0.62	43.35			9.90	10.44						
28	0.63	41.95	14.16				8.79	7.12				3.25
29	0.64	39.88									9.85	10.81
30	0.65	37.88			4.49*							
31	0.66	35.97				7.88*						
32	0.68	33.05					3.82		6.99	9.20		
33	0.69	31.48	6.08		6.37			3.63				
34	0.70	30.08		7.98*								
35	0.72	28.8						3.04			11.15	
36	0.73	27.39				3.27	13.27					
37	0.77	24.54	8.89	7.77	9.05	9.19	10.64	10.38	6.19	8.47	8.56	3.38
38	0.86	21.23	1.29	10.65	2.54	11.0	5.10	6.07	2.28			8.63
39	0.90	19.69	15.21		4.5		5.12	6.45	8.56	11.97	12.00	
40	0.96	15.66	8.75		11.35	7.61	8.41	8.27		11.69	12.55	11.99
41	0.97	14.72							10.65			8.77
42	0.98	13.80										10.82*
Number of individual specific bands			**2**	**1**	**1**	**3**	**1**	**0**	**1**	**1**	**0**	**1**
Percentage of polymorphic bands			**28.57**	**16.16**	**26.19**	**26.19**	**30.95**	**30.95**	**28.57**	**23.80**	**26.19**	**28.57**

* = accession specific ban

DISCUSSION

Most cultivated species have wild relatives that exhibit excellent tolerance to abiotic stresses and have obtained novel genes in their evolutionary history that have enabled them to occupy stressful environments. The study results showed that electromagnetic field has no effect on seed germination of winter wheat plants. Similar results were reported in barley (Mericle *et al*., 1966), in *Castanea sativa* (Ruzic *et al*., 1993) and in young sunflower and wheat (Fischer *et. al*, 2004). Rajendra *et al.* (2005) studied effects of power frequency electromagnetic fields on germination and protein content of *Vicia faba* and he suggested that exposure to power frequency electromagnetic fields did not cause any permanent damage, since the initial alteration under the magnetic fields in important housekeeping enzymes involved in the beginning of seed germination were returned to control values after eight days of germination. Similar results on germinating of Spruce seeds subjected to 50 Hz were recorded (Ruzic *et al*., 1998).

A highly significant increase in the percentages of abnormal pollen was observed in plants exposed to electromagnetic fields. A similar result was obtained by Bondar and Chastokolenko, (1988) where they found an increase in the percentage of sterile and semi sterile flower buds in *Vicia cracca* plants growing near high voltage electric power lines. It was indicated that the sterility was probably due to an increase in chromosome aberration frequency at various stages of meiosis in wheat plants (Hanafy, 2003).

In the exposed plants, the appearance of new bands in RAPD profiles reached a maximum of 15 bands in individual 10, while the disappearance of bands reached a maximumum of 29 bands in individual 8. Moreover, the exposed plants had the wide variation in the total number of bands ranged from a minimum of 23 bands to a maximum of 48 bands. The significant DNA fragmentation (damage) could be due to the leaks in the membranes surrounding lysosomes which release digestive enzymeslike DNAase and may explain the damage done to DNA after exposing to electromagnetic field (Diem *et al.* 2005; and Panagopoulous *et al*., 2007). Some workers have found that the damage of one base pair in the target sequence of the genome may result in a completely different RAPD profile, since each 10bp oligonucleotide primer only covers a very limited part of the genome (Zhang and Hashinaga, 1997).

The dendrogram showed considerable high genetic distances in individuals under the high voltage transmission line (ranged from 0.33 to 0.84), comparable to individuals of field free of power towers which clustered together with low genetic distances (ranged from 0.10 to 0.29). The evaluation of genetic variability parameters for all loci within each population confirmed the results obtained in the dendrogram. The study revealed an abundance of diversity within the exposed wheat individuals under the high voltage transmission lines and suggested that 50 Hz EMF acts as a stressing factor on wheat plants. The results of total soluble protein

content and protein profile revealed that the number of total bands and the polymorphic bands did not differ significantly between unexposed and exposed plants. However, the rate of mobility of most molecular masses in exposed plants was modified in exposed sites.

The protein profiles were examined four times and the same results were obtained on each occasion, that is to say the profiles of the seed proteins remained very similar between unexposed and exposed samples, but the overall mass of the proteins was consistently greater in the exposed samples. Kultz *et al.* (2005) suggested that stress proteins help damaged proteins to refold and to regain their conformations and molecular weight. However, my results are in disagreement with Walter (1997), MacGinitic (1994), Laberg (1997) and Hanfy *et al.* (2006) who reported that the electric field inhibits the biological properties of the membrane protein causing the amount of total protein to decrease, rather than increase after seed had been exposed, as a result of a decrease in the amount of nitrogen present. It was concluded that the genetic diversity in individuals of wheat plant grown under transmission lines increases, but seed germination and the amount of total soluble protein remain unchanged.

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