

Effects of permanent magnetic fields on *in vitro* growth of *Cymbidium* and *Spathiphyllum* shoots

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Abstract Magnetic fields affect biological systems. However, this is the first study on the effects of permanent magnetic fields (MFs) on the micropropagation of two ornamental plants, *Spathiphyllum* cv. i.e ‘Merry’ and *Cymbidium* Music Hour ‘Maria’. *Cymbidium* and *Spathiphyllum* shoots cultured in the ‘Miracle Pack’[®] culture system were exposed to MFs of different intensities, polarities, and duration of exposure. The results show that by increasing intensity from 5×10^{-6} Tesla (T) as the geo-magnetic field to 0.1, 0.15, and 0.2 T negatively influenced height and fresh mass of roots of *Cymbidium* plants (except for 0.1 T–S and 0.2 T–N treatments), but had no significant effect on other plantlet parameters. Long-term exposure (1, 2, or 3 mo) of *Cymbidium* shoots to 0.15 T–MFs negatively influenced plant height, positively affected the number of leaves (with the exception of 0.15 T–S—1 mo), and had no clear effect on other parameters compared to the control. MFs (0.1, 0.15, and 0.2 T), regardless of their polarity, increased chlorophyll content (SPAD value) and the number of leaves, but slightly decreased the dry mass of *Spathiphyllum* shoots.

Different exposure duration to 0.15 T (*i.e.*, 2, 4, or 8 wk) had no significant influence on *Spathiphyllum* plantlet development other than increasing the SPAD value. These two ornamentals could serve as model systems to study plant development, space production, yield maximization, and the development of new morphotypes essential for the floricultural market.

Keywords *Cymbidium* · *Spathiphyllum* · Magnetic field · Micropropagation · Miracle Pack’[®] culture system

Introduction

It is well known that all organisms are living under the influence of the Earth’s magnetic field (5×10^{-6} Tesla (T)), also termed the geo-magnetic field, or geoMF (Belyavskaya 2004). However, only few studies have shown how biological systems are affected by external MFs, in strengths lower or higher than geoMF (Atak *et al.* 2000, 2003, 2007; Belyavskaya 2004; Dhawi and Al-Khayri 2009). From those studies, it has been observed that MFs affect the metabolism and mechanism of growth of different plants based on the type of magnet used, the intensity of the MF, and the polarity, orientation, and duration of exposure. Some studies have shown that MFs affect the development of cells and tissues cultured *in vitro*, for example shoot and root formation rates of *Paulownia* tissue culture were increased when exposed to external MFs of 2.9–4.8 mT for 2.2, 6.6, and 19.8 s or 0.1–0.3 T compared to the control (Ham *et al.* 2004; Yayıcı and Alikamanoğlu 2005; Çelik *et al.* 2008). Similarly, Atak *et al.* (2007) found that both regeneration and growth of soybean (*Glycine max* L. Merrill) shoot-tip cultures exposed to MFs of 2.9–4.6 mT at various durations (2.2 or 19.8 s) increased relative to the controls. Our previous

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study reported that *in vitro* growth of *Phalaenopsis* was most enhanced in plantlets treated with 0.1 T-S (Van *et al.* 2011b). In the present study, the effects of MFs on micropropagation of two important commercial ornamental plants, *Cymbidium* Music Hour ‘Maria’ and *Spathiphyllum* cv. ‘Merry’, belonging to Orchidaceae and Araceae families, respectively, were studied for the first time as a new abiotic stress factor *in vitro*. The information gained could potentially fill in a current void concerning the effects of MFs on plant growth and development. The applied research applications are widespread, such as in space research.

Materials and Methods

Plant materials and culture conditions. Two explants were used. *Cymbidium* Music Hour ‘Maria’ shoots with three leaves, no roots, 4.5 cm in length, and with stems of similar size obtained from a mass of protocorm-like bodies (PLBs), and cultured *in vitro*. These PLBs originally derived from shoot-tip culture (Teixeira da Silva *et al.* 2006a). The second source of explant was terminal apices with three leaves, no roots, and 4.5 cm in length obtained from a mass of shoots derived from the *in vitro* culture of *Spathiphyllum* cv. ‘Merry’ shoot tips (Teixeira da Silva *et al.* 2005).

Twenty-five *Spathiphyllum* shoots were cultured per culture vessel (‘Miracle Pack’[®] (MP) culture system; Daikin Industries, Osaka, Japan) for 2 mo, or 3 mo in the case of *Cymbidium* under the following conditions: temperature ($25 \pm 1^\circ\text{C}$), photoperiod (16 h per day), light intensity ($45 \mu\text{mol m}^{-2} \text{s}^{-1}$; Plant Lux, Toshiba Co., Japan), CO_2 enrichment ($3,000 \mu\text{mol mol}^{-1} 24 \text{ h}^{-1}$). Three culture vessels (‘Miracle Pack’[®] culture system) were used for each treatment and all culture vessels were placed in the growth chamber.

