EFFECT OF MAGNETIC FIELD AND GAMMA RADIATION ON *PAULOWINIA TOMENTOSA* TISSUE CULTURE

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ABSTRACT

In this study, the in vitro tissue culture of the Paulownia tomentosa plant was established and a magnetic field which has 2.9-4.8mT flux density was applied to the culture at a velocity of 1m/s for 19.8 seconds. Additionally, gamma radiation of 10 and 25 Gy was applied as combined to the magnetic field. It was observed that magnetic field increased the capability of regeneration of Paulownia tomentosa cultures and supported the regeneration in a short time in comparison with the control group and applied radiation dose and decreased regeneration capability were re-supported when combined with magnetic field.

It was found that magnetic field has a positive effect on plant fresh weight, leaf number and chlorophyll amount on the 28th day of established cultures of P.tomentosa node explants and the negative effects on these parameters by radiation, when magnetic field and gamma radiation applied together, these parameters changed and aroused depending on the radiation doses.

Keywords: radiosensitivity, magnetic field, node culture, gamma rays, *Paulownia*

Introduction

Investigations of low magnetic field (LMF) which effect on biological systems have attracted the biologists. It is known that magnetic field is effective on the normal functions of the living (4, 6, 25). In cellular level, studies have shown that RNA and protein synthesis on G_1 phase are affected from magnetic field (MF) strength changes and cell division rate increases for cells exposed to magnetic field. (3, 9, 16, 18, 21).

It is alleged that in MF strength of 10^{-3}-10^{-2} Tesla, chemical reactions are affected by intermediate reactions affecting electron spin conditions and these effects have a potential to cause biological results (5, 9, 10).

It has been found that an increase occurs in chemical reactions of plants under magnetic field effect and magnetic field has a positive effect on photochemical activity, respiration ratio, and enzyme activity (13, 15, 21).

Studies made on various plants have shown that magnetic field was effective on seed germination. When seeds exposed to magnetic field, the germination was faster than control group and germination percentage was increased (6, 7, 15, 16, 21, 23).

In recent years, magnetic field has started to be applied on plants in addition to tissue culture techniques that are used for growing high economic valued plants faster and generating more products (4, 8, 14). *In vitro* techniques and their combined applications allow the production of high quality products and their selection.

Furthermore, the magnetic field studies combined applications of radiation were made and magnetic field has started to gain importance (20).

Radiation applications are often used on plants in developing varieties that are agriculturally and economically important and have high productivity potential (12). However, the harmful effect caused by radiation applications on biological systems has made it difficult to choose individuals in required characteristics. Magnetic field changes the effect of injury caused by radiation in oxidative way and partially protects the biological system from this kind of damages (24, 26).

Paulownia plant, which was cultured in vitro in our studies, has fine quality properties such as resistant to bending; cracking and rotting, and ability of avoid moisture (11, 28, 31). It has also became a desired plant since its habitat can extend from warm to tropical climates, and due to its tolerance to poor soil conditions (22).

Combined application of magnetic field and radiation in *in vitro* mutation study to be carried out on *Paulownia* tree which has a high economic value has a great importance regarding acquiring more material in a short time.

The objective of this study is to determine the effect of magnetic field and gamma radiation on *Paulownia tomentosa* tissue culture and to show the effect of magnetic field on gamma radiation sensitivity.

Materials and Methods

Plant material

P.tomentosa seeds were collected from Beijing, China. The seeds were pre-treated by incubating in distilled water at 40 ^{o}C for 10 minutes and at room temperature for 24 hrs. Then the seeds were removed from water and allowed to dry on filter paper. Dried seeds were inserted in Petri dishes containing 0.8% agar and left to germinate at 26 °C in a growth chamber with 16 hours light / 8 hours dark period. The plants were transferred to soil after 30 days of growth, and continued the incubation in growth chamber under the same conditions (3). After 3 months of incubation, the *Paulownia* seedlings were used to prepare tissue cultures (3, 11, 22).

