Research Article
Application of Bio-speckle Activity to Assess Seed Viability

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Abstract: This study presents an assessment method for seed viability, using bio-speckle technique. Bio-speckle is caused by moving of the biological material under highly coherent light. If this phenomenon can be measured by successive speckle patterns during the period of germination, it is possible to identify different activities of the seeds. Viable and non-viable *pisumsativum* seeds were illuminated by a helium-neon laser source of 7mW with wavelength of 632.8 nm. The speckle patterns were recorded by a digital colour charge-couple device camera and stored in the host computer for further analysis using Matlab. Two methods were used to obtain information of biological activities from these speckle patterns. It was observed that the seeds activities can be distinguished as viable seeds and non-viable seeds. The results indicate that bio-speckle can be used to assess seed viability.

Keywords: Bio-speckle, dynamic speckle, laser speckle, seed viability, viability assessment

INTRODUCTION

Bio-speckle is a phenomenon that organisms show a peculiar and dynamic granular appearance under highly coherent light. This phenomenon is caused by movements of the organisms, changes in the refractive index and rotatory power during the period of the detector working. Bio-speckle patterns contain valuable information of the organisms and this technique can be used in organism detection, e.g., detection of the seed viability. The major advantage of this technique is that it is non-destructive.

Bio-speckle was proposed as a new type of seed vigor test method in 1999 (Arizaga *et al*., 1999). Applying this method, it was proved that the moisture of the seeds affects the values of the bio-speckle pattern and such impact is low when moisture is less than 20% wet basis (Braga Jr *et al*., 2003). In 2005, previous work was extended in the detection of fungal contamination in seeds (Braga Jr *et al*., 2005). The bio-speckle technique has wide application in other fields, e.g., imaging blood flow of rodent brain (Cardoso *et al*., 2011), monitoring the maturation of fruits (Ansari and Nirala, 2013; Baranyai and Zude, 2009; Romero *et al*., 2009; Zdunek *et al*., 2007) and detecting the damage to fruits (Pajuelo *et al*., 2003).

Many qualitative and quantitative methods were developed to evaluate the activity of the speckle patterns. Inertia Moment (IM) method is based on the calculation of the second co-occurrence matrix in the time historyof the speckle patterns. Generalised Differences (GD) method can be applied to show the activity of the sample. Laser Speckle Temporal Contrast Analysis (LSTCA) method proposed by Liis to calculate the radio of average value of the speckle pattern to its standard deviation (Li *et al*., 2006; Nothdurft and Yao, 2005). Wavelet transformation is introduced by Bragefor biological feature isolation using bio-speckle laser image (Rabelo *et al*., 2011; Passoni *et al*., 2005; Braga Jr *et al*., 2007). Beside these methods, others have been used in the analysis of the speckle patterns, e.g., weight generalised differences (Arizaga *et al*., 2002), difference histograms (Fernández *et al*., 2003), fourier transform (Sendra *et al*., 2005), fuzzy mathematical morphology (Blotta *et al*., 2013), principal component analysis (Ribeiro *et al*., 2014).

As a new technique, it is important to investigate the relationship between the bio-speckle phenomenon and germination-associated biophysical and biochemical changes. As discussed in previous work, bio-speckle was regarded similar to tetrazolium text. For the advantage of its non-destructiveness, bio-speckle technique can be used during all the processes of germination for samples. It is developed to detect whether or not a seed germinate in the period of water uptake when seeds can be dried again. The seeds produce differing activities in different stages of germination. A curve of the intensity change of the bio-speckle patterns can be used to improve the accuracy of seed viable assessment.

The objectives of the present study are to:

- Investigate if bio-speckle, a fully noninvasive technique, can discern the speckle pattern of highly viable and dead seeds upon water uptake.
- Explore the biochemical basis of varying surface movement in the seeds.

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- Develop a method that can distinguish the viable seeds before germination.