Culture media. *Cymbidium* shoots were culture in sugar-free Vacin and Went basal medium (VW; Vacin and Went 1949) supplemented with Nitsch’s microelements (Nitsch and Nitsch 1967), 2 g L^{-1} tryptone (Bacto, Difco Laboratories, Sparks, MD), 0.1 mg L^{-1} β -naphthalene acetic acid (NAA; Nacalai Tesque, Kyoto, Japan), and 0.1 mg L^{-1} kinetin (Wako Chemicals Ltd., Tokyo, Japan). *Spathiphyllum* shoots were cultured in full-strength Murashige and Skoog (MS; Murashige and Skoog 1962), sugar-free medium. All media were adjusted to pH 5.3 or 5.5 (for *Cymbidium* and *Spathiphyllum*, respectively) by adding 1 N NaOH or 1 N HCl before autoclaving at 121°C for 17 min.

Preparation of ‘Miracle Pack’[®] culture system. The ‘Miracle Pack’[®] (MP) culture system was used as the culture vessel of choice for all experiments including the controls (as described in Tanaka *et al.* 1999, 2005). The substrate used in each MP was a $25 (5 \times 5) \text{ mm}^2$ joined-block of rockwool

(Grodan[®] Rockwool Multiblock[™], AO 18/30, Grodiana A/S, Denmark). The rockwool was sterilized in a dry sterilizer at 150°C for 2 h and placed in the MP when at room temperature. Sterilized medium (210 ml) was poured evenly over the rockwool block and 25 plantlets were inserted into the small holes ($\text{Ø } 5 \text{ mm} \times \text{d } 10 \text{ mm}$) in each multiblock[™], one plantlet per hole (Fig. 1).

Magnetic experimental device. Three types of permanent magnets with different intensities (0.1, 0.15, and 0.2 T; Kinki Magnet Co., Osaka, Japan) were used in this study. Each surface of the magnet has two polarities: North (N) and South (S). The magnetic experimental device used was as described by Tanaka *et al.* (2010). MP culture system contained 25 explants was placed directly on the surface of each magnet tested (Fig. 1).

Effects of intensity and polarity of magnetic fields on *Spathiphyllum* shoot development. Each cultured MP, containing 25 *Spathiphyllum* shoots and 210 ml full-strength MS medium, was placed directly on the North (N) or South (S) polarities of three types of magnets: 0.1, 0.15, and 0.2 T while the control had no extra-MF treatment other than natural geoMF. Plant growth was assessed after 8 wk of culture.

Effects of duration of exposure to magnetic fields on *Spathiphyllum* shoot development. In this investigation, six MPs containing 210 ml of full-strength, sugar-free MS medium, and 25 shoots/MP were exposed to N and S polarities of 0.15 T magnets for 2, 4, and 8 wk (Table 1). The control was exposed to geoMF ($5 \times 10^{-6} \text{ T}$) only. Data was recorded after 8 wk.

Effects of intensity and polarity of magnetic fields on *Cymbidium* shoot development. Twenty-five shoots were cultured per MP containing 210 ml of VW medium. Six culture vessels with a total of 150 explants were exposed to MFs at different intensities: 0.1, 0.15, and 0.2 T combined

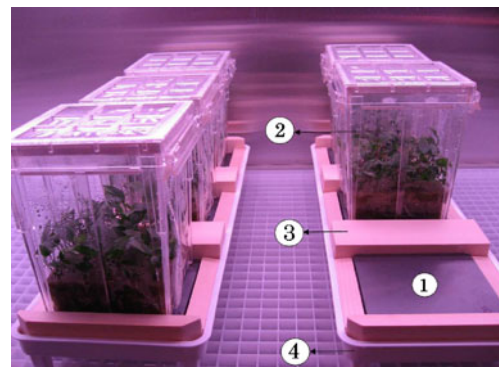


Figure 1. Practical magnetic field system used in this study. 1 Magnet (Kinki Magnet Co., Osaka, Japan); 2 Miracle Pack[®] culture system (BIO-U Co. Ltd., Japan); 3 wooden slab; 4 plastic tray.

Table 1. The sequence of treatment and non-treatment phases in the application of 0.15 T magnetic field on *Spathiphyllum* cv. Merry shoot development for different durations of exposure

Duration (weeks)	Control	0.15 T 2 W	0.15 T 4 W	0.15 T 8 W
Duration of exposure to MF (0.15 T–N/S)	0	2	4	8
Duration of exposure to GMF (as control condition)	8	6	4	0
Total time of culture	8	8	8	8

T Tesla; *MF* magnetic field; *GMF* geo-magnetic field (5×10^{-6} T); *N* North; *S* South; *W* week(s)

with different polarities: N or S. Controls with the same amount of medium and number of explants were exposed to non-external MF rather than geoMF. Both controls and treatments were cultured in a culture room under CO₂ enrichment. Growth parameters were recorded on the 90th day.

