Research Article

Acceleration Breaks the Cells Defense Mechanisms against Vibration in *Anthemis gilanica* Calli

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Vibration is a mechanical stress which happens in nature and affects many biological aspects of plants. In this research, the effect of acceleration and vibration was investigated on some physiological and biochemical responses of *Anthemis gilanica* in vitro. Calli were induced from leaf (LS) and root segments (RS) and were applied to different frequencies of vibrations (0, 50, and 100 Hz) and accelerations (1, 2, and 4 g) on the *A. gilanica* calli for 30 min. Results showed that vibration significantly increased relative water content (RWC), growth parameters, protein and proline contents, ascorbate peroxidase (APX), peroxidase (POX), and superoxide dismutase (SOD) activities and decreased total carbohydrate, malondialdehyde (MDA), H_2O_2 contents, and polyphenol oxidase (PPO) activity in both LS and RS calli. Inversely, increase of acceleration in vibrated calli decreased growth parameters, RWC, protein content, and POX activity and induced proline and carbohydrate accumulations, SOD, APX, and PPO activities as compared to vibration alone. Different responses of two callus types were observed, and the highest growth, protein content, and membrane stability were observed in LS calli as compared to RS calli. It found that high acceleration amplitude intensified the resonance effect of vibration by induction of lipid peroxidation and oxidative stress damage in *A. gilanica*.

1. Introduction

Plants in nature are motivated by various types of mechanical stimulations such as vibration, electromagnetic field, waves, sounds, and so on [1]. Mechanical forces can sense by cells and transfer to the nucleus through cytoskeleton and affecting many cellular activities [2] and biological characteristics of plants [3]. For example, cell division increased in some pea species for androgenesis induction using electroporation [4]. The direction of new cell wall is changed by mechanical stress [5]. Moreover, shoot length and thickness decreased in *Capsella bursa-pastoris* under mechanical stress [6].

Vibration is a kind of mechanical stress which can be classified into two categories, sinusoidal and random. Sinusoidal vibration is mostly used in biological studies [7, 8].

Amplitude (usually acceleration or displacement) and frequency are two parameters to define sinusoidal vibration. The amplitude can be constant or variable. When acceleration is used to define amplitude, its unit is usually g or meter per second squared (m/s^2). One g is equal to the acceleration produced by the earth's gravity and is equivalent to $9.8 \, m/s^2$ [9]. Recently, more consideration has been done to the impact of vibration at different frequencies and acceleration on biology. The physical and biochemical mechanisms by which cells can perceive and respond to vibrations are largely unknown. Vibration at different frequencies and accelerations can affect cellular fluid viscosity and induce matrix deformation, oscillatory motion against the gravitational force, and fluid shear stress [10]. The fluidity of cell membrane changes under vibration, and different physiological and biochemical responses have

happened in plant cells. For example, vibration at various frequencies induced growth and fiber accumulation in *Gerbera jamesonii acrocarpous* callus [11], secondary metabolite, and soluble protein content in *Camptotheca acuminata* [12], cell proliferation [13], and antioxidative enzyme activity in *Triticum aestivum* seedling [14]. Increased accumulation of reactive oxygen species (ROS) content is observed in wheat seedling [14] and *Arabidopsis* [15]. Also, the contents of sugar, ATP, and hormones are changed significantly under vibration [16, 17].

Anthemis gilanica Bornm. & Gauba is a medical plant belonging to Asteraceae family and is an endemic plant of Iran [18]. The cytotoxic activity of various *Anthemis* species against cancer cell lines is ascribed to the presence of different natural products such as sesquiterpenes lactones, and flavonoids with antimicrobial and antifungal activities [19–21]. So, access to specific conditions of *A. gilanica* cell culture with more growth and study of its antioxidant capacity is very valuable. Until now, some studies have been reported about the impact of different vibration frequencies on physiological and biochemical responses of plants [11, 14]. However, there is little information available about the mixed effect of vibration and acceleration on plant cell (Uchida and Yamamoto, 2002). Acceleration magnitude can induce cellular fluid shear stress [22] and affect various cellular responses under vibration. Antioxidative responses of the mixed impact of vibration and acceleration have never been studied before on any species. So, we studied the mixed effect of vibration and acceleration on cell growth induction and antioxidative responses in *A. gilanica* callus.

2. Materials and Methods

2.1. Callus Culture, Vibration, and Acceleration Treatments.
Seeds of *A. gilanica* were gathered from Rudsar, Gilan province of Iran. The seeds were sterilized in 70% (v/v) ethanol for one minute, then 15% (v/v) NaOCl for 10 min, and finally washed three times with sterile distilled water, and then were cultured on 1/2 MS medium [23] containing 3% sucrose and 7% agar-agar at $25 \pm 2°C$, pH 5.7, 60% relative humidity, and a 16 h artificial light (white fluorescent lamps giving light intensity $46 \, \mu\text{mol} \, \text{m}^{-2} \, \text{s}^{-1}$) pursued by the 8-hour dark period. After 4 weeks of culture, the 0.5-0.6 cm leaf (LS) and root (RS) segments were placed in a solid MS medium supplemented with 2,4-dichloro-phenoxyacetic acid (2, 4-D, $0.5 \, \text{mg} \, \text{L}^{-1}$) and kinetin (KIN, $0.5 \, \text{mg} \, \text{L}^{-1}$) for callus initiation. Calli (*ca*. 0.3–0.4 g) were subcultured to the MS solid medium with the same hormonal composition. Then, after one week, different frequencies of sinusoidal vibration (0, 50, and 100 Hz) with various accelerations (1, 2, and 4 g) along the Y axis were applied on callus tissues for 30 min, as is shown in Figure 1. Acceleration amplitudes and vibration frequency were generated using an electromechanical shaker composed of a power amplifier type SA-36, a signal generator, and a vibration exciter type DC-3200, China. The program was defined with a control Swept Sine Software. Calli are grown in a growth chamber with the same condition for three weeks and then were gathered for biochemical and physiological analyses.

