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EFFECT OF DELAY HARVEST ON SEED QUALITY AND GERMINATION OF THREE VARIETIES OF SOYBEAN (*GLYCINE MAX*) SEEDS

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Abstract

The loss in soybean seed quality owing to adverse environmental reasons is unavoidable, particularly in the tropics. Seed ability to germinate and produce a vigorous seedling is a significant characteristic for any seed-propagated crop. Seed deterioration is a harmful feature of agriculture crops which hindered quality seed of Soybean. Thus, the purpose of the study is to investigate the relationship between seed deterioration and physiological changes of delayed harvest soybean seeds. Three soybean varieties which are AGS-190, Cikurai, and Willis were used as planting in the field at Universiti Putra Malaysia as materials in this experiment. The seeds were harvested at harvest maturity HM (H1) demonstrating 95% of the pods have reached their mature brown color and 2-week delay after HM (H2). The experiment was conducted in a complete randomized design (CRD) with three replicates. The result showed that seed deterioration of larger seeded soybean varieties can be increased at a 2-week delay after harvest maturity stage. Seed quality of soybean was affected by field weather environment during harvest date. AGS190 was the most sensitive to adverse weather surroundings as shown by deterioration of seed quality at a 2-week delay after harvest maturity stage. Loss of seed viability and vigor demonstrated depending on harvest date and directly related to increase in the level of *phomopsis* sp. infection. Germination percentage, tetrazolium test seed viability and vigor are negatively correlated with electrical conductivity and *phomopsis* sp., while in small seeded varieties was less seed deterioration.

Key words : Soybean, seed quality, delay harvest, seed deterioration.

Introduction

Soybean [*Glycine max* (L.) Merr.] is the most significant oilseed in the world due to its high quality as a source of protein for human and animal diets (Rafiee *et al*., 2009; Afrakhteh, 2013). High germination capability and high vigorous seedling are important characteristic for any seed-propagated crop such as soybean and peanut. In addition, the supply of high quality seeds to farmers is a key factor to maintain crop production and food security. Many factors influence on seed quality. Climate change is main factor that give big impact on the quantity and quality of the seed production of worldwide, for example, the temperature and rainfall fluctuation. Previous studies by Mengistu and Heatherly (2006) and Gillen *et al*. (2012) reported that high temperature and humidity during seed production results in decreased germination and vigor of soybean seed. Changing on climate change increased the severity of some pathogens that resulted in seed-borne disease and eventually effects on seed quality. Mengistu *et al*. (2009) reported that in humid and hot environments, *Phomopsis* longicolla was identified as primary seed pathogen of soybean.

Planting time, harvesting time - early harvest or delay and infection of Phomopsis sp. or physical damage also effect on the quality of soybean seed (Thant, 2015). Seed harvested in delay harvest under humid environment caused the seed become aged in the field and consequently decline seed quality. The delayed harvest also related to seeds exposure to temperature, high relative humidity, and rainfall variations, which is negatively effect on seed quality. Diniz *et al*. (2013) reported that the harvest delayed in 30 days after stage R8 reduced germination and vigor of soybean. In addition, each cultivar responds differently to this condition. This demonstrates

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the significance of measuring the response of different varieties on seed quality in delayed crop conditions. Delay harvest can also be stressful to seeds, which can have altered responses soybean genotypes for high-quality seeds (de Lima *et al*., 2007). Therefore, identifying the stage of maturity at which seeds can be harvested without lowering their germination is a beneficial practice for farmers to maximize seed yield and quality dependent on their genotype. Nevertheless, this procedure can use with the objective of choosing. Therefore, this study was aimed to investigate the relationship between seed deterioration and physiological changes of delayed harvest soybean seeds.

