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Original Research

Effects of EMFs on Some Biological Parameters in Coffee Plants (*Coffea arabica* L.) Obtained by *in vitro* Propagation

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Abstract

In vitro coffee seedlings were exposed to an electromagnetic field (EMF) of 2 mT during 3 minutes in establishment, multiplication, and acclimazation phases. Shoot and root lengths and leaf pair numbers of treated groups increased when compared to control; in addition to SOD, CAT, and APX activities of *in vitro*-treated groups showed a decrease in levels. Four months after the magnetic treatment was applied, the same parameters were evaluated. Shoot lengths, root lengths, the pair of leaves numbers, and CAT activity increased in treated plants. APX activity decreased in treatment seedlings, whereas SOD activity did not show a difference between experimental groups.

Keywords: lead, cadmium, common buckwheat, seedling growth, flavonoids

Introduction

Electromagnetic fields (EMFs) are classified as non-ionizing radiation. However, it can cause damage depending on the power level, frequency, and the properties of exposed tissue. There is some evidence that it produces changes in the cell membrane's permeability and cell growth rate as well as interference with ions and organic molecules, such as proteins [1-4].

A potential link between magnetic fields and its effects on living organisms is the fact that magnetic fields cause oxidative stress, that is an increase in the activity, concentration, and lifetime of free radicals [5, 6]. Oxidative stress is a result of oxidative metabolites, free radicals, and reactive oxygen species (ROS), which are highly reactive by-products of normal metabolism and immune defense [7].

Some studies concerning the different magnetic field exposure times that show different biological responses and modulate enzyme activities have been recently carried out. Studies on oxidative stress have been conducted on plant cells, and the available literature has particularly directed their attention to the effects of magnetic or electromagnetic fields on the germination of seeds and plant growth and development [8-14].

Although the most common way for coffee propagation is by seedlings grown from seeds, germination loss during storage is considered one of the fundamental problems for propagation, which in turn makes more difficult the preservation of elite genetic resources [15].

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Therefore, the aim of this study was to determine the possible relationship between exposure to EMFs on growth; and superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX) activities; in coffee seedlings at different stages of *in vitro* growth and subsequent development of these seedlings.

Methods

Plant Growth Conditions

Micropropagation and Biochemical Identification of Coffee Varieties Methodolgy was done [16]. The embryos of *Coffea arabica* var. Catuai were grown during 6 weeks in the MS medium [17] supplemented with 30 g/l of sucrose, 6 g/l of agar, 25 mg/l of cysteine, casein 0.5 mg/l and the pH was adjusted to 5.6. Test tubes of 25×150 mm were used, each containing 10 ml of culture medium, for establishment phase. The plants were maintained under temperatures of 24ºC±1, relative humidity of 70%±5 and a photoperiod of 24 hours and 80 µmol/m^2·s, with fluorescent lamps. Coffee seedlings with 1 opposite pair of leaves were subcultivated in the same cultures medium and were maintained under temperatures of 24ºC±1, relative humidity of 70%±5, photoperiod of 16 hours and 80 µmol/m^2, reaching the multiplication phase after six weeks. At the end of this period, plantlets usually had 3 pairs of leaves and primary roots. For acclimatization phase, *in vitro* coffee plants were transplanted individually to 30 ml open pots (one per pot) and the substrate used was 1 soil: 1 organic matter (P_2O_5: 49.00 mg/l; K_2O: 55.20 mg/l, MgO: 12.09 mg/l, electric conductivity: 3 mS/cm^{-}, pH: 6.4) (v/v). Temperature of 26ºC±1, relative humidity of 85%±5, photoperiod of 19 hours, and 80 µmol/m^2·s were maintained during 4 weeks. Seedlings continued growing during four months at the end of the acclimation stage, in the same conditions without magnetic field exposure.

Magnetic Fields Exposure

To apply the electromagnetic treatment, a 40 cm long magnetizer solenoid that consisted of a 16-gauge copper wire (1.1 mm), with a total of 363 turns, was used. The intensity of the magnetic field was horizontally variable, changing from the ends toward the center.

The values of the magnetic induction (B) applied to seedlings were 2 mT, during a fixed exposure time of 3 minutes. The samples were placed randomly in a dielectric container and placed within the magnetic field at the end and center of the solenoid, in a homogeneous space.