2.2. Growth and Relative Water Content (RWC).
For detection of dry weight, the samples were put at 4°C for 72 h on five individual calli per treatment. RWC content was also defined according to [24].

2.3. Lipid Peroxidation and H₂O₂ Level.
The lipid peroxidation was defined based on malondialdehyde (MDA) content according to [25]. Hydrogen peroxide (H_2O_2) content was measured by [26], and the absorbance was read at 390 nm.

2.4. Proline and Carbohydrate Content.
Proline content of each sample was assayed based on [27], and the absorbance was recorded at 520 nm. Proline content of each sample was calculated from a standard curve. Carbohydrate content was determined based on [28].

2.5. Total Protein.
Total protein content was quantified based on the method of [29]. Absorption of the samples was measured at 595 nm using a standard curve of protein content and expressed as $\text{mg} \, \text{g}^{-1}$ FW. Bovine serum albumin (BSA) was used as a standard.

2.6. Antioxidant Enzyme Assay

2.6.1. Superoxide Dismutase (SOD) Activity.
SOD activity was estimated by monitoring its capacity to inhibit nitroblue tetrazolium (NBT) reduction at 560 nm. Isozymes of SOD were defined based on the photochemical staining method as described by [30]. For selective inhibition of various SOD isoforms, the gel was put in KCN (3 mM, inhibitor of Cu/Zn-SOD) and then in 5 mM H_2O_2 (inhibitor of Fe-SOD and Cu/Zn-SOD) for 20 min [31].

2.6.2. Peroxidase (POX) Activity.
Specific POX activity was estimated based on [32]. The absorbance was determined at 530 nm. The POX activity was determined as $1 \, \mu\text{mol}$ of benzidine oxidized per min per mg protein (unit, mg^{-1} protein). POX isoenzymes appeared by putting the gels in acetate buffer (0.2 M, pH 4.8) including H_2O_2 (3 %) and benzidine (4 %) in methanol (50 %) at room temperature until the brown color appeared [33].

2.6.3. Polyphenol Oxidase (PPO) Activity.
The activity of polyphenol oxidase was estimated based on [34]. The rise of absorbance was recorded at 430 nm. The PPO activity was detected as absorbance change per minute per mg protein (unit, mg^{-1} (protein)).

2.6.4. Ascorbate Peroxidase (APX) Activity.
The APX activity was determined by monitoring the reduction in absorbance at 290 nm because of ascorbate oxidation based on [35].

2.7. Statistical Analysis. Data from different experiments were analyzed by one-way analysis of variance (ANOVA) using SPSS (version 18) in a randomized complete block design. Data are the mean ± SE of four or five replications in each group. All data were conducted to analyze, and the significance of the differences among treatment means was examined using a least significant differences (LSD) test at the level of $P < 0.05$.

3. Results

3.1. Callus Growth and Tissue Water Content. Various frequencies of vibration and acceleration induced different responses in growth parameters of *A. gilanica*. Fresh and dry weight significantly increased in both LS and RS calli under vibration, and the highest growth was observed in LS than that of RS calli (Table 1). Vibration at 50 Hz frequency induced a 46.15% and 51.72% increase of fresh weight in LS and RS calli compared to the control, respectively. Application of acceleration to vibrated calli reduced growth parameters, and the increase of its dose decreased growth further. At 4 g acceleration and 50 Hz vibration, a 31.57% and 29.54% decrease of dry weight were observed in LS and RS calli compared to vibration alone, respectively. The LS and RS calli were yellow in color at control and green at 50 Hz and 100 Hz frequencies of vibration after three weeks of treatment application. With the increase of the acceleration level, the calli became light yellow and brownish, especially at 4 g acceleration and 100 Hz frequency.

RWC significantly went up with vibration intensified (50 Hz and 100 Hz), and a 19.55% and 27.23% induction in RWC was determined at 50 Hz frequency in LS and RS compared to the control, respectively (Table 1). An increase of the acceleration level decreased RWC in *A. gilanica* calli under vibration. At 4 g acceleration under 50 Hz vibration, LS and RS calli showed a 48.06% and 32.41% decrease of RWC compared to vibration alone, respectively.

3.2. Protein Content. Sinusoidal vibration and acceleration significantly changed the total protein content in *A. gilanica* calli. At 50 Hz and 100 Hz frequencies, vibration significantly enhanced protein content compared to control ($P < 0.05$) (Table 1). The frequency of 50 Hz resulted in an increase of 59.10% and 69.62% protein content in LS and RS calli compared to the control, respectively. Increase of the acceleration level from 1 g to 4 g reduced significant protein content under vibration compared to the control (1 g), and minimum protein was determined at 4 g. A 38.57% and 29.19% decrease of protein content was observed under 100 Hz vibration with 4 g acceleration in LS and RS calli as compared with vibration alone, respectively.

3.3. Proline and Carbohydrate Content. Significant increase in proline accumulation was observed with the rise of vibration frequency, and 100 Hz frequency showed a 41.30% and 50.33% increase in proline content in LS and RS calli compared to the control, respectively ($P < 0.05$) (Figure 2(a)). An increase in the acceleration level induced more proline accumulation than vibration alone in *A. gilanica*. At 100 Hz frequency and 4 g acceleration, LS and RS calli showed a 16.29% and 25.61% increase of proline content as compared to vibration alone, respectively.

Total carbohydrate content significantly decreased at 50 Hz and 100 Hz frequencies in both *A. gilanica* calli. Vibration induced a 21.11% and 13.48% decrease of total carbohydrate at 100 Hz in LS and RS calli, respectively. The mixed effect of acceleration and vibration caused a significant enhancement of total carbohydrate compared to the control, and a 30.56% and 27.88% increase of total carbohydrate was observed at 50 Hz with 4 g acceleration ($P < 0.05$) (Figure 2(b)).

