DATA MANAGEMENT FOR EVALUATING GERMINATION POTENTIAL OF COATED SOYBEAN SEEDS USING THERMAL IMAGING TECHNIQUE

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Abstract: *Soybeans are coated before planting to protect from pests during germination. Though different coating materials have been suggested for protecting soybeans such as benzyladenine, calcium, antimicrobial agents, food additives, ozone water treatment and chitosan but it is important to provide information on effectiveness of these materials. A study was undertaken to use thermal imaging technique for developing a germination predicting model for soybean crops. A greenhouse trials were conducted on 15 sets of experimental units with twenty five seeds in each unit. Two commercial polymers such as Precise seed finisher 1010 and SeedWorxAgShine Blue were used a coating for this research. Thermal images were acquired with an ICI 9640 infrared camera with a spectral range between 7µm to 14 µm and physical data were collected every day measuring the root and shoot length with a digital caliper. The thermal signatures were established and it was found that polymer coated seeds resulted in increasing the soybean sprouts health by imbibing water faster. The thermography has helped to identify the hypocotyl and radicle organ of germinated seeds with constant temperature difference. It was concluded that the temperature difference between seeds in the initial stage of the swelling process during germination gives a direct measure of the seed germination potential with the aid of thermal imaging.*

Keywords: *Thermal Imaging, Data Management, Soybean Germination, Polymers Coating*

1. Introduction

Soybean suffers attacks of various pests that result in considerable crop losses during germination and growing steps [1] [2]. Different coating materials have been suggested for protecting soybeans such as benzyladenine [3] calcium [4], antimicrobial agents [5] [6], food additives [7], ozone water treatment [8], and chitosan [9] [2]. However, none of these materials have been used successfully, and there is still need to do more research with different kind of coating materials. Successful coating for soybean seeds will have many other advantages other than protecting the seeds from pests. It can protect the soybean seeds during transportation and handling, and during the planting operations while improving the soybean seed appearance. Gesch et al., [10] demonstrated that using polymer-coated seed reduces the risk of poor soybean emergence in conservation tillage. It has been proposed that seed coatings can protect the seeds such as soybean when planted in cold, wet soils [11]. Zeng et al., [2] demonstrated the positive effects of using acetic acid as a fungicide treatment for soybeans. The effect of polymer coat with acetic acid on soybean seeds on germination will be studied in this research. Detecting the germination potential of seeds with thermal imaging will provide a fast method to choose seeds prior to field trials [12].

Even seed breeders will be able to evaluate the germination potential of the seeds quickly without performing the time-consuming germination trials. There has been a great interest in identifying and developing new methods to study the plant phenotyping non-invasively. This includes coupling of nonconventional optical imaging with computer vision as a tool to obtain an automated plant phenotyping. Such tools are believed to be performing much better than human eye and becoming a source for functional imaging [13]. Thermography is one such non-invasive imaging method which works on the principle of Planck's law, which describes the luminance of a black body in thermal equilibrium at a given wavelength, to measure temperature. Thermal imaging has been used to study the defects in stomatal regulation in Arabidopsis mutants [14], plant water content [15], plant damage in freezing temperatures [16], and in detection of scab spots in fruits 2 [17]. Thermal imaging has also been reported to successfully detect the biophysical and biochemical changes in seeds during imbibition and germination to predict whether the dormant seeds will germinate or die after uptake of water [18]. The objectives of this project was to study the effect of coating on soybean germination, and to develop an algorithm model to predict the germination potential of coated soybean seeds using thermal imaging technique.

2. Materials and Methods

2.1. Seed Coating

Soybean seeds were coated with a rotary pan technology using a tabletop lab seed treater (USC Seed Treating Solutions, Sabetha, KS, USA) with the two commercial seed coating polymers as follows: Polymer 1- Precise seed finisher 1010 (Bayer CropScience, Durham, NC, USA), and Polymer 2 - SeedWorxAgShine Blue (Aginnovation LLC, Walnut Grove, CA, USA). Both of these polymers are designed to increase the water uptake potential of seeds and to increase the flowability of the seeds inside the planting equipment. A total of five treatments were obtained by coating: Polymer 1, Polymer 1 + 1% Acetic Acid, Polymer 2, Polymer 2 + 1 Acetic Acid, and raw soybean seeds as a control. For each coating treatment, 1:1 (w/w) of seeds and polymer was used. The coated seeds were dried at room temperature until no change in mass observed to ensure full drying. To check the water uptake potential of the 5 abovementioned treatments, seeds were be placed in petridishes lined with water saturated germination paper (Anchor Paper Company, Saint Paul, MN, USA). Three replicates for each treatment with 25 seeds per treatment were used for this experiment. Weight of imbibed seeds were taken at the following time intervals: 2, 6, 12, 24, 48, and 72 h. Weight of raw seeds and imbibed seeds were used to calculate the percent water imbibition.