One week after the establishment and multiplication phase began the electromagnetic treatment was performed. In the case of acclimatization the electromagnetic treatment was performed at a time of transplantation. Fifty embryos and fifty plants were used for treatment with three replicates for each treatment.

Growth Parameters

At the end of each phases stem length, root length and leaf pairs number were evaluated. In the acclimatization phase after 2 weeks of growth survival percentage was determined.

Enzymes Extraction and Assay

Leaf samples of the control and EMF treated plants that were regenerated under culture conditions were taken and homogenized separately. Fresh coffee leaves were ground to a fine powder in liquid nitrogen. Aliquots of 200 mg of frozen leaf powder were transferred into 10 ml of extraction buffer containing 100 mM KH_2PO_4; pH 7.8; 5 mM ascorbate and 50% insoluble polyvinyl-polypyrrolidone. The homogenate was centrifuged at 13.000 g for 10 minutes and the supernatant was kept stored in separate aliquots at 80ºC, prior to catalase (CAT. EC. 1.11.1.6), L-ascorbate peroxidase (APX. EC 1.11.1.11), and superoxide dismutase (SOD. EC 1.15.1.1) analyses [18].

Superoxide dismutase activity was determined by the photochemical method of Dhindsa [19]. The reaction was started by adding 60 µM riboflavin to 50 µl of the extraction supernatant, 14 mM L-methionine, 75 µM nitrobluetetrazolium (NBT), 0.1 µM ethylenediaminetetraacetic acid (EDTA), 50 mM phosphate buffer (pH 7.8), and H_2O containing 2.0 ml reaction mix. Experimental tubes were incubated under 15 W lights for 7 minutes and then placed in the dark to stop the reaction.

Total catalase activity was determined in a spectrophotometer as described by Azevedo [20]. The reaction started 1,900 µl reaction mix: 100 mM phosphate buffer (pH 7.0) and 12.5 mM H_2O_2 containing substrate buffer added to 100 µl extraction supernatant and spectrophotometric measurements were taken at 240 nm wavelength. The activity was determined by monitoring the degradation of H_2O_2 at 240 nm over 1 minute against a plant extract-free blank.

Ascorbate peroxidase activity assay was performed according to the methods of Nakano and Asada [21]. 1.0 ml of reaction mix: 100 mM phosphate buffer (pH 7.0), 0.1mM H_2O_2 and 0.1 mM ascorbate containing the substrate buffer, and 100 µl of extraction supernatant was added. Spectrophotometric measurements were taken at 290 nm wavelength at 30ºC.

Total Protein Amount

Soluble protein amount was determined using the Bradford method [22].

Statistics

The experimental design was in a completely randomized way and the results were analyzed with Statgraphic 5.0 for Windows, comparing the values obtained for each of the treatments. A simple classification ANOVA was conducted (One-Way ANOVA), considering results obtained with

Table 1. Means of shoot lenghts, root lengths, and number of leaf pairs of in vitro propagated coffee plants (*Coffea arabica* cv. Catuai) treated with EMF (2 mT×3 minutes).

	Stem length (mm)	Root length (mm)	Leaf Pairs (Numbers)
Establishment phase Control	0.47±0.03b	0.24±0.02 b	0.33±0.02b
Establishment phase (2mTx3´)	1.57±0.05a	1.27±0.12 a	1.73±0.06a
Multiplication phase Control	0.72±0.04b	0.30±0.03b	1.24± 0.03b
Multiplication phase (2mTx3´)	1.99±0.07a	1.44±0.02[a]	3.98± 0.04a
Acclimatization phase Control	37.47±4.7b	64.92±4.5a	4.85±0.15b
Aclimatization phase (2mTx3´)	54.76±4.5a	82.32±4.7b	6 .72±0.16a

Different letters on the column represent the mean±SD of three replicates. P<0.05

confidence levels of 95% as significant, corresponding to significant differences (P≤0.05) and the differences between treatments that were significant were calculated using the t test.

Results and Discussion

Growth parameters such as shoot lengths, root lengths, and leaf pair numbers of *Coffea arabica* plants obtained by *in vitro* propagation exposed to EMF and compared to control group, are shown in Table 1.

