Peroxidase Activity in Cosmos bipinatus Seedling Under Sound Stress

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Abstract : The effect of sound on germination of *Cosmos* was investigated. Four different sound types including Triple gem chanting, sweet word, rock music and dirty pig word were used as abiotic stress conditions. After sterilization, seeds were placed on moisten paper towel in plastic containers under sound treatment for 7 days. Untreated seed experiment was used as control. Results showed difference in seedling growth. Peroxidase specific activity was used as stress marker in this study. The highest peroxidase activity in shoot extract was found in dirty pig word treatment (0.08 units/mg protein) while the lowest peroxidase activity of 0.058 units/mg protein was found in shoot extraction obtained from Triple gem chanting. Root extract from Rock music treatment showed the highest peroxidase activity (0.741 units/mg protein) whereas the lowest peroxidase activity of 0.261 units/mg protein was found in the control experiment. The K_m value for *O*-dianisidine and H_2O_2 obtained from shoot indicated that the rate of H_2O_2 detoxifying of shoots extract obtained from rock music and dirty pig word treatments was higher than other treatment. Optimum temperature and pH of the extract from shoot and root was 30-50°C and 40-50°C and pH of 5-6, respectively. Either shoot or root peroxidase isozyme consisted of POD 1, POD 2 and POD 3. The finding suggests that sound might have potential to be abiotic stress during germination state.

Keywords peroxidase, ROS, oxidative stress, abiotic stress

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1. Introduction

When plants are subjected to various abiotic stresses, some reactive oxygen species (ROS) are produced including superoxide ($O_2^{\bullet}$), hydrogen peroxide (H_2O_2), hydroxyl radicals ($^{\bullet}OH$) and singlet oxygen ($^1O_2$) (Arora et al., 2002). Under abiotic stresses, the extent of ROS production exceeds the antioxidant defense capability. This leads to an imbalance between the ROS production and the antioxidant defense of the cell, causing cells damage and reduction of growth and development. These activated oxygens cause oxidative damage to proteins, membrane lipids and nucleic acids (Apel and Hirt, 2004). Potential oxidative damage can be neutralized by scavenging or changing chemical identity of ROS. Plants have developed a complex antioxidant system both enzymatic and non-enzymatic detoxification systems to neutralize ROS and to protect cells from oxidative damage (Mittler, 2002). ROS scavenging enzymatic antioxidant system includes superoxide dismutase, ascorbic peroxidase, catalase, glutathione peroxidase and peroxidase (Mittler, 2004). Non-enzymatic antioxidants compose of ascorbic acid, β-carotene, flavonoids, α-tocopherols and glutathione (Ahmed et al., 2009). Peroxidases, heme containing enzymes that utilized H_2O_2 in the oxidation of various organic and inorganic substrates, are widely accepted as a stress enzyme (Bhaduri and Fulekar, 2012). Peroxidases play a vital role in various physiological processes, such as lignifications, suberization, auxin catabolism, wound healing and defense mechanism against abiotic and biotic stress. Plant peroxidases exist in many isomeric forms, some of which are constitutively expressed, while others are induced by environmental stresses and have been implicated in a number of cellular processes (Quesada et al., 1992).

Abiotic stress including UV-B radiation, water, high salinity, temperature extremes, mineral nutrient deficiency, metal toxicity, herbicides, fungicides, air pollutants, light, and temperature research area are driven by the hope of improving crop production. To date, the use of music is also in practice to reach the same expectation. However, there is no scientific report to support the benefit of music on agriculture productivity. This research was conducted to investigate the effect of various sounds on plant oxidative stress. This work is only a preliminary research on plant abiotic stress caused by sound treatment. The selected biological stress marker used in this study was peroxidase commonly used as an indicator on plant oxidative stress. The main objective of the present study was to evaluate the potential of sound as abiotic stress through peroxidase activity.

2. Materials and Methods

Plant material

Cosmos seeds were provided from Department of Horticulture, Faculty of Agricultural Production, Maejo University. After surface sterilization in 70% (v/v) ethanol for 3 min, the seeds were thoroughly rinsed with distilled water for 3 times and germinated in paper towel moistened with sterilized distilled water in a plastic container at room temperature in darkness for 48 hours and then germinated for 12 h in warm fluorescent light condition plus 12 h in dark cycle for 7 days. The selected sound of triple gem chanting, sweet word, rock music and dirty pig word at sound

level of 50 decibel were treated with *Cosmos* seeds for 24 hrs a day for seven days. The experiment was conducted in triplicates.