2.2. Greenhouse Trials

Greenhouse trials were conducted for all the 5 treatments with 3 replicates each in the greenhouse situated in North Dakota State University campus as showed in fig. 1. Twenty-five seeds from each treatment were sown in petridishes lined with water saturated germination paper. Data was collected every day measuring the root and shoot length with a digital caliper. Germination counts are recorded and classified as normal and abnormal seedlings according to the germination guidelines for soybean seeds by AOSA rules for testing seeds.

2.3. Imaging

2.3.1. Thermal imaging

Thermal images were acquired with an ICI 9640 infrared camera with a spectral range between 7µm to 14 µm, a pixel resolution of 640 × 480, a 14-bit dynamic range, thermal sensitivity of less than 50mK, and an accuracy of ±1 C. Seed imbibition rate plays as an early stage essential factor for seed germination. Several studies have shown coloration between seed temperature changes and seed imbibition. Preliminary potential capacity of treatments seeds germination evaluated based on temperature distribution of their surface seeds during the first hours of the swelling.

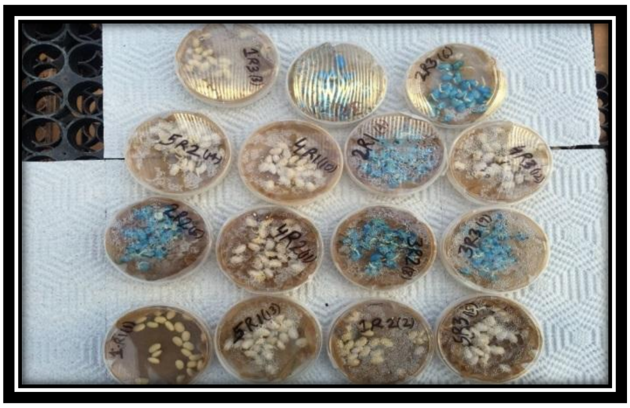

Fig.1. *The total number of treatments with three replicates set up in green house*

The radiation temperature differences between treatments show imbibition phase metabolic process after swelling as shown in fig. 2. These changes are so useful to evaluate seed germination and also indicate which seeds are more intensive affecting temperature differentiation. For water imbibition tests, the thermal images were taken at first hour in every minute and also one day after sowing hourly. Seed elongation is an early stage of the development of plants. The rates of elongation depend on species and genotype besides environmental conditions.

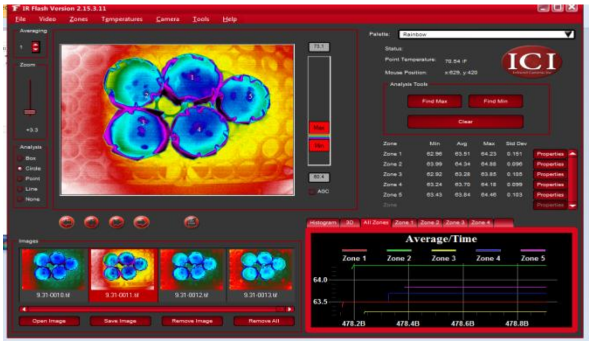

Fig. 2. *Thermal image of the seed swelling process.*

Large fluxes of metabolites move from the storage organs to the elongating zones, mainly the upper part growing towards the soil surface at seedling heterotrophic growth stage.