As can be seen from Table 1, EMF application induced regeneration of cultures. Statistical analysis showed that the effects of EMF exposure on shoot lengths, root lengths, and leaf pair numbers were significant at P≤0.05. Average shoot lengths and average root lengths increased in plants treated with EMF when compared to the control.

Perhaps the application of the electromagnetic field caused an increase in absorption of culture medium nutrients. It is directly involved in the metabolism of cells and the production of energy needed for cell division to increase the size and development of plantlets.

The promoting effect of magnetic fields on the elongation of the vegetative tissue has been observed in many species. Many authors have raised the electromagnetic field-induced cell elongation [23-25].

It has been presented by different studies that EMF is a factor that effects cell metabolism of meristem cells, it has a significant effect on mitosis and changes G1 phase of the plant cell cycle. Changes on metabolic reactions, cell signaling systems, cell cycle, transcription, and protein synthesis cause different biological responses on plant systems [26-28].

Based on recent research on different plants, EMF affects plant growth positively. Moreover, regeneration rate, plant fresh and dry weight, leaf number, length, shoot number, and rooting rate increased when compared to the control [29-32].

Also, the leaf pair number is critical for *in vitro* plantlets obtained during the acclimatization phase. Processes such as photosynthesis and transpiration take place in leaves. By photosynthesis, plants get the substances necessary to obtain metabolic energy to repair damaged structures and form other new elements useful for growth and development.

Therefore, the application of the 2 mT electromagnetic field during 3 minutes exerts a positive effect on the development of the leaf pair numbers, as evidenced by the results.

A significant increase in leaf area and leaf dry weight per tomato plant in the vegetative stage was also observed from seeds exposed to a magnetic field induced by an electromagnet (100 mT for 10 min and 170 mT for 3 min) [24].

Furthermore, the use of an EMF before the acclimatization phase probably establishes a long-term effect on the plants for further development. These results are demonstrated by evaluating the morphological parameters on 4-month-old coffee plants growing after EMF treatment compared to control (Fig. 1).

Statistical analysis revealed the mean values of root length of plants and leaf pair number of plants exposed to 2 mT during 3 minutes provided significant differences (P≤0.05) compared to control. Treated plants showed an average root length of 14.6 cm and average of 7.5 pairs of leaves. In the issue of case of shoot lengths, treated plants did not show differences with respect to control; however, it reached an average shoot length of 6.8 cm.

Results could be related to an electromagnetic field of low frequency (60 Hz) that can modify cationic flow through biological membranes, and change cellular metabolism and, therefore, this change remains in the plant physiological process.

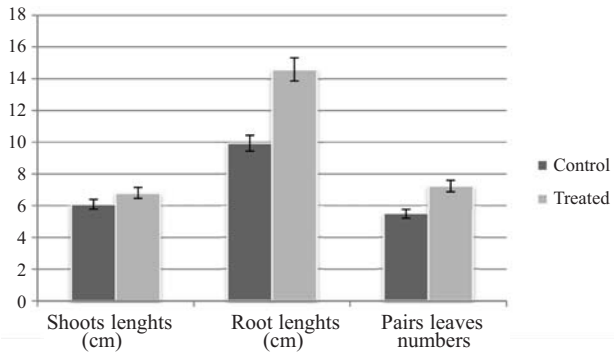

Fig. 1. Morphological parameters of 4-month-old coffee plants growing after EMF treatment (2 mT of induction for three minutes of exposure to magnetic field) compared to control. Vertical bars represent the mean±SD of three replicates. P≤0.05.

Several authors have noted that EMF can modify cationic flow through biological membranes based on the premise that the ionic flow is regulated by trasnmembrane voltage-dependent changes in the conformation of protein channels and processes that alter the ion flow, causing changes in cell metabolism [14, 32-34].

In addition, we observed that roots of plants exposed to a magnetic field were more highly developed, as shown in Fig. 2. This indicates that the magnetic field has a positive effect on root growth.

During the plant acclimatization phase, significant morphological and anatomical changes will take place due to the plants coming from storage in special conditions and as such, they must adapt to new environmental conditions. Therefore, an important parameter to take into account during the acclimatization phase is seedling root development. It should be noted that rhizogenic development plays an important role in coffee plants to ensure acclimatization of seedlings and future development. The magnetic fields promote root growth; these increased the absorption of nutrients and improve the absorption rate of minerals and water.