Preparation of crude enzyme

To assess the total peroxidase activity from root and shoot of *Cosmos* seedling, either root or shoot samples were extracted by homogenizing with cool mortar and pestle added with 50 mM sodium acetate buffer, pH 5.4 and addition of sea sand. The homogenate was centrifuged for 30 min at 16,000g at 4^{o}C. The clear supernatant was used for peroxidase activity assay according to Shannon et al. (1966).

Protein determination

Protein concentration was determined according to Bradford (1976) with bovine serum albumin as standard.

Peroxidase activity assay

Soluble peroxidase activity in primary leaves and root of 7-day old seedling were investigated. Peroxidase activity was determined by measuring colour development at 460 nm. The reaction mixture contained 0.1 ml of enzyme extract, 0.05 ml of 0.5% of *o*-dianisidine and 2.75 ml of 0.05 M sodium acetate buffer pH 5.4. The reaction started by adding 0.1 ml of 0.1 M hydrogen peroxide. Colour development was measured by spectrophotometer. When this assay was used, 1 unit of enzyme activity was defined as change in absorbance at 460 nm of 0.1 A unit per minute (1 unit = 0.1 A460/min). The specific enzyme activity was expressed as unit mg^{-1} protein. The K_m determinations for *O*-dianisidine were performed by using 100 µg soluble crude shoot extraction against various *O*- dianisidine concentrations of 0.6, 1.2, 1.8, 2.4 and 3×10^{-3} molar. The reaction was then started by adding 0.1 M hydrogen peroxide. While K_m value of H_2O_2 was determined by varied H_2O_2 concentrations of 0.6, 1.2, 1.8, 2.4 and 3×10^{-3} molar ageists 0.5% of *o*-dianisidine. The K_m values were determined from a double reciprocal plot of enzyme activity and substrate concentration.

Effect of pH and temperature on peroxidase activity

The effect of pH was determined by adjusting the rate of reaction over the range of 3-10 while the effect of temperature was obtained over the range of 30-100^{o}C under standard conditions.

Isozyme analysis

Non-denaturing polyacrylamide gel electrophoresis was performed in 7% separating gel and 5% stacking gel with modified method of Laemmli (1970). The peroxidase gel activity was stained by using *o*-dianisidine (Shanon et al., 1966) and TMBZ as substrates (Cucchi and Basaglia, 1998).

3. Results and Discussion

Protein content

Under abiotic stresses, the extent of ROS production exceeds the antioxidant defense capability of the cell, causing cells damage, growth inhibition and reduction of photosynthetic

ability. Imbalance between the ROS production and the antioxidant defense causes reduction of growth and development and injury of the cellular components of proteins, membrane lipids and nucleic acids. This study has investigated protein content and peroxidase activity under various sound conditions in *Cosmos* seedling. Results showed that the highest protein content in root was found in the control experiment, followed by sweet word and triple gem chanting treatments. In contrast, protein content in root of seedling was low under rock music and dirty pig word treatments (Figure 1). Similar results were also found in shoot which exhibited the highest protein content in triple gem chanting treatment and the lowest one in dirty pig words treatment (Figure 1). Demirevska-Kepova et al. (2004) have shown that after excessive supply of copper and manganese, barley seedling also showed reduction of chlorophyll and protein content. This indicated that sound treatment can cause abiotic stress during *Cosmos* seed germination which leads to the reduction of protein content under unpleasant sound condition.

