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Quantitative effect of musical sound on seed germination kinetics in *Pisum sativum*

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ABSTRACT

Germination of seed is a crucial step in a plant's life cycle. It is essential for emergence of seedling and finally producing viable offspring. Germination encompasses a wide number of events influenced by different factors and sound is not an exception. Sound is a vibration that propagates as an acoustic wave. Sound waves with different frequencies, intensities and amplitudes affect different phases of plant's life cycle including germination. Seed germination kinetics, particularly germination speed contributes a major role in the variability of germination. This work is aimed to analyse the effect of musical sound with specific spectral characteristics mainly Detrended Fluctuation Analysis scaling exponent on seed germination kinetics in *Pisum sativum*. Indian Classical music and Natural music was used to stimulate *Pisum sativum* seeds for 72 hours. Germination kinetics was evaluated in terms of Mean Germination Time, t20 and Germination Percentage. Experimental results showed that musical sound can speed up germination process in *Pisum sativum*. Extremely high statistically significant differences were observed in germination kinetics after musical sound treatment ($p<0.0001$). This new work will be valuable for improving germination performance in *Pisum sativum.*

Key words : *Acoustical complexity, Musical sound, Detrended fluctuation analysis scaling exponent, Spectral characteristics, Germination kinetics*

Introduction

Sir Jagadis Chandra Bose is one of the eminent Indian scientists in the field of physics and plant physiology. From his work we came to know that plant responses to sound stimuli along with other external environmental factors (Bose, 1902; 1926). Sound is a vibration that propagates as acoustic wave. The fundamental properties of acoustics are production and transmission of sound including its biological and psychological effects. Sound frequency less than 20 Hz is known as infrasound and greater than 20 kHz is ultrasound. The sound frequency range in between this two is audible sound. Musical sound is one kind of audible sound and is nothing but sounds arranged in an orderly sequence to produce a unified and continuous composition. Musical sound has its own spectral characteristics along with specific pattern of scaling or self-similarity which leads to a complete distinctive pattern in their Detrended Fluctuation Analysis (DFA) scaling exponent (α).

Plant starts life cycle with seed germination. Seeds are very much essential in rebuilding the pro-

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ductivity of a crop.Germination of seed is very complex phenomenon and controlled by different intrinsic and external factors. There are some scientific evidences showing the effects of sound waves on seeds and plants, but majority of these works deal with subsonic and ultrasonic sounds affecting the cellular and genetic level (Suslick, 1989; Joersbo and Brunstedt, 1992). Very little work has been done with audible sounds specially how seeds and plants are affected by music, but these studies did not look at acoustical complexities of musical sound.

As germination is one of the most important stages of the life cycle in plant, the present study aims to investigate the quantitative effect of musical sound with specific spectral characteristics on sprouting of *Pisum sativum*. *Pisum sativum* is a cool season vegetable crop with high nutritional value.It is widely consumed in India as sprouts and pulse as well and economically very demanding. The short life cycle of pea enables us to do more repetitive experiments.

Materials and Methods

Seed materials

Pisum sativum seeds (production place: Kolkata, India) with 12% moisture content were used for the experiment. At first the cracked, crushed and discoloured seeds were removed. The smallest as well as the largest seeds were also removed to maintain the uniformity of size. After that the sorted seeds of necessary number were counted for the experiments. As we cannot detect split or cracked seeds till the seeds soaked in water, an additional 15% seeds remain prepared for the experiments. Next, the seeds were rinsed in 1% bleach solution for 30 minutes for removing fungus and mold on the seeds. After that seeds were washed ten times in running water. Then the seeds were soaked in water for a long time and were placed in petri dishes. Before use the petri dishes were also rinsed in bleach solution to reduce contamination. Then the petri dishes were labelled. Music treatments were performed after imbibition period.

