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Letter to the Editor

Biochemical and physiological changes in plants as a result of different sonic exposures

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Abstract

The effects of two different sonic exposures on two vegetables, namely Chinese cabbage and cucumber at two growth stages, including seedlings and mature plants were investigated. The 3 h exposures included either 20 kHz sound waves or "green music" that comprised classic music and natural sounds such as those of birds, insects, water, etc. Analysis of variance between groups (ANOVA) was used to determine the appropriate statistics parameters for the different treatments. Both exposures caused significant elevations in the level of polyamines (PAs) and increased uptake of oxygen O_2 in comparison with the controls. For Chinese cabbage the highest PAs' levels were determined for both seedlings and mature plants that were exposed to "green music". The oxygen uptake in Chinese cabbage also increased as a result of sonic exposures, and the highest oxygen uptake was also observed after "green music" treatment. For cucumber, the highest content of PAs for both seedlings and mature cucumber plants was determined as a result of 20 kHz ultrasound exposure. 20 kHz exposure of mature plants also resulted in the highest level of oxygen uptake. No statistically significant differences in the vitamin C level were determined between the different sonic treatments and sham exposed vegetables.

Keywords: Sonic; Vegetables; Polyamine; O_2 uptake; Vitamin C

1. Introduction

In general, there exist relatively few studies describing the influence of music or sound exposure and plants.Although plant's growth after exposure to music and the fact that the "green music" (such music comprises classic music and natural sounds such as those of birds, insects, water, etc.) could promote plants' growth and enhance their infection-resistance to diseases and pests were recently reported [1,2]. However, these works have not attempted to explain the mechanism of such interaction. Therefore, a number of experiments was carried out between 1999 and 2001 to determine the relationship between the exposure of plants to sonic energy and the subsequent plant growth and their insect's susceptibility in South Korea and China. The preliminary results demonstrated that the sonic exposure, particularly to "green music", could increase the yield of the crops and control some insect pests in several vegetables, including Chinese cabbage and cucumber. It was also noted that an exposure to "green music" resulted in the change in the body color of green peach aphids [3,4].

This paper describes the results of a pilot study designed to examine biochemical and physiological changes in selected vegetables, namely cucumber and Chinese cabbage introduced by the exposure to 3 h of "green music" and 20 kHz ultrasound (continuous wave sound).

2. Materials and methods

As a convenient biochemical indicator PAs were selected. Polyamines mainly consist of Put (putrescine, 1,4-diaminobutane), Spd (spermidine), Spn (spermine), Cad (cadaverine), Agm (agmatine), and Dap (diaminopropane). They can be found in every plant and their

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concentration increases in rapidly growing tissues. PAs biosynthesis often increases greatly prior to growth and is related to hormones, level of senescence or ageing and fragility, light and stress. Also, PAs were reported to be able to stimulate and control growth in a number of higher order plants [5,6]. In addition, vitamin C is well known as an important component of vegetables and is considered to be one of the important indices of food quality [10]. One of the goals of our work was to study the influence of external acoustic energy on PAs and vitamin C, so the mechanism of interaction between ultrasound energy and plant tissue could be better understood. Several studies of plant physiology show that a high respiratory rates occur not only in fast-growing tissues, but also as a consequence of environmental stress, e.g. frost, injury or infection, or as an aging phenomenon. Physical injury to higher plant tissues often stimulates oxygen uptake because of increases in respiration (mitochondria-linked oxygen uptake) and in non-mitochondria activities (e.g. lipoxygenase, polyphenol oxidase, peroxidase) [7–9]. Hence, if the respiratory rate is raised during or after a sonic exposure, it is because the plant grows faster or it has suffered a physical injury. A few relevant reports [1,2] and the results of our preliminary research [3,4] indicate that the increase in a respiratory rate of the vegetables caused by the accelerated plants' growth was due to sound exposure. Another goal of our study was to determine whether exposure to external acoustic energy is capable of increasing or decreasing the rate of oxygen uptake in Chinese cabbage and cucumber. The relationship between acoustic exposure and plant growth is complex in that more often than not different vegetables have different responses to acoustic energy. Also, at different growth stages the same plants may have different responses to same acoustic energy. In the following our experimental methods are briefly described and the results obtained are discussed.