Materials and Methods

Three soybean varieties which are AGS-190, Cikurai, and Willis were planted in ladang 2 at Universiti Putra Malaysia. The period from planting until the last harvest was from January to May 2016. The seeds were harvested at harvest maturity (H1) demonstrating 95% of the pods have reached their mature brown color and 2-week delay after H1 (H2). The experiment was conducted in a complete randomized design (CRD) with three replicates. The treatment design was the combination of two factors (2- factorial experiment) varieties of (AGS-190, Cikurai, and Willis), and harvesting stages of harvest maturity (H1) and 2-weeek delay after harvest maturity (H2).

Germination percentage

Standard germination test was done according to the procedures described by ISTA (2006). Fifty seed per replication from each harvest stage were germinated in the plastic box size (28 × 22). It was oven dried in sterilized sand at 130°C. The seeds were considered germinated when the cotyledons completely emerged from the sand surface. After 7 days of sowing, final germination percentage was calculated using the following formula:

$$\text{Germination percentage} = \frac{\text{Number of seed germinated}}{\text{Total number of seed}} \times 100\%$$

Electrical conductivity

Electrical conductivity of the seeds was carried out using ISTA procedure (ISTA, 1995). Leachate was performed on 25 seeds obtained from each experiment unit plot. The seeds were weighted and soaked in 50ml of deionized water at room temperature. The samples were measured with EC meter, (model 4310, Jenway, UK). The unit of recording was ($\mu Scm^{-1}\ g^{-1}$).

Tetrazolium viability test

50 seeds from each variety and harvest date were presoaked in distilled water at 25°C for 18 hours. After removing water, the seeds were imbibed in 1% (w/v) 2,3,5 triphenyl tetrazolium chloride solution at 35°C for three hours in the dark (Moore, 1985). The 1% solution gave a distinct staining pattern based on preliminary tests. Tetrazolium seed viability test were evaluated by staining pattern and color intensity according to (AOSA, 2002).

Tetrazolium vigor test

Seed vigor was evaluated after differentiating viable seeds from the non-viable ones, the sustainable seed group can be reclassified into several vigor groups by the intensity of stain and staining pattern of different seed parts. The seed with uniform and superficial staining were termed as high vigor seeds according to (França Neto *et al*., 1998).

Seedling performance

Ten normal seedlings from each treatment were used to measure shoot length, root length, shoot dry weight and root dry weight of the seedling. The seedling root length was measured from the tip of primary root to the base of hypocotyl and seedling shoot length was measured from the base of primary leaf to the base of hypocotyl. The mean lengths of seedling shoot and root were expressed as centimeters (cm). For dry weight, the ten normal shoot and root of seedlings were dried in hot air oven at 70 ± 1°C for 24 h. The mean dry weights of seedlings shoot and root were recorded and expressed in milligrams (mg).

Pathogen Bioassay

Harvested seeds from three varieties were used for evaluation of *Phomopsis* sp. Seeds were sterilized with 1% sodium hypochloride and rinsed three times with sterile distilled water and plated on potato dextrose ager (PDA) media (McGee, 1986). 10 seeds were placed on each culture plate and the plates were incubated under fluorescent light at room temperature. *Phomopsis* sp. was evaluated based on colony morphological characteristics at 7 day after plating. The infection of *Phomopsis* sp. producing floccose, dense, wooly white aerial mycelia on PDA was recorded.

Statistical analysis

All data were analyzed using SAS software window version 9.4. One-way analysis of variance (ANOVA) at confident level p=0.05 was used to determine the different between harvest stage and variety and followed by Least Significant Difference (LSD) at confident level p=0.05 for mean comparison.

Results

Effect of Delay Harvest on seed quality and germination of three varieties of soybean

The result shows delay harvest had a significant effect on the seed quality and germination parameters of three varieties of soybean seeds (ANOVA, P>0.05). Electrical conductivity is significantly higher in H2 than H1 (table 1). In addition, among three varieties, EC of AGS190 is found higher in both H1 and H2 compared to other two varieties. The results on germination percentage showed significantly different (ANOVA, pÂ0.05) between harvest maturity (H1) and 2-week delay (H2) on the different soybean varieties. Germination percentage of 2-week delay harvest (H2) reduced nearly 50 % than harvest maturity. Moreover, Willis showed higher germination percentage in both H1 and H2 than AGS190 and Cikurai as presented in table 1.