3.4. H_2O_2 and Lipid Peroxidation Levels. Vibration significantly decreased H_2O_2 content in *A. gilanica* calli ($P < 0.05$), and 50 Hz frequency showed the minimum H_2O_2 level (Figure 3(a)). At 50 Hz vibration, a 19.04% and 23.07% decrease of the H_2O_2 level was observed in LS and RS calli compared to the control, respectively. Conversely, the mixed effect of acceleration and vibration significantly increased the H_2O_2 level, especially in RS calli. A 31.64% and 43.04% increase of the H_2O_2 level was observed at 100 Hz with 2 g and 4 g acceleration in RS calli compared to vibration alone, respectively.

MDA content was related to the H_2O_2 level and decreased under vibration compared to control (Figure 3(b)). LS calli indicated a lower MDA level than RS calli, and a 26.22% and 18.03% decrease of this parameter was observed at the frequencies of 50 Hz and 100 Hz compared to control. The mixed effect of acceleration and vibration significantly increased the MDA level as compared to control. In RS calli, a 36.61% and 84.15% increase of the MDA level were observed at 100 Hz with 2 g and 4 g acceleration as compared to vibration alone, respectively.

3.5. Antioxidant Enzymes Activities. The activities of antioxidant enzymes including SOD, POX, APX, and PPO under acceleration and vibration are shown in Figures 5–6, respectively. Vibration significantly induced SOD activity, and the maximum activity was found out at 50 Hz frequency compared to the control ($P < 0.05$) (Figure 4(a)). The mixed effect of vibration and acceleration strongly increased SOD activity, especially at 4 g acceleration compared to vibration alone. At frequency of 50 Hz, acceleration (4 g) induced a 42.94% and 24.43% increase of SOD activity in LS and RS calli compared to the vibration alone, respectively. Electrophoretic profiles of SOD showed different SOD isoforms (Figure 4(b)). Two SOD isoforms were observed in both LS and RS calli: an Mn-SOD isoform and Cu/Zn-SOD isoform. The isoforms were detected in the control and other treated plants, but the intensity of bands was higher in 4 g acceleration, especially at 100 Hz compared to control.

The activity of POX significantly increased under different frequencies of vibration, and 100 Hz frequency showed the optimum POX activity (Figure 5(a)). A 34.84% and 16.86% increase of POX activity was observed at 100 Hz frequency in LS and RS compared to control, respectively.

Callus induction

Four weeks in vitro seedlings → [callus image] → Application of sinusoidal vibrations (0, 50, and 100 Hz) with various accelerations (1, 2, and 4 g) for 30 min → Transfer of calli to in vitro culture room
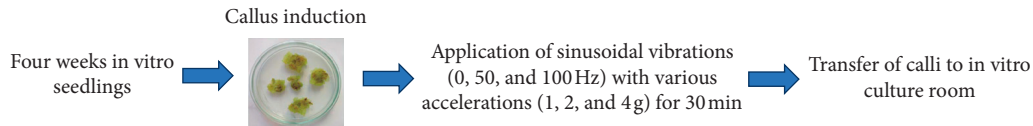
FIGURE 1: Depiction of application of vibration and acceleration treatments on *A. gilanica* calli.

TABLE 1: Effect of different sinusoidal vibrations (0, 50, and 100 Hz) and accelerations (1, 2, and 4 g) on growth parameters, relative water content (RWC), protein, and total carbohydrate of two *Anthemis gilanica* callus sources.

Callus source	Vibration (Hz)	Acceleration (g)	Fresh weight (g/plant)	Dry weight (g/plant)	RWC (%)	Protein (mg/g FW)
LS	0	1	4.91 ± 0.131^{cd}	0.39 ± 0.031^{d}	49.6 ± 1.53^{b}	29.1 ± 2.67^{cd}
		2	—	—	—	—
		4	—	—	—	—
	50	1	6.92 ± 0.25^{a}	0.57 ± 0.023^{a}	59.3 ± 1.44^{a}	46.3 ± 2.55^{a}
		2	5.22 ± 0.33^{c}	0.52 ± 0.043^{ab}	51.5 ± 1.40^{b}	40.1 ± 1.04^{b}
		4	4.24 ± 0.22^{d}	0.39 ± 0.029^{d}	30.8 ± 1.52^{d}	32.3 ± 1.39^{c}
	100	1	5.90 ± 0.34^{bc}	0.51 ± 0.025^{b}	57.3 ± 1.24^{a}	39.4 ± 1.29^{b}
		2	5.43 ± 0.32^{c}	0.46 ± 0.032^{bc}	50.3 ± 1.32^{b}	32.7 ± 1.06^{c}
		4	$4.13 \pm 0.29d$	0.44 ± 0.029^{c}	38.7 ± 3.29^{c}	24.2 ± 1.52^{d}
RS	0	1	3.22 ± 0.12^{c}	0.29 ± 0.014^{d}	51.4 ± 2.08^{bc}	23.7 ± 1.61^{bc}
		2	—	—	—	—
		4	—	—	—	—
	50	1	4.52 ± 0.14^{a}	0.44 ± 0.014^{a}	65.4 ± 1.14^{a}	40.2 ± 2.28^{a}
		2	4.22 ± 0.19^{a}	0.38 ± 0.029^{b}	58.2 ± 1.18^{b}	25.9 ± 1.77^{b}
		4	4.03 ± 0.15^{b}	0.31 ± 0.030^{c}	44.2 ± 1.15^{cd}	20.5 ± 1.62^{c}
	100	1	3.91 ± 0.24^{b}	0.35 ± 0.021^{bc}	61.3 ± 1.21^{ab}	27.4 ± 1.21^{b}
		2	2.83 ± 0.83^{c}	0.28 ± 0.022^{cd}	55.8 ± 1.19^{b}	22.1 ± 1.04^{bc}
		4	3.01 ± 0.17^{c}	0.31 ± 0.012^{c}	40.5 ± 1.17^{d}	19.4 ± 1.52^{c}

Values are given as mean $\pm$ SE ($n = 5$) in each group. Different letters indicate significant differences at $P < 0.05$ (LSD). LS, leaf segments; RS, root segments.