The Chinese cabbage and cucumber were exposed in three different greenhouses to "green music" (G.M.), 20 kHz continuous wave ultrasound (U.S.), and natural background sound (control) respectively. The vegetables were exposed to 3 h per day of "green music" and 20 kHz ultrasound in $3\ \mathrm{m} \times 3\ \mathrm{m} \times 2.2\ \mathrm{m}$, vegetable greenhouses. 20 kHz was selected as the exposure frequency because this frequency lies outside that of human hearing and did not represents any annoyance to the farmers working in the greenhouse. Also, 20 kHz frequency lies relatively close to the highest frequency components in the green music spectrum, which makes the comparison between those two exposures easier.

A 200 W tape recorder/power amplifier unit (KGM-60F, S. Korea) with associated loudspeakers was used for green music exposure while 300 W ultrasonic generator (KW-506, S. Korea) was used to produce 20 kHz continuous wave. The generator was capable of generating frequencies beyond 20 kHz and therefore, it was used with a 20 kHz low pass filter. The tape recorder and ultrasonic generator were positioned in the corners of the two greenhouses respectively. The third greenhouse was used as control. Equivalent exposure level (Leq) of the green music was determined to be 75 ± 5.54 dBA (here "75" is the mean value of numerous data of sound pressure level and "5.54" is the standard deviation), and the sound pressure level (SPL) of the CW, 20 kHz ultrasonic wave was kept at the level of 75 dBA. Both measurements were carried out in the center of the greenhouse room. Because the greenhouse volume ($3\ \mathrm{m} \times 3\ \mathrm{m} \times 2.2\ \mathrm{m}$) was relatively small all plants were exposed to a relatively uniform level of acoustic energy.

The exposed vegetables were sampled twice: after 15 days (15 d) when they start to grow the second genuine leaves and after 70 days (70 d), that is when the plant reached its early maturity. As discussed below, analysis of variance between groups (ANOVA) was used to determine the appropriate statistic parameters for each treatment.

3. Results and discussion

In the discussion below, the following notation is used:

- F describes: F statistic, $F =$ (variance between groups)/(variance of random),
- df denotes: degrees of freedom in ANOVA,
- P is probability in ANOVA analysis, e.g. $p < 0.01$ or $p < 0.05$ means the difference between groups is significant, and the level of confidence is over 99% or 95% respectively; "Significantly" means notable difference in statistics between groups,
- d refers to days after exposure.

3.1. Polyamines content

Fig. 1 presents the results obtained for Chinese cabbage: PAs content of 15-d seedlings was significantly higher after 20 kHz and green music exposure than that determined for control (genuine leaf: $F = 538.28$, $\mathrm{df} = 2, 12$, $p < 0.01$; cotyledon: $F = 3720.11$, $\mathrm{df} = 2, 12$, $p < 0.01$; stem: $F = 2183.97$, $\mathrm{df} = 2, 12$, $p < 0.01$). For 70-d mature Chinese cabbage vegetable PAs content was significantly higher after green music exposure than that determined after 20 kHz ultrasound treatment and that measured in control ($F = 1157.28$, $\mathrm{df} = 2, 12$, $P < 0.01$). The primary PAs detected were conjugated spermidines (Spd). Among all three different treatments, the green music exposure also resulted in the highest growth rate and fresh weight.

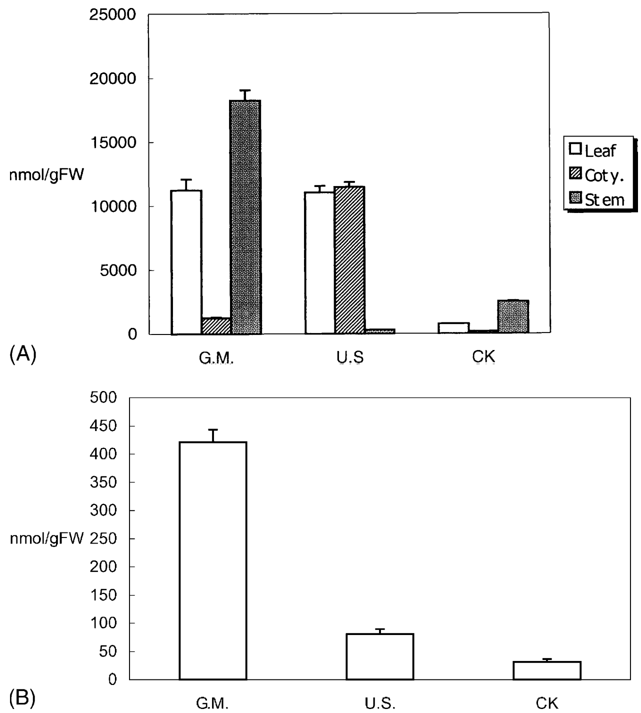

Fig. 1. Polyamine content (nmol/gFW) of Chinese cabbage seedlings: (A) 15 d and (B) mature plant (70 d) as a result of different acoustic treatments. Error bars represent the standard deviations of the means of polyamine contents.