Table 2 showed that delay harvest reduced the tetrazolium (TZ) test seed viability and TZ seed vigor of compared to H1. Results show that Willis is the highest followed by Cikurai and AGS190.

Effect of Delay Harvest on early seedling growth of three varieties of soybean

The early seedling growth performance of seeds harvested at harvest maturity (H1) and 2-week delay harvest (H2) showed no significantly different on shoot length (ANOVA, P>0.05) as presented in fig. 1. This study also found that no significant difference among soybean varieties. However, there is a significant difference in root length which is root length in AGS190 and Willis at H1 in higher than H2. On other hand, root length of Cikurai is higher in H2 than H1 (fig. 2). Both H1 and H2 do not effect on the shoot and root dry weight as presented in table 3. The highest root and shoot dry weight is found in AGS190 followed by Cikurai and Willis.

Table 4 presented that Percentage of *Phomopsis* infection at H2 significantly higher than H1 (ANOVA, P<0.05). AGS190 have the highest percentage of *Phomopsis* infection in both HM and H2 followed by Cikurai and Willis.

Correlation analysis between seed viability and *Phomopsis* infection

Correlation analysis was conducted to study the relationship between seed quality and *Phomopsis* infection. Fig. 3A revealed the negative correlation between germination percentage and percentage *Phomopsis* infection (R2=0.82). The results indicated that higher *Phomopsis* infection would decrease germination percentage, which is 22% *Phomopsis* infection reduced the germination percentage to 76.7%. There is a positive relationship between electrical conductivity and percentage *Phomopsis* infection with R2 is 0.68 as shown in fig. 3B. In addition, TZ for seed viability and vigor showed a negative correlation with

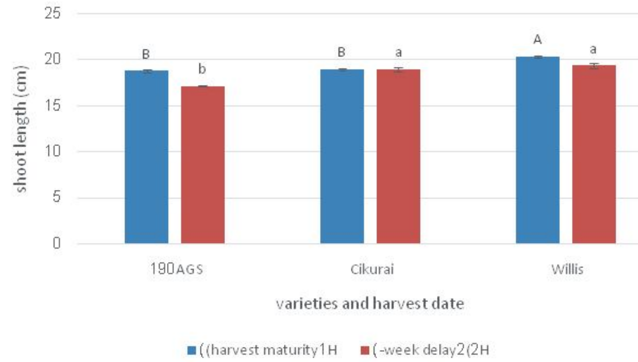

Fig. 1 : Shoot length of three varieties of soybean seeds at two harvest date, H1 (Harvest Maturity) and H2 (2-week delay). Each point is given as mean ± standard error. Means in the same bars with different alphabet(s)/arteris are significantly different at p=0.05 according to LSD comparison test.

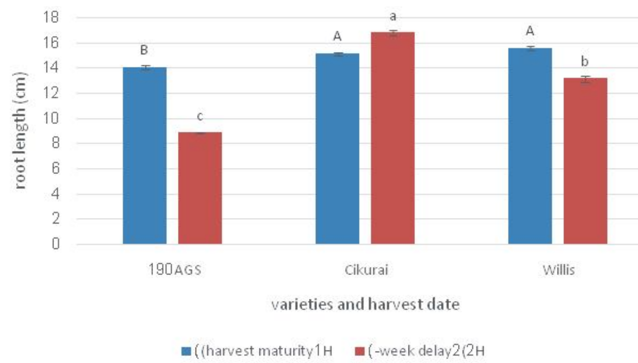

Fig. 2 : Root length of three varieties of soybean seeds at two harvest date, H1 (Harvest Maturity) and H2 (2-week delay). Each point is given as mean ± standard error. Means in the same bars with different alphabet(s) are significantly different at p=0.05 according to LSD comparison test.

Table 1 : Electrical conductivity and germination percentage of three varieties of soybean seeds at two harvest stage.