POX activity was higher in RS calli than that of LS calli. The mixed effect of acceleration and vibration significantly decreased POX activity at 2 g and 4 g acceleration. At 100 Hz frequency, POX activity induced a 34.82% and 22.88% decrease of POX activity at 2 g and 4 g acceleration in RS calli as compared with vibration alone, respectively. The electrophoretic profiles of POX revealed four isoforms in LS calli and two isoforms in RS calli. POXs from 1 to 4 were observed in LS calli (Figure 5(b)). The POX1, POX2, and POX3 were identified in control and vibrated plants with or without acceleration, but the intensity of the mentioned bands was lower at 4 g acceleration as compared to the control. POX4 was observed just in control and disappeared under vibration and acceleration. In RS calli, POX1 and POX2 were observed in all vibration treatments and control; however, the intensity of bands was higher at 50 Hz and 100 Hz frequencies. Application of acceleration decreased the intensity of bands, especially at 4 g acceleration.

The activity of APX significantly increased in both LS and RS calli of *A. gilanica* ($P < 0.05$) (Figure 6(a)), and the maximum activity was determined at 50 Hz frequency of vibration compared to the control. The mixed effect of acceleration and vibration increased more APX activity than that of vibration alone. Frequency of 50 Hz with 4 g acceleration caused 46.34% and 58.33% increase of APX activity in LS and RS calli comparing to vibration alone, respectively.

PPO activity significantly decreased under vibration and 1 g acceleration, and minimum activity was found out at 50 Hz frequency ($P < 0.05$) (Figure 6(b)). A 54.16% and 57.14% decrease of PPO activity was identified at 50 Hz frequency in both LS and RS calli compared to the control, respectively. The mixed effect of acceleration and vibration increased the PPO activity compared to vibration alone, especially in RS calli, and an 84.61% and 129.23% increase of PPO activity was observed at 50 Hz frequency with 2 g and 4 g acceleration as compared to vibration alone, respectively (Figure 6(b)).

4. Discussion

This study was performed to establish the effect of acceleration on vibration tolerance mechanisms in *A. gilanica* callus. Growth alteration is the most distinct plant reaction to stress, and the growth level changes between plant species, genotypes, and organs. In this research, vibration significantly increased fresh and dry weight, RWC, and protein content, and high acceleration decreased these parameters in response to vibration. Kang et al. [12] showed that the growth and protein content of *Camptotheca acuminata* calli improved under vibration. Hassanpour et al. [36] also reported the positive relation of growth and RWC content under vibration, which may be related to osmolytes accumulation [37]. Salami et al. [38] displayed that growth induction in *Matricaria chamomilla* L. callus can be associated with an increment in protein, proline, total phenol content, and antioxidative enzyme activities. Mechanical vibration
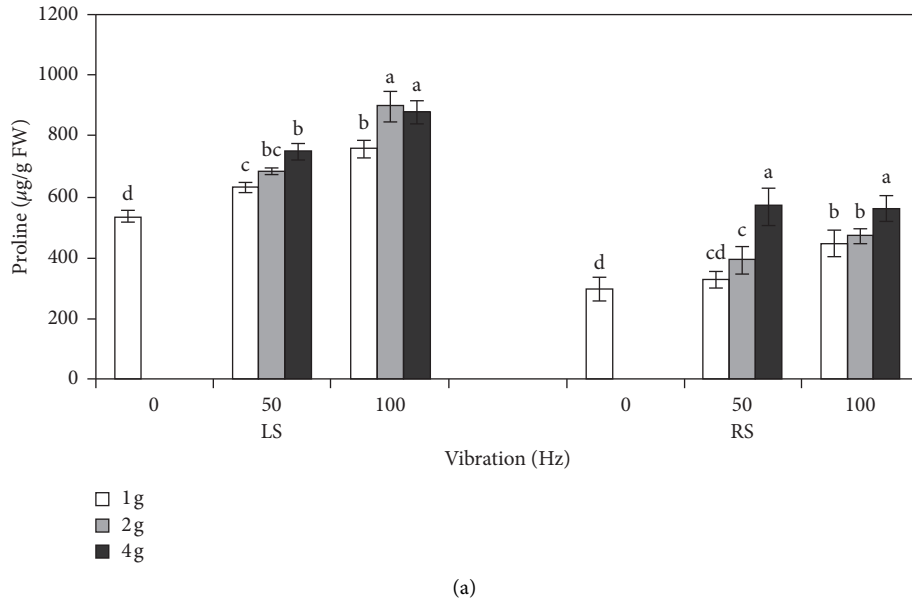
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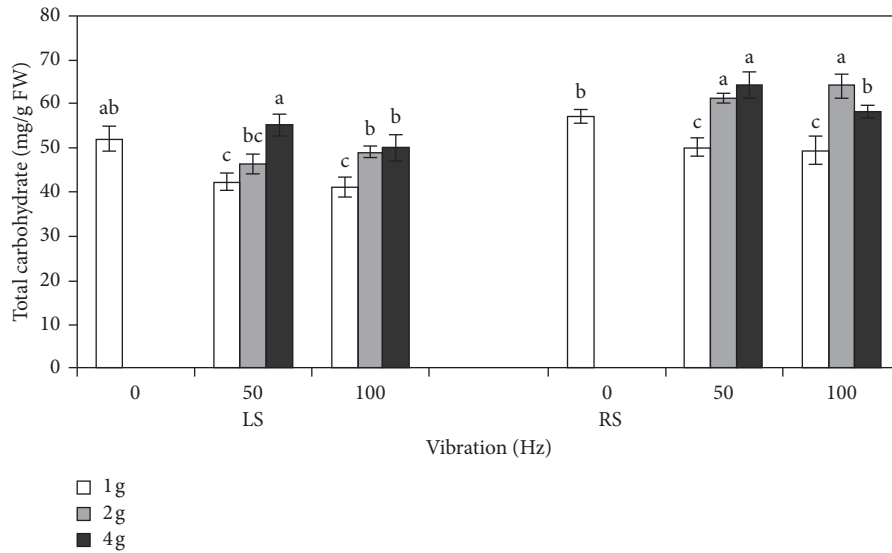
(b)

