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Proline Accumulation in Response to Magnetic Fields in Date Palm *(Phoenix dactylifera* L.)

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Abstract: Proline accumulation is a common biochemical indicator for assessing environmental stress in plants. The objective of this study was to determine the effect of various doses of two types of magnetic fields on date palm (*Phoenix dactylifera* L. (cv. Khalas) seedlings based on proline accumulation. The first type involved static magnetic field (SMF) generated by an electromagnetic circuit set at 10, 50, and 100 mT for 30, 60, 120, 180, 240 and 360 min. The second type involved alternating magnetic field (AMF) generated by a magnetic resonance imaging (MRI) device set at 1.5 T for 1, 5, 10, and 15 min. Following exposure to radiation, the seedlings were grown in potting soil for 4 weeks after which proline analysis was conducted. The results showed a significant two-way interaction between SMF intensity and exposure duration. At the lowest intensity, 10 mT, proline concentration increased in response to longer exposure durations reaching a maximum at 240 min. Beyond this duration, reduction in proline concentration occurred. In contrast, at 50 and 100 mT, proline concentration decreased as the exposure duration was increased. AMF significantly reduced proline concentration after as short as a 1-min exposure. This study would facilitate investigations related to stress physiology and *in vitro* selection of date palm tissue culture.

Keywords: Date palm, magnetic field, plant, proline, stress.

INTRODUCTION

Magnetic fields (MF) induce electric potential that exerts cellular stress causing biochemical, physical and physiological changes in cellular structures and functions in living systems. In higher plants, MF has been shown to induce stress effects [1-3]. Although the role of MF is insufficiently understood, recent studies have shown that MF increases nitrogen and ions uptake by plants [4-6] and plant chlorophyll content [7]. In addition, MF has been shown to increase the release of free radicals that are known to damage cellular macromolecules and their activities [3, 8-10].

During adaptation to various types of environmental stress, plants accumulate cellular solutes [11-13]. The cellular solutes include quaternary amino acid derivatives such as proline, glycine betaine, alanine betaine, and proline betaine [14]. Among these, proline is the most commonly studied as a biochemical indicator of stress [15]. Proline has been shown to protect plants against damages caused by free radicals by scavenging the radicals and stabilizing of macromolecules [16-19]. Under abiotic stress proline is accumulated in cells at concentrations of a few millimolar, depending on the species and the extent of stress [20, 21]. High accumulation of cellular proline in plants under abiotic stress, up to 80% of the amino acid pool, compared with 5% under normal conditions, has been documented in a number of plant species [22-24]. In addition, proline acts as a source of energy, carbon, and nitrogen, which enhances tissue recovery, and the relief of stress effects [25]. Proline accumulation has been extensively studied as a common metabolic response of higher plants to drought and salinity stress [20, 26-29].

In studies on date palm (*Phoenix dactylifera* L.), an economically important species adapted to environmental stress factors associated with the hot arid regions where it is predominately grown, proline accumulation was observed in callus cultures subjected to salinity [30] and drought conditions [31]. However, we have not encountered any literature relating to the effect of magnetic field on proline accumulation in plant systems. The objective of this study was to assess proline accumulation in date palm seedling in response to various exposures to static and alternating magnetic fields, to measure the ability of date palm seedlings to tolerate MF stress. Application of MF treatments could facilitate investigations related to stress physiology and *in vitro* selection in date palm.

MATERIALS AND METHODS

Date palm seeds (cv. Khalas) were surface sterilized with 1% sodium hypochlorite for 5 min, soaked in water for 24 h at 37°C then germinated on moist filter paper placed in 9-cm Petri dishes. Two-week-old seedlings were subjected to either static magnetic field (SMF) or alternating magnetic field (AMF).

The SMF was applied using an electromagnetic circuit, constructed by Dr. Essam Hassan, Electrical Engineering Department, King Fahd University for Petroleum and Miner-

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als, (KFUPM) Saudi Arabia. The magnetic circuit consisted of two coils each of 480 turns per coil wound on carbon steel through which was passed variable currents to achieve a range of magnetic field intensities. The distance between dipoles was 10 cm diameter to accommodate the Petri dishes. The seedlings were exposed to 10 mT, 50 mT and 100 mT of MF for durations of 0, 30, 60, 180, 240 and 360 min. The experiment was set up as a two-factor factorial design. The main factors were magnetic field intensity at three levels and exposure duration at six levels. Each treatment was replicated 7 times and each replication consisted of a single seeding. A total of 126 seedlings were analyzed.