Varieties	Electrical conductivity ($\mu Scm^{-1}g^{-1}$)		Germination (%)	
	H1	H2	H1	H2
AGS190	$88.7\pm3.2^{A*}$	$103.8\pm2.1^{A**}$	$76.7\pm1.5^{C*}$	$48.7\pm1.8^{C**}$
Cikurai	$41.7\pm1.2^{C*}$	$52.3\pm1.5^{C*}$	$82.7\pm0.8^{B*}$	$72.0\pm1.9^{B**}$
Willis	59.5 ± 1.6^{B}	61.5 ± 2.1^{B}	$92.7\pm0.4^{A*}$	$86.0\pm0.7^{A**}$
LSD $_{0.05}$	6.6	5.8	3.1	4.8

Means (± standard error) within a column/row followed by same letters/ asterisk are not significantly different at p=0.05

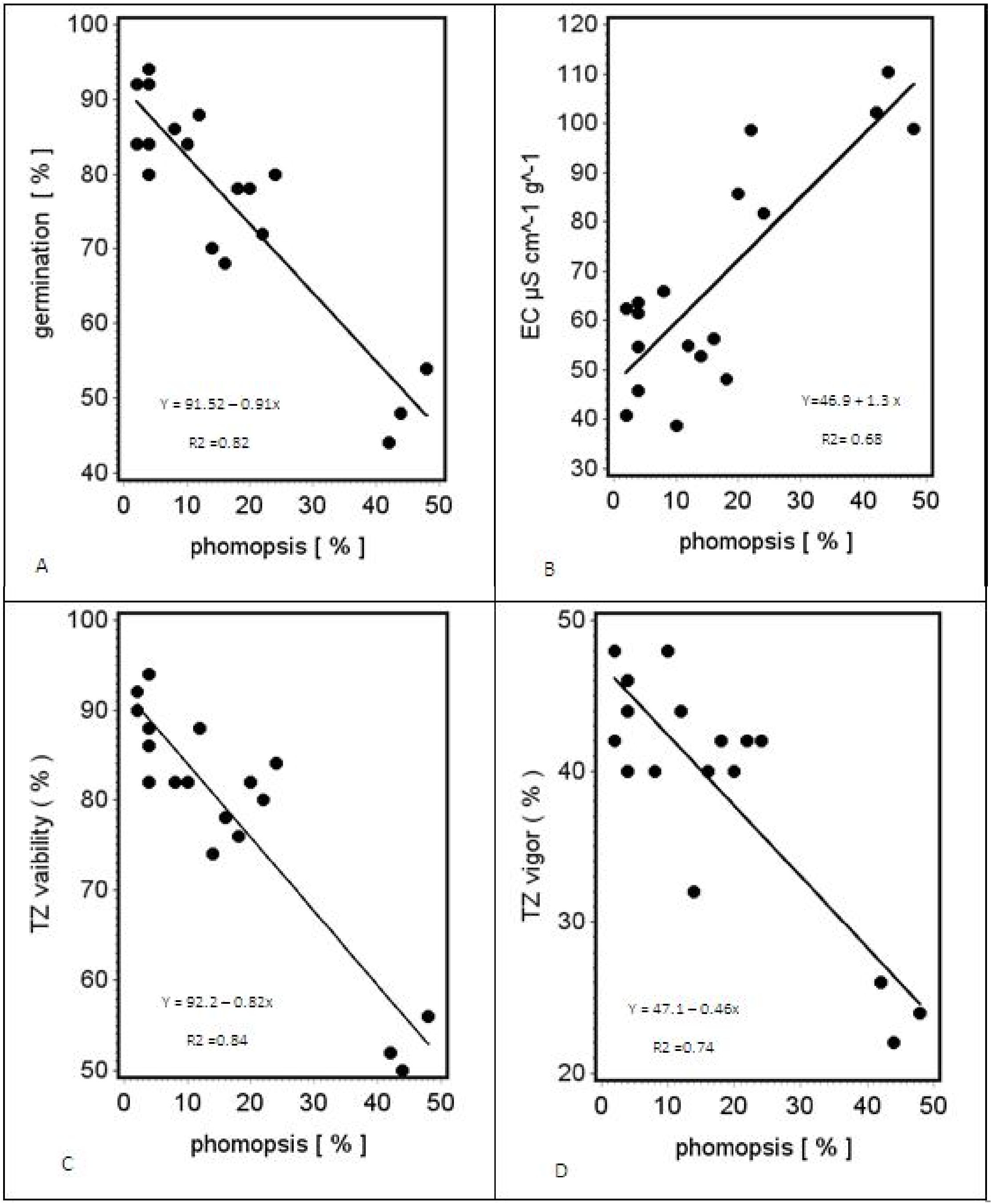

Fig. 3 : Correlation analysis between seed viability and vigor tests and Percentage *Phomopsis* infection. A: Germination percentage, B- EC electrical conductivity, C-TZ tetrazolium test seed viability, D-TZ tetrazolium test seed vigor of three varieties of soybean seeds at two harvest date.

percentage *Phomopsis infection* with R2 is 0.84 and 0.74, respectively (fig. 3C -D). The result of TZ for viability and vigor was lower due to greater *Phomopsis* infection percentage in the seed of soybean, and this is expected as a result of the negative relationship.

Discussion

The result demonstrates that 2-week delay harvest after harvest maturity stage reduced the quality of soybean seed which eventually decreased germination percentage. The findings showed that AGS190 shows lower germination percentage than other two varieties for the 2-week delay. This is probably due to more prolonged exposure to field weather condition. Similar finding was

Table 2 : Tetrazolium viability and vigor test of three varieties of soybean seeds at two harvest stage.

