In Vitro Cell.Dev.Biol.—Plant (2015) 51:233–240
DOI 10.1007/s11627-015-9675-z

INVITED REVIEW

How do magnetic fields affect plants *in vitro*?

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Received: 25 April 2014 / Accepted: 21 January 2015 / Published online: 19 May 2015 / Editor: John Finer

Abstract This mini-review aims to assess how magnetic fields (MFs) have been shown to affect *in vitro* plant growth and development and the practical uses of this technology. Magnetic or electromagnetic fields have shown effects on morphogenesis from different initial explants; on growth-related parameters of *in vitro* shoots, roots, somatic embryos, and callus; and on the photosynthetic pigment profile, level of stress-induced alanine production, activity of stress-related enzymes, and endogenous levels of cytokinins and auxins. These effects have depended in part on the intensity and duration of exposure of the applied field and in part on the species and *in vitro* conditions, such as explant type or medium consistency. *In vitro* growth and development has been manipulated in a series of species, including field crops (soybean, alfalfa, wheat), herbs and medicinal plants (mojito mint, peppermint, spearmint, *Calendula officinalis*), horticultural crops (potato, sugar beet, wild *Solanum* spp.), fruits (beach plum), ornamentals (hybrid *Cymbidium*, hybrid *Phalaenopsis*, duckweed, *Krainzia longiflora*, *Spathiphyllum*), a weed (*Haplopappus gracilis*), and trees (cork oak, *Paulownia* sp.). MFs thus have the potential of being used to manipulate the growth and development of plants *in vitro* and serve as a novel system to open up novel avenues of research in plant science.

Keywords Growth and development · Magnetic field · Metabolism · Micropropagation · Organogenesis · Tissue culture

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Magnetic Fields and Plant Life

All life on Earth is under the influence of the Earth's geomagnetic field (mean GMF = 5×10^{-5} Tesla [T]), which is generated from the combination of several magnetic fields (MFs) and a dipole on the surface of the Earth, while being influenced by atmospheric flow, crust flow, and other local electric anomalies (Olsen *et al.* 2010). The impact of external MFs or electromagnetic fields (EMFs) on plant life has been studied since the early 20th century (Savostin 1930). An MF is a vector field in the neighborhood of constant magnets or electric currents that is specified by both direction and strength and is characterized by magnetic flux density (measurement in T) and MF strength (measurement in amperes [A]/meter [m]). An EMF is generated from the acceleration of charged particles and has two components: an electric field surrounding all charged particles and an MF produced by the motion of charged particles (Macintyre 2000).

Experiments on the influence of MFs or EMFs have shown that they are able to have positive effects on the growth, development, and metabolic processes of plants, mainly in early stages of germination or seedling growth (Hirota *et al.* 1999; reviewed in Vasilevski 2003; Belyavskaya 2004; Galland and Pazur 2005). These effects depended on the type of magnet; the intensity, polarity, and orientation of the MF; and the duration of exposure. Despite knowledge of these concepts, studies on plant morphogenesis and growth under *in vitro* conditions were only conducted and published after the turn of the millennium, except for two pioneering experiments (Dijak *et al.* 1986; Celestino *et al.* 1998).

In this review, we present information from all studies that have applied MFs to manipulate plant growth and development *in vitro*. Water can be magnetized then used to alter plant growth and development, but magnetized water has not been used in *in vitro* systems (Teixeira da Silva and Dobránszki 2014). As a nonchemical stressor, the effective use of MFs

would provide a nontoxic means to increase productivity, change growth form, or provide a sterile study platform for the mechanisms underlying MF-related stress responses in plants. The review is divided into two sections: the first is a summary of the literature that covers all studies *in vitro* that have employed MFs, and the second presents hypotheses and possible mechanisms by which MFs affect plant growth and development *in vitro*.