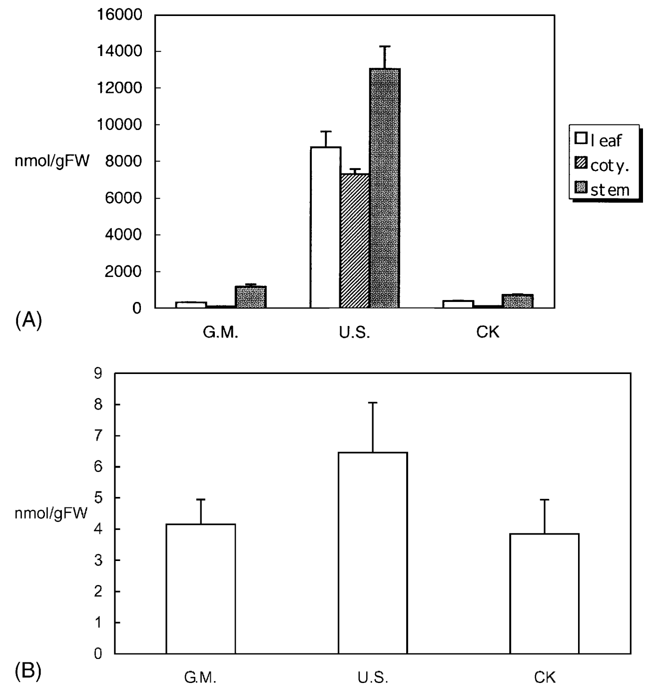

Fig. 2. Polyamine content (nmol/gFW) of cucumber seedlings: (A) 15 d and (B) mature plant (70 d) as a result of different acoustic treatments. Error bars represent the standard deviations of the means of polyamine contents.

In Fig. 2 the results related to cucumber 15-d seedling and 70-d mature plant are summarized. It shows that after the exposure to 20 kHz acoustic wave, the observed PAs content for both cucumber seedling and mature plant was significantly higher than that measured after the exposure to green music and control (genuine leaf: $F = 475.43$, $\mathrm{df} = 2, 12$, $P < 0.01$; cotyledon: $F = 3673.51$, $\mathrm{df} = 2, 12$, $P < 0.01$; stem: $F = 477.63$, $\mathrm{df} = 2, 12$, $P < 0.01$; and mature plant: $F = 7.20$, $\mathrm{df} = 2, 12$, $P < 0.01$). Again, the main PAs detected were the conjugated spermidines (Spd).

The results presented in Figs. 1 and 2 indicate that both green music and 20 kHz ultrasound exposures can promote the vegetables to secrete polyamines, especially Spd (spermidine). PAs level in the sonic treated seedlings, including cotyledon, genuine leaf and stem was determined to be significantly higher than that observed in control or sham samples. Hence, the sonic treated seedlings can increase PAs secretion during they exposure to green music or 20 kHz ultrasound.

The synthesis of spermidine Spd in Chinese cabbage has already been reported [11]. In this context, the results presented here appear to support the notion that sonic exposures, particularly green music exposure enhances Spd syntheses in Chinese cabbage. For this reason, possible further studies should also focus on some enzyme activity in Chinese cabbage, which affects Spd synthesis.

The results depicted in Figs. 1 and 2 also showed that PAs concentration in the seedlings of both vegetables examined was higher than the PAs level measured in mature plants. These results seem to further indicate that higher concentration of PAs takes place in rapidly growing tissues of seedlings. It should also be noted that different vegetables had different responses to the same sonic treatment. Specifically, for Chinese cabbage, the PAs content after exposure to green music was the highest for both seedlings and mature plant. But in the case of cucumber the exposure to 20 kHz ultrasound resulted in the highest PAs level.

3.2. O_2 uptake in three treatments

The influence of the acoustic energy exposure on the oxygen uptake in the treated vegetables is presented in Figs. 3 and 4.

