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The Effects of an Artificial and Static Magnetic Field on Plant Growth, Chlorophyll and Phytohormone Levels in Maize and Sunflower Plants

By

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With 5 Figures

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Summary

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In the present study the effects of a continuous static magnetic field (SMF) on growth and concentration of phytohormones and chlorophylls were investigated in maize and sunflower plants. SMF was applied in two directions; parallel to gravity force (field-down) and anti-parallel (field-up). Chlorophyll concentrations decreased in maize plants, but increased in sunflower in SMF of either direction. Root dry weight decreased in maize and increased in sunflower plants. The changes of dry weight in stem and leaf were not significant ($p \geq 0.05$). The root length decreased in both plant species. Leaf and stem length increased in maize plants in SMF of either direction. Leaf length did not change in sunflower, whereas stem length rose in field-down application of SMF. Concentrations of gibberellic acid-equivalents (GAs), indole-3-acetic acid (IAA) and trans-zeatin (t-Z) increased in sunflower plants under field-up application of SMF, whereas they decreased in SMF of the opposite direction. The concentration of phytohormones decreased in maize plants in SMF of either direction.

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Zusammenfassung

TURKER M., TEMIRCI C., BATTAL P. & EREZ M. E. 2007. Die Effekte von künstlichen statischen Magnetfeldern auf Wachstum sowie Chlorophyll- und Phytohormongehalte von Mais und Sonnenblumen. – *Phyton* (Horn, Austria) 46 (2): 271–284, 5 Abbildungen. – Englisch mit deutscher Zusammenfassung. In der vorliegenden Studie wurden Effekte von kontinuierlichen statischen Magnetfeldern (SMF) auf das Wachstum sowie die Phytohormon- und Chlorophyllgehalte von Mais und Sonnenblumen untersucht. SMF wurde in zwei Richtungen angewendet, parallel (nach unten) und antiparallel zur Schwerkraft (nach oben). Chlorophyllgehalte von Maispflanzen waren in SMF beider Richtungen verringert, während sie bei Sonnenblumen erhöht waren. Das Wurzeltrockengewicht von Mais war verringert und jenes von Sonnenblumen erhöht. Änderungen im Spross- und Blatttrockengewicht waren statistisch nicht signifikant ($p > 0,05$). Die Wurzellänge nahm bei beiden Arten ab, Blatt- und Sprosslänge nahm bei Mais im SMF beider Richtungen zu. Bei Sonnenblumen veränderte sich die Blattlänge nicht, während die Sprosslänge im nach unten orientierten SMF zunahm. Die Konzentration von Gibberellinsäureäquivalenten (GAs), Indol-3-Essigsäure (IAA) und trans-Zeatin (*t-Z*) nahmen bei Sonnenblumen im nach oben orientierten SMF zu, und im nach unten orientierten ab. Bei Mais nahmen die Konzentrationen der Pflanzenhormone im SMF beider Richtungen ab.

Introduction

The changes in living systems exposed to magnetic fields have attracted the attention of biologists, molecular biologists, physicists and chemists. The role of magnetic fields (MF) and their influence on the functions of organisms are still insufficiently investigated. Under the effect of MF, various changes are brought about in the process of growth and development. Some changes would be advantageous to organisms, but others would be unfavourable. To overcome disadvantages, it may be required to exploit some other environmental factors which substitute for MF in some properties. The effects of magnetic fields were reported to be dependent on the direction of the magnetic field (KATO 1988). It was particularly the south pole that stimulated the growth and many metabolic functions in plants. A strong magnetic field had harmful effects on plants (DUNLOP & SCHMIDT 1969). Today many researchers are engaged in studying biological effects of weak magnetic fields (RUŽIČ & al. 1993). MF affects various growth processes such as seed germination, shoot and root growth, flowering, chlorophyll quantity, and crop yield (ATAK & al. 2003). It was demonstrated that root growth was enhanced when seedlings were grown in a magnetic field alternating at 40–160 Hz. Furthermore plants grown at 240–320 Hz showed a reduction in primary root growth. MF may elicit a biochemical change in either the root or the seedling as a whole (MURAJI & al. 1998). The root growth rate of *Raphanus sativus* was enhanced under 60 Hz magnetic fields (SMITH & al. 1993). The treatment of onion with a permanent magnetic field accelerated sprouting and extended the leaf

length (NOVITSKY & al. 2001). Phytoferritin in plastids which is an important compound for photosynthesis was decreased by a magnetic field (BELYAVSKAYA 2001). In contrast, chlorophyll content was increased by magnetic fields (ATAK & al. 2000, NOVITSKY & al. 2001, OLDACAY 2002).