Effects of Magnetic Fields on Plant Micropropagation and *In Vitro* Development

Several studies have shown that MFs can affect the development of cells and tissues cultured *in vitro*. Shoot and root formation rates of *Paulownia* tissue culture increased when nodes were exposed to external MFs (2.9–4.8 microTesla [mT] for 2.2, 6.6, and 19.8 s or 0.1–0.3 T during the culture period) compared to the control (Ham *et al.* 2004; Yaycili and Alikamanoglu 2005; Çelik *et al.* 2008). *Paulownia tomentosa* and *Paulownia fortunei*, in response to an MF of 2.9–4.8 mT applied for 19.8 s, increased the shoot regeneration percentage, fresh weight, length, leaf number, and chlorophyll (chl) content after 28 d of culture from nodal sections of 3-mo-old seedlings (Yaycili and Alikamanoglu 2005). Shoot formation of *P. tomentosa* exposed to a magnetic flux density of 2.9–4.8 mT for 2.2 s increased from 61.9 to 82.5%; the contents of total chl, chl *a*, and chl *b* increased; and the total RNA content of the treated tissues doubled compared to the control (Çelik *et al.* 2008). However, in contrast to the earlier results of Yaycili and Alikamanoglu (2005), increasing the exposure time to 19.8 s decreased the regeneration percentage to 45% and was accompanied by a decrease in chl and RNA contents. Similarly, Atak *et al.* (2003) found that both regeneration and growth of soybean (*Glycine max* (L.) Merr.) shoot-tip cultures exposed to MFs (2.9–4.6 mT) for 2.2 and 6.6 s increased 87 and 74%, respectively, relative to the control (62%), while rooting percentage increased 26 and 36%, respectively, relative to the control (14%). Chl content of leaves was the highest after exposure for 2.2 s. However, exposure for 19.8 s decreased all parameters relative to the control.

Belyavskaya (2004) indicated that a weak magnetic field (WMF; 100 nT–0.5 mT) affected the development of cells and tissues of sugar beet (*Beta vulgaris* L. var. *saccharifera*) and two cultivars derived from *Haplopappus gracilis* (Nutt.) A. Gray cultivated *in vitro*. The cell index of sugar beet culture started to decrease 11 d after exposure to WMF and was most decreased (47% decrease) compared to GMF (control) on the 13th day of culture. However, by the 24th day of culture, there were no significant differences in the cell number of cultures exposed or not exposed to WMF. Callus production of both strains of *H. gracilis* decreased by 15% compared to GMF after a 5-d-long culture under WMF and by 14 or 21% after 10-d-long cultivation under WMF, depending on the strain. No differences between WMF and GMF were detected in callus cell number after 5 d, but it was lower after 10-d-long cultivation in WMF than in GMF. The regeneration of plants from the callus of peppermint (*Mentha*×*piperita* L.), spearmint (*Mentha spicata*), mojito mint (*Mentha villosa*), and *Calendula officinalis* L. was studied after exposing callus to MF with different intensities, including 0.4×10^{-4} T (GMF), 3×10^{-4} T high-intensity static MF, and 0 T for 22 to 96 h (Criveanu and Taralunga 2006). *Mentha* species had the highest developmental response (regeneration and growth dynamics) in response to 3×10^{-4} T while *C. officinalis* responded best to 0 T MF. Both stimulating and inhibiting effects at near 0 T MF (*i.e.*, "super weak" MF) conditions were detected in *in vitro* shoot-tip and nodal segment cultures of 'Desirée' potato (*Solanum tuberosum* L.) on root, stem, and leaf growth, depending on the exposure period (Rakosy-Tican *et al.* 2005). If cultures were kept for 14 d at near 0 T MF, no significant effect was detected, but exposure for 28 d stimulated the growth of roots (by about 50%) and leaf surfaces (about a 40% increase in leaf length and a 37% increase in leaf width), increased the leaf number/shoot (from 9 to 12), and doubled the photosynthetic pigment content. Even after two to three subcultures under normal GMF conditions, plants continued to form large leaf surfaces. However, when experiments were repeated, vegetative growth was inhibited, suggesting the role of the initial explants in response to MF. When examining the responses of other wild *Solanum* species (*S. chacoense* [Chaco potato], *S. microdontum*, *S. verrucosum*), vegetative *in vitro* growth was inconsistent: it could be inhibited, stimulated, or unchanged, regardless of the species. The stimulation of *in vitro* vegetative growth was connected to variation in the variable component of MFs at the beginning of growth, presumably during cell expansion. The values of GMF and geoelectrical fields affected the *in vitro* callus development of *Krainzia longiflora* (Corneanu *et al.* 2004) by affecting the mitotic division spindle. Exposure to twofold GMF reduced callus formation only slightly but a screened (*i.e.*, dampened) geoelectrical field significantly affected the development of calli. When GMF was natural, a screened geoelectrical field decreased callus diameter from 38.4 to 34.8 mm in clone α and from 15.3 to 9 mm in clone β. However, twofold GMF increased callus diameter from 42.7 to 45.3 mm in clone α but decreased it from 19.4 to 11.2 mm in clone β. Shoot number/explant was decreased by the geoelectrical field at each GMF level (*i.e.*, at natural GMF and at twofold GMF) and the percentage of necrosis doubled in clone β when twofold GMF and screened geoelectrical field were applied. Photosynthetic pigment contents (chl *a*, chl *b*, and carotenoids) were reduced when callus was exposed to twofold GMF, but the level of reduction depended on the type of callus. Under normal GMF, isoperoxidase activity was higher (by as much as 123%) in

