Journal of Plant Process and Function, Vol. 6, No. 22, Year 2018

Comparison of static and electromagnetic field effects on redox system of soybean (*Glycine max* L. Merrill) seedlings

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(Received: 21/05/2016-Accepted: 04/01/2017)

Abstract:

The effects of magnetic fields (MF) on germination and early growth parameters, redox state and the activity of antioxidant system were investigated in soybean (*Glycine max* L. Merrill) seedlings. The seeds were imbibed in water and then were exposed to a 30 mT static magnetic field (SMF) and a 10 kHz electromagnetic field (EMF) for 4 days, each 5 h. Water uptake of seeds increased after 40 and 120 minutes of the treatment with MF. In comparison with the control group, exposure to EMF significantly increased germination percent of the seeds and vigor index I, II of seedlings while SMF significantly increased vigor index II but had no effect on seed germination. The ratio of fresh weight to dry weight and ferritin content of both MF-treated groups was significantly higher than those of the control group. Treatment with EMF improved radical scavenging capacity of seedlings and reduced membrane leakages. Exposure to MF also protected seedlings from the risk of increase of internal calcium content. The results provide us with a new approach for application of magnetic fields in order to promote growth of strategic crops.

Keywords: Electromagnetic field, Ferritin, *Glycine max*, Soybean, Static magnetic field.

Introduction

Recent reports about beneficial effects of magnetic field (MF) on germination of seed and growth parameters of certain crops have introduced MF as one of more environmental friendly techniques which fulfill the requirements of organic agriculture (Florez *et al*., 2007; Cakmak *et al*., 2010; Shine *et al*., 2011). Perception of MF by plants can be explained by three mechanisms: radical-pair mechanism, ion cyclotron resonance mechanism, and ferrimagnetism (Galland and Pazur, 2005). Ferrimagnetism is the most plausible mechanisms for bio-effects of MF, however this model has not been tested experimentally in higher plant cells. Magnetic fields interact with certain atoms, in particular iron, which is an essential micronutrient and an abundant ferromagnetic element in plant cells (Hajnorouzi *et al*., 2011). Despite necessity of iron for normal growth and development, plants avoid iron overload toxicity and strictly control its homeostasis by different mechanisms. Some reports have shown that the adverse bio effects of the magnetic fields are usually mediated by oxidative stress, possibly due to either the direct production of reactive oxygen species (ROS) mediated by iron (Fenton reaction) or by an increase of their longevity due to a reduction in the level of antioxidant enzymes (Lobreaux and Briat, 1991; Sahebjamei *et al*., 2007).

Ferritins are a class of ubiquitous iron storage proteins, found in all living kingdoms (Briat *et al*., 2010). The role of ferritin is to concentrate iron (as many as 4500 ions of iron per molecule) to an effective level that matches the cellular need (Goto *et al*., 2000). In the case of very high concentrations of iron, ferritin has a protective function by sequestering the iron inside the protein thus performing a detoxification function (Fourcroy *et al*., 2004).

The present study was undertaken in order to elucidate the mechanism(s) of plants response to MF by comparing the effects of static magnetic field (SMF, 30 mT) and EMF (10 kHz) on early growth characteristics, calcium, iron and ferritin content, and antioxidant system of wheat seedlings.

Materials and methods

Plant materials, treatments, and biochemical assays: Soybean seeds (*Glycine max* L. Merrill) uniform in size and shape were surface sterilized by subsequent washing with detergent, sodium hypochlorite (containing 5% active chlorine), and 70% EtOH. The seeds were rinsed three times with double distilled water and were imbibed in distilled water for 6 hrs. Then, the seeds were wrapped in layers of moistened filter papers and were allowed to germinate in darkness. During germination period they were treated with or without

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EMF (10 kHz) and SMF (30 mT) for 4 days, each 5 hrs. Generation of EMF and SMF was performed by locally designed apparatuses as previously described (Abdollahi *et al*., 2011; Payez *et al*., 2013). The seeds were oriented in the apparatus perpendicular to the magnetic fields. The intensities of SMF and EMF were selected based on the fact that EMF of 10 kHz is usually generated by high-frequency inductive power distribution, which is routinely used in industry of electricity and SMF of 30 mT is the minimum level of MF used in magnetic therapy equipment (Dawson *et al*., 1998; Sakhnini and Dairi, 2004). Control seeds were kept far enough from the MF producing apparatuses but under similar condition in terms of temperature and humidity. They were only exposed to local geomagnetic field (47 ± 5 μT, according to The Geophysics Institute of Tehran University).