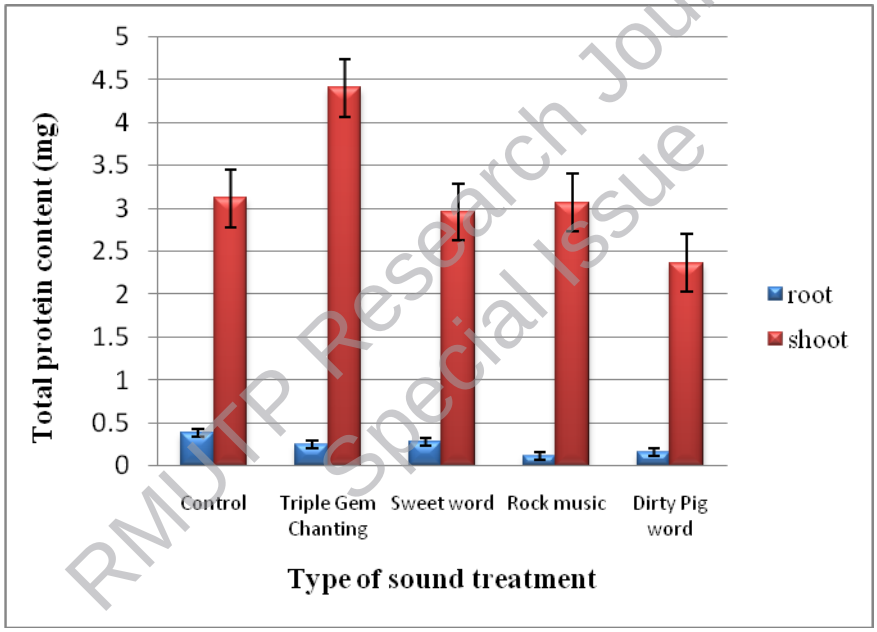

Figure 1. Total protein content in root and shoot of *Cosmos* seedling under various sound treatments. The vertical base indicates standard errors/(n=3).

Peroxidase activity

Peroxidase is an important component of antioxidative defense mechanism as it is responsible for the quenching of toxic H_2O_2. In order to investigate the effect of sound stress on the oxidation of *o*-dianisidine by peroxidase in the presence of H_2O_2, the formation of oxidized *o*-dianisidine was determined at 460 nm. After the germination of *Cosmos* seeds under various sound conditions, the root and shoot extractions were used as sources of soluble protein. Results showed that the lowest specific root peroxidase activity of 0.261 units/mg protein was found in the control

seedlings and the highest interaction between peroxidase and H_2O_2 was observed in rock music seedling treatment with the value of 0.741 units/mg protein (Figure 2). However, there is some points to be noted that seedling of Triple gem chanting and sweet word treatments had better growth rate than others, especially the root length (data not shown). This, therefore, caused the break of root at removal from paper towel. In addition, this evidence also activated peroxidase activity in response to wound healing. As a result, those of relax sound experiments showed higher peroxidase activity than the control treatment.