The AMF was applied using a magnetic resonance imaging device (General Electric, USA). The device provides an alternating magnetic field frequency ranging from 0.01 to 63000 Hz, 220 V current, and magnetic flux of 1.5 T (1500 mT). Two-week-old seedlings were treated for 0, 1, 5, 10 and 15 min. The experiment was designed as a single-factor (AMF) with five levels (duration of exposure). Each treatment was replicated 7 times and each replication consisted of a single seeding. A total of 35 seedlings were analyzed.

After exposure, seedlings were grown individually in a 20-cm plastic pot containing potting mix: 1 soil: 1 peat moss: 1 vermiculite (volume) and maintained in a greenhouse under natural light at a minimum temperature of 30°C and maximum of 41°C with relative humidity of approximately 50%. The plants were watered as needed to ensure the seedlings were not stressed for moisture and grown for an additional 4 weeks to obtain sufficient tissue growth for proline analysis.

Proline was extracted and measured according to Bates [32]. Briefly, fresh leaf tissues (0.5 g per sample) were homogenized in 10 ml 3% (m/v) cold aqueous sulphosalicylic acid. The homogenate was filtered through Whatman No. 2 filter paper. In a test tube, 2 ml of the filtrate was mixed with 2 ml of acidic ninhydrin and 2 ml of glacial acetic acid then incubated in a 100^{o}C water bath for 1 h. After fast cooling in an ice bath, 4 ml of toluene was added and it was vigorously shaken. The toluene phase, containing the chromophore (colored complex) was aspirated and the absorbance of this phase, using samples of 1 ml, was determined at 520 nm using, a UV/VIS spectrophotometer (Model V-530, Jasco Inc, USA).

An analysis of variance (ANOVA) was performed and the statistical difference between means was determined using the least significant difference (LSD) procedure applied at 5%. The standard deviation for each treatment mean was also calculated.

RESULTS

Proline concentration was significantly affected by the intensity of the SMF and the duration of exposure to SMF as indicated by the significant two-interaction (Table **1**). Static magnetic field increased proline significantly, from 1.22 µmol/g in the control to 1.57 µmol/g with seedling exposure to 10 mT for 240 min, which was the highest proline concentration. With a greater exposure time proline concentration decreased from its peak concentration, significantly after 360 min of exposure (Fig. **1A**). There was a similar reduction in proline concentration when SMF intensity was increased to 50 mT or 100 mT, regardless time of exposure (Fig. **1B**, **C**).

Table 1. Analysis of Variance of the Proline Concentration in Date Palm Leaves Exposed to Two Types of Magnetic Fields for Varying Durations

Factor	df	MS	F	P
Static Magnetic Field				
Intensity	2	6.3809	931.56	0.0001
Time	5	0.5740	83.80	0.0001
Intensity x Time	10	0.4142	60.47	0.0001
Error	108			
Alternating Magnetic Field				
Time	4	1.32293	148.891	0.0001
Error	30	0.0092		