The electric components of phytotrons generate electromagnetic fields that may act as an environmental factor influencing plant growth and morphogenesis (CELESTINO & al. 1998).

Plants are seriously influenced by environmental factors. Plants develop a cell wall with a complicated but well-organized structure. The plant cell wall, thus, plays an important role in resisting the gravity force and supporting the plant body under $1 \times g$ on earth (HOSON 1998). Hypergravity treatment inhibits elongation growth of stem organs in various plants (SOGA & al. 1999a) and decreases the cell wall extensibility in mesocotyls of maize (SOGA & al. 1999b). The opposite changes are expected to occur under microgravity conditions. Water submergence stimulates growth and the cell wall loosening and activates the metabolism of the cell wall polysaccharides, which is partly explained by the microgravity effect due to buoyancy in rice coleoptiles (MASUDA & al. 1994). Also, MF changed the characteristics of the cell membrane, influenced cell reproduction, protein biosynthesis, and enzyme activities (GOODMAN & al. 1995). RNA and protein synthesis in the G_1 phase of the cell reproduction cycle were affected by MF resulting in a decrease of germination and an inhibition of seedling growth (GOVORUN & al. 1992, FOMICHEVA & al. 1992).

Static magnetic fields (SMF) may cause the Lorentz force on moving ionic particles in plants. When charged particles move vertically towards MF, they meet the magnetic field force. Therefore, the direction of ionic particles' velocity and the value of the current are important in terms of the effect of the Lorentz force. The cell membrane acts as a condenser because of the lipids in its structure (GLASER 2000). The Lorentz force in field-up and down directions has similar effects on moving ionic particles. However, there are several factors influencing plant growth and metabolism. Beside the Lorentz force, MF may have some other effects on plant morphology and physiology. Therefore, it is important to investigate the effects of MF on plant growth and development directly.

Phytohormones are a group of naturally occurring organic substances which influence physiological process at low concentrations. The processes comprise mainly growth, differentiation and development. They influence the appearance of organelles and nutrients through the plant and also enhance plant resistance to adverse environments (DAVIES 1995). For example, gibberellins contribute to plant resistance to gravity probably via the construction of a proper cell wall (HOSON & al. 2002). Although the role of phytohormones in higher plants has been studied extensively, there are limited reports in the literature about the effect of MF on phytohormones.

In order to survey how a magnetic field affects plants, maize and sunflower were incubated under controlled SMF and also in the normal geomagnetic environment. This paper describes the effects of SMF on the development and growth (root, stem and leaf length, width), on fresh and dry weight, and on chlorophyll and phytohormone content of maize and sunflower plants. In published studies, plants have been exposed to magnetic fields ranging from 15 μT to 250 mT (FLÓREZ & al. 2006, BELYAVSKAYA 2004). In the present study, 150 gauss (15 mT) was selected as a value of SMF considering those literature values. The direction of SMF was chosen as field-up and field-down regarding stem and root growth and flux and nutrient distribution.

Material and Methods

Plant Material Seeds of sunflower (*Helianthus annuus* L. cv. NSH 712) and maize (*Zea mays* L. cv. OSSK 644) were sown in pots containing cleaned soil and sand (2:1).