Characteristics of music input

Music is composed of a complex time series comprising some fundamental elements of physics - pitch, rhythm, dynamics, timber and textures, but in real life music is something much more than that. Music is a mode of communication between human beings as well as between other living creatures. For the present study Indian Classical music (based on Raag Bhairavi) and Natural music (with sounds of song birds, insects, water, wind etc.) were used. Two audio clips (Indian Classical music and Natural music) of 3 min each were taken. All the signals were digitized at the rate of 22050 samples/sec 16 bit format. Each three minutes signal was divided into three equal segments of 60 seconds each. This was done to see the change of complexity in each time window for each clip. The DFA scaling exponent was calculated for each of the time window of 60s. DFA method is used to determine the self- affinity of a signal and the value is expressed as DFA scaling exponent α. The scaling exponent gives an estimate of the amount of long-range correlations (LRTC) present in the time series data. Spectral analysis including waveform, spectrogram and power plot were done for both music clips to represent the frequency and amplitude of musical sound versus time.

Experimental design for music treatment

To understand the effects of musical sound on seed germination, Indian Classical music and Natural music stimuli was used to *Pisum sativum* seeds as audio signal during the germination period. The study was based on randomised control design. Experiments were conducted in three groups: Indian Classical music treatment group, Natural music treatment group, and control group. Identical growth chambers were used for each group. Each growth chamber had an identical speaker inside. The difference between conditions was whether the musical sound was on (treated) or off (control) and what musical sound was played through the speaker. Music was continuously played using music player and the player continuously replayed for a period of 72 hour through the speaker by adjusting the volume at 80 dB. The pieces of music and volume of the pieces remain constant throughout the whole experimental period. Four replicates for 20 seeds were used for each trial. Experiments were repeated four times. Watering was done in such a way that the seeds were neither over-watered nor under-watered. The environmental conditions of the test room (continuous dark condition, temperature 26 ± 2 ^{o}C, and humidity 66 ± 6%) remain same for all the treated and untreated sets of seeds.

Germination Tests

The germination tests were carried out in our experiments. A seed is an embryo enclosed in a seed testa which contains necessary nutrients for sprouting. After imbibition in water the seed swells and testa splits, which are the indications of seed germination. When the embryo axes protruded 2.0 mm, the seed was considered as a germinated seed. The number of sprouted seeds was counted every 12 hour intervals over 72 hour long runs. Throughout each trial seeds were also photographed in their petri dishes. Germination kinetics was evaluated in terms of Mean Germination Time (MGT), t20 and Germination Percentage. MGT is an index for germination speed.t20 is the time taken to reach 20% of total seed population.Germination percentage indicates the viability and potential of a seed. The MGT and Germination Percentage were calculated as follows:

$$\text{Germination percentage} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds}} \times 100 \quad (1)$$

$$\text{Mean Germination Time} = \frac{\Sigma\,(nt)}{\Sigma N} \quad ..\,(2)$$

Where, n is the number of newly germinated seeds at time t, t is the germination time of newly germinated seeds, and N is the total number of germinated seeds.

Statistical Analysis

Data were analysed by using one-way and two-way analysis of variance (ANOVA). At P<0.05 level, the treatment effects were considered to be significant.

Results and Discussion

DFA and Spectral analysis

As discussed earlier that each music has specific spectral characteristics along with a distinct pattern of self-similarity. DFA scaling exponent (α) quantifies self-similarity and correlation properties of time series. The DFA scaling exponent of Indian Classical music and Natural music was calculated and the corresponding α value was 1.592 and 0.502 respectively. These values indicate that Indian Classical Music had higher long-range temporal correlation (LRTC) compared to Natural Music. Spectral analysis of Indian Classical music and Natural music were presented in Figure 1. The figure indicates the differences between Indian Classical music and Natural music from different perspective, mainly the changes of amplitude over a certain amount of time (waveform) (Figure 1a), the strength or loudness of the music signal over time at various frequencies of a particular waveform (Spectrogram) (Figure 1b) and the intensity or power level of the music signal on a logarithmic scale (Power plot) (Figure 1c).