Fig. 3 shows that O_2 uptake in Chinese cabbage treated with green music was the highest among the three exposures described. In this case, the O_2 uptake of 15-d seedlings and 70-d mature plant were significantly higher than that of control (15-d seedling: $F = 19.08$, $\mathrm{df} = 2, 21$, $p < 0.01$; 70-d mature plant: $F = 83.76$, $\mathrm{df} = 2, 21$, $p < 0.01$). This result appears to indicate that Chinese cabbage was more sensitive to green music treatment.

As shown in Fig. 4 cucumber exhibited different behavior: for 15-d seedlings the O_2 uptake was the

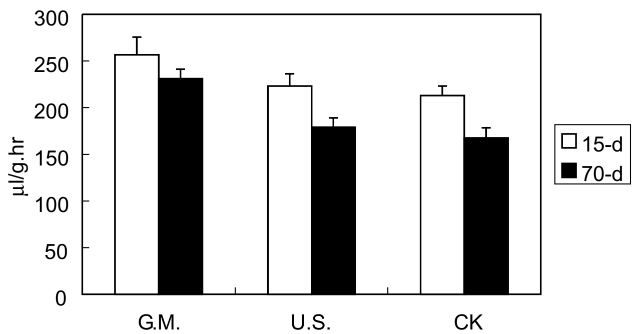

Fig. 3. Oxygen uptake (l/g h) in Chinese cabbage as a result of different acoustic treatments. Error bars represent the standard deviations of the means of oxygen uptake.

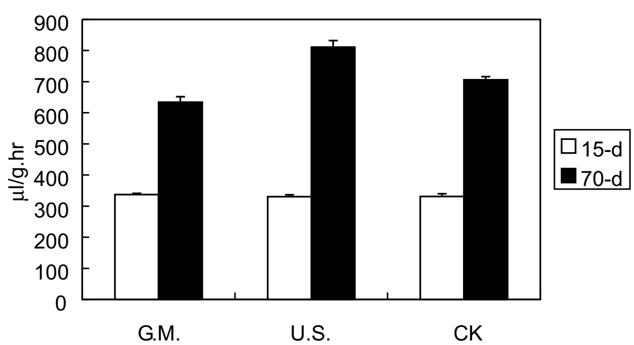

Fig. 4. Oxygen uptake (l/g h) in cucumber as a result of different acoustic treatments. Error bars represent the standard deviations of the means of oxygen uptake.

highest after the green music exposure, whereas 20 kHz ultrasound exposure resulted in the lowest O_2 uptake. On the other hand the results for all three exposure schemes have not shown any statistical significance ($F = 2.18$, $df = 2, 21$, $P > 0.05$). It is interesting to note that for 70-d mature plant the results was opposite: the highest level of oxygen uptake was observed for 20 kHz ultrasound exposure, then control and lastly green music. The O_2 uptake for U.S. exposure has shown statistically significant difference in comparison with that determined for sham and green music ($F = 283.69$, $df = 2, 27$, $p < 0.01$) exposure. The results presented above seem to indicate that cucumber growth at its later stage (70-d) could be influenced by 20 kHz ultrasound exposure, however, they do not offer any explanation why the younger, 15 d seedlings failed to respond to the same exposure.

Overall, the results of this work appear to indicate that Chinese cabbage growth is relatively sensitive to G.M. as the level of PAs and oxygen uptake increased following the G.M. exposure. Unfortunately, the data collected failed to provide more specific information about the relationship between PAs and O_2 uptake. The results of the cucumber exposure showed that this vegetable is more sensitive to 20 kHz U.S. exposure. Based on the outcome of this study it would appear that different vegetables produce different responses to different acoustic exposures.

The amount of the Vitamin C content was also investigated and is briefly summarized in Section 3.3.

3.3. Vitamin C content

The analysis of variance showed no statistically significant difference in vitamin C content as a result of different acoustic exposures examined in this work for both vegetables. For Chinese cabbage (15-d seedling and 70-d mature plant) the highest level of vitamin C was measured after the exposure to green music, then 20 kHz sound and lastly, sham or control. A slightly different behavior was determined for cucumber plants. Here, the highest level of vitamin C was observed following green music exposure, then control and lastly 20 kHz acoustic wave.

These results tell us the content of vitamin C is not easily affected by sound wave energy. This is because in comparison with PAs and O_2 uptake, vitamin C is relatively inactive in plants. Thus, if acoustic energy can promote the growth of some plants (here: Chinese cabbage and cucumber), the more active molecules such as PAs will first be affected. A more quantitative study of the relationship between the sound exposed, sonic-active molecules (like PAs) and other molecules, which are relevant to the crop yield is being prepared.

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