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Evaluation of Chlorophyll Contents and Peroxidase Activities in *Helianthus annuus* Genotypes Exposed to Radiation and Magnetic Fields

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Abstract: The effects of γ-radiation and magnetic fields (MF) on chlorophyll contents and peroxidase The effects of γ-radiation and Magnetic fields (MF) on chlorophyll contents and peroxidase activities of two sunflower genotypes Nantio and AS508, have been investigated. For the irradiation, ^{137}Cs up to 150 Gy has been used while 5 mT MF have been applied with the different exposure times. Treatments have been applied as alone and combinated on the seeds of genotypes. After the treatments, seedlings have been grown and, chlorophyll contents and peroxidase activities of young seedlings have been determined. Irradiation has decreased germination rates and chlorophyll contents while it has increased peroxidase activities of both genotypes, but seeds have been more radiosensitive in Nantio than in AS508. MF has different effects on the parameters than the radiation. It has also decreased germination rates but increased chlorophyll contents and decreased peroxidase activities. On the other hand, the results of combinated exposures have shown that MF exposure has reduced the effects of radiation on all parameters that have been examined.

Key Words: Chlorophyll, *Helianthus Annuus*, Magnetic Fields, Peroxidase, Radiation

Introduction

Radiation is one of the best known physical mutagens used the generation of genetic variations in the plant breeding. Magnetic fields (MF) can also be used for the same purpose due to their biological effects (Matsuda *et al.*, 1993). There are a lot of biological effects include carcinogenesis that have 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 the effects of MF formed on the biological systems is difficult, since most of the biological structure are heterogeneous (Goodman *et al.*, 1995).

Radiation dissociates the atoms of water molecule and causes the generation of hydroxyl radicals that are the most reactive. Therefore it enhances the free radical concentration in the living cells (Leibovitz and Siegel, 1980). Increasing the concentration of free radicals creates oxidative stress and induces peroxidase activities (Gutzeit, 2001). Plant peroxidases (EC 1.11.1.7) are important in diverse cellular functions such as lignin and hormone biosythesis, and detoxification of hydrogen peroxide (Conroy *et al.*, 1982).

This study has aimed to examine the nature of the effects of a MF with 5 mT strength and γ-radiation up to 150 Gy on peroxidase activities and chlorophyll contents of two different *H.annuus* genotypes, Nantio and AS508.

Materials and Methods

The two sunflower genotypes used in the study, Nantio and AS 508, are widely grown in Turkey. For each genotype, we used 300 seeds obtained from different generations.

(i) Mutagen Exposures: For the exposure of γ-radiation on the seeds, ^{137}Cs has been used. According to the testing exposures, GR_{50} dose -that reduces the height of seedlings at the rate of 50%- of γ-radiation has been determined 150 Gray (Gy) for both genotypes. Therefore the starting dose of γ-radiation exposure has been applied at the level of 100 Gy. 300 seeds obtained from each genotype in a polyethylene bag have been incubated at Room Temperature (RT) in the γ-radiation source for 13 and 19.5 minutes to apply 100 and 150 Gy doses respectively. After the exposure, the seeds have been stored at +4°C until the sowing. For the exposure of magnetic fields, maximum MF levels that have not effect on germination rate of each genotype have been selected as the threshold value. AS 508's seeds have been incubated at RT for 5 seconds (1x -threshold value for AS508) and 15 seconds (3x); Nantio's seeds have been incubated at RT for 15 seconds (3x -threshold value for Nantio) and 45 seconds (9x) in the 5 mT magnetic fields. For the combinated exposures, MF have been exposed after the radiation.

(ii) Sowing: Sowing has been performed at greenhouse conditions. All of the seeds have been sowed at the beginning of April while the temperature of soil has been 10°C. Seeds have germinated after approximately 10 days from sowing.

(iii) Clorophyll Content: Chloroplasts have been extracted from the leaves of 15-day-old *H. annuus* genotypes seedlings. An extraction of leaf pigments has been done with 80% acetone and the absorbance has been measured at 663 and 645 nm with UV spectrophotometer for chlorophyll a and b respectively, and chlorophyll contents have been calculated according to the method of Arnon (1949).

(iv) Peroxidase Activities: Peroxidase activities have been determined by using the Birecka and Miller's method. H_2O_2 and guaiacol have been used as substrats for peroxidase. Activities have been measured at 470 nm with UV spectrophotometer (Birecka and Miller, 1974).