Growth Environment

The body material of the coil was made of several layers of wood laminated and glued to each other. The dimensions of the coil were as follows: the length 1 m, the inner radius 0.1 m, the outer radius 0.14 m. The coil was located in a vertical position up to 0.2 m above the ground supported by wooden chocks. The plants were placed in the coil vertically 0.3 m above the bottom of the coil, where a uniform magnetic field was obtained (Fig. 1). Three plants were germinated in each pot. The length of the pots was 0.1 m and plants were grown up to a maximum of 0.27 m. To avoid the heating effect of the current, the inside and outside of the coil was covered by a capillary tube made from plastic through which water ran continuously. The current source was provided by a DC power supply (Keithley 2410c, 1100 V Sourcemeter). The variation in the field strength along the height of the coil was as indicated in Fig. 1. A uniform magnetic field was selected as a region in which plants were grown and the magnitude of the field did not deviate by more than 2 % from the center value at any point. The number of the turns of the wire was 25000 and its diameter was 1 mm. The static continuous magnetic field in the axial centre of coil was measured as 150 gauss (15 mT) with a gauss meter (Walker Scientific Inc. MG-4D Gaussmeter). The current in the coil was measured as 0.491 A. SMF was applied in two directions; parallel to the gravity force (field-down) and anti-parallel (field-up). The plants in the coil were illuminated by a fluorescence lamp for 12 h ($20\text{--}30 \mu\text{mol m}^{-2} \text{s}^{-1}$), with $25 \pm 2^\circ\text{C}$ temperature, and 85 % humidity. The light source was fixed in 0.2 m distance above the coil. Control plants were kept out of coil in the same growing conditions as the experimental plants. The plants were kept under magnetic field conditions for 15 days when plants had 4–5 leaves.

The Determination of Chlorophyll Content

Chlorophyll was extracted from leaves of 15 days old plants with 80 % acetone and the absorbance value was measured at 663 and 645 nm wavelength in a UV-160 Shimadzu spectrophotometer. Chlorophyll a, chlorophyll b and total chlorophyll were calculated according to WITHAM & al. 1971.

The Determination of Phytohormones

Extraction, purification, and isocratic HPLC (High Performance Liquid Chromatography) analyses were performed according to the modified methods of BATTAL & al. 2004 and KURAISHI & al. 1991. Two grams of leaves were ground into powder with liquid nitrogen. Then cold methanol was added and tissue samples were homogenized in an Ultra Tissue Lyser (Ultrasonic Processor Jenway LTD.) at 4 °C for 1 h. The homogenization process was continued at 4 °C for 24 h in dark. The samples were filtered through filter paper (Whatman No: 1) and the supernatant was transferred into clean vials. The residues were reprocessed and combined with the former supernatant. The supernatants were filtered through PTFE filters (0.45 µm) and methanol was removed under reduced pressure. Then the extracts were re-dissolved in 0.1 M potassiumdihydrogenphosphate (KH_2PO_4 ; pH=8.5) buffer and centrifuged at $14534 \times g$ for 1 hour at 4 °C. The extracts were passed through polyvinylpyrrolidone (PVPP, Sigma Chemical Co. UK) and Sep-Pak C_{18} (Waters) cartridges. The hormones absorbed by the cartridge were eluted with 80% methanol-water (v/v) and the extracts were collected in vials. Phytohormones were separated by an isocratic HPLC-system consisting of 6000A pump (Waters), UV detector (Unicam), µ-Bondapak column.

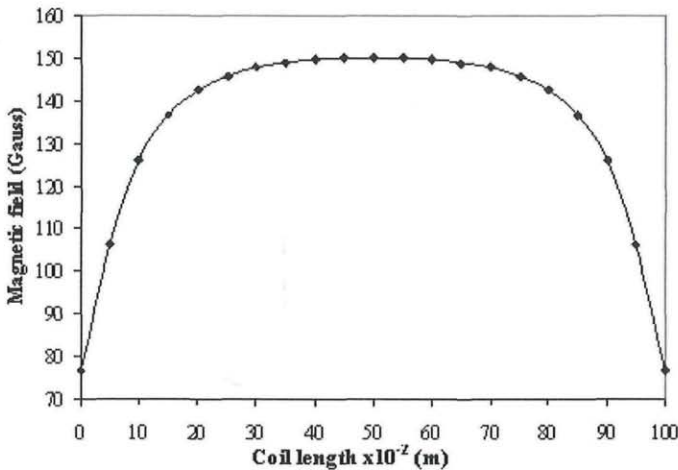


Fig. 1. The magnetic field measured in the coil. The uniform area was selected as between 0.30–0.70 m where plants were grown.