anthocyanin-type callus but under twofold GMF, its level was significantly higher (by as much as 369%) in normal, green callus.

A low EMF (0.02, 0.05, or 0.15 V, applied for 20 h in an initial range of 1–44 h) applied directly after protoplast isolation stimulated somatic embryogenesis from mesophyll protoplasts of alfalfa (*Medicago sativa* L.; Dijak *et al.* 1986). The highest number of somatic embryos developed when an EMF of 0.02 V was applied for 20 h. An alfalfa clone ('Regen S') that was not embryogenic under control conditions could produce somatic embryos in the same developmental pattern as the embryogenic clone 'Rangelander'. In this case, EMF appeared to have the effect of overcoming the recalcitrance of 'Regen S' to embryogenesis. EMF stimulated development by improving the aggregation of protoplasts caused by the establishment of protoplast polarity. Exposure of three different embryogenic lines of cork oak (*Quercus suber* L.) somatic embryos to extremely low-frequency MF (50 Hz, 15 μT) for 8 wk affected the morphogenic response. The number of detachable somatic embryos decreased, but the decrease was significant only in one of the three genotypes ('G3.27') examined (Celestino *et al.* 1998). Neither germination nor the percentage of plant formation from somatic embryos was influenced by extremely low sinusoidal EMF. However, under suppressed GMF (0 T), germination percentage and plant formation increased significantly (from 44.6 to 58.3% and from 5.7 to 15.3%, respectively), although how GMF was suppressed was not explained.

When duckweed (*Spirodela oligorrhiza* (Kurz) Hegelm.) cell culture was exposed to sinusoidally varying MF (60 and 100 Hz/0.7 mT), there were specific metabolic stress effects (Parola *et al.* 2005). Alanine was produced only under EMF conditions, suggesting that EMF treatment altered the biochemical processes in which free radicals were involved and that alanine production was a stress-induced response. The addition of vitamin C, a free-radical scavenger, reduced the accumulation of alanine. A similar result, *i.e.*, enhanced alanine production, was found by Monseline *et al.* (2003) when duckweed plants (*S. oligorrhiza*) were exposed to sinusoidally varying MF (SVMF) (60 and 100 Hz/0.7 mT) for 24 h. Monseline *et al.* (2003) demonstrated that a variety of different stresses (anoxia, air exposure, salinity, hyperosmosis, hypertonic stress, heat shock, freezing, cold shock, heavy-metal stress, starvation, water-stress deficiencies in nitrogen and phosphorous, EMF, microgravity, red/far-red pulses) caused the production and accumulation of alanine in response to free-radical production; conversely, the production and accumulation of alanine was reduced by the suppression of free radicals. They proposed, therefore, that alanine production and accumulation is a universal stress signal in living organisms. The average fresh weight of 28-d-old embryos increased when mature zygotic embryos of wheat (*Triticum aestivum* L.) 'Flamura-85' were exposed to (more specifically, run under) MFs with different flux intensities between 2.9 and 4.8 mT, at 1 m/s for 2.2 and 19.8 s (Alikamanoglu and Sen 2011). The percentage increase depended on the exposure period: it was 25% after 2.2 s exposure and 30% after 19.8 s exposure. Similarly, some biochemical parameters were also affected by MF treatment: the amount of total protein increased by 12 and 14% and chl content increased by 32 and 35% after exposure to MF treatment for 2.2 and 19.8 s, respectively. The activities of stress-related enzymes such as superoxide dismutase (SOD), catalase (CAT), peroxidase (POX), and ascorbate peroxidase (APX) also increased. The activity of total SOD increased by 62 and 88% after exposure to MF treatment for 2.2 s and 19.8 s, respectively. After 19.8 s, POX activity increased by about 80%, CAT activity by 73%, and APX activity by 62%. The endogenous production of reactive oxygen species (ROS) is a general response to environmental stresses. Induction of the antioxidant enzyme system in plants, as a defense against ROS, is the part of the plant's defenses and acclimatization process under unfavorable or suboptimal conditions (i.e., stress conditions) to maintain plant growth (Boguszewska and Zagdańska 2012).