Figure 2: Effect of different sinusoidal vibrations (0, 50, and 100 Hz) and accelerations (1, 2, and 4 g) on proline (a) and total carbohydrate (b) accumulation of *A. gilanica* calli. Bars indicate mean ± SE ($n = 4$) in each group. Different letters indicate significant differences at $P < 0.05$ (LSD).

induces some signals such as cytoplasmic Ca^{2+} and protein kinases [39, 40], which can have impact on gene expression associated with antioxidant system induction, lipid peroxidation decline, osmoregulation, cell division, and growth [2]. Unlike animal cells, there are no data about acceleration effects on plant cells. In osteoblast cells, high-frequency vibration (20 Hz) with low acceleration (0.05 g) increased cell proliferation and at 60 Hz and 0.13 g induced metabolic activity [13]. Also, bone formation is promoted by low acceleration [10]. High accelerations usually cause a higher force and can shift the resonance frequency of vibration [41]. It seems that an increase of the acceleration level from 1 g to 4 g increases the mechanical resonance of the vibration on cells and decreases protein content and cell growth. On the

other hand, decreased growth by mechanical stress may be due to the disruption of hormone hemostasis in the cells [42, 43].

Proline is a common compound in response to different abiotic stresses [37, 44]. Besides their role in osmoregulation, proline has a key role in stabilizing subcellular structures and scavenging of free radicals under stress. Indeed, proline accumulation can indicate the level of plant tolerance to stress conditions [45, 46]. Also, proline and ROS can facilitate the activation of Ca^{2+} channels along with K^+ channels under mechanical stress [17]. In this research, vibration increased significantly proline accumulation, and with an increase of the acceleration level, this parameter induced especially in LS calli (Figure 2(a)). These results
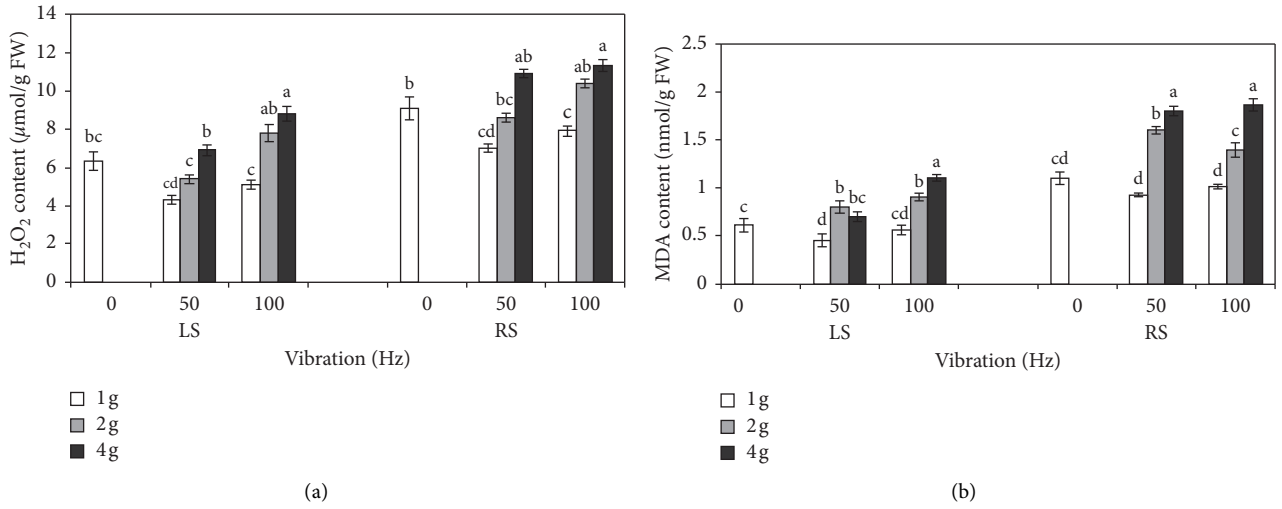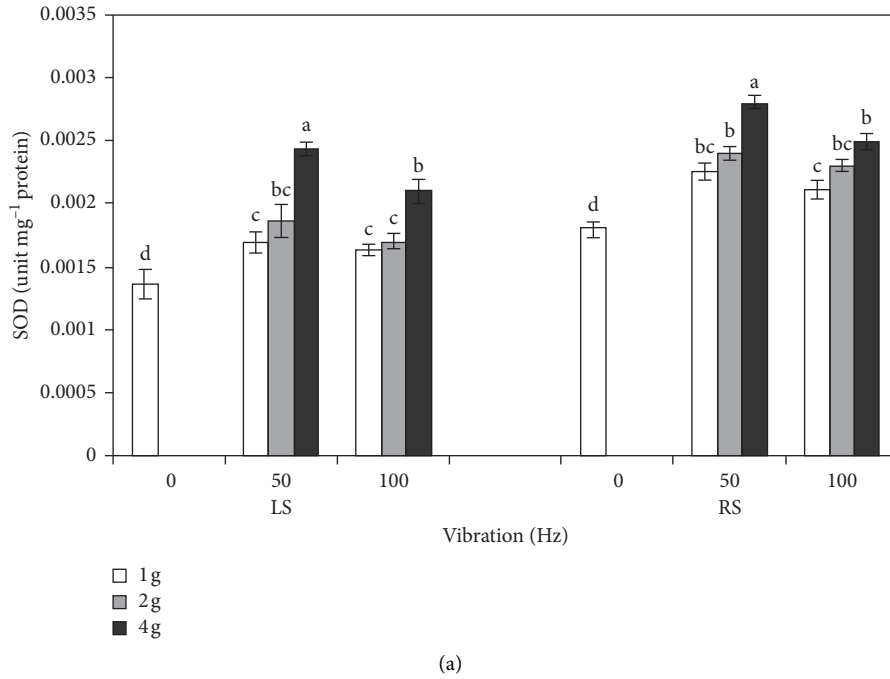
(a)