THEORETICAL BASIS

Different types of the experiment samples show varying characteristics of bio-speckle activities. While some present either fast or slow changes in the bio-speckle phenomena and some bear no change at all. The following two methods are introduced to characterize such phenomena.

Method: To show the time evolution of the bio-speckle, successive speckle patterns are registered. The area of every seed in the successive patterns is selected to build a new image. This resulting image is Time History of the Speckle Pattern (THSP). The rows of THSP represent different points on the speckle pattern and the columns represent intensity change of a specific point at an interval. The activity of the sample occurs as intensity changes along the rows.

The activity of bio-speckle is related to the sharp of a horizontal row in the THSP. When the activity of the speckle phenomenon is low, the THSP shows horizontally elongated shape. When there is no activity, the THSP shows no variation in the horizontal direction. As expected, when the phenomenon is active, the intensity variations of THSP are fast.

The co-occurrence matrix which used to value the THSP, is defined in Eq. (1):

$$M_{co} = [N_{ij}] \tag{1}$$

The intensity value i and j are an individual point in consecutive speckle patterns and N is the number of occurrence of i and j in a series of speckle patterns. This matrix is usually used to characterize the texture of the image. In the spatial case, its principal diagonal is related to homogeneous regions and the non-zero elements far from the principal diagonal represent high contrast occurrences.

When the intensity does not change, the only nonzero values of this matrix belong to the principal diagonal. As shown in the experiment samples, intensity values change over time and the number N outside the diagonal increases.

To quantitatively measure this, the co-occurrence matrix needs to be normalized first (shown in Eq. 2). Each row of this matrix is divided by the number of times that the first gray level occurred and the sum of each row equals to 1:

$$M_{ij} = \frac{N_{ij}}{\sum_j N_{ij}} \tag{2}$$

The normalized matrix is an approximation to the transition probability matrix between intensity values in the THSP. The spread of this normalized matrix can be obtained by the sum of the matrix values multiply by the squared of the distance to the principal diagonal.

The moment of inertia IM is the second-order moment of the matrix with respect to that diagonal in the row direction, as shown in Eq. (3):

$$IM = \sum_{ij} M_{ij} (i-j)^2 \tag{3}$$

The occurrences in the diagonal do not contribute to the increase of the IM value, while the occurrences of the non-diagonal elements add more weights to the values of IM.

GD method: The differences between nonconsecutive frames are taken into account by assigning each pixel in the bio-speckle patterns a value $GD(i, j)$:

$$GD(i, j) = \sum_k \sum_l |I_k(i, j) - I_{k+l}(i, j)| \tag{4}$$

The number k and l are indices spanning all the possible numbers in the consecutive patterns and this procedure is called Generalized Differences (GD). Every value $I_k(i, j)$ is subtracted from all other values in the same location, so the result is not affected by the order of appearance of $I_k(i, j)$. GD values depend on the activity of bio-speckle patterns. When all $I_k(i, j)$ values are not change obviously, the GD value is low. When the $I_k(i, j)$ values are distributed as the time changes, the GD value is high. The GD pattern constructed from $I_k(i, j)$ can be used to show the activities of different regions. Further, GD method can lower the noise efficiently, which usually occurs in one speckle pattern. So, GD method has the advantage of identifying relevant information.

MATERIAL AND METHODS

As shown in Fig. 1, the experiment equipment consists of a low-power (7mW) He-Ne laser (632.8 nm wavelength), a digital color Charge-Coupled Device (CCD) camera and a host computer. A low power laser was used as the light source to minimize the effect of the irradiation on sample activities. The acquired patterns with a resolution of 900×600 pixels were digitized to 8bit (256 grey levels) and stored 256 patterns with the frame rate of 15 fps. The speckle patterns are stored one time for each hour in one day and these speckle patterns were processed in Matlab.