Statistical Analysis

The data were expressed as mean \pm standard error (SE). For statistical analysis the SPSS/PC package (SPSS/PC+, Chicago, IL, USA) was used. For all parameters, means and SE were calculated according to standards methods. The significance level was accepted at $p \leq 0.05$. All analyses were carried out with three different plants as triplicate.

Results and Discussion

SMF Applied on Maize in Field-up Direction

Early senescence was seen on primary leaves as an indication of chlorophyll loss and concomitant leaf yellowing. The number of roots was greater but root thickness less in experimental plants than those in controls. Stem thickness ($2.8 \pm 0.2/2.6 \pm 0.3$ mm), internode length ($5.1 \pm 0.5/4.1 \pm 0.8$ cm), the size ($1.2 \pm 0.1/1.0 \pm 0.8$ cm in width) and number of leaves (4/3) were greater in SMF-applied plants than in controls.

SMF Applied on Maize in Field-down Direction

Plants were seriously affected by SMF in field-down application. Chlorosis and necrosis were seen on leaves related to decrease in chlorophyll content (Table 1). Stems shriveled due to low turgor pressure. Roots were thicker than control, whereas the number of roots was less than control. Leaf width was greater ($1.1 \pm 0.2/1.0 \pm 0.2$ cm) but stem width was less than control ($2.5 \pm 0.8/2.9 \pm 0.2$ mm). The magnetic field may have resulted in inhibited root elongation due to thicker cell walls.

SMF Applied on Sunflower in Field-up Direction

Primary leaves turned yellow. The number of roots was less than in controls. Internode space ($6.6 \pm 0.4/1.9 \pm 0.5$ cm), stem thickness ($6.5 \pm 0.4/6.1 \pm 0.1$ mm), root length ($14.8 \pm 1.8/11.3 \pm 1.5$ cm), number of leaves (5/4) and leaf width ($1.7 \pm 0.1/1.0 \pm 0.1$ cm) were greater in experimental plants than in controls. The finding was in accordance with DAVIES' 1996 work who found increased stem diameter in two plant species under a 60 Hz MF. Similar events may have happened in the static continuous MF.

SMF Applied on Sunflower in Field-down Direction

Stem growth increased. However, stem collapsed, which may be attributed to low turgor pressure. Secondary leaves turned yellow and a necrotic zone was seen on the margin. Leaf width ($1.6 \pm 0.2/1.6 \pm 0.1$ cm), and root length ($15.0 \pm 2.6/11.3 \pm 1.8$ cm) were greater than in controls, but stems were thinner ($2.2 \pm 0.3/2.5 \pm 0.4$ mm).

It was reported that an artificial electric field altered Ca, Mg, Mn and Fe levels in plants (NECHITAILO & GORDEEV 2001). In the present study, the data may provide an argument for ion transport deterioration. Ionic and structural heterogeneity of cells, tissues and organs of plants are associated with a spectrum of electrical characteristics such as bioelectric potentials, electrical conductance and bioelectric permeability. The electrical properties of the cell membranes and organelles, which maintain energy and substance exchange with the environment, are important determi-

nants of cell function. Enzymes and other biologically active substances have a powerful charge at molecular level (NECHITAILO & GORDEEV 2001).

The Effects of SMF on Dry Weight of Plants

Dry weight of the plants was seriously influenced by SMF. Statistically significant changes ($p \leq 0.05$) were seen on root growth. Field-up and field-down directions of SMF caused a decrease in root dry weight in maize (Fig. 2a), but an increase in sunflower plants (Fig. 2b). DAVIES 1996 reported increased root and stem dry weight in radish and barley plants under a MF of 60 Hz. Root dry weight in maize plants was more influenced by the field-down direction than by the field-up application. Stem dry weight also decreased (Fig. 2a). Under microgravity, cell wall constituents decreased in level and molecular mass, which appeared to contribute to an increase in cell wall extensibility (HOSON & al. 2000). The same effects may play a role in SMF because of changes in ion and nutrient transport.