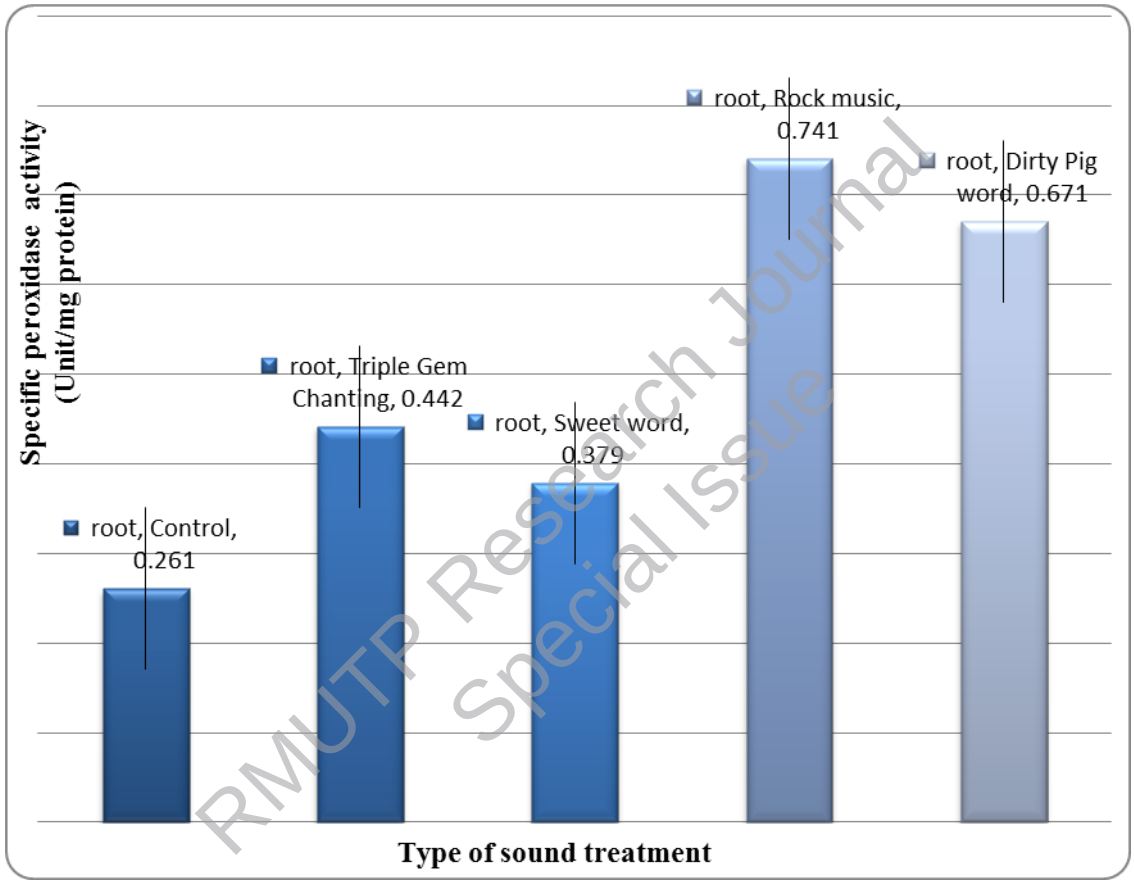

Figure 2. Specific peroxidase activity of root extracted from *Cosmos* seedling under various sound treatments. The vertical base indicates standard errors/(n=3).

On the other hand, the peroxidase specific activity in shoot seedling of the control experiment was higher than those from Triple gem chanting and sweet word experiments (Figure 3). Shoot seedling obtained from dirty pig word and rock music treatments showed high amount of H_2O_2 in their extraction as indicated by high level of interaction of peroxidase and its substrate (Figure 3). The increase of peroxidase activity also indicated a stress marker under abiotic stress in *Phaseolus vulgaris* (Cuyper et al., 2002), *Vigna radilata* (Karuppanapandian et al., 2006), sweet

potato (Kim et al., 2007) *Populus przewalskii* (Lei, 2008), tomato (Ammar et al., 2008), *Ficus religiosa* L. (Smitha et al., 2009) and *Vicia faba* (Zhang et al., 2009).

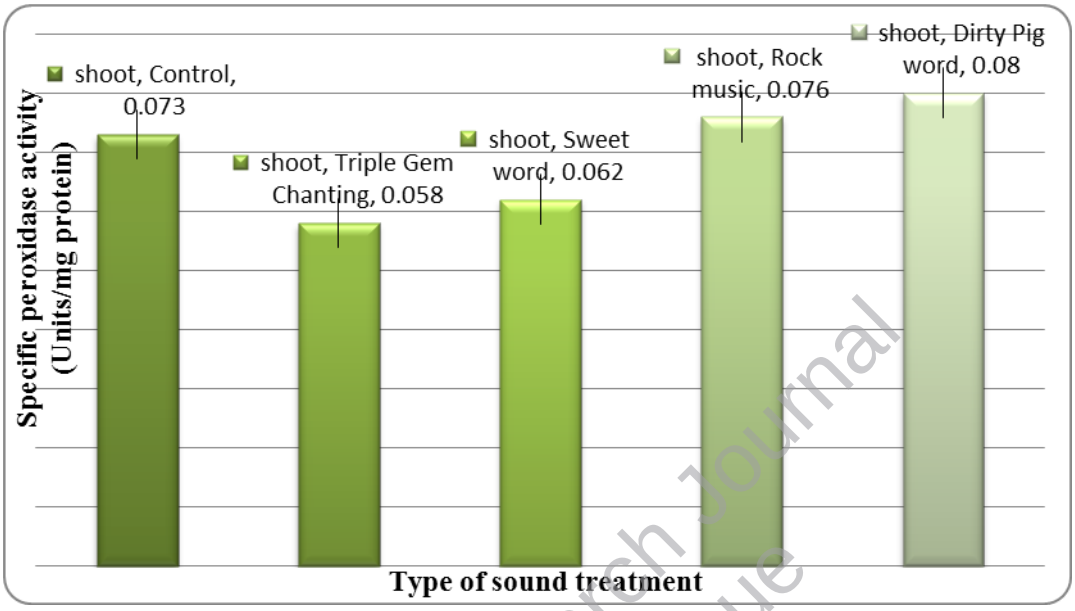

Figure 3. Specific peroxidase activity of shoot extracted from *Cosmos* seedling under various sound treatments. The vertical base indicates standard errors/(n=3).