Pisumsativum seeds are the experimental samples, which were submitted to standard germination tests for quality control. A total of 80 seeds are divided into two
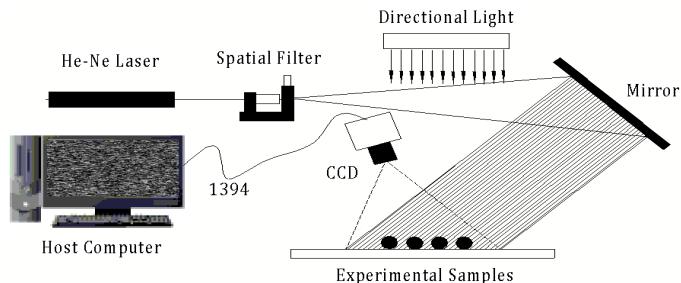
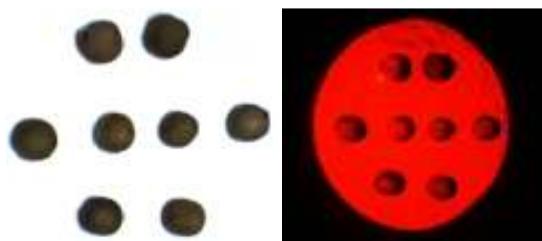Fig. 1: Illumination and image capture experimental system

Fig. 2: Visible pattern and speckle pattern recorded during the experiments

Fig. 3: The seed area in the speckle pattern

categories (40 seeds each type), namely viable seeds and nonviable seeds, for different treatments. The visible type seeds were stored at 5°C for 3 days and the nonviable type seeds were treated at 100°C for 3 days. Root lengths were recorded 5 days after sowing so that the bio-speckle phenomenon can be quantified.

The seeds were put on filter papers in Petri dishes with12-cm diameter. Enough space between adjacent seed is ensured so that individual seeds did not interfere. Distilled water thermostatted of 24°C was injected into the dishes and the same level of the filter paper moistening was maintained till the end of the experiment.

Figure 2 shows the visible and speckle pattern recorded in the experiments. The visible patterns were used to ascertain the seed area and the speckle patterns were used to calculate the variance of the samples.

RESULTS AND DISCUSSION

The variance analysis was presented for both methods: IM and GD method. The values in both methods represent the intensity of seed activities.

The variance analysis of the IM method for the viable samples and non-viable samples was performed. As shown in Fig. 3, the seed area was first applied the image segmentation method before THSP was used. The THSP and M_{co} that calculated by IM method is shown in Fig. 4. At the same time, the IM values of all the samples were recorded.

It was found that there were differences between the mean values for the two kinds of samples. As shown in Fig. 5, the difference of the average values for
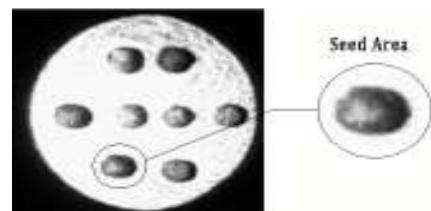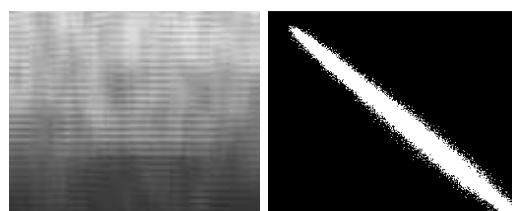

(a) THSP of the seed area   (b) M_{co} of the seed area

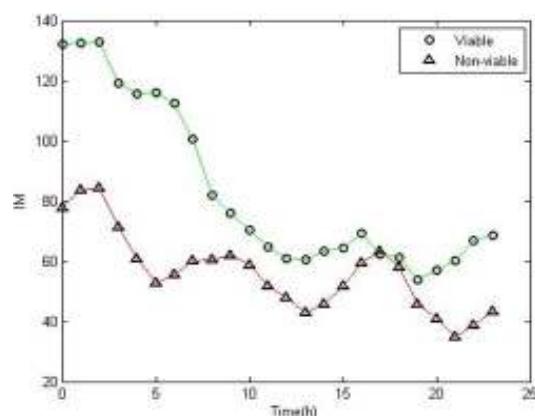Fig. 4: THSP and M_{co} of the seed area in speckle pattern

