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Bio-speckle Method for Garden Pea Seed Nondestructive Classification

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Abstract. Bio-speckle is a non-destructive method applied widely in detection of seed viability.This paper explored the application of the Generalized Difference Method(GD),a bio-speckle image processing algorithm,in classifying garden pea seed viability quantitatively and qualitatively.The experiment was conducted for recording the gray-scale images of the bio-speckle images obtained by illuminating the active materials with laser light.The GD value calculated by THSP figure could depict the tendency of the seed viability.The experiments prove that the method can effectively improve the classification results of garden pea seed obtained by aging chamber at different levels.

1.Introduction

Bio-speckle is based on optical phenomenon, occurring during illumination of the sample by laser light (or coherent light). In the case of biological samples, the intensity distribution diagram evolves and fluctuates in time. In recent years, the technique is widely used in agricultural, biomedical fields.

In agriculture production, the growth and development of the seed directly affects crop yields and economic benefits. Therefore, the detection of seed viability has become one of the highly focused issues. In recent years, a large number of researchers detect and evaluate the quality of the seeds by traditional methods. For instance, ATP Determination, Enzyme Activity Determination, Glucose Metabolism Determination, Respiration Rate Determination, Conductivity Method, Germination Rate Determination, Freezing and Accelerated Aging Method. Although these traditional testing methods can accurately and intuitively predict the seed viability, these methods also have flaws, including heavy workload, poor reproducibility, long measurement period, environmentally sensitive, causing injury to the seeds, etc. By contrast, bio-logical speckle is gradually developed into a convenient and effective new technology to detect the viability of seed because of its non-invasion, non-destruction, and real-time testing. Thus it grows a series of modern analyzing algorithm of bio-speckle pattern, such as Generalized Differences Method (GD Method), Inertia Moment Method (IM Method), Laser Speckle Contrast Analysis Method(LASCA Method), Temporal Contrast Method(TC Method), Fujii Method.

Briers created the bio-speckle measurement techniques by applying biological speckle technology into animal and plant tissues in 1970s.Then the research on seed vigor based on bio-speckle has became popular since Roberto A. Braga proved that it can be used in seed detection through his experiment in 2001.In 2002, Yiwen Ma also certified bio-speckle is a kind of effective method for seed test by contrast the speckle image of dormant and germinating bean seed [1]. In 2003, Robeto A. and Braga Jr. used GD method shown the differences in bio-speckle images among different vigor level of seeds clearly and then they studied how the humidity effected the experiment by using IM method. They also detected whether the bean seed contain fungus or not one year later [2].In 2011, Rabelo proved that the IM value is different between healthy bean seed and the fungal one by using IM method and frequently analysis. Arturv also studied the bean seed and confirmed the fungal bean has different IM value in 2014 [3]. Margarita Fernández calculated the limits value to distinct living and dead seeds by analyzing THSP figure using Difference Histogram method. The dead and living areas of maize seeds have been distinguished by the mathematical model designed by Peisi Wang and Kun Bi using bio-speckle method combined with Generalized Difference Method [6].

Because of the wide application of bio-speckle technique, the five methods cannot meet production demand, as a result, there have been many studies about improved and innovative methods. A fast LASCA method which was proposed by Bries could reduce image processing time. Another innovative LASCA method aimed to noise reduction was found by Dunn, called SLASCA. The

LASCA method with a better flow velocity value and accuracy was analyzed by Le called tLASCA.

IM method is the most widely used one among the five methods. The main step of IM method is to obtain IM value from THSP figure. In 2011, Braga RA, Silva BO assumed a method with a better result than IM method, called AVD. Braga also proved that the AVD value is better than IM when the THSP figure lacking of high-frequency data [4]. Besides, another alternate value named WE witch is more accuracy for low-frequency data was found by Passoni I and Dai Pra A.Except algorithm optimization, there are many other optimizations for those factors which affect bio-speckle experiment. Yuan analyzed the relationship among sensibility, noise and the exposure time of CCD camera. How the different CCD exposure time affects the experiment was studied by Choi [12]. Smausz used static scatter to find how light source affect the result. Voiler putted up a method to reduce the noise.

Although there are many advantages for bio-speckle method and the accuracy of this technology has been developed with depth study, its shortcomings still remain. Lacking of uniform standard for all kinds of seeds classification is a primary problem. That is because the bio-speckle phenomenon is complex, different light source, moisture of samples and the slightly shake of equipment, which will affect experiment results. Besides, the bio-speckle data redundancy also adds a degree of difficulty to the experimentation process.