During germination period the water uptake (WU) by seeds was determined at intervals of 40, 80, 120 mins and 5 hrs (Shine *et al*., 2011). After 4 days treatment with or without MF, seed germination was determined (ISTA, 1985) and radicle emergence from the seed was scored as positive germination. Seedling length, fresh and dry weights were measured and vigor indices I and II were calculated (Shine *et al*., 2011). In order to determine membrane leakage the samples were incubated for 30 mins in double distilled water at 25 °C and then were boiled for 15 mins. The ratio of electrical conductivity of water before and after boiling was defined as electrolyte leakage (EL) (Safari *et al*., 2012).

The level of damage to the membranes was determined by thiobarbituric acid through measuring its complex with malondialdehyde (MDA) at 532 nm. Nonspecific absorption for proteins and sucrose (600 and 440 nm, respectively) were subtracted and the MDA content was calculated using its absorption coefficient of 157 $mM^{-1}cm^{-1}$. Radical scavenging capacity of the samples was assessed as the percentage of inhibition of a stable free radical, 1, 1-diphenyl-2-picrylhydrazyl (DPPH) at 517 nm. For measurement of antioxidant enzymes activities samples were homogenized in 50 mM Tris-maleate buffer (pH 6.0) and centrifuged at 12,000 ×g for 20 mins at 2 °C. The supernatant was used for enzyme assay. Activity of CAT was monitored by decomposition of H_2O_2 at 240 nm by spectrophotometer (Cintra 6, GBC, Australia). Activity of PO was monitored by peroxidation of guaiacol at 470 nm (Hajnorouzi *et al*., 2011; Rajabbeigi *et al*., 2013). Activities of the enzymes were expressed against protein content of the extract. Protein content was determined by the method of Bradford (1976), using bovine serum albumin (BSA) as a standard.

Extraction and quantification of ferritin was adapted from an indirect ELISA protocol method described by Lukac *et al*. (2009), using an ELISA kit (Pishtazteb Zaman Diagnostics, Tehran, Iran). The contents of iron and calcium in the samples were measured in acid-digested ashes by an atomic absorption spectrometer (Shimadzu AA-670, Japan).

Statistical analysis: All of the experiments were carried out with at least three independent repetitions. Statistical analysis was performed using SPSS 16.0 (SPSS, Chicago, IL, USA) and the significance of differences between treatments was evaluated using LSD test at level of $p \leq 0.05$.

Results and discussion

Exposure to EMF significantly increased the germination percent of seeds (117%) as well as length, fresh weight, vigor indices I, and II of soybean seedlings of seeds 115%, 117%, 134%, and 119% of the control, respectively) (Fig. 1a, b, c, e, f). Exposure to SMF had no significant effects on soybean seed germination, but significantly increased fresh weight, dry weight, and vigor index II of their seedlings (132%, 116%, and 115% of the control, respectively) (Fig. 1b, c, d, f).

Enhanced performance of early growth characteristics of crop seedlings after treatment with MF has been reported previously by researchers dealing with various crop seeds (De Souza *et al*., 2006; Florez *et al*., 2007; Cakmak *et al*., 2010; Bilalis *et al*., 2011). It has been supposed that increased accumulation of water in the seeds after treatment with MF may activate the germinating enzymes, which accelerate the germination in treated seeds (Vaezzadeh *et al*., 2006; Vashisth and Nagarajan, 2010). Shortly after exposure to MF (40 min) water uptake of soybean seeds significantly increased, and the rate of increase was more pronounced in SMF-treated (76%) companed to EMF-treated (17%) seeds (Table 1).