It can be found by experiments carried out by Barnes that barley root growth was increased under the action of electromagnetic fields, due to the increase of micro flows of H^+ ions, producing sensory changes in the absorption of other ions such as Na^+, K^+, Ca^{++}, and Cl^-, and this influenced the root growth, which could be related to the results [35].

The effects of EMF on specific enzymatic activities in different growing phases of *in vitro* propagated coffee plants exposed to 2 mT of electromagnetic field during three minutes are shown in Fig. 3.

SOD activities decreased significantly in acclimazation phase of coffee plants with 2mT electromagnetic exposure during 3 minutes compared to control ($P \leq 0.05$). In other phases, the electromagnetic fields had no effect on SOD-specific activities (Fig. 3a).

Based on the results shown in Fig. 3b, CAT activities of *in vitro* propagated coffee plants exposed to EMF decreased significantly in acclimazation, multiplication, and rooting phases when evaluated compared to control ($P \leq 0.05$). CAT-specific activity showed its highest value in plants for 4-month-old seedlings growing after EMF treatment.

In the same way, APX activity was higher in the acclimazation phase and 4-month-old seedlings. At multiplication and acclimazation phase as well as 4-month-old seedlings after EMF treatment the APX-specific activity decreased with significant differences between the coffee plants exposed to EMF compared to control plants. ($P \leq 0.05$). In the establishment phase, differences in the specific activity of APX were not observed (Fig. 3c).

Many experimental data demonstrate various effects of magnetic fields on the plants. The effects of magnetic fields on germination of seeds and growth of plants have