Effects of duration of exposure to magnetic fields on Cymbidium shoot development. In this experiment, six MPs containing 210 ml of VW medium, and 25 explants/MP were exposed to N and S of a 0.15 T magnet for 1, 2, and 3 mo (Table 2). For comparison, and simultaneously, the control was an MP not exposed to extra MFs. Data were recorded on the 90th day.

Experimental design and statistical analysis. In each treatment, three vessels were used. Each vessel contained 25 plants. Each sample was placed randomly within the culture chamber and the experiment was repeated in triplicate. Therefore, for each treatment, a total of $3 \times 3 \times 25 = 225$ explants were used.

For all treatments, plantlet growth was assessed by the following parameters after 8 or 12 wk of culture for *Spathiphyllum* or *Cymbidium* shoots, respectively: plant height (cm), length of the longest root (cm), number of leaves, number of roots, fresh mass of shoots (mg), fresh mass of roots (mg), dry mass of shoots, and dry mass of roots (mg). Chlorophyll (chl) content of the third leaf counting downwards from the plantlet apex was measured by a chl meter (SPAD-502, Minolta Co., Japan) and reported as the SPAD value (Teixeira da Silva *et al.* 2005). For all parameters tested, data analyses were carried out using IRRISTAT version 3.0. Following ANOVA, Duncan's multiple range test at $P=0.05$ was applied to test for differences between the means.

Table 2. The sequence of treatment and non-treatment phases in the application of 0.15 T—magnetic field on *Cymbidium* Maria 'Music hour' shoot development for different durations of exposure

Duration (months)	Control	0.15 T—1 M	0.15 T—2 M	0.15 T—3 M
Duration of exposure to MF (0.15 T–N/S)	0	1	2	3
Duration of exposure to GMF (as control condition)	3	2	1	0
Total time of culture	3	3	3	3

T Tesla; *MF* magnetic field; *GMF* geo-magnetic field (5×10^{-6} T); *N* North; *S* South; *M* month(s)

Results

Effects of intensity and polarity of magnetic fields on Spathiphyllum shoot development. The effects of exposure to different intensities and polarities of MFs (0.1 to 0.2 T–N/S) on the *in vitro* growth of *Spathiphyllum* shoots are shown in Table 3 and Fig. 2. The chl content (single photon avalanche diode; SPAD value) and number of leaves increased while the dry mass of shoots decreased after exposure to any external MF (0.1–0.2 T–N/S), more than the geoMF. The average fresh mass of shoots exposed to 0.1 T–S, 0.15 T–S, and 0.2 T–N was significantly higher than that of the control. However, other shoot/root parameters of *Spathiphyllum* plantlets, namely plant height, number of roots, fresh and dry mass of roots were not significantly different from the control.

Effects of duration of exposure to magnetic fields on Spathiphyllum shoot development. Short- or long-term exposure to external MFs, regardless of polarity, had no effect on the *in vitro* growth of *Spathiphyllum* shoots (Table 4, Fig. 3) in terms of these growth parameters (*i.e.* plant height, root length, number of leaves, fresh and dry mass of roots) compared to the control (*i.e.* geoMF). The SPAD value of explants exposed to geoMF (control) was clearly lower than that of explants exposed to externally applied MFs. However, among treatments, different exposure durations and polarities did not affect the SPAD value of all treated shoots. The number of roots formed on explants exposed to an MF of 0.15 T–N significantly decreased compared to the control, regardless of the exposure duration. Among them, 0.15 T–N–2 wk showed the least number of roots. The greatest fresh and dry masses of shoots were observed in shoots exposed to MF of 0.15 T–N for 1 mo.

Table 3. Effects of intensity and polarity of magnetic fields on *Spathiphyllum* cv. Merry shoot development

MF intensity	PH (cm)	RL (cm)	SPAD ^v value	Number		FM (mg)		DM (mg)	
				Leaves	Roots	Shoot	Root	Shoot	Root
Control (GMF)	4.7 ^a	9.8 ^{bc}	44.1 ^c	4.8 ^b	2.0 ^{ab}	223.2 ^c	78.1 ^{ab}	23.2 ^a	6.8 ^{abcd}
0.1 T-N	4.7 ^a	10.2 ^{ab}	46.4 ^{ab}	5.2 ^a	2.1 ^a	230.9 ^{bc}	81.5 ^a	20.5 ^{bc}	6.7 ^{abcd}
0.1 T-S	4.8 ^a	10.2 ^{ab}	46.8 ^a	5.1 ^a	1.9 ^b	242.5 ^{ab}	79.7 ^{ab}	20.9 ^{bc}	6.3 ^{cd}
0.15 T-N	4.7 ^a	10.8 ^a	47.2 ^a	5.0 ^a	1.9 ^{ab}	234.9 ^{bc}	81.3 ^a	20.6 ^{bc}	7.2 ^a
0.15 T-S	4.9 ^a	9.3 ^c	45.5 ^b	5.1 ^a	1.9 ^{ab}	249.4 ^a	76.6 ^{ab}	21.3 ^{bc}	6.7 ^{abcd}
0.2 T-N	4.7 ^a	9.6 ^{bc}	46.8 ^a	5.1 ^a	2.0 ^a	238.8 ^{ab}	81.7 ^a	21.9 ^b	7.0 ^{ab}
0.2 T-S	4.7 ^a	9.4 ^{bc}	46.7 ^a	5.0 ^a	2.1 ^a	234.0 ^{bc}	73.4 ^b	20.1 ^c	6.4 ^{bcd}