Decreases were observed in callus growth rate (from 0.181 to 0.175), relative growth rate (from 1.441 to 0.655), and relative callus growth rate (from 0.052 to 0.022) when mature bread wheat (*Triticum aestivum* L.) embryos were exposed to MF (8.8 and 17.6 T); this negative effect became more pronounced as the MF increased (Kahrizi *et al.* 2013).

EMF in the range of 48–115 kA/m was applied to *in vitro* nodal segment culture of beach plum (*Prunus maritima* Marshall; Yan *et al.* 2009). Increasing field strength stimulated regeneration and growth from explants with 2.3-folds more sprouts (shoot buds) being induced when an EMF of 97 kA/m was applied for 10 min. The multiplication rate was 5.2- or 3.1-folds higher than the control, depending on the auxin content of the culture medium. The multiplication rate was higher if medium contained 0.2 mg/l indole-3-butyric acid (IBA) instead of 0.2 mg/l α-naphthaleneacetic acid (NAA) in addition to 2.0 mg/l zeatin, and longer *in vitro* roots were produced from plants exposed to EMF of 97 kA/m, increasing the survival during acclimatization, although survival was not quantified.

However, studies on the effects of MFs on micropropagated plants remain scanty, especially with permanent magnets that have moderate intensities (1 mT to 1 T). This range of MFs can hypothetically be used widely in practical tissue culture chambers, as shown by results for ornamental orchids (hybrid *Phalaenopsis* and *Cymbidium*) and *Spathiphyllum* (Tanaka *et al.* 2010; Van *et al.* 2011a, 2011b, 2012; Tables 1 and 2). However, the effects of MFs on plant growth and development are species-specific, and the exact properties of MFs (*i.e.*, intensity, polarity, duration of exposure, and type of magnet) are variables that still need to be individually tested for different genotypes. In these studies, increasing the MF intensity from

Table 1. Effects of intensity and polarity of magnetic fields on *Phalaenopsis*, *Cymbidium*, and *Spathiphyllum* cultured *in vitro* (Tanaka *et al.* 2010; Van *et al.* 2011a, b, 2012)

Magnetic field intensity	*Phalaenopsis* PLB proliferation		*Phalaenopsis* plantlet growth	*Cymbidium* plantlet growth	*Spathiphyllum* plantlet growth
	Solid medium	Liquid medium			
0.1 T—S	No effect	Greatest increase in FW and DW of PLB clusters; decreased number of *neo*-PLBs	Significantly enhanced plant growth after 3 mo of exposure (best treatment)	Inhibited shoot elongation; highest Chl contents[z]	Increased Chl contents and number of leaves; no other significant effect
0.1 T—N	No effect	Increased FW and DW of PLB clusters; decreased number of *neo*-PLBs	Increased maximum leaf length, FW of shoots; FW of roots and leaf width	Inhibited shoot elongation; no other significant effect	Increased Chl contents and number of leaves; no other significant effect
0.15 T—S	Decreased FW of PLB clusters	Decreased number of *neo*-PLBs	Highest number of roots	Inhibited shoot elongation; decreased Chl contents; no other significant effect	Increased Chl contents and number of leaves and FW of shoots
0.15 T—N	Decreased FW of PLB clusters	Decreased number of *neo*-PLBs	Inhibited the emergence of new leaf	Inhibited shoot elongation; decreased Chl contents; no other significant effect	Increased Chl contents and number of leaves; no other significant effect
0.2 T—S	Decreased total number of *neo*-PLBs	Increased DW of PLB clusters; decreased number of *neo*-PLBs	Highest Chl contents	Inhibited shoot elongation; increased Chl contents; no other significant effect	Increased Chl contents and number of leaves; decreased DW of shoots
0.2 T—N	Increased FW and DW of PLB clusters	Increased DW of PLB clusters; decreased FW of PLB clusters and number of *neo*-PLBs	Inhibited the emergence of new leaf	Inhibited shoot elongation; no other significant effect	Increased Chl contents and number of leaves; decreased DW of shoots