Effect of musical sound on germination of pea

Figure 2 shows visual differences in early germination stages of *Pisum sativum* seed. Seeds exposed to musical sound sprouted faster than the untreated

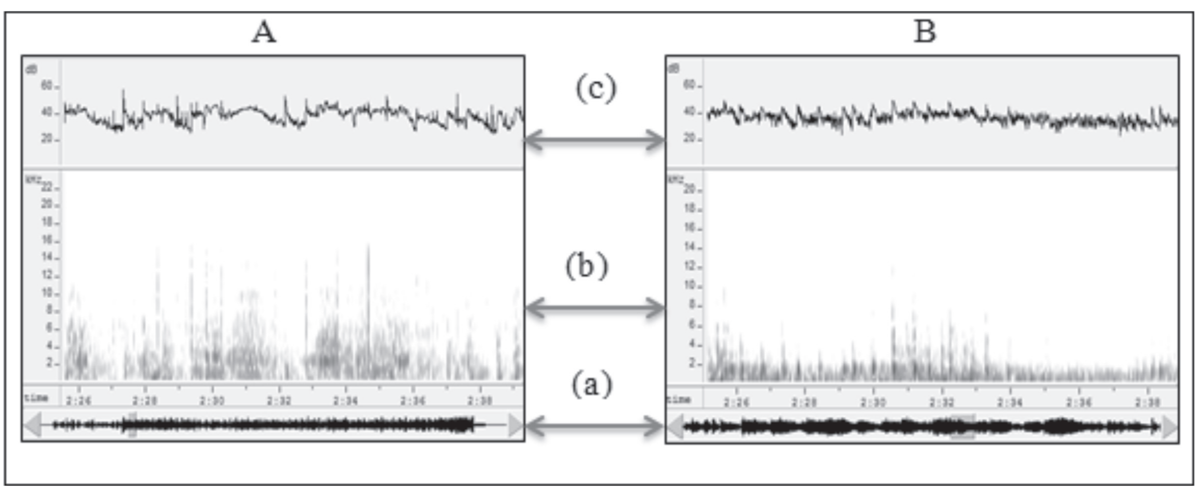

Fig. 1. Spectral analysis of A. Indian Classical Music and B. Natural Music showing (a) wave form (b) Spectrogram and (c) Power plot

control. Figure 3 shows the photographs of control and two different musical sound treated *Pisum sativum* seeds sprout after 48 hours (trial 4, Replicate 1). Significant differences have been observed in seed germination parameters among the three different seed groups. MGT was determined for all the treated groups and relative control groups. The Indian Classical music treated seeds germinated with a lowest MGT of 42.41 hours, Natural music treated seeds showed 45.33 hours of MGT and the untreated control group germinated with highest MGT of 52.20 hours. The Indian Classical music and Natural music treatments produced significant ($P<0.0001$) decreases of 18.75% and 13.16 % in MGT compared to the control. The t20 was also reduced after music treatment. Figure 4 shows plots of the percentage of seeds sprouted versus time of both the music treated groups and control group averaged over all replicates of four trials. The percentages of germinated seeds were significantly higher after music treatments at different assessed time (24 hr, 36 hr, 48 hr, 60 hr, 72 hr) ($P<0.0001$).

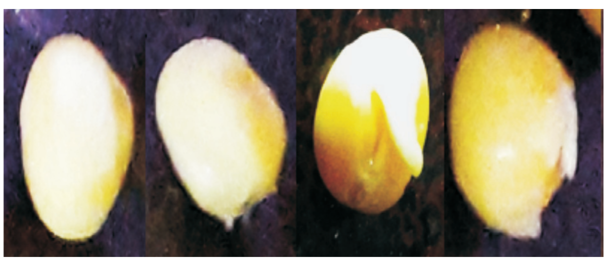

Fig. 2. Visual differences in early germination stages of *Pisum sativum* seed

Throughout the experimental period the performance of Indian Classical music was better than Natural music. These findings are in congruence with the findings of Creath and Schwartz (2004) who reported that music exposure had significant effect on number of sprouted seeds. The germination rate was significantly increased after music treatment when compared to untreated control. The results of the present study are also similar with Vanol and Vaidya (2014); Roy Chowdhury and Gupta (2015) as they stated that musical sound exposure showed faster sprouting. The results are in accordance with outcomes of Takahashi *et al.* (1991); Uchida and Yamamoto (2002), Wang *et al.* (2003) who investigated that audible sound with specific frequencies and intensities significantly increased the germination rate and other germination indexes in different plant species. Increased germination rate along with reduced MGT was also revealed by Chuanren *et al.*(2004) and Cai *et al.* (2014). For the first time the present study revealed impact of DFA scaling exponent on MGT. Interestingly a lower MGT was associated with higher DFA exponent (Figure 5). Researchers have already investigated the critical role of DFA in predicting climate change (Ivanova and Ausloos, 1999), revealing organization of DNA nucleotides (Peng *et al.*, 1994), understanding brain dynamics (Banerjee *et al.*, 2016) and many more.