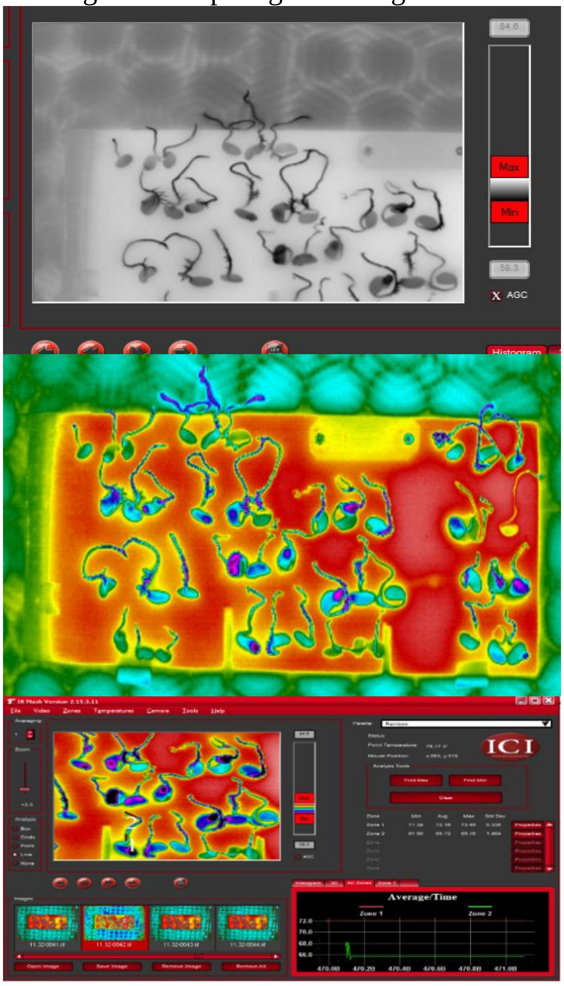

Fig. 3. *Identifying seed hypocotyl and radicle by thermal imaging*

While the elongation radical also receives metabolites from seed reserve hydrolysis, and absorb water and minerals from the growth support at this time. From this physiological point of view, the difference temperature between radicle and hypocotyl was measured to evaluate thermal discontinuity as a discrimination factor between seedling organs (fig. 3).

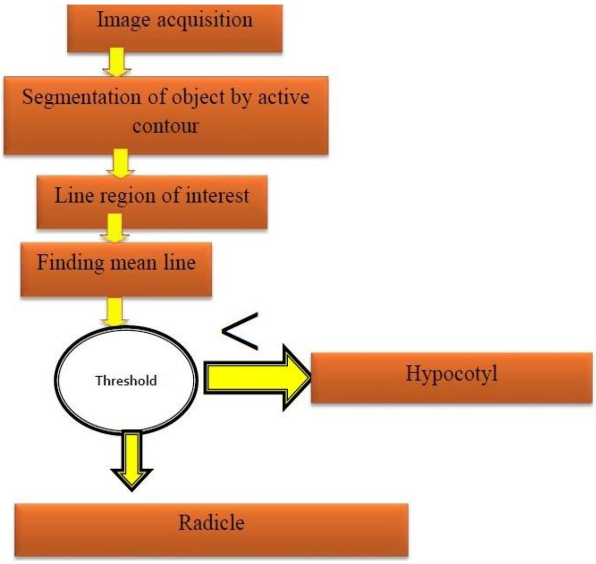

Fig. 4. *Steps of algorithm development for identifying hypocotyl and radicle length*

The steps of algorithm development to identify the hypocotyl and radicle length are shown in fig. 4.

2.3.2. Digital RGB Imaging

Germination is usually represented by a cumulative curve where the completion of radicle protrusion is polled as a function of time in in a population of individual seeds following imbibition. Therefore RGB images from each treatment were collected by Sony NEX 5R camera daily. Seed elongation was measured by image processing method with the Fiji image processing package based on image J. Images were first imported to the Image J software and then threshold value was applied to differentiate the shoot from the background followed by setting the scale, and finally the length measurement using the scale set up in the previous step as shown in fig. 5.

To identify germinated seeds, 2mm elongation of seed root was as an indicator for germination. Radicle elongation stages of germination was measured by image processing developed algorithm and compared to manual measuring.

2.3.3. Statistical Analysis

The data was analyzed using analysis of variance, and an F-protected LSD ($P \leq 0.05$) was calculated for comparisons of main effect means by using MINITAB 17 software.