Means within a column followed by the same letter are not significantly different at $P=0.05$ by Duncan's multiple range test. Total 63 samples were used

^v Estimated chlorophyll content in the third leaf, counted from the apex of the plantlet by a SPAD chlorophyll meter

PH plant height; RL root length; FM fresh mass; DM dry mass

Effects of intensity and polarity of magnetic fields on Cymbidium shoot development. The development of shoots (treatment and control) is shown in Table 5 and Fig. 4. Statistical analysis indicates that the SPAD value, fresh mass of shoots/roots, and dry mass of shoots/roots were randomly influenced by the application of various MF intensities combined with two polarities. There was no significant difference between treated and untreated explants by MFs in terms of root length (except for shorter roots in 0.1 T-N/S), number of leaves (except for fewer leaves in 0.1 T-N), and number of roots (except for fewer roots in 0.2 T-S). Increasing intensity (from geoMF to 0.1, 0.15, and 0.2 T), regardless of polarity, negatively influenced plant height. MFs of 0.15 T, regardless of their polarity, negatively affected chl content (reported as SPAD value), and fresh mass of roots of plantlets. The SPAD

value of shoots exposed to 0.1 T-S was the highest. Negative effects on fresh mass of roots were also found in 0.1 T-N and 0.2 T-S treatments. *Cymbidium* shoots exposed to 0.2 T-S had fewer roots than in other treatments and the control. The highest dry mass of roots was found in explants exposed to 0.2 T-S – MF. The highest fresh mass of shoots was found in 0.2 T-S, while the highest dry mass of shoots was found in 0.15 T-N.

Effects of duration of exposure to magnetic fields on Cymbidium shoot development. The growth parameters of treated and untreated shoots after 3 mo are detailed in Table 6 and Fig. 5. Long-term exposure of *Cymbidium* shoots to external MFs negatively influenced plant height. However, they positively affected number of leaves of treated shoots

Figure 2. Effects of intensity and polarity of magnetic fields on *Spathiphyllum* cv. Merry shoot development. T Tesla; N North; S South.

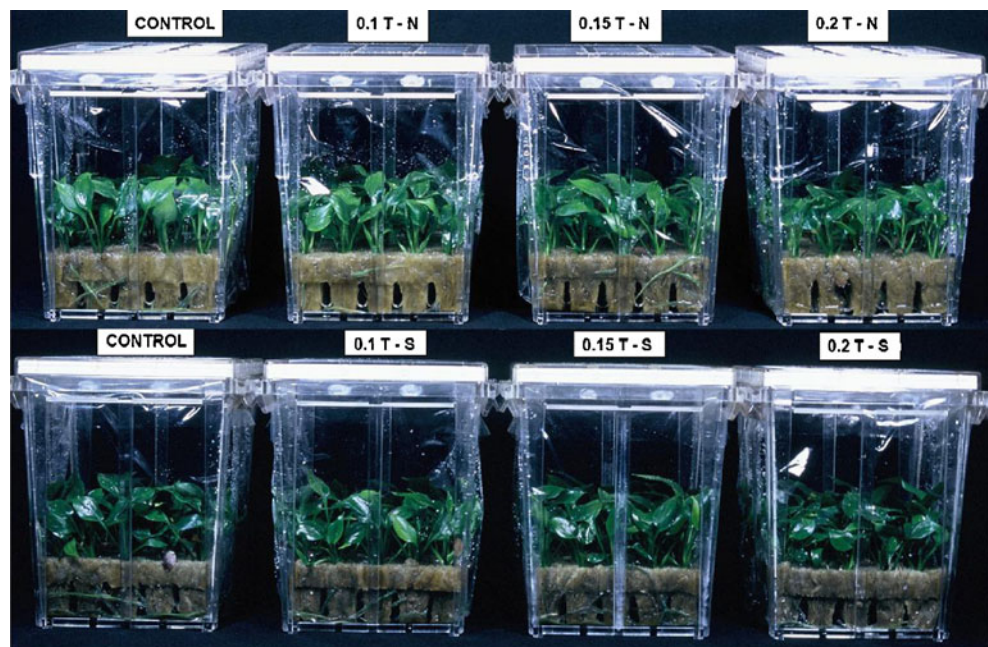


Table 4. Effects of duration of exposure to magnetic fields on *Spathiphyllum* cv. Merry shoot development