Stem and leaf dry weight of maize was also decreased in both, field-up and field-down directions of SMF (Fig. 2a), but the changes were not prominent. Electric stimulation changed nitrogen, phosphorus and potassium levels in the above-ground plant segment (NECHITAILO & GORDEEV 2001). Our finding may indicate an unexpected nutrient distribution in stem and leaf in the two plant species. Stem dry weight of sunflower plant was quite similar in either direction of the magnetic field and in controls, whereas leaf dry weight was similar among the plants incubated under field-up SMF and controls, but less in the field-down direction (Fig. 2b).

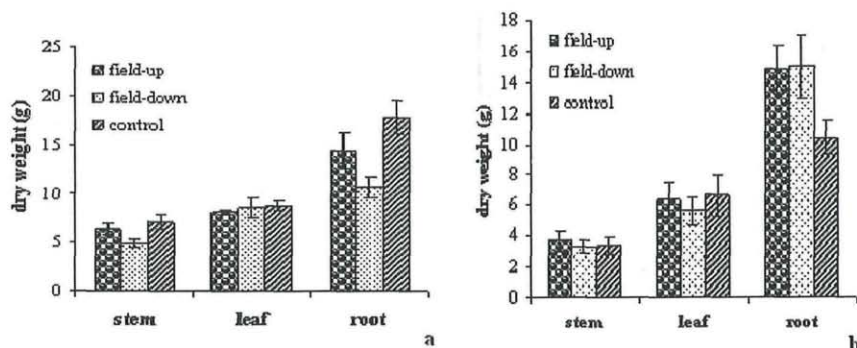


Fig. 2. Stem, leaf and root dry weight of maize (a) and sunflower (b) plants. Values are the means of triplicates \pm SE.

Extreme conditions may cause biological abnormalities such as impairment in the synthesis of important biological substances and a disturbance of normal metabolic waste elimination. It has been also reported that MF altered key cellular processes such as gene transcription and enzyme synthesis (PHILLIPS 1993).

The resonance electrical polarization of plant tissues accelerates both the transport of photosynthates, minerals and water and the influx of assimilates to storage organs of plants (SHEVTSOV & al. 2000). We may speculate that similar mechanisms apply for SMF.

The Effects of SMF on the Length of Plants

Stem, leaf and root length of maize plants were influenced under SMF. Slight changes in stem length between experimental and control plants were observed. However, leaf length of maize plant increased significantly in experimental plants compared to controls (Fig. 3a). Our data are in good agreement with DAVIES' 1996 work who found increased plant length in two plant species under a 60 Hz MF.

Root length decreased in the plants in either direction of SMF (Fig. 3 a,b). It was reported that gravitational acceleration decreased the growth rate of maize coleoptiles and mesocotyls by decreasing the cell wall extensibility via an increase in the molecular mass of matrix polysaccharides (SOGA & al. 2003). It can be speculated that the effect of MF may slower root growth in a similar way.

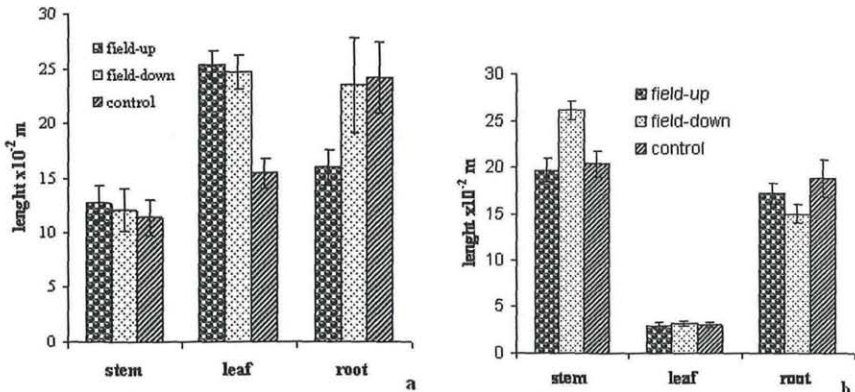


Fig. 3. Stem, leaf and root length of maize (a) and sunflower (b) plants. Values are the means of triplicates \pm SE.