The preliminary report in abiotic stress under various sound conditions indicated that *Cosmos* seedlings developed their defense mechanism by formation of ROS and created the oxidative stress. Therefore, there were some differences on peroxidase activity between types of sound under investigation as indicated by peroxidase specific activity. In addition, the peroxidase K_m value (catalytic efficiency) of each treatment was also obtained in order to confirm the purpose of this study.

K_m determination of shoot peroxidase

Prior to determining K_m of *O*-dianisidine, peroxidase activity was determined by using constant H_2O_2 concentration at 0.1 molar with various concentrations of *O*- dianisidine 0.6, 1.2, 1.8, 2.4 and 3×10^{-3} molar. The results from Line Weaver Burk reciprocal plot indicated that the best K_m value of 0.116 mM could be obtained from shoot extract of rock music treatment, followed by K_m value of 0.157 mM from dirty pig word experiment (Table 1). The K_m value for H_2O_2 also indicated that the rate of H_2O_2 detoxifying in these two sources was higher than others. This might implied that oxidative stress was ongoing in *Cosmos* cell during germination period under rock music and dirty pig word treatments.

Table 1. Determination of *Cosmos* K_m values under sound treatments

Treatment	K_m for *O*- dianisidine (mM)	K_m for H_2O_2 (mM)
Control	0.239	0.290
Triple gem chanting	0.241	0.240
Sweet word	0.266	0.272
Rock music	0.116	0.182
Dirty pig word	0.157	0.222

Biochemical property of *Cosmos* peroxidase

Optimal temperature for *Cosmos* shoot peroxidase was 30-50°C while optimal temperature of root peroxidase was 40-50°C (Figure 4). The optimal temperature of *Cosmos* was in a range of previously reported peroxidase, such as 40-50°C for cabbage and radish peroxidases, 30-40 for *Allium sativum*, *Ipomoea batatas*, *Raphanus sativus* and *Sorghum bicolor* and 40°C for tobacco (Bania and Mahanta, 2012).

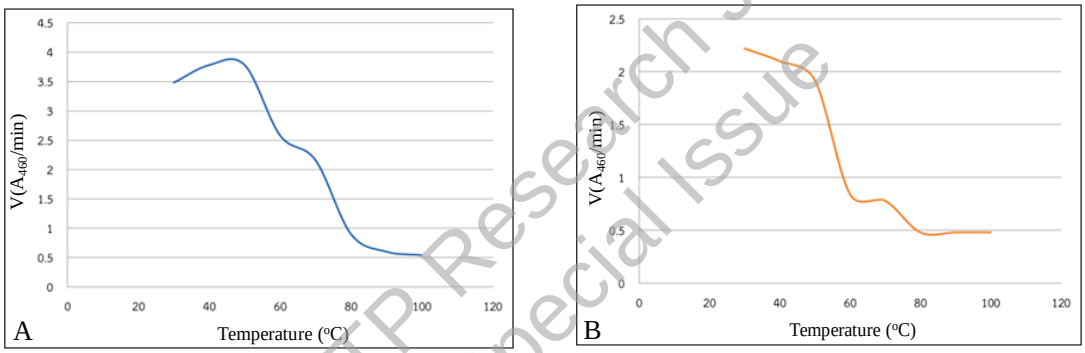

Figure 4 Temperature profile for *Cosmos* peroxidase (A) shoot (B) root

Optimum pH of either *Cosmos* shoot or root peroxidase for the oxidation of *O*-dianisidine was 4-5 as indicated in Figure 5. The optimal pH of several plant peroxidases were between 4-6, such as pH 4.5-6 for corn root (Mika and Luthje, 2003), pH 5 for potato and tomato (Suha et al., 2013), pH 5.5-6 for arracacha (Menolli et al., 2011), pH 6 for cabbage and radish, pH 7 for tobacco (Bania and Mahanta, 2012), and pH 6 for carrot and eggplant (Suha et al., 2013). Peroxidase optimal pH variations might represent efficient regulatory mechanism *in vivo* to shift optimum conditions from one isoenzyme to another and pH preference for different processes (De Marco et al., 1999).