Exposure duration	PH (cm)	RL (cm)	SPAD ^v value	Number		FM (mg)		DM (mg)	
				Leaves	Roots	Shoot	Root	Shoot	Root
Control (GMF)	5.47 ^a	9.39 ^{ab}	40.97 ^b	5.20 ^{abc}	2.19 ^b	205.71 ^{bc}	75.97 ^a	18.78 ^{bc}	6.76 ^{ab}
0.15 T-S-2 W	5.52 ^a	8.81 ^b	41.99 ^{ab}	5.24 ^{ab}	2.01 ^{cd}	208.88 ^{abc}	69.83 ^a	18.20 ^c	6.12 ^{bc}
0.15 T-N-2 W	5.48 ^a	9.39 ^{ab}	43.30 ^a	5.11 ^c	1.91 ^c	211.15 ^{ab}	72.85 ^a	19.32 ^b	6.59 ^{abc}
0.15 T-S-1 M	5.41 ^a	9.15 ^{ab}	43.19 ^a	5.29 ^a	2.29 ^a	211.35 ^{ab}	74.92 ^a	18.48 ^c	7.04 ^a
0.15 T-N-1 M	5.52 ^a	9.59 ^a	44.05 ^a	5.12 ^c	1.96 ^{de}	215.65 ^a	74.83 ^a	20.39 ^a	6.70 ^{abc}
0.15 T-S-2 M	5.44 ^a	9.28 ^{ab}	43.61 ^a	5.15 ^{bc}	2.11 ^{bc}	202.19 ^c	73.61 ^a	19.57 ^b	6.10 ^{bc}
0.15 T-N-2 M	5.44 ^a	9.85 ^a	43.40 ^a	5.19 ^{abc}	2.07 ^c	206.56 ^{bc}	75.00 ^a	19.50 ^b	6.67 ^{abc}

Means within a *column* followed by the same *letter* are not significantly different at $P=0.05$ by Duncan's multiple range test. Total 63 samples were used

^v Estimated chlorophyll content in the third leaf, counted from the apex of the plantlet by a SPAD chlorophyll meter

W weeks; *M* months; *PH* plant height; *RL* root length; *FM* fresh mass; *DM* dry mass

(with the exception of shoots exposed to 0.15 T-S for 2 mo). Other parameters did not differ significantly from the control, in general. Plantlets exposed to MFs of 0.15 T-S for 3 mo had slower growth than the control in terms of plant height, root length, SPAD value, fresh, and dry masses of shoots/roots.

Discussion

Little quality information exists in the literature concerning the effects of MFs on plant growth. This study was thus conducted to determine the effects of MFs on *in vitro* shoot development of two important ornamental plants: *Cymbidium* Maria Hour 'Music' and *Spathiphyllum* cv. Merry. Our results

indicate clear variations in some plant growth parameters between the treatments and the control.

MFs, in the range of intensities tested, *i.e.*, from 0.1 to 0.2 T, independent of polarity and exposure duration, increased the chl content of the third leaf of *Spathiphyllum* shoots. Conversely, the chl content the third leaf of *Cymbidium* shoots when exposed to 0.15 T MF for 3 mo, and independent of polarity decreased. The same effect on plant growth was confirmed by several studies on different plants. *Phalaenopsis* plantlets grown *in vitro* indicated that MF (0.1, 0.15, and 0.2 T) had no effect on the chl contents of treated plantlets (Van *et al.* 2011b). Atak *et al.* (2000) determined that MFs of 3–4 mT increased the chl content in *Paulownia* sp., compared with the control. Studies by Atak *et al.* (2003, 2007) and Çelik

Figure 3 Effects of duration of exposure to magnetic fields on *Spathiphyllum* cv. Merry shoot development. *T* Tesla; *N* North; *S* South; *W* weeks.