Chl chlorophyll, *DW* dry weight, *FW* fresh weight, *N* north, *PLB* protocorm-like body, *S* south, *T* Tesla

[z] Estimated chlorophyll contents in the third leaf, counted from the top downward, of the plantlet by a SPAD chlorophyll meter

Table 2. Effects of duration of exposure to magnetic fields (MFs) of 0.15 T, N or S pole on *Phalaenopsis*, *Cymbidium*, and *Spathiphyllum* cultured *in vitro* (Tanaka *et al.* 2010; Van *et al.* 2011a, b, 2012)

Explant	Effects of magnetic field of 0.15 T—N or S pole on plants treated for different exposure times
Phalaenopsis PLBs (liquid medium)	When the duration of exposure to MFs increased from 2 to 7 wk, the number of newly formed PLBs decreased while the FW and DW of PLB clusters increased. Longer duration of exposure to MF of 0.15 T—S pole (7 wk) resulted in the highest FW and DW of PLB clusters.
Spathiphyllum plantlets	Short- or long-term (2, 4, 8 wk) exposure to MF of 0.15 T—N or S pole had no significant effect on *Spathiphyllum* plantlet growth except for increased Chl contents[z] when compared to the control.
Phalaenopsis plantlets	*Phalaenopsis* shoots continuously exposed to MFs of 0.15 T—N or S for 3 mo had the greatest total FW of shoots and roots and total DW of shoots and roots compared to shorter exposure times (*i.e.*, 1 or 2 mo).
Cymbidium plantlets	Increasing exposure time (1 *vs.* 2 or 3 mo) to MF of 0.15 T—N or S pole resulted in decreased Chl contents of the treated *Cymbidium* plantlets and had no other significant effect on plant growth parameter such as plant height, number of leaves, total FW, and FW of shoots and roots.

Chl chlorophyll, *DW* dry weight, *FW* fresh weight, *N* north, *PLB* protocorm-like body, *S* south, *T* Tesla

[z] Estimated chlorophyll contents in the third leaf, counted from the top downward, of the plantlet by a SPAD chlorophyll meter

GMF (5×10^{-6} T) to 0.1–0.2 T, north or south pole resulted in positive (highest chl content) and negative (inhibition of leaf emergence) effects on *Phalaenopsis in vitro* plantlet growth (Van *et al.* 2011b; Table 1), but had no clear influence on *Cymbidium* plantlet development other than negatively affecting plant height. Testing the same level of MF (0.1–0.2 T, north or south pole), external application of MF increased chl content of the third leaf (reported as SPAD value) and the total number of leaves but slightly decreased total dry weight of *in vitro Spathiphyllum* plantlet shoots (Table 1; Van *et al.* 2012).

The proliferation of *Phalaenopsis* protocorm-like bodies (PLBs) was affected not only by the application of external MFs (0.1, 0.15, 0.2 T; north or south pole) but also by the substrates used (Tanaka *et al.* 2010; Van *et al.* 2011a). An MF of 0.1 T north or south pole significantly increased the fresh and dry weights of PLB clusters after culture in liquid medium for 2 mo. When a similar experimental design was used to test the same cultivar on solid medium, the fresh and dry weights of PLB clusters increased when 0.2 T north pole MF was applied, although an MF of 0.1 T north or south pole had no effect (Table 1; Tanaka *et al.* 2010; Van *et al.* 2011a). The difference may be because liquid medium by itself is more sensitive and active under exposure to MF than solid medium (Pang and Deng 2008; Teixeira da Silva and Dobránszki 2014).

The *In Vitro* Milieu for Understanding the Possible Mechanistic Response to Magnetic Fields

How do intensity, polarity, and duration of exposure of MFs affect plantlet development *in vitro*? The effects of MFs on plants have been studied widely over at least 20 years. However, the results have usually been inconsistent and at times even contradictory, and there is still no clear mechanistic explanation for how MFs affect plant growth and development. Some reports have shown negative effects while others have shown positive effects or no effects. MF intensity and duration of exposure affect plant growth to different degrees (Alikamanoğlu *et al.* 2007; Atak *et al.* 2007). Most research on this topic has concentrated on the effects of a WMF (100 nT–0.5 mT) or "super weak" MF (magnetic vacuum)—as in space-like conditions—where MF intensity is nearly 0 nT and has usually focused on the effects of seed germination and seedling development under the action of MFs.