(b)

FIGURE 3: Effect of different sinusoidal vibrations (0, 50, and 100 Hz) and accelerations (1, 2, and 4 g) on H_2O_2 (a) and MDA contents (b) of *A. gilanica* calli. Bars indicate mean ± SE ($n = 4$) in each group. Different letters indicate significant differences at $P < 0.05$ (LSD).
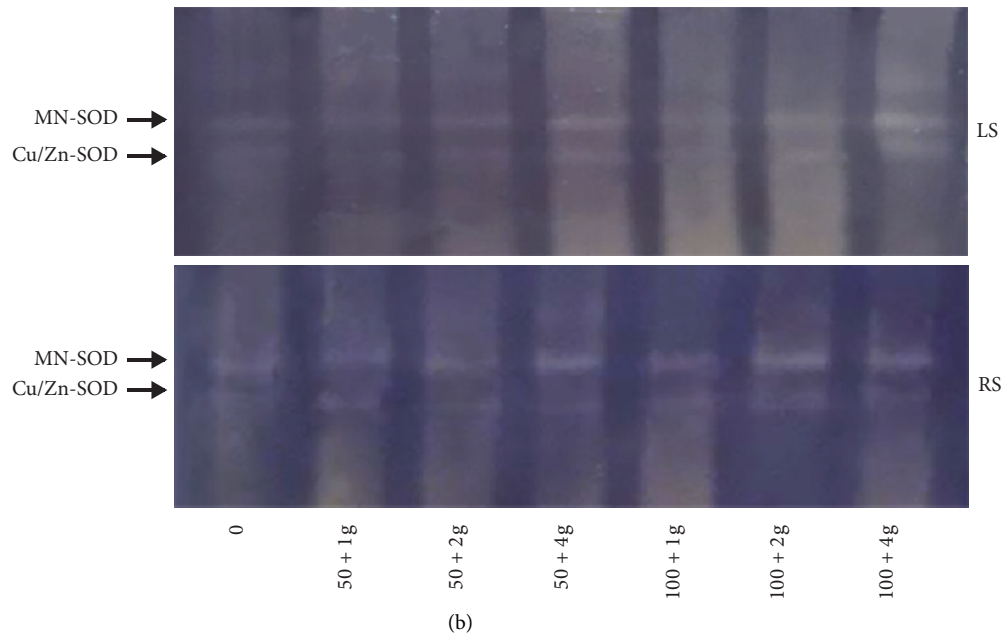

(a)

FIGURE 4: Continued.

FIGURE 4: Effect of different sinusoidal vibrations (0, 50, and 100 Hz) and accelerations (1, 2, and 4 g) on the activities (a) and isoform patterns (b) of SOD enzyme in *A. gilanica* calli. Bars indicate mean ± SE (*n* = 4) in each group. Different letters indicate significant differences at *P* < 0.05 (LSD).

suggested that acceleration can act as an osmotic adjustment cause and affects intracellular osmotic pressure. Increased pyrroline-5-carboxylate synthase activity and following proline content have been reported earlier in tobacco suspension cells [37]. It seems that vibration under higher acceleration can induce more oxidative stress than vibration alone, and osmolyte accumulation may relate to stress tolerance in *A. gilanica* calli.

Carbohydrates serve as an energy source under stress and provide carbon skeletons for both catabolic and anabolic reactions. Also, carbohydrates preserve membrane and protein structures and prevent oxidative stress injury [47]. Rolland et al. [48] showed that soluble sugars could activate crosstalk between hormonal signaling and gene expression in plants. In this study, vibration reduced total carbohydrate content at 50 Hz and 100 Hz frequencies, and with an increase of the acceleration level, the carbohydrate accumulation increased in comparison with control (Figure 2(b)). Increased carbohydrate content has been reported early in *Dendranthema morifolium* [49] and *chrysanthemum* [50] under mechanical stress. It seems that an increase of carbohydrates under acceleration may act as a factor in cell protection against oxidative damage. Furthermore, this might have resulted from the increased calli area, which induced light absorption and photosynthetic rate, consequently leading to induction in the overall metabolite contents. On the other hand, enhancement of carbohydrate content aids the *A. gilanica* calli to maintain their water balance and growth in a better way.

Different abiotic stresses lead to the production of more ROS constitutes including O_2^-, H_2O_2, and OH^- in plants [51, 52], and the cellular damages are revealed in the form of degradation of biomolecules such as proteins, lipids, and DNA, which ultimately amalgamate in oxidative stress and cellular death. MDA is the most frequent biomarker of lipid peroxidation that determines oxidative stress in cells [53, 54]. In this research, H_2O_2 and MDA levels significantly decreased under vibration, especially at 100 Hz in both LS and RS calli, and with an increase of the acceleration level, the mentioned parameters increased as compared to vibration alone (Figure 3). The decreased MDA level has been previously notified in *Triticum aestivum* [14] and *Nicotiana tabacum* under vibration [37]. The reduction of MDA content may illuminate that vibration can diminish oxidative damage and maintain membrane stability. There are no data about the effect of high acceleration on plant cell membrane damage. It seems that the reduction of membrane stability in *A. gilanica* calli can be related to higher production of ROS constitutes and following oxidative damage under the high acceleration level.