The results of this study revealed that musical sound can promote the speed of *Pisum sativum* seed germination by increasing germination capacity and reducing germination time and these effects are very much music specific.

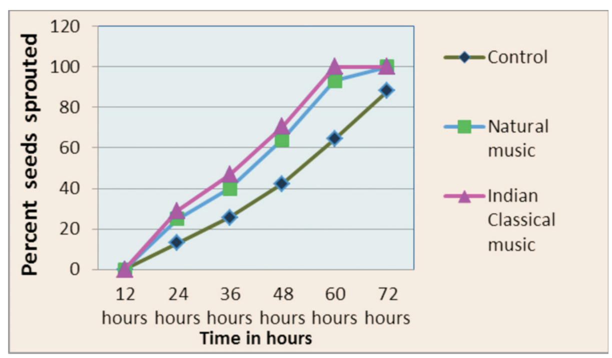

Fig. 4. Percentage of seeds sprouted versus time for Indian Classical music, Natural music and control averaged over all experimental trials.

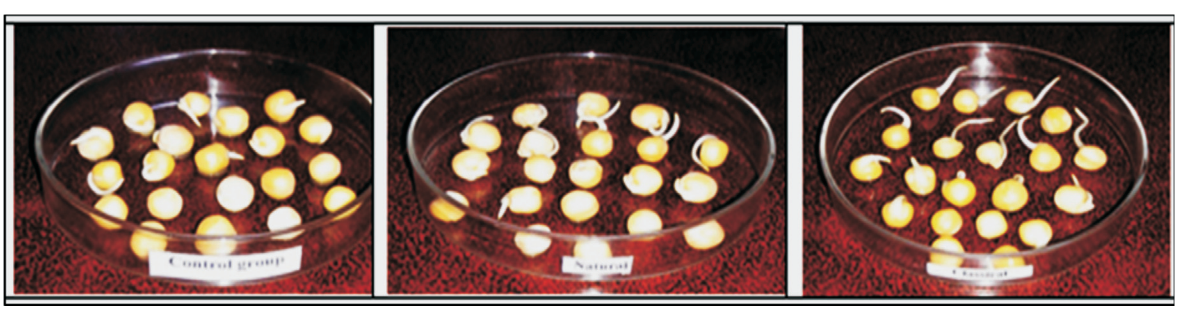

Fig. 3. *Pisum sativum* seeds sprout after 48 hours

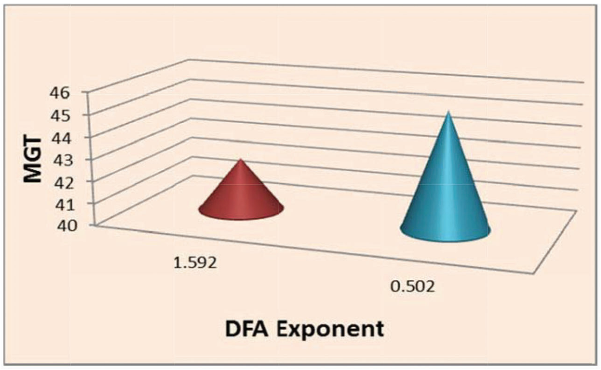

Fig. 5. Graph showing effect of DFA Exponents on MGT of *Pisum sativum*.

Conclusion

The domain of response of seed germination to audio signal specially to musical acoustics is still unexplored. We present here a serious attempt to ascertain quantitatively a contribution of the musical sound of different acoustical complexities on seed germination. It will not be irrelevant to mention that no serious work has been reported in this particular field by any physicist and botanist till date. It is inferred from this study that definitely musical sound plays an important role in *Pisum sativum* seed germination kinetics. Not only this, musical stimuli of different acoustical complexity has distinguishing effects on seed germination as well.This work has interesting possibilities in biophysics for improving seed germination.

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