Varieties	Tetrazolium viability test (%)		Tetrazolium vigor test (%)	
	H1	H2	H1	H2
AGS190	82.0±0.7^{B*}	52.7±1.1^{C**}	41.3±0.4^{B*}	24.0±0.7^{C**}
Cikurai	85.3±2.1^{B*}	76.0±0.7^{B**}	43.3±1.5^{AB*}	38.0±1.9^{B**}
Willis	90.7±1.1^{A*}	85.3±1.1^{A**}	46.0±0.7^{A*}	43.3±1.1^{A**}
LSD $_{0.05}$	4.3	3.0	3.0	4.1

Means (± standard error) within a column followed by same letters/ asterisk are not significantly different at p=0.05

Table 3 : Shoot dry weight and root dry weight of three varieties of soybean seeds at two harvest date H1 (Harvest maturity) and H2 (2-week delay).

Varieties	Shoot dry weight (mg)		Root dry weight (mg)	
	H1	H2	H1	H2
AGS190	248.6±2.2^{A*}	219.8±1.0^{A**}	29.3±0.4^{A*}	28.7±0.4^{A*}
Cikurai	73.8±0.7^{B*}	74.3±0.8^{B*}	14.8±0.3^{B}	13.5±0.4^{B}
Willis	63.9±0.4^{C**}	59.0±1.4^{C*}	16.3±0.9^{B**}	14.4±0.3^{B*}
LSD $_{0.05}$	4.0	3.3	1.9	1.1

Means (± stander error) within a column followed by same letters/ asterisk are not different at p=0.05.

Table 4 : *Phomopsis* sp. infection percentage of three varieties of soybean seeds at two harvest date H1 (harvest maturity) and H2 (2-week delay).

Varieties	***Phomopsis* sp. infection (%)**	
	H1	H2
AGS190	22.0±0.7^{A*}	44.7±1.1^{A**}
Cikurai	5.3±1.5^{B*}	16.0±0.7^{B**}
Willis	3.3±0.4^{B*}	8.0±1.5^{C**}
LSD $_{0.05}$	3.0	3.4

Means (± stander error) within a column/row followed by same letters/ asterisk are not different at p=0.05.

reported by Diniz *et al*. (2013), germination and vigor of soybean seeds harvested at 30 days after harvest maturity were reduced.