Higher antioxidant activity is positively associated with an increase in stress tolerance in cells [12, 14]. ROS signals activate several different defense pathways such as antioxidant enzymes in cell organelles. Peroxisomes contain ROS regulatory enzymatic systems including CAT, ascorbate-glutathione cycle, and SOD in both peroxisomal matrix and membrane [55]. SOD is a key antioxidant enzyme to catalyzing of the toxic superoxide radical level to molecular oxygen and H_2O_2, and other antioxidative enzymes such as CAT, POX, and APX complement the process of ROS elimination by transforming H_2O_2 into water and molecular oxygen [55, 56]. In this study, SOD activity increased under vibration, and SOD activity continuously induced with an increase of the acceleration level, especially at 4 g (Figure 4(a)). Band intensity of Mn-SOD and Cu/Zn-SOD also increased at 4 g acceleration (Figure 4(b)). The increased
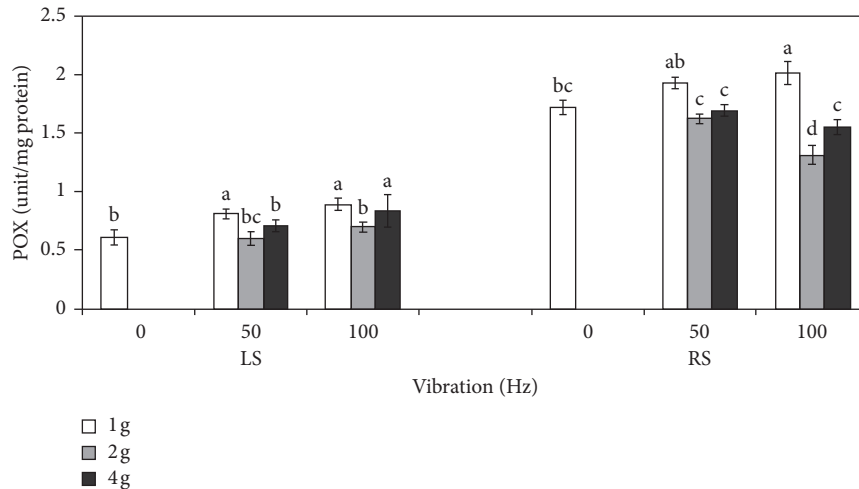
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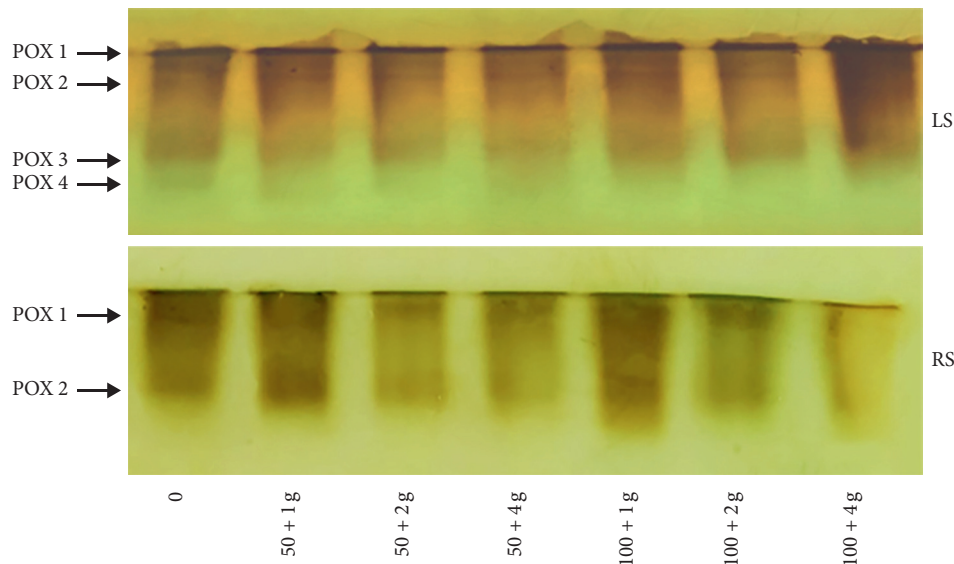
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FIGURE 5: Effect of different sinusoidal vibrations (0, 50, and 100 Hz) and accelerations (1, 2, and 4 g) on the activities (a) and isoform patterns (b) of POX enzyme in *A. gilanica* calli. Bars indicate mean ± SE ($n = 4$) in each group. Different letters indicate significant differences at $P < 0.05$ (LSD).

SOD activity might be considered as an efficient system to protect cell organelles such as chloroplasts from ROS and has been reported in *Actinidia chinensis* Planch. [57], *Triticum aestivum* [14], and *Mentha pulegium* [58]. Hassanpour et al. [36] displayed different SOD isoforms, including Cu/Zn-SOD, Mn-SOD, and Fe-SOD, which are responsible for O_2^- scavenging under vibration. It seems that an increase of acceleration levels together with vibration imposed more stress than vibration alone in *A. gilanica* calli, and cells need more SOD activity for O_2^- radical scavenging.

POX, APX, and CAT are the main enzymes responsible for H_2O_2 scavenging under abiotic stress [59, 60]. Enhanced activity of antioxidant enzymes could protect plant cells against oxidative injury [61, 62]. Our results showed that vibration improved POX and APX activities in both LS and RS calli as compared to the control (Figures 5(a) and 6(a)),

indicating that the antioxidant defense system could keep H_2O_2 at a low level and improve calli growth. An increase of the acceleration level caused an increment of APX activity and a decrease of POX activity under vibration (Figures 5(a) and 6(a)). The intensity of POX1, 2, and 3 bands markedly decreased with the increase in the acceleration level, especially at 4 g, and POX4 also disappeared under vibration (Figure 5(b)). Decreased POX activity and band intensity may show that this enzyme has the low duty or is inactivated under high acceleration and can be considered as one of the causes of growth reduction.