According to above information, we cannot put the bio-speckle method to every kind of seed. More actual experiments should be performed to evaluate the feasibility of seed viability detection. Next, the paper will be aimed at the application of the Generalized Difference Method in the detection and classification of garden pea seed viability.

2.Material and Methods

2.1 Equipment

The equipment consist of laser light, spatial filter, reflector, CCD camera and computer (Fig.1).

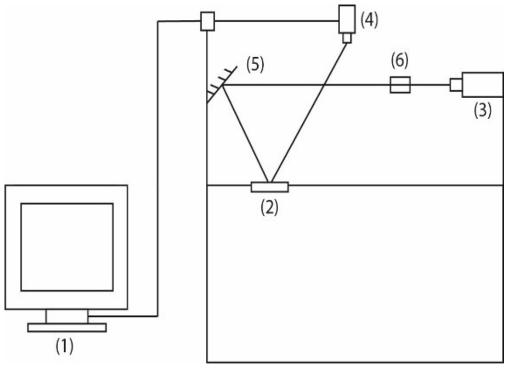


Fig. 1 The bio-speckle equipment

(1)computer (2) samples (3)laser light (4) CCD camera (5) reflector (6) spatial filter

Samples was illuminated by reflected light of He-Ne red laser beam (SPL-HN7.0R, 632.8nm, 7mW) which through a spatial filter (GC0-01M) and two consecutive polarizers (GCL050003) to keep a suitable light intensity. Then the videos of 512 frame were collected by CCD camera, and the 512 bio-speckle images (1280×960 pixels) were obtained by one video (Fig.2). The gray-scale images of them were used in the experiment. The changes of these 512 pictures can be described by a GD value, and the values of different times can use to depict the tendency of the seed viability.

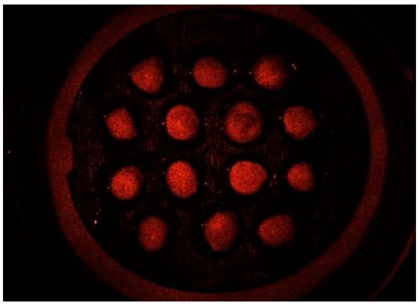


Fig.2 the bio-speckle images obtained by CCD camera

2.2 Seed sample

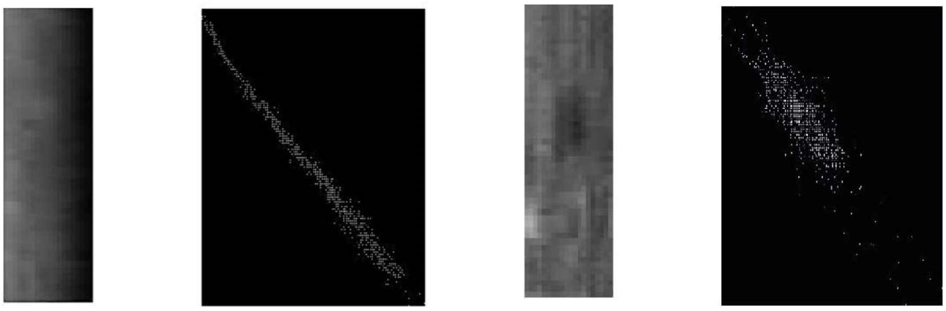
250 seeds were used in this experiment and 150 of them were baked in ovens under 60°C for 3, 6, 9 days respectively, in order to acquire 3 levels of seed viability. 50 seeds were baked in ovens under 100 ºC for 3 days as dead seeds. Eventually, these seeds were divided into 5 levels (dead, alive, 3, 6 and 9 days aging) and each of levels contained 50 samples. Then the experiment began in an environment of 25°C. Samples were placed in the dishes which should injected water (25°C) twice a day to keep the filter paper wet. The videos were collected from 9:00 am to 21:00 pm every hour in the first day and then only two videos at 9:00 and 21:00 pm were needed in the last few days. The experiment was continued 5 days until the seeds geminated.