Electrical conductivity values indicate the level of membrane integrity of seed. High values of electrical conductivity show high electrolyte leakage from the membrane which lead to low quality of seed. This study found that delay harvest increased the EC of soybean seeds. AGS190 had the highest EC than other two varieties for the 2-week delay. As a result, vigor of soybean seed reduced. According to Vieira (1994), seed deterioration was positively correlated with electrical conductivity of seed. Seed that produces a high amount of electrolyte leaked into the deionized water is said to be the low vigor seed. Furthermore, higher greater EC value indicates a seed of less quality as a result of the weak membrane which encourages the passage of leachates out of the imbibed seed. This positive association demonstrates that *Phomopsis* infection will destruct the cell membranes in the seed. The increased electrical conductivity level of seed in this finding is positively correlated with the *Phomopsis* infection (fig. B).

Soybean seeds harvested after harvest maturity exhibited adverse effects on seed physiological valuations. Based on results of tetrazolium tests, seed viability and vigor, this study found that seed deterioration is faster in AGS190. This might be owing to its larger seed size and soft seed coat features. Seed coat is one of the leading essential reasons for the quality of seed because it is the seeds primary defense against adverse environmental conditions (Mohamed-Yasseen *et al*., 1994). Compared with Cikurai and Willis have small seed size, and hard seed coat, therefore, being more resistant to field weathering than AGS190. TeKrony *et al*. (1984) also found that small seed size and hard seed coat improved soybean seed quality. Moreover, varieties with hard seed coat are more resistance to field weathering. Thant *et al*. (2017) also stated that seed viability and vigor decreased in delay harvest seeds in both cultivars.

Delayed harvest reduced seedling growth of all three soybean varieties. It might be associated to the failure of activation enzymes responsible for the mobilization of storage reserves to ensure germination and seedling growth (Mohammadi *et al*., 2012). Seedling characters' like seedling length and dry weight varied with harvest times and may be strongly influenced by seed vigor. High vigorous seeds have a higher capacity to produce healthy seedlings, germinate rapidly, uniformly and withstand environmental adversity after sowing (Ajouri *et al*., 2004). Thant *et al*. (2017) also stated that seedling length and dry weight decreased in delay harvest seeds in both cultivars.

The infection of *phomopsis*sp. was occurred higher in AGS190 variety than the other varieties. It indicates that large-seeded soybean was more natural to infect with fungus inoculums because of large space area (Raeisi, 2011). While Willis recorded the lower level of infection may be due to it has small seed. In the humid, wet tropical environment of Malaysia, the loss of viability and vigor seem to be associated with environmental reasons. Some studies reported that effect of environmental conditions and soybean varieties on *phomopsis* sp. infection in Malaysia (Gutema, 2006; Raeisi, 2011; Thant, 2015).

Seed germination and tetrazolium test for viability and vigor showed a negative relationship with *phomopsis* sp. infection. The infection of *phomopsislongicolla*was negatively correlated with seed healthy that showed in soybean seed germination (Mengistu and Heatherly, 2006). Raeisi *et al*. (2011) also reported that *phomopsis* sp. infection was negatively correlated with seed germination and tetrazolium test.

High electrical conductivity value shows high amount electrolyte leached out from imbibed seed showing lower seed quality. Electrical conductivity presented a positive correlation with *phomopsis* sp. infection in soybean varieties. During the electrical conductivity exhibited positive relationship. According to Vieira *et al*. (1999), the electrical conductivity values are positively correlated with seed deterioration, so more values of EC displays lower seed quality. In this study, EC was the maximum in AGS190 seeds and also in 2-week delay, due to higher infection of *phomopsis* sp. Raeisi *et al*. (2011) also reported that *phomopsis* sp. infection was positively correlated with electrical conductivity test.

Conclusion

As conclusion, delay seed harvest reduces the seed quality which eventually reduce the germination performance, increased the pathogen infection and reduced on early seedling growth of all three soybean varieties.

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