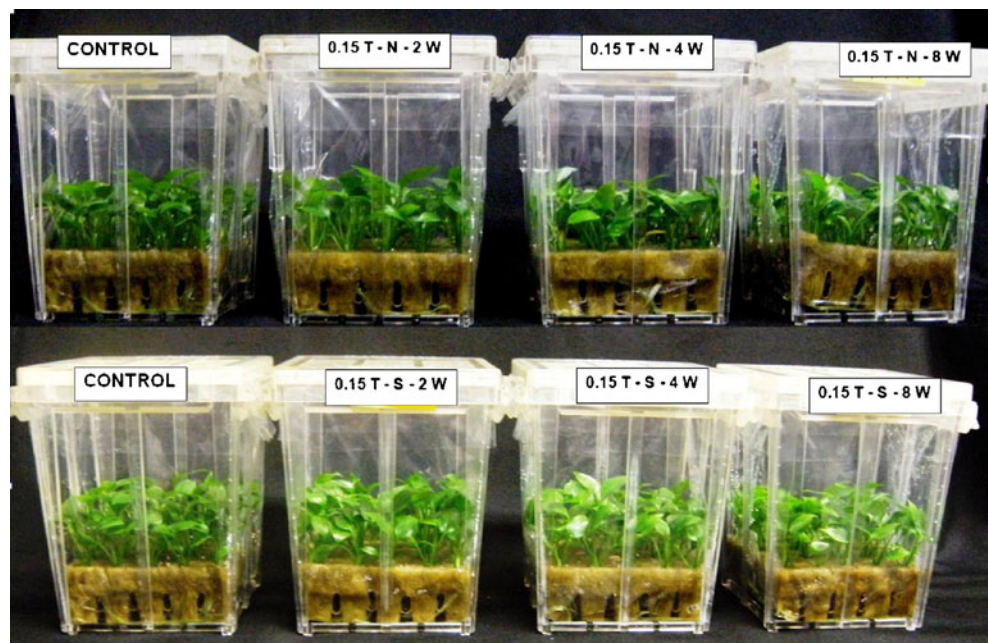


Table 5. Effects of intensity and polarity of magnetic fields on *Cymbidium Music Hour* ‘Maria’ shoot development

MF intensity	PH (cm)	RL (cm)	SPAD ^v value	Number		FM (mg)		DM (mg)	
				Leaves	Roots	Shoot	Root	Shoot	Root
Control (GMF)	8.71 ^a	1.30 ^a	53.14 ^d	5.71 ^{abc}	2.17 ^a	453.94 ^b	176.90 ^a	35.74 ^c	6.70 ^b
0.1 T–S	7.85 ^d	1.12 ^b	56.27 ^a	5.67 ^{bc}	2.21 ^a	425.44 ^c	181.44 ^a	40.86 ^{ab}	7.43 ^a
0.1 T–N	8.43 ^b	1.12 ^b	54.16 ^c	5.46 ^d	2.23 ^a	449.83 ^b	155.81 ^b	34.84 ^c	6.22 ^{bcd}
0.15 T–S	8.18 ^c	1.27 ^a	52.06 ^e	5.77 ^{ab}	2.13 ^a	456.04 ^b	156.29 ^b	35.37 ^c	6.02 ^{cd}
0.15 T–N	8.44 ^b	1.31 ^a	52.10 ^e	5.69 ^{abc}	2.17 ^a	455.44 ^b	158.60 ^b	43.62 ^a	6.72 ^b
0.2 T–S	8.40 ^b	1.25 ^a	55.09 ^b	5.83 ^a	2.00 ^b	472.44 ^a	147.98 ^b	37.40 ^{bc}	5.78 ^d
0.2 T–N	8.35 ^b	1.28 ^a	53.08 ^d	5.60 ^c	2.15 ^a	456.92 ^b	184.08 ^a	35.61 ^c	6.37 ^{bc}

Means within a column followed by the same letter are not significantly different at $P=0.05$ by Duncan’s multiple range test. Total 63 samples were used.

^v Estimated chlorophyll content in the third leaf, counted from the apex of the plantlet by a SPAD chlorophyll meter

PH plant height; RL root length; FM fresh mass; DM dry mass

et al. (2008) on the effects of MFs of 2.9–4.6 mT on *Paulownia* node cultures and soybean tissue culture at different exposure times, also confirmed that the total chl content was increased by MFs. Dhawi and Al-Khayri (2009) emphasized that MFs in the range of 10–100 mT and exposure for 30–360 min increased photosynthetic pigments significantly in date palm (*Phoenix dactylifera* L.) seedlings whereas high doses (1.5 T, >10 min) had a negative effect on the content of photosynthetic pigments.

The mechanisms of the interaction of MFs with biological systems are still not well understood. However, the theory that the “magnetic field effect” acts in photosynthesis, may partially explain the interaction of MFs with intermediate ionic pairs. The increase in chl content of plants exposed to MFs could be explained by the properties of magnetized water and the oriented movement of the paramagnetic substance under

external MF (Yaycılı and Alikamanoğlu 2005; Atak *et al.* 2007; Çelik *et al.* 2008; Dhawi and Al-Khayri 2009; Yan *et al.* 2009). Chloroplasts contain Mn^{2+} which plays an essential role in photosynthesis is a paramagnetic substance. When the external MF (*i.e.*, 0.1–0.2 T) is applied, Mn^{2+} which is moving non-direction in water is oriented in the same direction as the applied field and tends to move in to the MF. This interaction absorbed energy therefore could affect chloroplasts and disturb the pigment synthesis (Commoner *et al.* 1956; Theg and Sayre 1979; Dhawi and Al-Khayri 2009). MFs also affect ions in the humid environments of plants and allow those ions to absorb MF energy and mobilization. Increasing ion mobility and ion uptake improved under MFs leads to better photostimulation, explaining higher chl contents and SPAD values in this study. Moreover, MFs have the ability to change the properties of water, thus magnetized water increases chl content in leaves

Figure 4 Effects of intensity and polarity of magnetic fields on *Cymbidium Music Hour* ‘Maria’ shoot development. T Tesla; N North; S South.