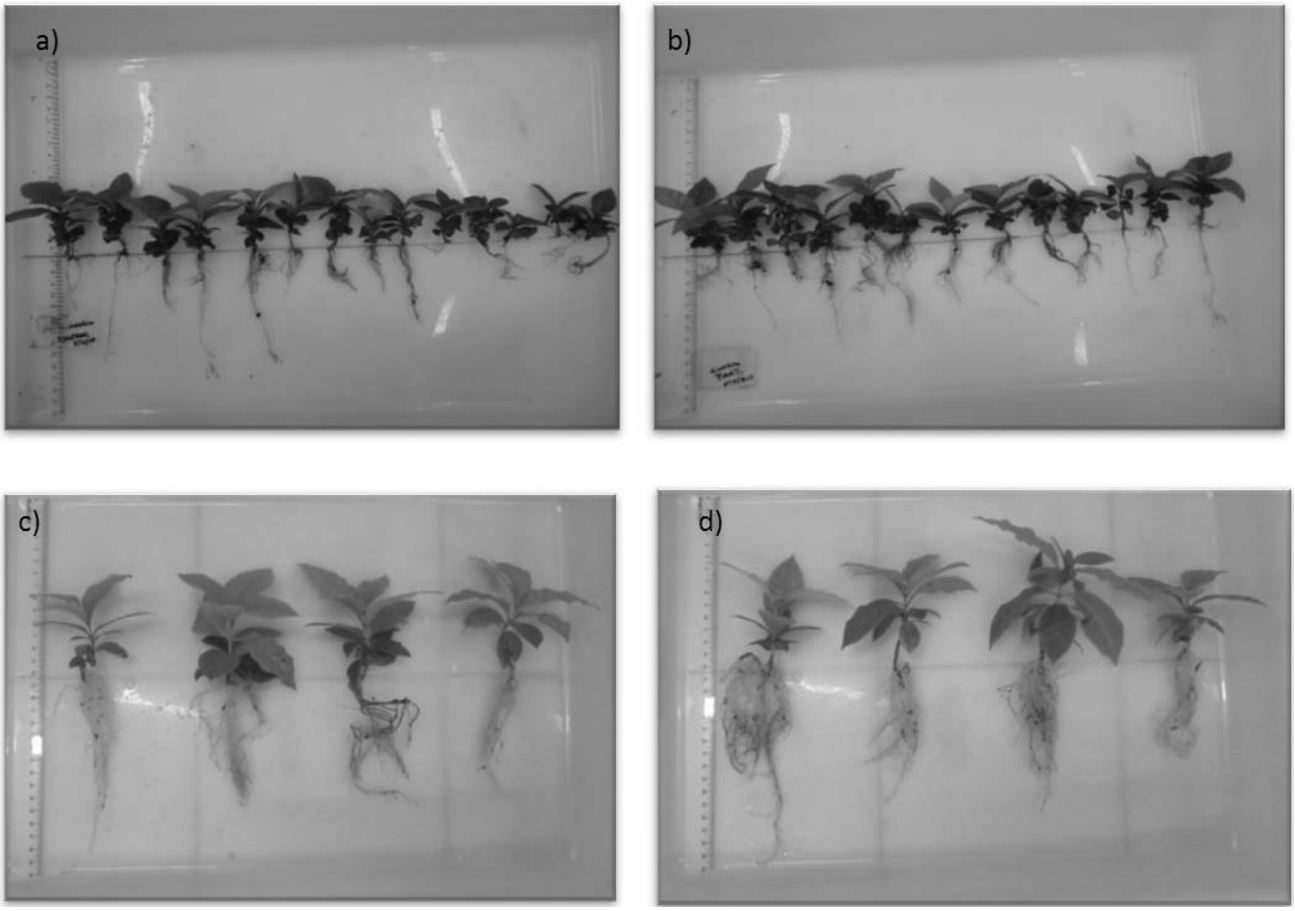

Fig. 2. Comparison of seedlings of *Coffea arabica* var Catuai exposed to 2mT×3 minutes magnetic field with control; a) acclimatization stage control, b) acclimatization stage treated, c) 4-month-old plants control, d) 4-month-old plants after EMF treatment.

been the subject of much research. Nevertheless, the exact mechanism of interactions with living cells is still unclear [9, 36, 37].

The results obtained in this research may be related to the fact that normally, plantlets are stressed during the *in vitro-ex vitro* transition due to changes on environmental conditions such as light and relative humidity [38]. As a result, plantlets suffer from abiotic stress that frequently is manifested by dehydration and photo-oxidation, which causes changes in the electron transfer chain and thus in redox systems. Light reactions are the most important source of ROS in illuminated mesophyllic cells [39]. Low generation of ROS (presumably O_2^-) in plantlets treated with EMF ensures their good growth.

In the plant cell there are enzymatic systems that protect them against H_2O_2 and other harmful ROSs. These enzymatic systems include SOD, CAT, APX, and glutation reductase (GR).

SOD converts superoxide radicals to hydrogen peroxide and CAT converts H_2O_2 to water and oxygen. SOD catalyzes the dismutation of superoxide to H_2O_2 and O_2, and plays an important role for protection against superoxide-derived oxidative stress in plant cells. Detoxification of cellular H_2O_2 by the activity of the Asada-Halliwell scavenging cycle is an important step in the defense mechanisms against active oxygen species. APX catalyzes the reaction of ascorbic acid with H_2O_2, and catalase also can reduce H_2O_2 to water [40].