Changes in gene expression levels and stress-related responses. There are also some studies on the effects of very high MF (>10 T) on gene expression in *Arabidopsis thaliana* (L.) Heynh. (Paul *et al.* 2006). An MF of 15 T induced the expression of an *Adh*/GUS transgene in both the roots and the leaves of an *A. thaliana* transgenic line (Paul *et al.* 2006). Comparative analysis of gene expression patterns in a subsequent experiment (Paul *et al.* 2006) showed that MF induced changes in gene expression when 3-wk-old plants were exposed to MF (MFs of 14 T for 2.5 h or of 21 T for 2.5 and 6.5 h). Out of 8000 genes examined, 114 were differentially expressed (2.5-folds more than the control after exposure to 21 T MF), and many of them were related to stress responses (stress-related genes or transcription factors) and ion transport functions. However, in other cases when there were stress-related changes, such as the accumulation of alanine, there was also a simultaneous increase in the activity of stress-related enzymes (ATX, CAT, POX) (Monseline *et al.* 2003; Parola *et al.* 2005; Alikamanoglu and Sen 2011) after exposing tissues to MFs with an increasing order of magnitude of mT (0.7 mT to axenic culture of duckweed and 2.9 or 4.8 mT to mature wheat embryos), although no further experimental proof exists about whether and how these enzymatic changes are connected with changes in gene expression.

Changes in endogenous levels of plant growth regulators. MFs (2.9–4.6 mT for 2.2, 6.6, or 19.8 s) increased cytokinin and auxin synthesis in *Paulownia* tissue culture, increasing shoot regeneration and root induction (Atak *et al.* 2003; Çelik *et al.* 2008). An analogous process may have caused the observed effect of MFs on the development of *Phalaenopsis* plantlets (Van *et al.* 2011b), but future studies would need to assess endogenous levels of plant growth regulators over the entire developmental period.

Changes in growth, development, and biomass production. A positive influence of MFs was observed during micropropagation shoot-tip culture of soybean (Atak *et al.* 2007) and nodal segment culture of *Paulownia* (*P. tomentosa* and *P. fortunei*; Yaycili and Alikamanoglu 2005; Alikamanoğlu *et al.* 2007) and beach plum (Yan *et al.* 2009). *P. tomentosa* plants exposed to 2.9–4.8 mT for 19.8 s had higher plant fresh weight, leaf number, and chlorophyll (chl) content after 4 wk of culture than control plants not exposed to MFs (Yaycili and Alikamanoglu 2005; Alikamanoğlu *et al.* 2007). The fresh weight of plantlets regenerated from soybean shoot tips exposed to MF of 2.9–4.6 mT for 2.2 and 19.8 s was higher than that of controls (Atak *et al.* 2007). Similarly, beach plum sprouts resulting from nodal segment culture showed higher fresh and dry weights and height when treated with an MF of 97 kA/m for 10 min (Yan *et al.* 2009). In the Van *et al.* (2011b) study, within the range of MFs tested (*i.e.*, 0.1–0.2 T), the greatest influence on *Phalaenopsis* was when plantlets were exposed to MFs of 0.1 T south. In a previous, closely related study (Van *et al.* 2011a) in which *Phalaenopsis* PLBs were exposed to different MFs, 0.1 T south also had the greatest influence on the fresh and dry weights of regenerated PLBs (Table 1). However, a plausible explanation or mechanism for these effects was not (and could not be) provided, and it continues to be a difficult experimental parameter to standardize, measure, or test.