Fig. 5. *Steps for the length measurement using RGB Imaging*

3. Results and discussion

A. Germination and Water Imbibition

The water imbibition test was conducted to determine the water absorption potential for all the five treatments for 3 days. During the first 6 h, the raw soybean seed absorbed the least amount of water (17%) as compared to all the 4 coated treatments: Polymer 1 (32%), Polymer 1 + Acetic Acid (39%), Polymer 2 (45%), Polymer 2 +Acetic Acid (35%). The similar trend continued throughout the 3 d time interval, where the water imbibition percent for raw soybean seeds was 79 % less as compared to the averaged water imbibition percent of all 4 coated treatments (fig. 8). The water imbibition percent directly affected the seedling germination; more the water imbibition percent, faster the seedling emergence (fig. 6 and fig. 7). The raw soybean seeds resulted in only 12% germination; whereas, all the coated treatments resulted in greater than 80% germination at the end of 3 d.

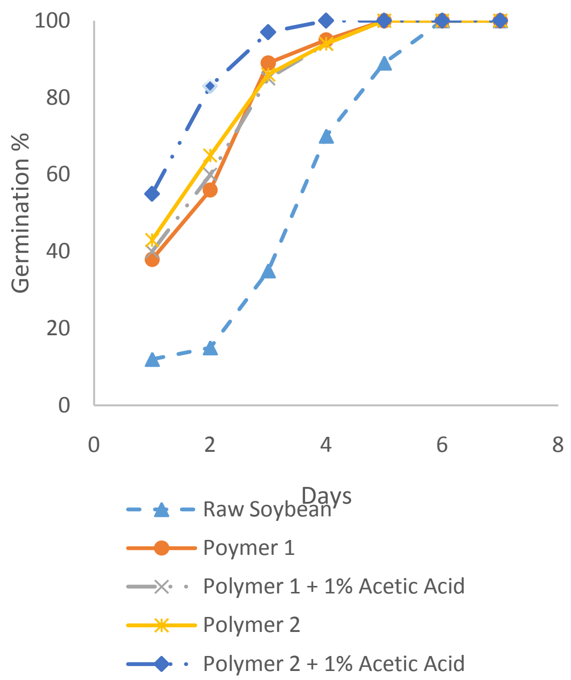

Fig. 6. *Effect of coating treatments on soybean seed germination*

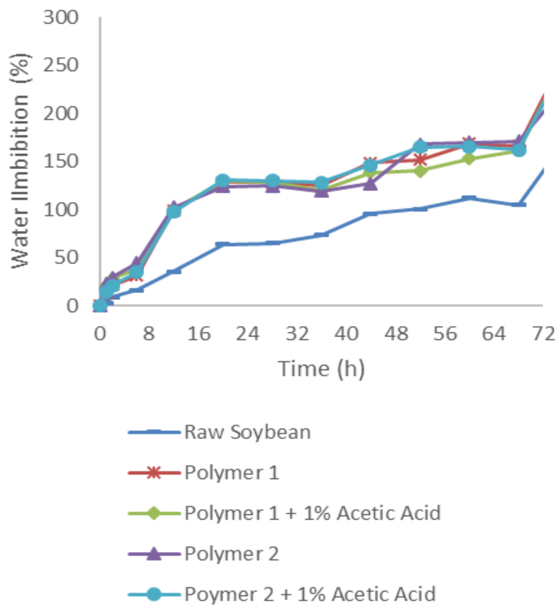

Fig. 7. *Effect of coating treatments on water uptake potential of soybean seed*

Furthermore, the seedlings emerged from the coating treatments resulted in statistically higher weights as compared to the raw soybean seeds at the end of 3 and 7 d. When averaged across all the time intervals from day 1 to day 7, all the coated treatments resulted in 2 g higher weight than the raw soybean seeds (fig. 8). No and co-authors (2003) observed the similar trend of improving soybean sprouts health by the chitosan treatment on soybean seeds.

For both Polymer 1 and Polymer 2, addition of acetic acid did not result in statistically significant germination percent, water imbibition percent, and seedling weight. This suggests that the polymer helps in absorbing the moisture faster, and thus improves the quality of soybean sprouts; furthermore, addition of acetic acid as a fungicide did not affect the both the germination and seedling weight.