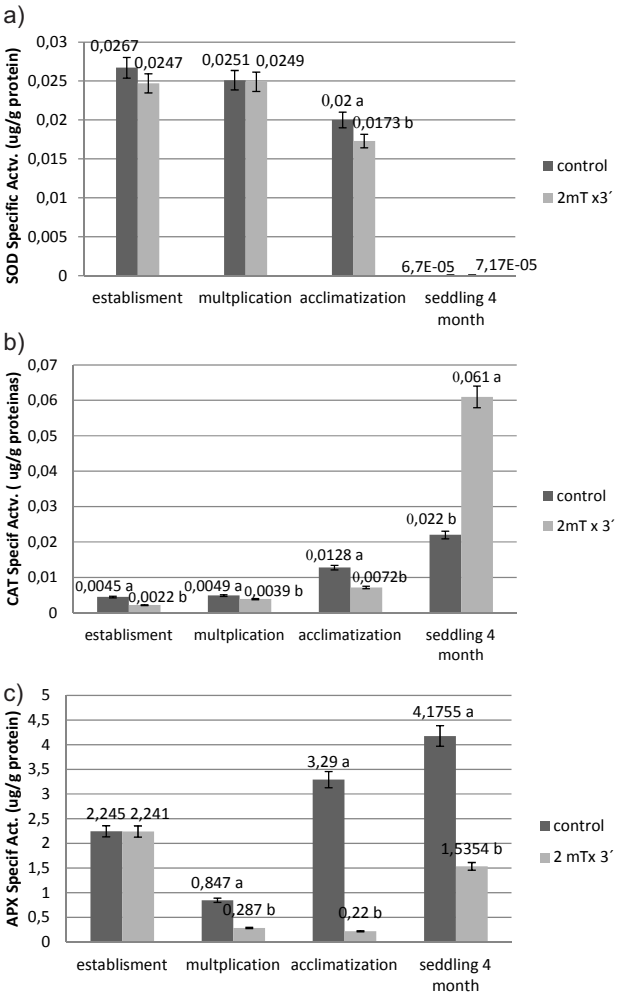

Fig. 3. Specific Activities of superoxide dismutase (a), catalase (b), and ascorbate peroxidase (c), in different growing phases of *in vitro*-propagated coffee plants exposed to 2 mT of electromagnetic field during three minutes. Vertical bars represent the mean±SD of three replicates. P≤0.05.

Several hypotheses try to explain cellular responses of magnetic and electromagnetic field on biological systems. One is the energy level alterations and changes on electron spin conditions of ionic formed atom and molecules which present paramagnetic properties [41, 42].

Free radicals are highly reactive transient chemical species characterized by the presence of an unpaired electro. Superoxide (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radicals (OH^-), and singlet oxygen ($^1O_2$) are the major reactive oxygen species produced by the metabolic reactions in plant cells, which have multiple defense systems against such oxidative stresses [42-46].

In leaf tissues, $^1O_2$ photoproduction from $^3O_2$ in chloroplasts has been demonstrated using the fluorescence and spin probe DanePy [40]. The superoxide radical contains an unpaired electron with paramagnetic properties. It is highly reactive and has a short lifetime, probably affected by a prolonged and continued exposure to electromagnetic fields [47-49].

A magnetic field in the range of 1-20 mT can split the Zeeman energy levels of a radical pair and provide an alternate reaction pathway that can change the observed rate or alter product distribution [41]. On the other hand, De Certaines [42] suggest that enzymatic reactions involving free radicals are sensitive to magnetic fields. Fast reaction in the order of 20 nseg or less can occur. Therefore, a magnetic field may be expected to have a minimal effect on the concentration of the final product.

Evolution of radical pair spin dependent magnetic field is shown by others authors. In particular, it has been found that decreasing triplet products yields magnetic densities in the range of 10-50 mT and to values above 200 mT the yield of products increased [50-52].

The results of inhibition of specific enzyme activities by low-level electromagnetic fields provides indirect evidence supporting the hypothesis presented above.

Researchers assume that magnetic fields between 10^{-3} to 10^{-2} Tesla can affect chemical reactions by changing electron spin locations and in this manner they have the potential to cause biological effects [24, 53, 54].

4-month-old seedlings growing after EMF treatment showed a higher metabolic efficiency compared to control. Increasing morphological parameters and CAT activity in 4-month-old plants after EMF treatment could be related to increased photosynthetic activity. CAT enzymes specifically act in the routes of C2 amino acid production as well as in the increment of metabolites to ensure plant growth.

The C2 oxidative photosynthetic carbon cycle acts as a scavenger operation to recover fixed carbon lost during photorespiration by the oxygenase reaction of rubisco. The vast amounts of hydrogen peroxide released in the peroxisome are destroyed by the catalase action, whereas the gly-

oxylate undergoes transamination. The amino donor for this transamination is probably glutamate, and the product is the amino acid glycine [55].

These results agree with several researchers who have reported that EMF reduces negative effects of stress plant factors [56-59].

Conclusions

Electromagnetic fields 2 mT induction and three minutes of exposure on *Coffea arabica* seedlings improved growth parameters in all development stages. In addition, the seedlings showed a decrease in SOD, CAT, and APX activities during *in vitro* culture. These results suggest that electromagnetic fields could increase the general metabolism on coffee plants and act on antioxidant enzymes of coffee seedlings obtained by *in vitro* propagation, improving plant growth and productivity, allowing EMF applications in the future.

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