Culture conditions

Node sections of 3-month-old seedlings were removed and surface-sterilized by keeping in 70% ethanol for 1 min and then in 10% commercial bleach (commercial bleach contains about 4% sodium hypochloride) for 10 min. They were then washed 3 times with distilled water and dried on sterile filter papers. The node explants were cultivated onto MS (17) medium supplemented with 0.1 mg/ml NAA, 1 mg/ml BA and 30 g/l sucrose (11).

Gamma radiation and magnetic field experiment

Paulownia node explants were irradiated with Cs-137 source in IBL 437 C irradiation facility belonging to Our Leukemia Children Foundation (dose rate/min 10 Gy) with doses of 0, 5, 10, 25 and 50 Gy of gamma rays. Immediately after irradiation, node explants were aseptically transferred into sterile fresh medium, placed in a growth chamber at 26 °C with 16 hours light / 8 hours dark period. GR_{50} dose that decreases the plant fresh weight 50%, were determined on the 28th day. GR_{50} dose found 16 Gy (**Fig.1**). According to this result 10 and 25 Gy doses were determined for gamma radiation.

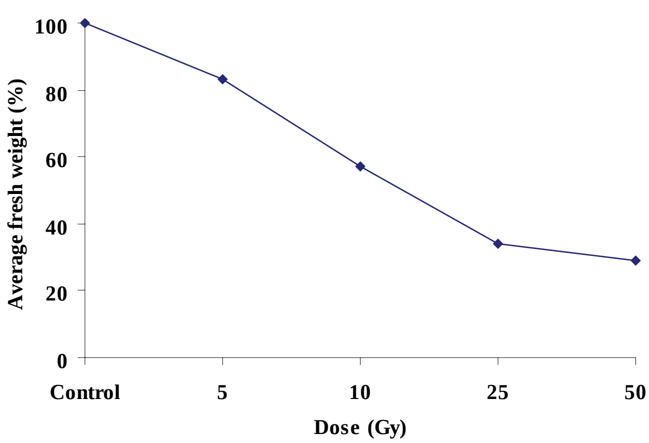

Fig.1. Effect of gamma radiation on fresh weight of *P.tomentosa*. GR_{50} dose:16 Gy

According to Yaycili and Alikamanoglu (29) obtained results for *Paulownia tomentosa,* magnetic field application with 2.9-4.8 mT magnetic flux for 19.8 second gave the best result on plant regeneration. Therefore the plant tissue cultures have been exposed to magnetic field for 19.8 seconds at 2.9-4.8 mT magnetic flux.

For magnetic field and radiation combination, *Paulownia* node explants were irradiated with doses 0, 10 and 25 Gy of gamma rays than passed at 2.9-4.8 mT magnetic flux density with a speed of 1 m/s for 9 times, exposing them to the MF for 19.8 seconds. Subsequently, the explants were aseptically transferred to sterile culture tubes, placed in a growth chamber at 26 °C with 16 hours light / 8 hours dark period.

At the 28th day, control and experiment shoots that were regenerated from *P.tomentosa* node explants and exposed to radiation and MF were measured for leaf number and fresh weight. Statistical evaluations were done according to variant analysis. The statistically meaningful fresh weight analyses comparisons were done using Student-Newman Keuls analysis (30).

Chlorophyll content

28-day old plant leaves were extracted in 80% cold acetone and the absorbance of the extract were determined at 663 and 645 nm in a Schimadzu UV-160 spectrophotometer. Chlorophyll a, b and total chlorophyll quantities were calculated in accordance with Arnon method (2).

Results and Discussion

At the 3rd day, regeneration level of *P.tomentosa* node explants that were exposed to MF increased in comparison with that of controls (**Table 1**). In these cultures, at the 7th day, the percentage of regeneration was equalized with the controls.