Rate of water uptake in longer periods of exposure to MF however, was more or less identical to the untreated groups (Table 1). Similar promotional and fast effects of SMF on water uptake in soybean seeds have been observed by Shine *et al*. (2011). They reported that exposure to SMF with magnitudes lower than 50 mT had no effects on water uptake while magnitudes higher than 200 mT were detrimental for soybean seeds. Effect of nanosecond-scale static and alternating electric field in the gating dynamics of human aquaporin 4 has been investigated and it has been shown that electric field affects the dipolar orientation of the histidine-201 residue in the selectivity filter which governs the dihedral angle, and hence influences water self-diffusion (Reale *et al*., 2013). To the best of our knowledge however no sound proof of similar hypothesis on the influence of magnetic field on aquaporins has been provided, as yet. If indeed aquaporins were sensitive to the magnetic field, it would give us a clue, as to what is the exact mechanism of increased water uptake of soybean seeds under MF treatments.

Exposure to EMF and SMF reduced calcium content of soybean seedlings to 89% and 75% of the control, respectively (Fig. 2a). It appears that membrane-associated Ca^{2+} ion transport represents a crucial role at which MF could come into play. By applying

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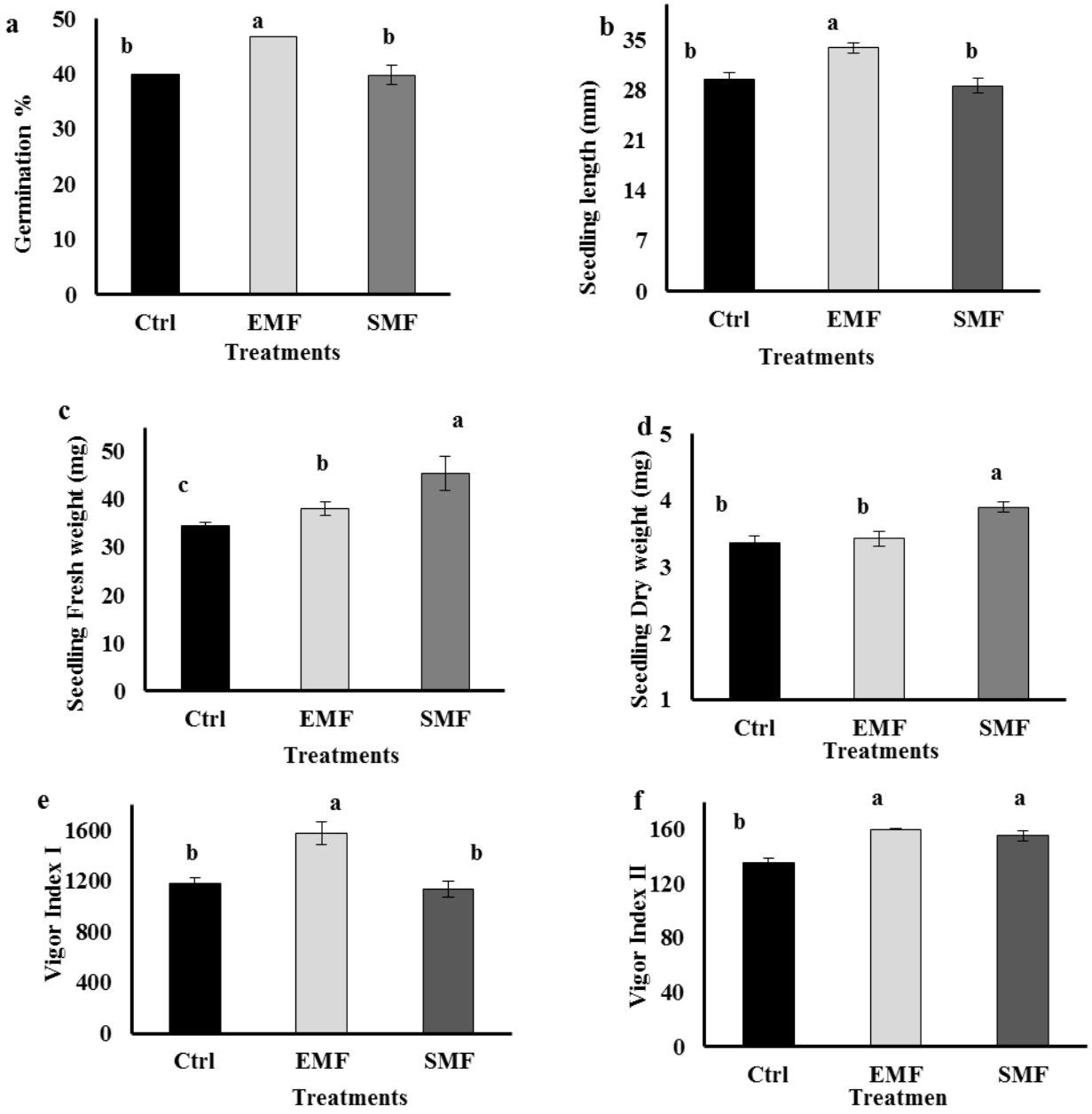