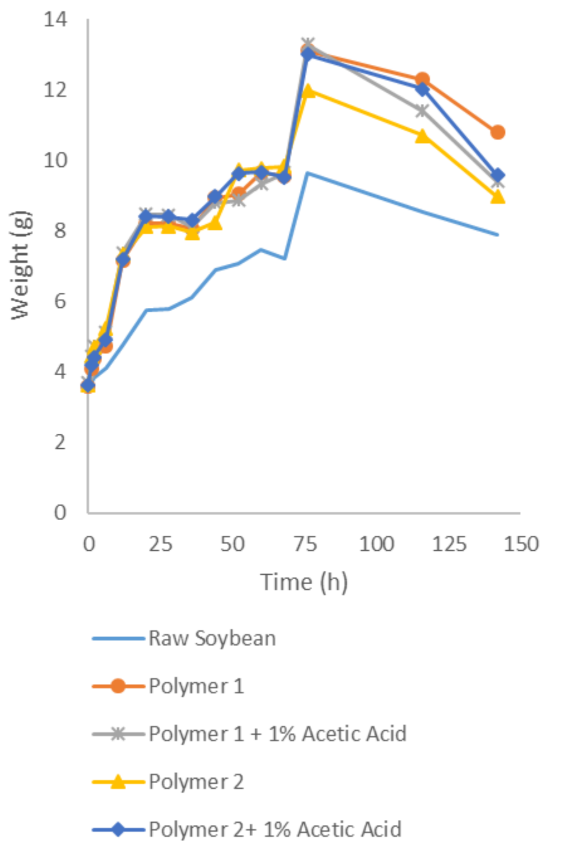

Fig. 8. *Effect of coating treatments on soybean seedling weight*

B. Thermal Imaging

The robustness of the thermal difference during the seedling growth was observed in fig. 9. The thermal gradient along the seedling was observed at the beginning and end of the hypocotyl organ. Polymer 1 + 1% acetic acid coated seedlings exhibited the highest thermal difference between the radicle and hypocotyl followed by the polymer 2.

The other treatment seeds showed similar trend with the thermal difference during the first hour. The higher the thermal difference the better is the length elongation of both root and shoot of the seeds. When statistical analysis was performed using one way ANOVA followed by the Fisher's LSD for the multiple comparisons, polymer 1 + 1% acetic acid treatment was significantly different from all of the other treatments.

The polymer 2 treatment was also significantly different from other treatments, but there was no significant difference observed between the other four treatments at 60 minute time interval.

Smaller differentiation of temperature shows limited germination capacity while high level germinated seeds show larger temperature differentiation.

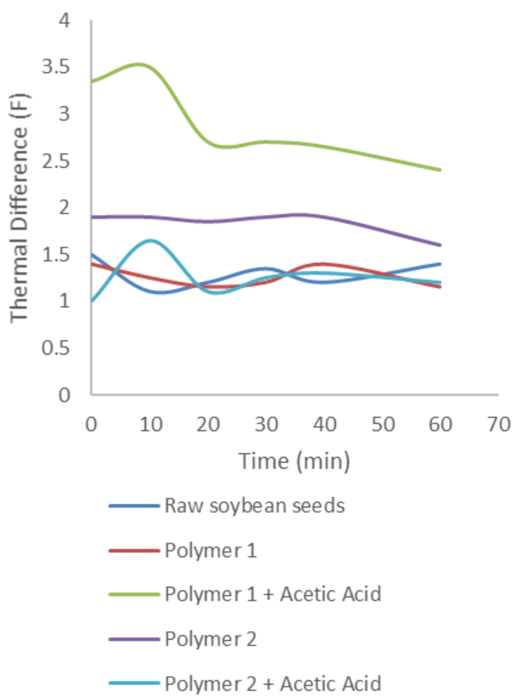

Fig. 9. *Time evolution of the difference of temperature (Thyp- Trad) between seedling organs (radicle and hypocotyl) during time*

The change in the radiation temperature of the seeds in the first imbibition (44 minutes) was observed in fig.10. All the treatments showed lower temperature compared to the ambient temperature. The raw soybean seeds showed the least temperature difference when compared with the coated treatments but it was not significantly different to other treatments.