Table 1: The Effects of Radiation, Magnetic Fields and the Combined Exposures on the Germination. Control Germination Values for Each Genotype are Given as * Symbol. Values in the Same Column with Different Supercript Letters are Significantly Different at P≥0.05 for AS508, at P≥0.01 for Nantio, Based on Duncan's Multiple Range Test

Genotypes	Exposures γ- Radiation	MF	Percentage of Germination
AS508	–	–	*95,67[a]
		1x	92,67[ab]
		3x	94,33[a]
	100 Gy	–	80,67[c]
		1x	86,67[abc]
		3x	78,33[c]
	150 Gy	–	78,00[c]
		1x	86,33[abc]
		3x	84,00[c]
NANTIO	–	–	*92,67[a]
		3x	63,33[bcd]
		9x	74,33[b]
	100 Gy	–	58,33[d]
		3x	65,67[bcd]
		9x	43,33[e]
	150 Gy	–	60,00[cd]
		3x	62,67[bcd]
		9x	70,67[bc]

Table 2: The Effects of Radiation, Magnetic Fields and the Combined Exposures on the Chlorophyll Contents. Control Chlorophyll Values for Each Genotype are Given as * Symbol. Values in the Same Column with Different Supercript Letters are Significantly Different at P≥0.01 for Both Genotypes, Based on Duncan's Multiple Range Test

Genotypes	Exposures γ-Radiation	MF	Chlorophyll a	Chlorophyll b	Total Chlorophyll mg/gfw
AS508	-	-	*0,850±0,098[a]	*0,700±0,039[ab]	*1,549±0,133[ab]
		1x	0,906±0,091[a]	0,732±0,027[a]	1,638±0,118[a]
		3x	0,913±0,036[a]	0,737±0,067[a]	1,649±0,051[a]
	100 Gy	-	0,770±0,081[ab]	0,611±0,105[abcd]	1,381±0,186[abc]
		1x	0,751±0,038[ab]	0,657±0,019[ab]	1,407±0,051[abc]
		3x	0,727±0,168[ab]	0,585±0,062[bcd]	1,312±0,211[bc]
	150 Gy	-	0,505±0,017[c]	0,481±0,010[d]	0,986±0,026[d]
		1x	0,634±0,044[bc]	0,560±0,049[cd]	1,193±0,094[cd]
		3x	0,638±0,026[bc]	0,585±0,025[bcd]	1,222±0,050[cd]
NANTIO	-	-	*0,666±0,095[bc]	*0,505±0,145[bc]	*1,170±0,238[cd]
		3x	0,864±0,099[ab]	0,751±0,164[ab]	1,615±0,259[ab]
		9x	0,773±0,088[ab]	0,635±0,084[bc]	1,407±0,163[bcd]
	100 Gy	-	0,673±0,086[bc]	0,505±0,056[bc]	1,178±0,105[cd]
		3x	0,697±0,056[bc]	0,578±0,101[bc]	1,275±0,144[bcd]
		9x	0,922±0,076[a]	0,480±0,184[c]	1,402±0,147[bcd]
	150 Gy	-	0,514±0,026[c]	0,494±0,093[bc]	1,007±0,096[d]
		3x	0,863±0,162[ab]	0,705±0,030[abc]	1,568±0,191[abc]
		9x	0,931±0,045[a]	0,912±0,026[a]	1,842±0,034[a]

Table 3: The Effects of Radiation, Magnetic Fields and the Combined Exposures on the Peroxidase Activity. Control Activity Values for Each Genotype are Given as * Symbol. Values in the Same Column with Different Supercript Letters are Significantly Different at P≥0.01 for Both Genotypes, Based on Duncan's Multiple Range Test

Genotypes	Exposures		Peroxidase activities ΔA_{470} $min^{-1}g^{-1}fw$
	γ- Radiation	MF	
AS508	–	–	*30,533±1,350^{ef}
		1x	29,067±5,065^{f}
		3x	30,400±1,311^{ef}
	100 Gy	–	40,867±1,700^{d}
		1x	34,267±1,290^{e}
		3x	35,333±1,171^{e}
	150 Gy	–	109,667±2,212^{a}
		1x	81,467±1,563^{b}
		3x	50,067±1,986^{c}
NANTIO	–	–	*20,967±0,862^{f}
		3x	17,833±0,404^{g}
		9x	18,733±0,568^{fg}
	100 Gy	–	47,933±1,150^{b}
		3x	30,233±1,404^{e}
		9x	33,233±0,513^{d}
	150 Gy	–	109,500±1,058^{a}
		3x	40,533±1,823^{c}
		9x	48,433±1,350^{b}