3. Data Analysis

3.1 Time History of the Speckle Pattern (THSP)

The THSP figure consists of one row from all the bio-speckle images (Fig.3a, 3c). Fig.3a shows the THSP figure of a low viability level seed, and a row of 20 pixels was selected in one bio-speckle image and 50 images were included. Fig.3c shows the THSP figure of a high vigor level seed, the obtaining method is the same as Fig.3a. As can be seen in the two THSP figures, the intensity of the figure has connection with the dynamic of the bio-speckle image. The low vigor HTSP figure changes slowly and the high one opposite. In order to express this kind of changes, the co-occurrence matrix of THSP can be used, it can reflect this relationship intuitively (Fig.3b, 3d). Compared two figures, the distance of the white points away from the main diagonal reflect the seed vigor. The larger distance is, the more vigor of the seed is.

GD is a value calculated by THSP figure, it can be used to express the sum variation of THSP.



(a) (b) (c) (d)

Fig.3 THSP figure and the co-occurrence matrix image

(a)THSP figure of a low vigor level seed. A row of 20 pixels was selected in one bio-speckle image and 50 images were included. (b)The co-occurrence of the (a) seed. (c) THSP figure of a high vigor level seed. Obtaining method is the same as (a). (d) The co-occurrence of the (c) seed.

3.2 GD method for bio-speckle image analysis

THSP consist of one row from all the bio-speckle image, but sometimes, the whole information of THSP pictures are needed in the experiment. Therefore, the variation of all THSP pixels are statistical analysis as:

$$D_G(S_P) = \sum_{i=0}^{n-1} \sum_{j=i+1}^{n-1} |x_i - x_j| \tag{1}$$

DG is the final value of GD method, i and j are different times. |xi - xj| means that the difference of the same THSP point in different time.

Different biological viability levels lead to changes in bio-speckle image, and the superposition of these changes reflect the seeds viability. GD value is the quantization of this superposition, the result shown that GD image will more light if the biological more activity (fig.4).

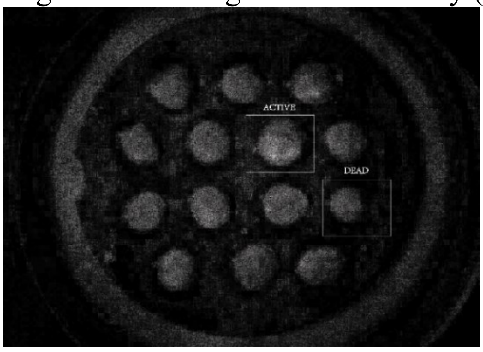


Fig.4 Different parts in GD image

4. Result and Discussion

Five levels were selected based on samples root length (no roots-level 5, 1~4 mm root length-level 4, 5~9 mm root length-level 3,10-15 mm root length-level 2, 11-16 mm root length-level 1), and the average bio-speckle values were calculated in every viability level.

Fig.5 presents the result of GD methods. In order to analysis more clearly, the bio-speckle value was normalized within 100 and processed with B-spline method.

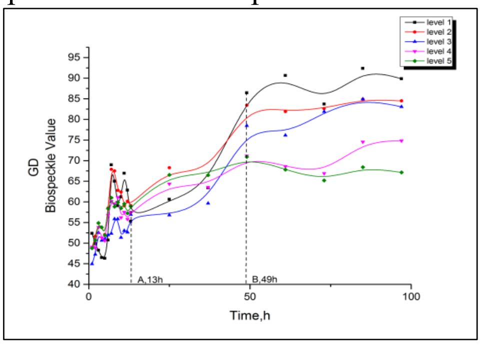


Fig.5 the curve of GD methods of 5 seeds vigor value

It is clear that, the bio-speckle value is nonlinear relationship with time, only an upward trend preformed and the low vigor curse perform a low GD value. Before the point A, the GD values change dramatically and erratically, this is mainly because the seed coat uptakes water and swelling in the beginning. The property causes every level seeds show the strong vigor and cannot be distinguished.

Then the changes become gently until point B, the five vigor levels can be basically distinguished after 49 hours.

The experiment shows that GD method can be used in garden pea seed nondestructive classification, but it is important to pretreat the seed coats in the light of the water uptake and swelling property. Besides, the coat of garden pea seed sticks the body tightly, it is hard to separate them at the beginning. 49 hours are needed to draw a conclusion in the experiment, a shorter time will be wasted without the seed coat.

Summary

This paper demonstrates that GD is a useful method for garden pea seed nondestructive classification.The GD value calculated by THSP figure could depict the tendency of the seed viability and could effectively improve the classification results. In the experiment,it is hard to separate the coat of garden pea seed and the body at the beginning.Pretreating the seed coats according to the water uptake and swelling property can shorten the test time.

Acknowledgments

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