Belyavskaya (2004) mentioned that prolonged exposure of pale flax (*Linum bienne* L.) plants and lentil (*Lens culinaris* L.) roots to WMF may have different biological effects at the cell, tissue, and organ levels than shorter exposures. In the Van *et al.* (2011a) study, exposure to different durations of an MF of 0.15 T had some effects on the growth of *Phalaenopsis* plants. In particular, plantlets exposed to an MF for 3 *vs.* 1 or 2 mo had significantly higher total fresh and dry weights of shoots and roots and had more leaves, but the same treatment inhibited shoot elongation of *Cymbidium* plantlets (Van *et al.* 2012; Tables 1 and 2). As for *Spathiphyllum*, exposure to 0.15 T had no significant influence on plantlet development other than increasing the chl content (SPAD value), with longer exposure giving higher chl levels (*i.e.*, 8 wk>4 wk>2 wk) (Van *et al.* 2012; Tables 1 and 2). Another closely related study showed that *Phalaenopsis* PLBs exposed to 0.15 T at either the north or south pole for a longer period of time, *i.e.*, 7 *vs.* 2 wk, resulted in greater biomass of newly formed PLBs (Van *et al.* 2011a). MFs can thus be applied to increase biomass in *Phalaenopsis* PLBs and plantlets. Were MFs to be applied to other crops, greater biomass accumulation, productivity, or yield might be possible, making tissue culture laboratories more productive per unit area. At a wider scale, in a system where space restrictions apply (such as in a space station), the induction of greater yield of harvestable plant material would have obvious advantages. In contrast to *Phalaenopsis*, *Paulownia* tissue cultures were inhibited when exposed to an MF of 2.9 to 4.8 mT for longer than 6.6 s (Yaycili and Alikamanoglu 2005). Atak *et al.* (2003) reported similar inhibition of soybean tissue culture when exposed to 2.9–4.6 mT for 2.2, 6.6, or 19.8 s.

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 decreased in the third leaf of *Cymbidium* shoots exposed to 0.15 T MF for 3 mo, regardless of polarity (Van *et al.* 2012; Tables 1 and 2). The same effect on plant growth was confirmed by several studies on different plants. Studies by Atak *et al.* (2003, 2007) and Çelik *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.

The Van *et al.* (2012) study found 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* (except for reducing the dry weight of *Spathiphyllum* shoots) and *Cymbidium* plant growth compared to the control, except for plant height (*i.e.*, stem elongation) of *Cymbidium* shoots (Tables 1 and 2). However, Ham *et al.* (2004) found that an increase in MF from 0 to 0.15 T increased multiplication rate and rooting of *in vitro Paulownia* plantlets. The Van *et al.* (2011a) study on the effects of duration of exposure of 0.15 T (north or south) on *Phalaenopsis* PLB proliferation showed that longer exposure (7 *vs.* 2 wk) resulted in greater biomass of newly formed PLBs and smaller average number of newly formed PLBs. An MF of 0.1 T south (*vs.* 0.15 T or 0.2 T, north or south) resulted in the greatest fresh and dry weights of regenerated PLBs (Table 1). Regarding *Phalaenopsis* plantlet growth *in vitro*, exposure to MFs of 0.15 T for 3 mo (*vs.* 1 or 2 mo) of either polarity resulted in the highest total fresh and dry weights of shoots and roots (Van *et al.* 2011b).

Conclusion

The purpose of this review is to synthesize how MFs or EMFs can influence plant growth and development *in vitro*. Based on advances achieved to date, both MFs and EMFs have been

shown to influence the *in vitro* development and growth of plants. Regardless of the actual mechanism at work, the following positive aspects of MFs can be noted: (a) MFs, when applied to small explants as a constant force, have the ability to stimulate growth, and (b) the *in vitro* environment can serve as an ideal test condition since it requires minimal space and limited material and can be reproduced with confidence (see Tanaka *et al.* 2010, for a model system). Even though there are positive aspects, some points of caution are advised: (a) cytogenetic and molecular analyses are urgently required to assess whether the changes caused to plant growth and development also irreversibly alter the cellular and genetic mechanisms of plants, and (b) researchers are advised about extrapolating *in vitro* results and scaling-up to greenhouse or field experiments since *in vitro* explants are often in close proximity to the applied MF. The response of plants after *in vitro* growth may differ and if the force of the MF is removed at the time of acclimatization then a plant may respond by reverting to a wild-type state, or the *in vitro* effect of the MFs may be lost or become diluted. Therefore, extension of systematic studies conducted under an *in vitro* milieu might facilitate and promote better understanding of a plant's behavior in response to MFs, particularly from a theoretical viewpoint. It would help to identify and understand how MF-related techniques can improve growth and yield and how the direction of plant development can be regulated by using such a biophysical technology.

Acknowledgments The authors thank Dr. Pham Thanh Van for initial feedback on the first version of the manuscript and for assisting with the development of Tables 1 and 2.

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