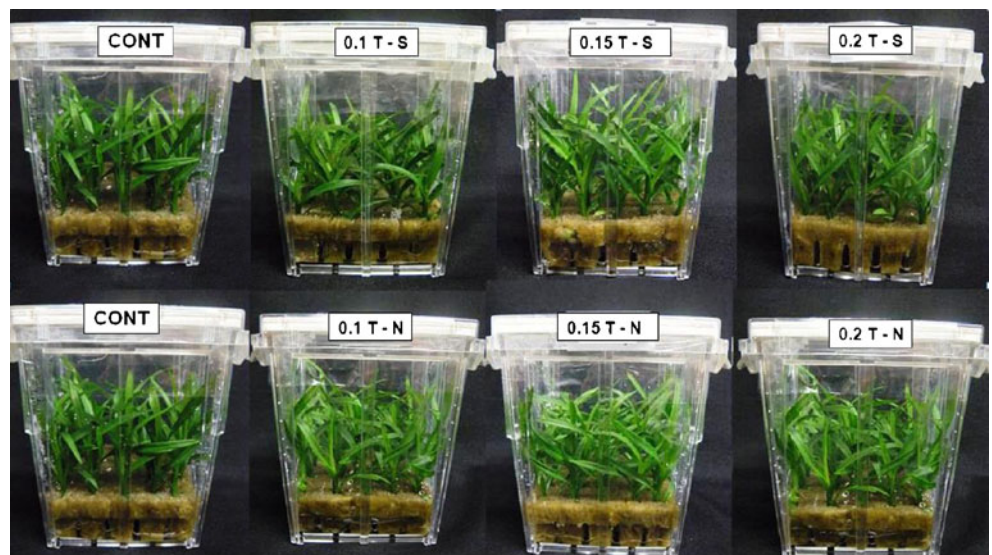


Table 6. Effects of duration of exposure to magnetic fields on *Cymbidium* Music Hour ‘Maria’ shoot development

Exposure duration	PH (cm)	RL (cm)	SPAD ^v value	Number		FM (mg)		DM (mg)	
				Leaves	Roots	Shoot	Root	Shoot	Root
Cont. (GMF)	8.9 ^a	0.8 ^a	47.1 ^{bc}	5.6 ^c	1.7 ^a	498.3 ^{ab}	87.2 ^a	38.0 ^{bc}	4.4 ^a
0.15 T-S-1 M	8.5 ^c	0.7 ^{bc}	49.2 ^a	6.0 ^a	1.5 ^d	499.8 ^{ab}	58.9 ^b	38.8 ^b	2.6 ^c
0.15 T-N-1 M	8.5 ^c	0.8 ^a	46.3 ^{cd}	5.9 ^b	1.6 ^{bc}	473.1 ^{cd}	83.7 ^a	37.6 ^{bcd}	4.2 ^a
0.15 T-S-2 M	8.3 ^d	0.7 ^c	46.4 ^{cd}	5.6 ^c	1.7 ^{ab}	468.2 ^d	88.4 ^a	36.7 ^d	4.3 ^a
0.15 T-N-2 M	8.7 ^b	0.6 ^d	47.9 ^b	5.9 ^b	1.7 ^{ab}	512.1 ^{ad}	82.3 ^a	41.4 ^a	4.2 ^a
0.15 T-S-3 M	8.3 ^d	0.8 ^{ab}	45.7 ^d	5.9 ^b	1.7 ^{ab}	474.0 ^{cd}	67.1 ^b	37.1 ^{cd}	3.5 ^b
0.15 T-N-3 M	8.5 ^c	0.8 ^a	45.8 ^d	6.0 ^a	1.7 ^a	486.3 ^{bc}	60.0 ^b	38.4 ^b	3.2 ^b

Means within a *column* followed by the same *letter* are not significantly different at $P=0.05$ by Duncan’s multiple range test. Total 63 samples were used

^v Estimated chlorophyll content in the third leaf, counted from the apex of the plantlet by a SPAD chlorophyll meter