Polyphenol oxidases (PPOs) catalyze the oxidation of some phenols to chinones and produce brown or black pigments in plant tissues. In our research, PPO activity decreased markedly under vibration alone but induced with an increase of the acceleration level in both LS and RS calli
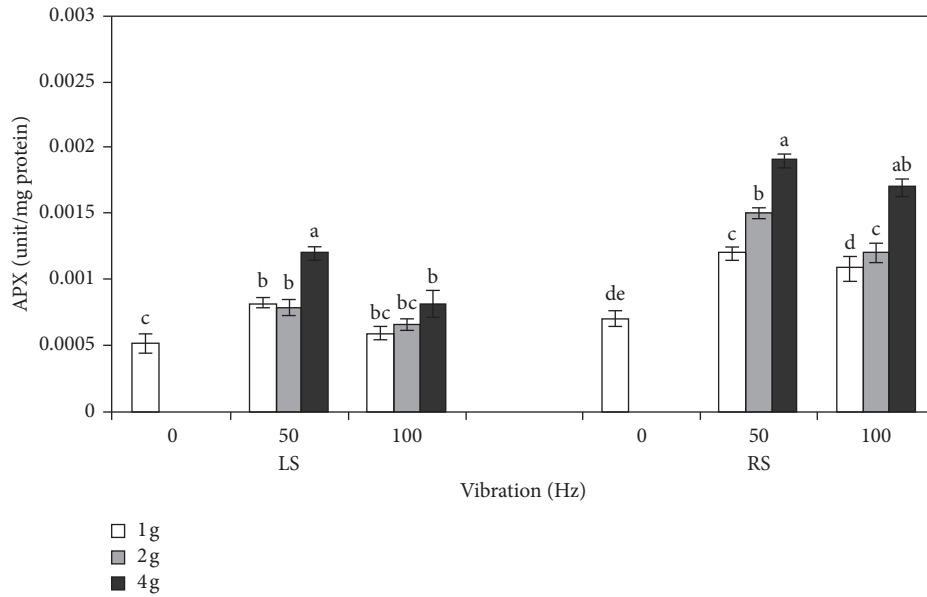
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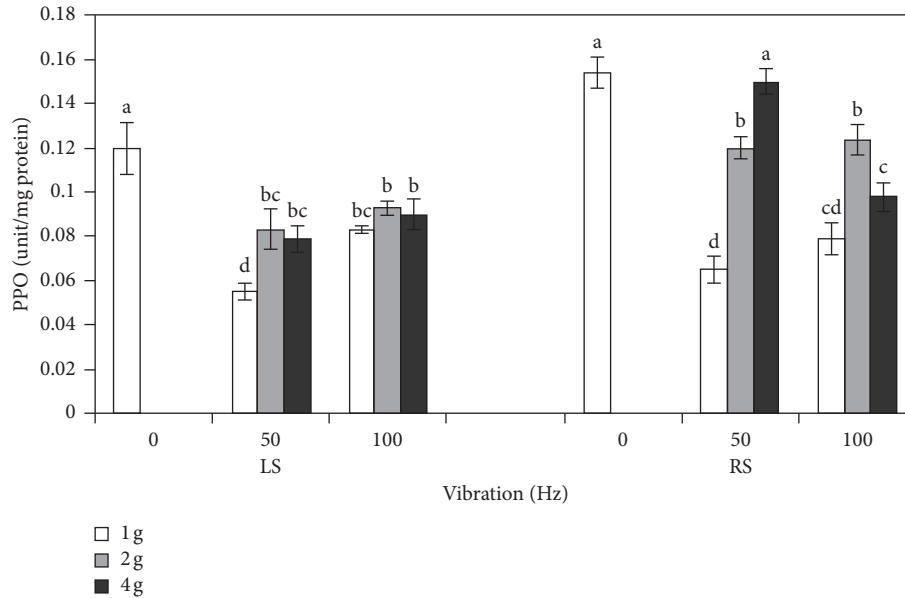
(b)

FIGURE 6: Effect of different sinusoidal vibrations (0, 50, and 100 Hz) and accelerations (1, 2, and 4 g) on APX (a) and PPO (b) activities in *A. gilanica* calli. Bars indicate mean ± SE ($n = 4$) in each group. Different letters indicate significant differences at $P < 0.05$ (LSD).

(Figure 6(b)). In contrast to our results, Wu and Lin [63] showed that ultrasound vibration markedly induced PPO activity and polyphenol production in *Panax ginseng* cells and can be related to enzymatic browning and membrane permeabilization. It seems that acceleration maximized oxidative stress damage, reduced growth, and induced calli browning in *A. gilanica* calli by induction of PPO activity.

5. Conclusions

Understanding of the mechanisms by which plant cells change their defense mechanisms for stress tolerance is still limited. However, the identification of the effective frequency of vibration and acceleration as a new triggering antioxidant mechanism and growth will help advance our knowledge in this field. The increase of the acceleration level decreased growth and protein content and induced browning of the callus tissue, especially at 4 g acceleration in RS calli. Proline and carbohydrate contents, lipid peroxidation, and some oxidative enzyme activities were also induced under high acceleration. Although the effective frequency of vibration can induce some defense responses such as antioxidative enzymes and osmolyte accumulation in callus cells, the presence of high acceleration intensified

the vibration resonance effect on cell organelles by reduction of RWC and concomitant disruption of cell adaptive mechanisms through induction of membrane and oxidative damage. However, there is effective acceleration for induction of growth and defense mechanisms, which needs to be investigated in the future.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

SS participated in the bench experiments and contributed to analyze data. V. N. and H. H. have designed and provided overall supervision of the study and organized the article. All authors read and approved the final draft of the article.

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