TABLE 1

Number of explants and regeneration percentages at 3rd, 5th, 7th, 14th days in *P. tomentosa* tissue cultures exposed to 10 and 25 Gy gamma radiation and 2.9-4.8 mT MF for 19.8 seconds

Treatment Groups	Number of explants	Regeneration (%)			
		3rd day	5th day	7th day	14th day
Control	30	53.3	73.3	100	100
MF	30	76.6	100	100	100
10Gy	30	36.6	60	83.3	87.5
10Gy+MF	30	43.3	66.6	87.5	91.6
25 Gy	30	23.3	50	79.2	79.2
25 Gy+MF	30	33.3	56.6	83.3	83.3

In the cultures irradiated with 10 Gy radiations, the regeneration percentage was 83.3% and this percentage was increased from 83.3% to 87.5% when applied together with magnetic field. When the radiation dose increased to 25 Gy, the regeneration percentage has decreased according to control. But regeneration percentage has increased from 79.2% to 83.3% by magnetic field application.

When the number of leaves was compared in regenerated *P.tomentosa* cultures at 3- 28th days, it was observed that MF influenced leaf development. The plants regenerated from cultures that were exposed to both of radiation and MF, possessed more leaves in comparison with radiation experiment groups (**Table 2**).

At day 28, average fresh weight of the plants regenerated in *P.tomentosa* cultures was measured and according to control, it was found that the weight increased in relation with MF

TABLE 2

Number of explants and leaves at 3rd, 5th, 7th, 14th, 21st, 28th days in *P. tomentosa* tissue cultures exposed to 10 and 25 Gy gamma radiation and 2.9-4.8 mT MF for 19.8 seconds

Treatment Groups	Number of explants	Average of the leaf numbers					
		3rd day	5th day	7th day	14th day	21th day	28th day
Control	30	1.59	3.34	4.9	8.5	11.19	12.1
MF	30	2.07	4.2	5.85	9.8	13.27	14.13
10Gy	30	0.79	1.88	2.79	5.08	6.71	7.33
10Gy+MF	30	0.95	2.04	2.96	5.88	7.17	7.96
25 Gy	30	0.33	0.71	1.46	4.04	4.08	4.25
25 Gy+MF	30	0.58	1.29	2.13	3.04	6.04	6.38

application. (**Table 3**). A statistical analysis showed that time of MF application was significant on plant fresh weight.

TABLE 3

Number of explants and average fresh weight in regenerated plants in 28-day-old *P. tomentosa* tissue cultures exposed to 10 and 25 Gy gamma radiation and 2.9-4.8 mT MF for 19.8 seconds

Treatment Groups	Number of explants	Average fresh weight (mg)
Control	30	539.1±151 a*
MF	30	656.7±192 b
10Gy	30	388.1±187 c
10Gy+MF	30	480.6±175 a
25 Gy	30	174±96 d
25 Gy+MF	30	237.8±114 d

* Those that were not represented with the same letter show that the differences in the plant fresh weights noticed between controls and plants exposed to gamma radiation and MF appear to be significant to a level of 0.05 by Student-Newman-Keuls method.

± Standard Deviation

It has been found that the fresh weight decreased by 10 and 25 Gy gamma radiation applications and has increased by combined magnetic field application.

The amount of experiment and control plants chlorophyll was determined spectrophotometrically by measuring the absorbance at 645 and 663 nm. When compared to the controls, an increase in the total chlorophyll amounts was detected in plants regenerated in the cultures exposed to MF for 19.8 seconds. In the cultures that were exposed to radiation and MF for 19.8 seconds, chlorophyll a, b and total chlorophyll content was higher than radiation experiment groups (**Table 4**).

In the current study, the effects of gamma radiation and MF (alone and together) were determined on the cultures prepared from node explants of *P.tomentosa*.

Gamma radiation applications are very important in mutation breeding in order to develop required features of plants and increasing the genetic variability. The many mutant varieties, which are resistant to diseases, cold, saltiness and with high quality, have been developed (12). Physiological damage, gene mutation and chromosome mutation as a result of gamma radiation are generally restricted to the plant regeneration. For this reason, mutagens are required that results in low plant injury but in high genetic effects for mutation breeding.

Magnetic field is effective on the cell division rate and regeneration of explants. It was previously shown that MF significantly influences the cell metabolism and mitosis in plant meristem cells (5, 9). In another study with soybean tissue cultures, number of shoots, root formations, fresh weight and chlorophyll contents of plants regenerated from the explants treated with MF were examined and it was found that MF had a positive effect on regeneration in soybean tissue cultures (4).