Fig. 5: The IM values of viable and non-viable samples

the two types is significant in the first ten hours. In viable samples, the IM values remain at high level for few hours and then dropped sharply. The curve of the viable samples reached a minimum at 14 h and the value showed no significant difference beyond that point. Compared with viable samples, non-viable samples reached the balance point earlier. The curve of the non-viable samples shows a small peak which did not occur in the curve of the viable samples. This is
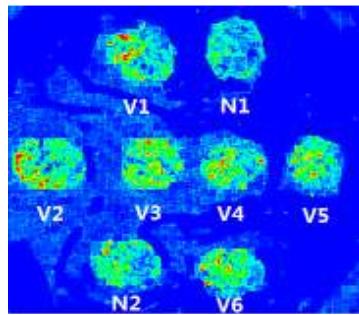
Fig. 6: The pseudo-colour image after illumination and image processing by GD method
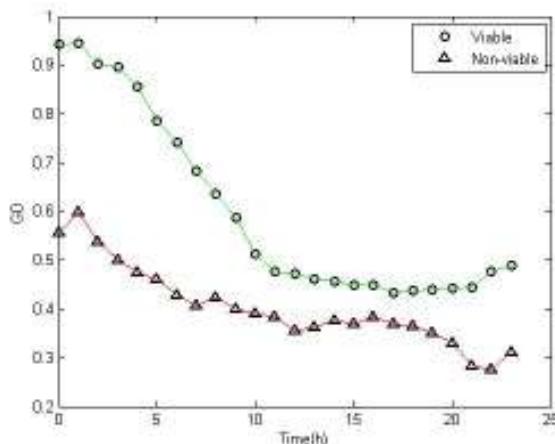

Fig. 7: The GD values of viable and non-viable samples

caused by the change of seed capsules of non-viable samples. The non-viable seeds capsules have a better water permeability. This change leads to an increasing movement of the samples during the water up take period. A threshold of moisture content is needed for a seed to germination. The seeds below this threshold failed to germinate. So, the viable seeds show more activities for a prolonged period of time. After the period of water uptake, the difference of the activities between viable seeds and non-viable seeds is nearly constant. This demonstrates that the IM method can be used to detect the viability of the seeds.

As shown in Fig. 6, the result of GD method is displayed as the pseudo-colour image. In the pseudo-colour image, the red areas represent for higher activity regions and the blue areas represent for lower activity regions. The type of the samples is determined by the germination text. The V samples denote viable and the N samples denotes non-viable. A significant difference between the two is the average of the activity for all sample areas.

The GD value is related to the number of the speckle patterns. To normalize the results, GD value is divided by a particular parameter and it is convenient to measure when the result is between zero and one. Figure 7 show that, the result of GD method is similar to the IM method, but the curve of GD method is more

smoothly. This is because the noise in adjacent patterns is reduced by the GD method. It can be concluded that the GD method can be used to detect the viability of the seeds.

In conclusion, results indicate that the bio-speckle technique can be used as a methodology to evaluate the viability of seeds. The application of bio-speckle in viability assessment is the main objective of this study, but this technique could be further applied to characterize other processes in biological samples. It is critical to determine the meaning of the activities in a certain biological phenomenon before applying bio-speckle technique. Hence, further research in this direction is needed.

CONCLUSION

Bio-speckle technique was applied to assess seed viability and the result of bio-speckle experiments can be measured by IM method and GD method. The results from the two methods show the consistency. This analysis shows that it is possible to distinguish the viable and non-viable seeds.

More data of the extensive experiment is required to validate the proposed method for assessing seeds viability. Bio-speckle technique is a simple, inexpensive and fast detection method. It is a promising method in the viability assessment for various agricultural specimens.

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