Fig. 1. The effects of EMF and SMF treatments on the seed germination and growth parameters of soybean seedlings, a: germination percent, b: seedling length, c: fresh weight (FW), d: dry weight (DW), e: vigor index I, f: vigor index II. Data are means obtained from three independent repetitions, each at least with 50 samples of seeds and ten samples of seedlings ± SD. Different letters indicate significant differences at $p \leq 0.05$ according to LSD test.

radioactive ^{45}Ca on plant plasma membrane vesicles, Baureus Koch *et al*. (2003) showed that suitablecombinations of static and time varying magnetic fields directly interact with the calcium channel proteins in cell membrane. It has been also shown that extremely low EMF (50 Hz, maximum of 41.7 to 43.6 mT) and moderate SMF (up to 0.8 mT and 1.4 mT) increase cell surface negative charges thereby affect the N-H in plane bending and C-N stretching vibrations of peptide linkages, and change the secondary structures of membrane proteins (Ikehara *et al*., 2003; Calabro *et al*., 2013). Examination of these hypotheses and clarification of the exact mechanism(s) of calcium reduction in soybean seedlings after MF treatment were out of goals of the present study and need further investigations. Calcium regulates diverse cellular processes in plants as a ubiquitous internal second messenger by conveying signals received at the cell surface to the inside of the cell through spatial and temporal concentration changes that are decoded by an array of Ca^{2+} sensors. Elevated concentrations of free cytosolic calcium has been reported to be involved in signal transduction leading to the hypersensitive response (HR) and cell death under pathogens attack as well as abiotic stresses (Xu and Heath, 1998; Liu *et al*., 2005). Therefore it is likely that decrease of calcium