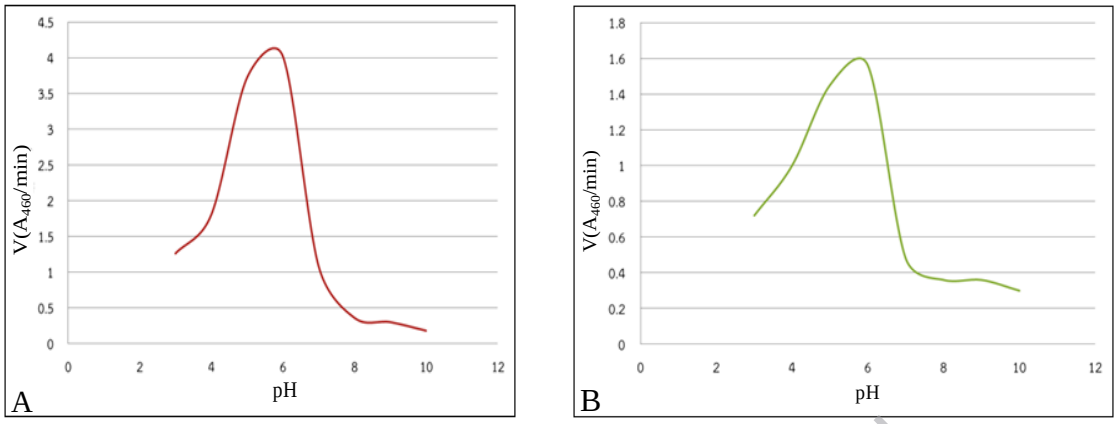

Figure 5 pH profiles for *Cosmos* peroxidase (A) shoot (B) root

Isozyme analysis

Both *Cosmos* shoot and root peroxidase isozyme analysis was revealed by using *O*-dianisidine and TMBZ as substrates. Both staining methods gave an identical profile which indicated that *Cosmos* peroxidase consisted of three isozyme: POD 1, POD 2 and POD 3 (Figure 6 and 7). No new isozyme band appeared for sound treatment samples in comparison to untreated sound. This occurrence was also found in *Phaseolus vulgaris* under copper and zinc (Cuypers et al., 2002) and *Ctenanthe setosa* peroxidase isozyme under dought stress (Terzi and Kadioglu, 2006). In addition, under heat stress, no peroxidase pattern variation in kentucky bluegrass was reported (He, 2010).