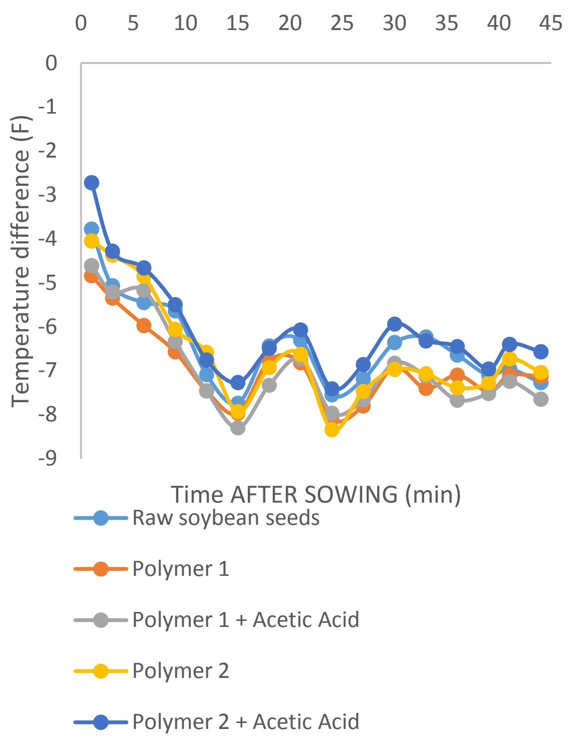

Fig.10. *Changes in radiation temperature of soybean seeds in the first 45min of the swelling process*

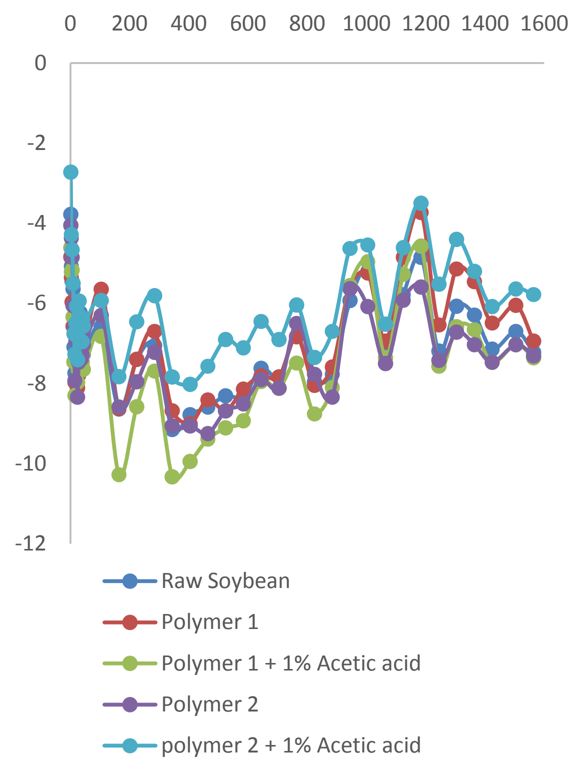

Fig. 11. *Evolution of soybeans seeds temperature during imbibition*

This further illustrates that the coating treatment proved beneficial for seedling health. The thermal signature of fig. 11 displays a larger variation of temperature for higher temperature of the water and larger surface of contact between the water and the seed.

This is made possible by maximizing the surface contact between the seeds and the water supply. The statistical analysis performed at 824 minutes showed polymer 2 alone and in combination with 1% acetic acid absorbed moisture was significantly different from other treatments. But at 1600 minutes, only polymer with 1% acetic acid found to be significantly different from the other treatments.

Conclusions

The study was undertaken to evaluate the germination potential of coated seeds and concluded that polymer coated seeds resulted in increasing the soybean sprouts health by imbibing water faster. Furthermore, addition of acetic acid did not adversely affect the germination and seedling health. This study suggests that the addition of acetic acid during the coating process will protect the seeds from pests while improving the seedling health; however, field trials are necessary to support this conclusion.

With the aid of thermography, the hypocotyl and radicle organ of germinated seeds are identified by the constant different temperature between these two organs. Furthermore, this study concluded that the temperature difference between seeds in the initial stage of the swelling process during germination gives a direct measure of the seed germination potential with the aid of thermal imaging.

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