The largest increase in the stem length of sunflower plants was seen in the field-down direction of SMF (Fig. 3b). Transduction of hormone signals activates the metabolic processes and results in elongation growth of cells. The resonance stimulation of radical polarization causes an acceleration of the axial elongation of cells (SHEVTSOV & al. 2000). The promotion of cell elongation under MF may also relate to an increase of the osmotic pressure in the cell (NEGISHI & al. 1999). The stem length was quite similar in field-up direction and controls. There were no significant changes among leaf length of the plants under SMF of field-up and field-down

direction and controls. Root length of the plants decreased in experimental plants compared to the controls (Fig. 3b). The change was obvious in field-down direction.

The data obtained in our study suggest that the observed effects of SMF on plant development may be related to disruptions in different metabolic systems.

Phytohormone Levels

The concentration of gibberellic acid-equivalents (GAs) in maize leaves decreased under SMF condition of either direction. The largest decrease was observed in the field-down direction of SMF. In sunflower leaves, GAs concentration was lowest in the field-down direction of SMF, but in field-up direction slightly greater than in the controls (Fig. 4a). Both directions of SMF might activate the enzymes which degraded GAs.

The indole-3-acetic acid (IAA) concentration in maize leaves was strongly decreased by SMF. In sunflower leaves, in contrast, the IAA concentration was greater in field-up direction of SMF compared to controls, but less in field-down direction (Fig. 4b).

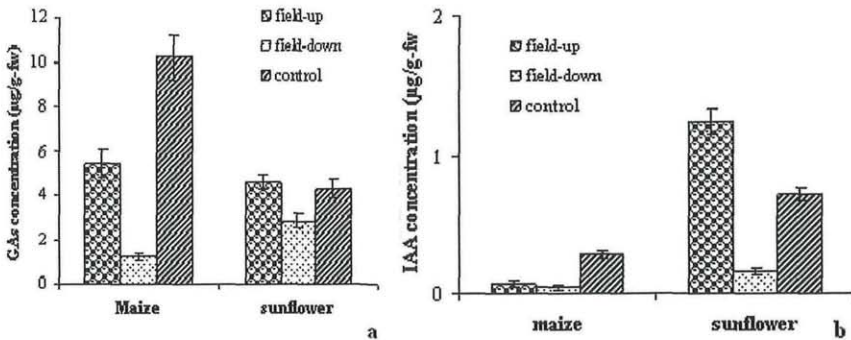


Fig. 4. Gibberellic acid-equivalents (GAs) (a) and indole-3-acetic acid (IAA) (b) concentrations in maize and sunflower leaves. Values are means of triplicates \pm SE.

The trans-Zeatin (t-Z) concentration in maize leaves was slightly decreased by SMF. trans-Zeatin concentration in sunflower leaves was strongly increased in the field-up direction and decreased in the field-down direction of SMF compared to controls (Fig. 5). SMF of either orientation might have prevented IAA synthesis in maize plants. However, IAA seemed to have been stimulated in field-up direction, but inhibited in field-down direction in sunflower plants. The different effect of SMF on IAA might be related to the metabolism and assimilation type of the plants as C_3 (sunflower) and C_4 (maize) plants. Static magnetic fields may operate by affecting the endogenous level of plant growth substances, or by affecting the activities of senescence-retarding substances such as cytoki-

nins. It has been reported that senescence-associated genes are affected e. g. by abscisic acid (ABA) and jasmonates (REINBOTHE & al. 1994, BUCHANAN-WOLLASTON 1997, WEAVER & al. 1998).