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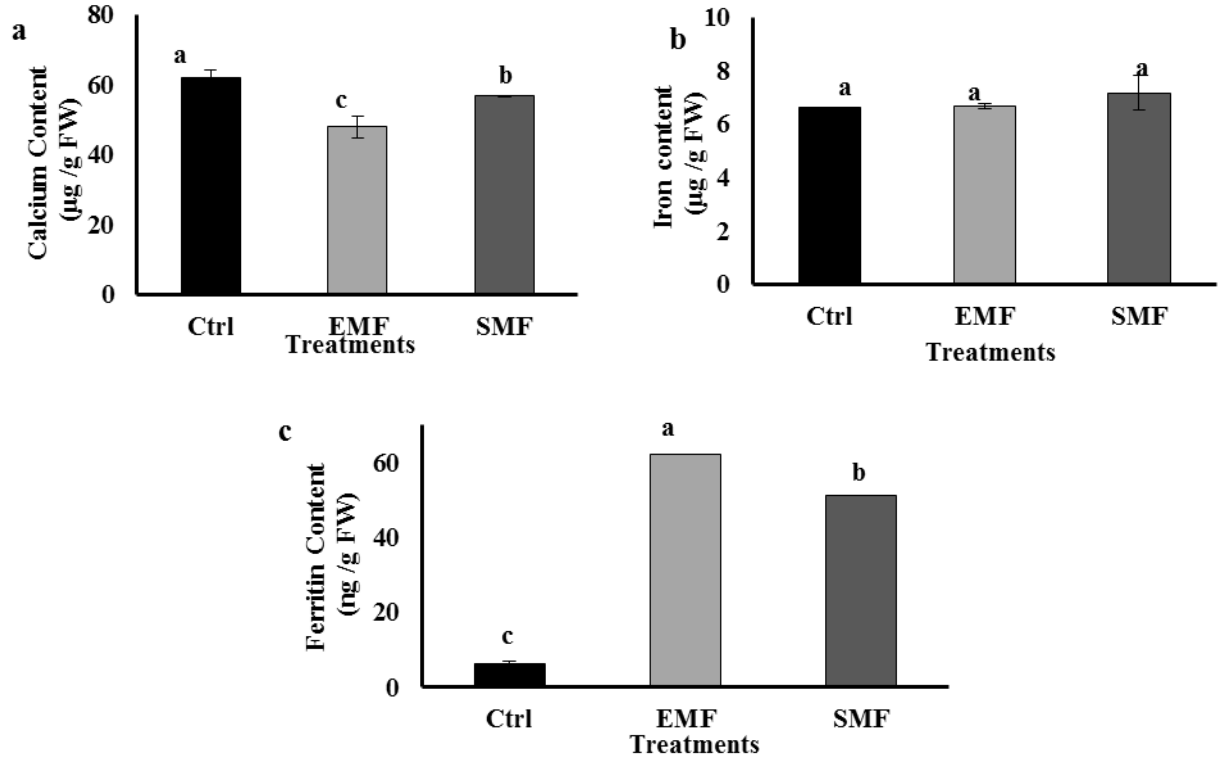


Fig. 2. The effects of exposure to EMF and SMF on the content of calcium (a), iron (b), and ferritin (c) contents of soybean seedlings. Data are means obtained from three independent repetitions, each at least with and ten samples ± SD. Different letters indicate significant differences at $p \leq 0.05$ according to LSD test.

Table 1. Effects of EMF and SMF treatment on Water uptake of soybean seeds

	Water uptake (%)			
Soaking time	40 min	80 min	120 min	5 h
Ctrl	1.94 ± 0.00 ^{a}	4.57 ± 0.04 ^{a}	5.33 ± 0.30 ^{a}	8.35 ± 0.10 ^{a}
EMF	2.26 ± 0.03 ^{b}	4.68 ± 0.28 ^{a}	6.19 ± 0.27 ^{ab}	8.76 ± 0.11 ^{a}
SMF	3.41 ± 0.01 ^{c}	5.27 ± 0.27^{a}	6.63 ± 0.30 ^{b}	8.18 ± 0.62 ^{a}

Data show mean ± SD of four different experiments with 50 seeds each. In each column, different letters indicate significant differences at $P \leq 0.05$ according to LSD test.

Table 2. Effects of EMF and SMF treatments on the antioxidant system activity and membrane integrity of soybean seedlings

Treatments	CAT (ΔAbs 240 / mg protein)	PO (ΔAbs 470 / mg protein)	RSC (DPPH %)	LPO (mM MDA / gFW)	Membrane electrolyte leakage (%)
Ctrl	3.41 ± 0.16 ^{c}	4.29 ± 0.31 ^{a}	49.81 ± 0.67 ^{b}	7.89 ± 0.53 ^{a}	34.49 ± 2.53 ^{c}
EMF	2.04 ± 0.10 ^{a}	8.01 ± 0.47 ^{c}	60.46 ± 3.16 ^{c}	11.26 ± 0.57 ^{c}	20.52 ± 2.29 ^{a}
SMF	2.32 ± 0.03 ^{b}	6.41 ± 0.21 ^{b}	25.57 ± 1.90 ^{a}	10.01 ± 0.41 ^{b}	28.29 ± 1.63 ^{b}

Data show means ± SD of three different experiments with 10 seedlings each. In each column, different letters indicate significant differences at $P \leq 0.05$ according to LSD test.

content of soybean seedlings by MF in the present study provided them with postponed programmed cell death and improved their growth characteristics.