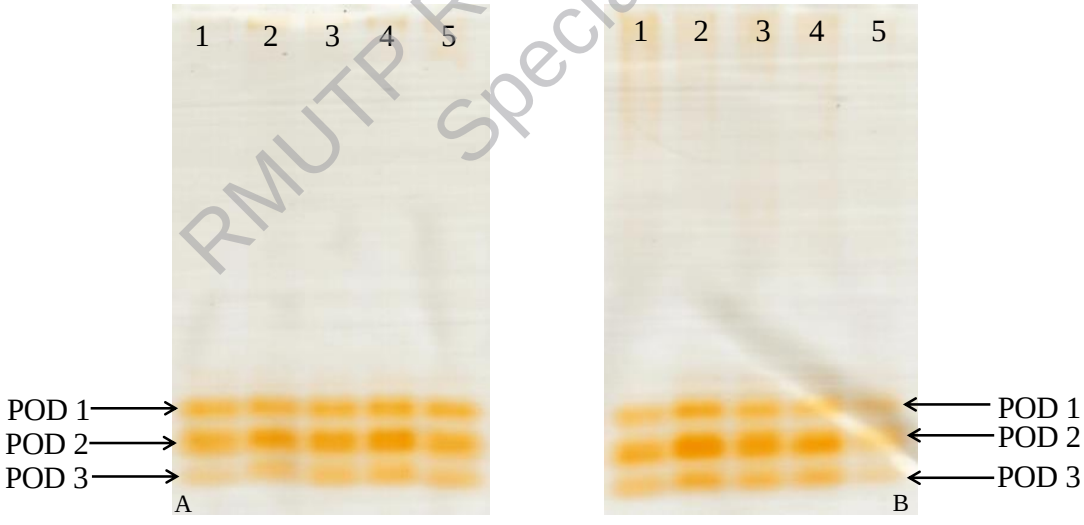

Figure 6 *Cosmos* peroxidase activity staining by using *O*-dianisidine as substrate in (A) shoot (B) root; lane 1 control, lane 2 triple gem chanting, lane 3 sweet word, lane 4 rock music and lane 5 dirty pig word

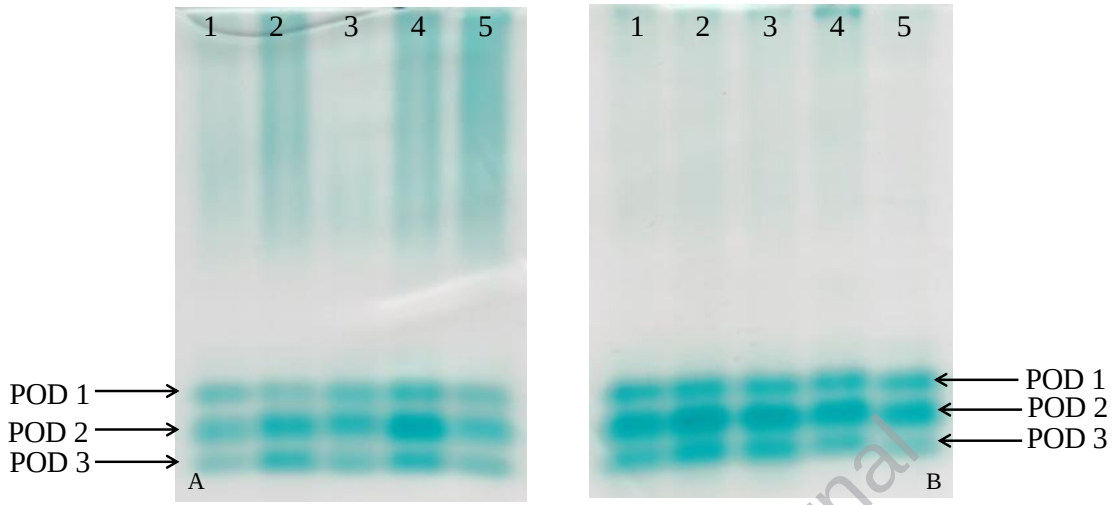

Figure 7 *Cosmos* peroxidase activity staining by using TMBZ as substrate in (A) shoot (B) root; lane 1 control, lane 2 triple gem chanting, lane 3 sweet word, lane 4 rock music and lane 5 dirty pig word

4. Conclusion

The first report in abiotic stress under various sound conditions indicated that *Cosmos* seedlings developed their defense mechanism by formation of ROS and created oxidative stress. Therefore, peroxidase activity of unpleasant sound was higher than normal condition. High level of peroxidase activities on abiotic stress were also found on sulfur dioxide, ozone, and ultraviolet radiation treatments in sweet potato (Kim et al., 2007) toxic concentrations of aluminum in rice seedling (Sharma and Dubey, 2007) low temperature treatment in strawberry leaf tissue (G**ű**len et al., 2008) and NaCl salinity in canola (Bybordi et al., 2010). The differences between peroxidase activity in root and shoot seedling might indicate peroxidase isozyme action on H_2O_2 scavenging. This is also supported by peroxidase pH optima. The different amount of H_2O_2 in each experiment indicated the differences of ROS development among selected sound treatments. This might indicate that sound can be recognized as abiotic stress. However, this study needs to further investigate several plant species and sounds in order to make decision on effect of sound on plant physiology. However, there is no scientific report on effect of sound on plant physiology, especially on productivity. There are uses of music in some orchards in order to gain more productivity. The similar experiment should be further conducted to find the relationships between damage oxygen species and antioxidant enzymes in various sound conditions. This will also be obtained on other stages of plant development.

5. Acknowledgements

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