Results and Discussion

(i)Germination: Number of the seeds sowed for each exposure are 300. Radiation exposures have decreased the percentage of germinations in the both of genotypes at statisticaly significant level (for AS508 p≥0.05; for Nantio p≥0.01) (Table 1). MF exposures have also reduced the germination rate at the level of 1x for AS508 and level of 3x and 9x for Nantio. Therefore, the combinated exposures have showed that MF has also reduced the radiation effect. After the 100 Gy exposure of radiation, MF exposures (1x for AS508 and 3x for Nantio) have been effective to reduce the radiation effect, while MF exposure has reduced the effect of 150 Gy radiation exposure, at all doses.

(ii)Chlorophyll: The results of the effects of γ-radiation, MF and combined exposures on the chlorophyll contents of the AS 508 and Nantio genotypes have been given in Table 2. Gamma radiation has negative effect on the chlorophyll content for the both sunflower genotypes while the Nantio genotype has been more stable against to irradiation. However, MF exposure has increased the amounts of chlorophyll a, chlorophyll b and total chlorophyll. These results are statistically significant at the level of 0.01. Furthermore, the results of combined exposures has indicated that MF exposures supressed the γ-radiation effects.

(iii)Peroxidase Activities: The results of peroxidase activities of the samples have been given in Table 3. It has been observed that peroxidase activities of the irradiated samples have been induced while MF exposures have decreased the peroxidase activities of the both genotypes. On the other hand, γ-radiation has lost activity because of MF combination.

All results are also statistically significant at the level of 0.01.At the normal conditions, the percentanges of germination in the both of genotypes is the same. The activitiy of peroxidase in non-treated tissues of AS 508 is about half times higher than that of Nantio. Chlorophyll content of AS 508 is also slightly higher than Nantio's.

The results presented above clearly have showed that seeds are more sensitive to radiation in Nantio than in AS 508, as is revealed by the germination rate and peroxidase activity (Table 1 and 2). The seeds of both genotypes exposed to MF are tolerable to radiation effect on germination, chlorophyll content and peroxidase activity (Table 1, 2 and 3).

In generally, irradiation has increased the retardation of seedling growth, the degradation of chlorophyll and activation of peroxidase. MF exposure has also retarded Nantio's seedling growth, but has no effect on the AS 508 growing. However MF exposure has increased the amount of chlorophyll contents of both genotypes at any level, but it has no significant effect on the peroxidase activities.

It has generally been agreed that radiation-induced cellular damage is brought about through indirect effects by formation of various toxic molecular species, including free radicals and peroxides that are generated tissues. A range of responses to enviromental stresses has been reported to exist between drought-tolerant and susceptible plants as well as between salt-tolerant susceptible plants and these differences have been explained in terms of radical metabolisms. Radiation exposures increase in the activities of some antioxidant enzymes that are response elements of oxidative stress (Wada *et al.*, 1998).

The data obtained previously has indicated that MF alone or combined with γ-radiation has stimulated root and shoot development and secondary root formation in the soyabean plants (Keul *et al.*, 1994). Therefore, some possitive effects of MF and electromagnetic fields on the growth of different plants have been shown previously (Vakharia *et al.*, 1991; Formicheva *et al.*, 1992; Singh *et al.*, 1993; Danilov *et al.*, 1994; Namba *et al.*, 1995; Carbonell *et al.*, 2000).

The biological effects of MF depend on the energy level, exposure time, distance of target from energy source and structure of organism (Goodman *et al.*, 1994). It has been reported that there are some negative effects of MF on the biological system including carcinogenesis at the high MF energy level or at close around the energy source (Goodman *et al.*, 1995). In general, MF alters the electron spins of molecules, especially ionic forms. Some studies have suggested that MF exposure could be due to both the increase in the concentration (Jajte, 2000) and oscillating of free radicals (Scaiano *et al.*, 1995). MF are known to affect radical pair recombination and they may increase the concentration of oxygen free radicals in living cells (Jajte, 2000). Increasing the concentration of free radicals creates oxidative stress, enhances stress response and some biological reactions, such as DNA damage occurs under this condition (Gutzeit, 2001).

The reason of the increase in the oscillating of free radicals is the transformation of the radicals into more stabile and less reactive forms under the low MF energy (Scaiano *et al.*, 1995). The mechanism could explain the reducing effect of MF on the oxidative effect of radiation. The data obtained from this study may be helpful to explain the mechanism of the biological effects of MF.

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