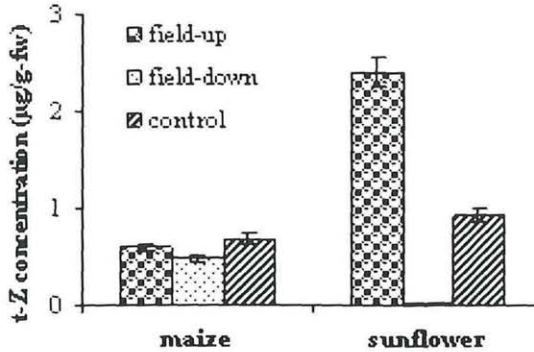


Fig. 5. trans-Zeatin (t-Z) concentrations in maize and sunflower plants. Values are means of triplicates \pm SE.

While auxins (such as IAA) affect the DNA replication, cytokinins (such as t-Z) are effective on some events in mitosis. Cells cannot enter mitosis when cytokinin is not available (KUBO & KAKIMOTO 2000). As a result it can be concluded that the endogenous phytohormone level decreased in maize in the field-down direction of SMF, whereas they increased in the field-up direction in sunflower plants. As a consequence, the changes in GAs, cytokinin and auxin level may strongly be influenced by SMF.

MIYAMATO & al. 2001 reported that exogenous kinetin caused increasing levels of endogenous ABA and jasmonates in the segment of oat plants under microgravity condition. The loss of chlorophyll was also enhanced by hypergravity conditions. Similar results may be expected by the effect of SMF.

Chlorophyll Content

Chlorophyll a and total chlorophyll content decreased in both directions of the static magnetic field in maize plants. Chlorophyll b content remained unchanged in the field-up direction, whereas it decreased in the field-down direction of SMF. In contrast, chlorophyll a, chlorophyll b and total chlorophyll contents of sunflower increased significantly in the field-up direction of SMF. Chlorophyll b and total chlorophyll contents increased in the field-down direction of SMF, whereas chlorophyll a slightly decreased compared to controls (Table 1).

Table 1. Concentrations of chlorophyll a, chlorophyll b and total chlorophyll in maize and sunflower leaves ($\text{mg}\cdot\text{g}^{-1}\text{-FW}$). Values are means of triplicates \pm SD.

	Maize			Sunflower		
	Control	Field-up	Field-down	Control	Field-up	Field-down
Chlorophyll a	1.53 ± 0.08	1.01 ± 0.02	1.40 ± 0.04	0.55 ± 0.01	1.35 ± 0.07	0.47 ± 0.02
Chlorophyll b	1.04 ± 0.05	1.06 ± 0.09	0.84 ± 0.01	0.20 ± 0.02	0.53 ± 0.01	0.36 ± 0.01
Total Chlorophyll	2.57 ± 0.25	2.07 ± 0.30	2.24 ± 0.56	0.75 ± 0.02	1.88 ± 0.03	0.83 ± 0.02

The t-Z concentration was found to be greatest where chlorophyll content was also greater. It has been well known that cytokinins are effective in inhibiting or delaying the loss of chlorophyll (THIMANN 1977, UEDA & al. 1981). Based on these results, it was postulated that SMF may have inhibited senescence by decreasing the endogenous level of senescence-promoting substances (MIYAMATO & al. 2001). MF caused a decrease in rRNA synthesis in root cell nuclei, phytoferritin in plastids and an increase in lipid content due to decreased enzyme synthesis. Mitochondria were the most sensitive organelles to MF application (BELYAVSKAYA 2001). The metabolic reactions occurring in plastids are similar to the reactions in mitochondria. Similar effects of SMF might be expected in both organelles.

In conclusion, the present study suggests many different effects of SMF on plants depending on the species and the direction of SMF. The negative effect of SMF in both directions was seen on dry weight of maize plants, whereas a prominent increase in roots of sunflowers was detected. Mostly, phytohormone contents decreased in both directions of SMF application. However, the field-up direction caused a significant increase in IAA and t-Z contents. The total chlorophyll content decreased in maize plants, whereas it increased in sunflower plant in both directions of SMF application. The different findings depend not only on the variety of plants but also on the different responses developed by plants exposed to adverse environmental factors (MURAJI & al. 1998, ATAK & al. 2000, NOVITSKY & al. 2001, BELYAVSKAYA 2001, OLDACAY 2002, ATAK & al. 2003). More investigations are needed to elicit the specific effect of MF on plant metabolism.

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