M months; *PH* plant height; *RL* root length; *FM* fresh mass; *DM* dry mass

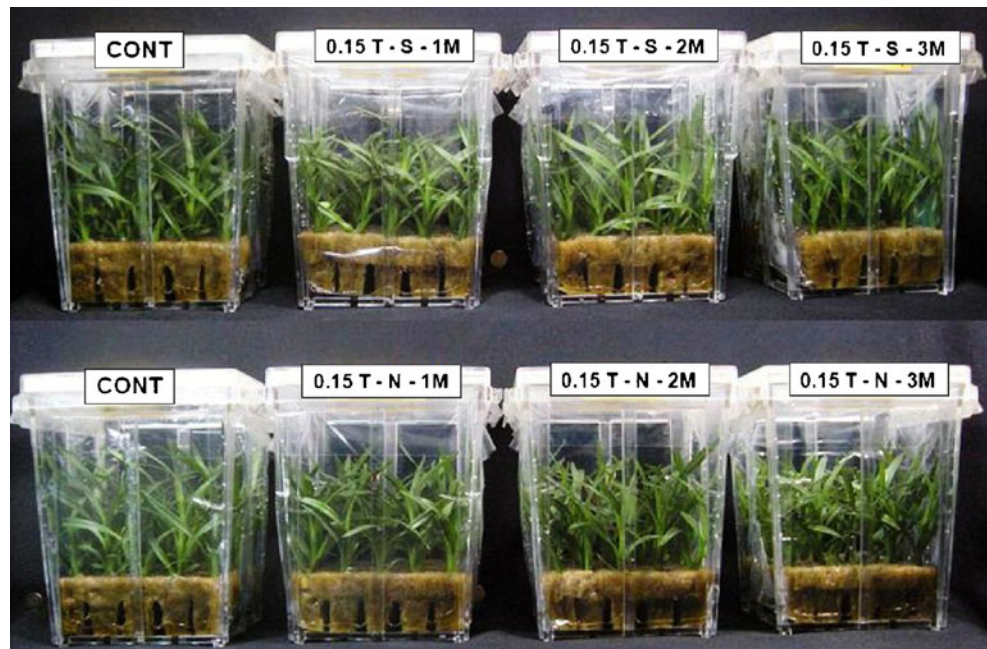
(Pang and Deng 2008; Dhawi and Al-Khayri 2009), also observed for *Cymbidium* and *Spathiphyllum* (Tables 3, 4, 5, and 6).

Our study demonstrates that an increase in intensity from 0.1 to 0.2 T or short- or long-term exposure to MFs of 0.15 T did not have a significant effect on *Spathiphyllum* and *Cymbidium* plant growth compared to the control, in general (except for plant height of *Cymbidium* shoots). Conversely, Ham *et al.* (2004) found that increase MF from 0 to 0.15 T increased the multiplication rate and rooting of *in vitro* Paulownia plantlets. Our previous study on the effects of duration of exposure of 0.15 T-N/S on *Phalaenopsis* protocorm-like bodies proliferation showed that longer exposure duration (7 wk *vs.* 2 wk) resulted in greater biomass of newly formed PLBs and smaller

average number of newly formed PLBs; and the MF of 0.1 T-S (*vs.* 0.15 T, 0.2 T-N/S) resulted in the greatest fresh and dry weights of regenerated PLBs (Van *et al.* 2011a). At plantlet development state, long-term exposure to MFs of 0.15 T for 3 mo, apart from differences in polarity, had the greatest influence on *Phalaenopsis* plant development in terms of total fresh mass of shoots and roots, and total dry mass of shoots and roots (Van *et al.* 2011b).

As can be appreciated from the above, although there are studies demonstrating the effects of MFs on plant growth, a plausible explanation has not yet been given. Our results provide further evidence of the effect of permanent MFs on horticultural plant growth. However no clear explanation about the mechanism is yet available, although it adds to a growing

Figure 5 Effects of duration of exposure to magnetic fields on *Cymbidium* Music Hour ‘Maria’ shoot development. *T* Tesla; *N* North; *S* South; *M* months.



body of evidence that abiotic factors strongly influence morphogenesis in ornamental plants (Teixeira da Silva *et al.* 2006b).

This is the first detailed study on the effect of MFs on ornamental plants *in vitro*. MFs (0.1, 0.15, and 0.2 T) affected *Cymbidium* and *Spathiphyllum* shoot development, although the magnitude of the effect depended on the different intensity, polarity, and duration of exposure. These MFs, regardless of polarity and exposure duration, increased chl content (SPAD value) of *Spathiphyllum* shoots. However, this parameter decreased in *Cymbidium* plants exposed to MFs of 0.15 T for 3 mo at both polarities. Different exposure duration to 0.15 T had no significant influence on *Spathiphyllum* plantlet development. External MFs (0.1, 0.15, and 0.2 T) increased the number of leaves and decreased the dry mass of shoots of treated plants compared to the control.

The effects of MFs on *Cymbidium* shoot development were non-specific. Increasing intensity (from $\text{geoMF} < 0.1 < 0.15 < 0.2$ T) negatively influenced *Cymbidium* plant height and fresh mass of roots (except for 0.1 T–S and 0.2 T–N), and had no significant effect on the other plantlet parameters compared to the control. Long-term exposure of *Cymbidium* shoots to MFs negatively influenced plant height, positively affected the number of leaves (with the exception of 0.15 T–S for 2 mo), and had no effect on other parameters when shoots were exposed to MFs for different durations, compared to the control.

These two ornamentals could serve as model systems to study plant development in systems where gravitational and anti-gravitational forces are pertinent such as space studies. Where positive effects have been shown, such as increased leaf production, this could serve as an excellent way of increasing yield of leafy vegetables, or increasing the leafy nature of ornamental plants and thus has extensive potential applications.

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