Belyavskaya et al. (5) and Grundler et al. (10) reported that MF caused significant changes in RNA and protein synthesis in the G_1 stage of cell cycle in plants, and that a MF with strength of 3.5 mT caused an increase in mRNA and total RNA levels. It is also, known that a MF in the range of $10^{-3}10^{-2}$ T can start chemical reactions by affecting electron spin positions (5, 10, 18)

In our study with MF, the regeneration rate in *P.tomentosa* increased at the 3rd day after the application of MF in comparison with the control experiments. In *P.tomentosa*, at the 7th day there was no regeneration difference left between the controls and the explants that were exposed to MF. The regeneration rate in *P.tomentosa*, which exposed with gamma radiation and MF, increased in comparison with the groups, which exposed with gamma radiation alone.

In this study, the leaf numbers of plants regenerated in culture environment were determined. It was found that average plant leaf number increased in cultures exposed to magnetic field in contrary with control group. For cultures irradiated with gamma radiation, it was found that leaf number decreased according to the applied dose and this physiological damage in M_1 generation caused by radiation has been somewhat removed when applied together with magnetic field.

In *P.tomentosa* tissue cultures, the fresh weight differences of those which exposed to 10 Gy gamma radiation together with the MF and 25 Gy gamma radiation together with the MF were increased according to 10 and 25 Gy doses. Statistical studies showed that fresh weight of *Paulownia* plants was influenced

TABLE 4

Chlorophyll a, b and total chlorophyll content in the leaves of 28-day-old *P. tomentosa* tissue cultures exposed to gamma radiation and MF

Treatment Groups	Chlorophyll contents (mg/g fresh weight)		
	Chlorophyll a	Chlorophyll b	Chlorophyll total
Control	0.496±0.007	0.216±0.003	0.711± 0.01
MF	0.608± 0.02	0.250±0.002	0.858±0.022
10Gy	0.284±0.011	0.128±0.006	0.412±0.017
10Gy+MF	0.338±0.014	0.159±0.007	0.497±0.021
25 Gy	0.275±0.006	0.103±0.001	0.378±0.007
25 Gy+MF	0.322±0.016	0.142±0.006	0.464±0.022

±Standard Deviation

by the application of the gamma radiation and the MF and there were significant differences among the results ($P<0.05$). The fresh weight differences between control and treatment group exposed to 10 Gy gamma radiations together with the magnetic field were not significant. These statistical results showed that magnetic field was affected on the regeneration of explants, which were irradiated with gamma rays. Magnetic field can repair the damage produced in plants by exposing to 10 Gy radiation doses and the fresh weight of the treatment group was close to the control group. However, positive effect of magnetic field was lower on plants, which exposed to 25 Gy gamma radiation dose.

An increase in chlorophyll a, b and total chlorophyll levels was observed in *Paulownia* plants that were exposed to MF. When compared to the controls, while the total chlorophyll increased 20.6 % in *P.tomentosa* plants exposed to MF.

The chlorophyll amounts of plant leaf regenerated by radiation application were decreased. This decrease occurred in the amount of chlorophyll were increased due to combined application of magnetic field and radiation.

It was demonstrated that low-intensity MF application increased protein and chlorophyll levels in onion plant (19), and it increased chlorophyll a, b and total chlorophyll levels in *Paulownia* (3).

Radiation causes oxidative injury by accelerating free radical generation in living systems. The primary damage induced by ionizing radiation is modified in enzymatic repair processes (1). Because of the protein biosynthesis and enzyme activities are changed with magnetic field (27) only small damage remains to be realized in a biological change in the combined application of magnetic field and radiation. Magnetic field may be partially protects the biological system for this kind of injuries (24, 26).

In our study it has been found that magnetic field changes the effect of gamma radiation in a positive way as a result of combined application of magnetic field and gamma radiation. Combined applications of magnetic field and gamma radiation on future studies to be carried out on mutation breeding would provide producing plants, which are desired to be developed and faster.

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