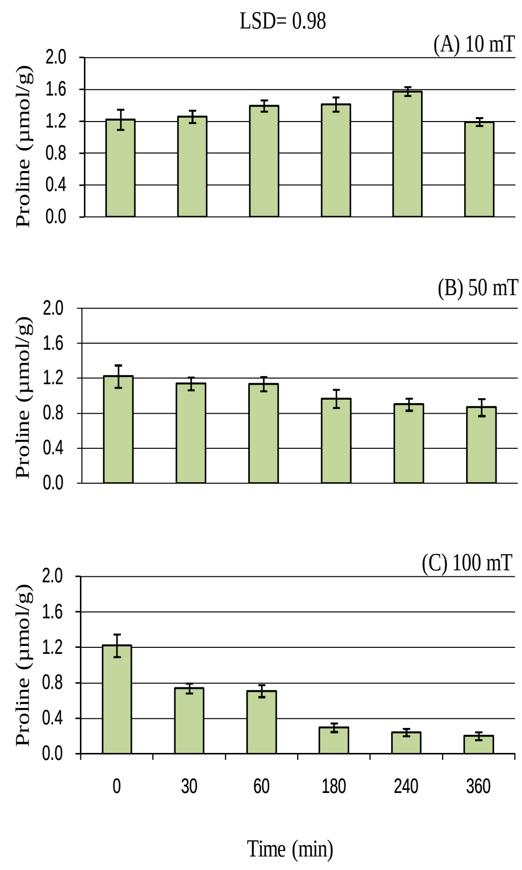

Fig. (1). Effect of a static magnetic field on cellular proline concentration (umol /g fresh leaf wt) in seedlings of date palm. Exposed to different field strengths **A**: 10 mT, **B**: 50 mT, **C**: 100 mT) for varying durations of time (30, 60, 180, 240 and 360 min). Bars are standard deviations of the means.

Significant reductions in proline concentration occurred in response to AMF (Table **1**), with a greater reduction as the duration of exposure was increased (Fig. **2**). AMF reduced proline concentration with as short as 1 min of exposure of seedlings.

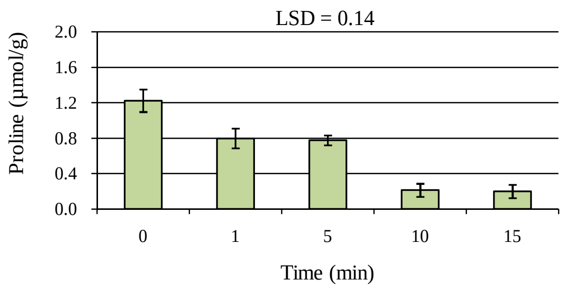

Fig. (2). Effect of alternating magnetic field on proline concentration (umol /g fresh leaf wt) in seedlings of date palm. Exposed to (1500 mT) for varying durations of time (1, 5, 10 and 15 min). Bars are standard deviations of the means.

DISCUSSION

In the present study SMF increased proline accumulation at beginning of the exposure to stress followed by a decrease due to accumulation of its product, when exposure time was prolonged an inverse response was noted analogues to Belyavskaya study [2]. This pattern of response is similar to that of chlorophyll in soybean (*Glycine max L.*) reported by Atak *et al.* [7], in that low intensity of MF, or short period of exposure to MF, increased chlorophyll concentration, whereas high intensity MF and long exposure to MF reduced the concentration. A similar pattern of response, MF can cause an inconsistency in the function of antioxidant enzymes in (*Nicotiana tabacum* L.) [10]. Ghanati *et al.* [3] also observed that a magnetic field led to a decrease of phenylalanine-ammonialyase activity and phenolic compound concentration in (*Ocimum basilicum*) in response to exposure to SMF.

The decrease in the cellular concentration of proline at high doses of MF could be explained by proline consumption by 'caged reaction', whereby proline reacts with the free radicals that are enhanced during exposure to MF [8, 9]. Proline consumption in 'caged reaction' oxidizes proline to various compounds [33] and this process can protect plant tissue from potential damage [19].

In addition, the proline pathway could be shifted by MF exposure, through oxidation of proline to glutamate or forming glutamic acid *g*-semialdehyde [19]. Shift in cellular metabolic pathways following exposure to MF was noted by Ghanati *et al.* [3] who reported that under stress of SMF, plants shifted their metabolism from biosynthesis of phenolics to the production of essential oils.

Although proline accumulation was disturbed, growth of date palm seedlings proceeded normally regardless of the type of magnetic field tested. This may be explained by the greater ability of seedling plants to recover from stress than older plants [27].

CONCLUSION

It is concluded that proline accumulation in date palm seedlings is affected by exposure to MF. Proline concentration in date palm increased significantly under salinity [30] and drought stress [31], so that exposure to low intensity MF may be a convenient method to select for tolerance to these abiotic stresses. At high intensity of SMF or AMF, the proline mechanism appears to fail, yet as exposed seedlings develop normally, this suggests that other protective mechanisms may be involved, perhaps involving oxidizing enzymes [3, 10] or another cellular solute such as alanine [1].

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