While total iron content of MF-treated soybean seedlings was identical to that of the control, their ferritin content was remarkably (9-10 folds) higher than the controls (Fig. 2 b, c).

When considering the effects of MFs at the molecular scale, the iron cage protein ferritin is an obvious candidate because it has the highest net magnetic moment of all proteins (Cespedes and Ueno, 2009). A multilayered system of genetic controls has evolved to regulate ferritin synthesis under the control of both iron and oxygen (Lescure *et al.*, 1991), which emphasizes the key role played by ferritin in controlling iron and oxygen interactions. Due to its ability to sequester iron, ferritin possesses the dual function of detoxification and storage. Studies on plant ferritin functions and regulation of their synthesis revealed strong links between these proteins and protection against oxidative stress (Ayala-Vela *et al*., 2008).

Treatment with EMF and SMF remarkably decreased the activity of CAT (40.2% and 32% respectively) in comparison with the control plants

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(Table 2).

Such reduced activity of CAT of various crop seedlings exposed to magnetic fields has been reported previously by workers (Aleman *et al*., 2014; Sahebjamamei *et al*., 2007; Serdyukov and Novitskii, 2013). Aliquot of hydrogen peroxide which was not detoxified by CAT was detoxified by other enzymes, in particular PO. Due to low activity of CAT, treatment with EMF and SMF significantly increased the activity of PO up to 1.9 and 1.5 folds of the control plants, respectively (Table 2).

As shown in Table 2, EMF treatment caused a significant increase (21.4%) in the radical scavenging capacity of soybean seedlings, compared to the control group, while SMF treatment lowered this content to 49% (Table 2). The rate of membrane lipid peroxidation of EMF and SMF-treated seedlings was respectively 42.7% and 27% higher than those of the control group (Table 2). Also, increase of membrane lipid peroxidation of different plant species in the treatment with magnetic field also has been reported in earlier studies (Sahebjamei *et al*., 2007). However, MF treatment of soybean seedlings lowered membrane electrolyte leakage, in comparison with the control plants emphasizing on reinforcement of membrane integrity of seedlings in these treatments (Table 2).

Primary function of MF in biological systems is the induction of electrical charges and currents. Although formed in normal cell metabolism, free radicals are potentially damaging and can initiate chain reactions to form new free radicals (Sahebjamei *et al*., 2007). Hydrogen peroxide, one of the very toxic ROS, initiates the Fenton and Haber–Weiss reactions that are catalyzed by iron and results in the generation of the more reactive hydroxyl radicals. The hydroxyl radicals initiate self-propagating reactions which in turn lead to the peroxidation of membrane lipids (Bowler *et al*., 1992). Scavenging and detoxification of H_2O_2 can be achieved by either non-enzymatic antioxidants or scavenging enzymes, e.g. CAT and PO. Although all H_2O_2 scavenger enzymes act in a cooperative or synergistic way for the survival of the cell even under normal conditions, it is more likely that among ROS scavenger enzymes, CAT is the key enzyme that effectively eliminates H_2O_2, thereby regulating the activity of PO. This clearly explains why the reduction of CAT activity (the accumulation of H_2O_2) was accompanied by the increase of PO activity in the soybean seedlings exposed to MF. Nevertheless, the remained ROS in a dual action resulted in peroxidation of membrane lipids, and on the other hand triggered other antioxidant system, ferritin, that by sequestering iron and preventing Fenton reaction can function upstream of all other scavengers.

Conclusions

The results presented here confirm the stimulatory effects of SMF of 30 mT and EMF of 10 kHz on early growth stages of soybean seedlings. Although exact mechanism(s) of this function remained to be elucidated by further investigations, it is obvious that at least a part of MF effects, on soybean seedling is mediated by calcium and iron homeostasis.

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