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Seed Quality of Soybean in Relation to *Phomopsis* Seed Decay in Malaysia

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ABSTRACT

Phomopsis Seed Decay (PSD) is detrimental for seed quality in soybean. In this study, three varieties of soybean were evaluated to determine their sensitivity to PSD and its influence on seed quality. These varieties were planted in a factorial experiment with three replication in two plant densities and two seasons in University Putra Malaysia. The incidence of *Phomopsis* was determined using culture plate method. Seed quality was tested using standard germination test, tetrazolium test and electrical conductivity. Most colonies were morphologically similar with *Phomopsis longicolla*. Infection to *Phomopsis* and seed viability was significantly different between soybean varieties and plant densities in two seasons. The AGS 190 with 46% showed the most percentage of *Phomopsis* in higher plant density in the second season. Pershing had 10% infection in lower plant density in the first season and showed more tolerance to *Phomopsis* in this study. Higher plant density caused higher infection to PSD, lower percentage of seed viability and more electrical conductivity. Standard germination and tetrazolium test were 76 and 72% in higher plant density respectively. Electrical conductivity was 83 $\mu S\ cm^{-1}\ g^{-1}$ in higher plant density whereas it was 68 $\mu S\ cm^{-1}\ g^{-1}$ in lower plant density. *Phomopsis* incidence showed negative correlation with seed viability and it was positively correlated with electrical conductivity. Based on these relationships, Pershing with its low *Phomopsis* infection showed the highest seed viability. The values for viability were 84 and 82%. This study indicates that the incidence of *Phomopsis* seed decay is usually dependent on field environments and planting densities. The severity of infection is also dependent on the variety.

Key words: Soybean, *Phomopsis* seed decay, seed germination, plant density, tetrazolium test

INTRODUCTION

Soybean is one of the most important oil-seed crops, with a valuable source of protein that cultivated in many of countries. *Phomopsis* Seed Decay (PSD) is detrimental for seed quality in soybean that reduces the quality of seeds as planting material and infected seed mostly do not germinated (Hartman *et al*., 1999). This disease also suppressed yield but the losses vary among years and regions (Wrather *et al*., 2001). The reduction of yield is happened because the seed severely remains small and light in harvesting time (Tenuta, 2010). Among fungi, belong *Diaporthe/Phomopsis* complex (DPC), *Phomopsis longicolla* is the most aggressive seed pathogen

(Duvnjak *et al*., 2007) and it is endemic in soybean production areas worldwide. Significant economical losses reported by this species of DPC (Baird *et al*., 2001; Bradley *et al*., 2002). This fungus overwinters on infected soybean straw in the field or may be seed-borne. Pods may become infected at any time during their development but most of seed infections occur and reveal after the yellow pod stage of physiological maturity (R7) (Wrather *et al*., 2004; Mengistu *et al*., 2009). Prolonged wet periods after flowering and pod set, favor the infection and development of pod and stem blight. As pods mature, the fungus grows from the wall of the pod to the seed. Seed infection is greatly increased if harvesting of the crop is delayed during warm wet weather (Mengistu *et al*., 2007).

The seeds that severely infected are usually elongated, shriveled, cracked and may sound white and chalky and sometimes seed maybe infect but it does not show any symptoms (Hartman *et al*., 1999). The viability and vigour of seed and grain yield of the seed decreased by this pathogen and the emergenced seed is weak in the field (Tenuta, 2010). Mengistu *et al*. (2009) found low (-37) but significant negative correlation between *P. longicolla* that had been recovered from soybean plant and seed germination in irrigated systems.

Chin *et al*. (1993) reported that *Phomopsis* infection occurred more in higher plant densities, in fact pathogen is dispersed in higher densities by splashing, so the infection is transferred more on other side of plant or other plants. But Feritas *et al*. (2002) observed a significant interaction between variety and plant density for *Phomopsis* infection.

The objective of this study is to indicate relation between seed infection by PSD and soybean seed quality in different varieties in two plant densities which was conducted in two seasons in Malaysia.

MATERIALS AND METHODS

Three varieties of soybean, AGS190, Dieng and Pershing were evaluated in experimental field 2 of University Putra Malaysia (UPM) in two densities. This research was conducted in factorial experiment with three replications and two planting dates. Two densities included normal plant density (30 plant m^{-2}) and double plant density (60 plant m^{-2}). Two planting dates carried out in two different season of Malaysia in 2009. At the first season, experiment was laid out at 25 January in 2009 and the period for field experiment lasted until the early of May. The second experiment that carried out in second season was started from the end of August until early of December of 2009.

The experiments carried out in the location of field, that soybean had been planted in previous years, so naturally infection was available based on sampling of soil. The percentage of PSD infection was determined using culture plate method based on Morphological characterization. This method identifies different species of *Diaporthe Phomopsis* complex that cause PSD, based on colony and spore specification (McGee, 1986).

Determination of percentage infection and identification of *Phomopsis* species: The samples including harvested seeds were surface-sterilized in 1.3% sodium hypochlorite for 1 min. Then, they were rinsed in distilled water and were placed on Potato Dextrose Agar (PDA). The pH of PDA was adjusted to 4.5 with lactic acid. *Phomopsis* infection percentage was determined after incubation for 7 days at 25°C in the 12/12 light and darkness. It was done based on recordings of infected seeds. For making pure culture of each sample, a small piece of growing mycelium was

incubated in water agar. After 2-3 weeks, DPC species for each sample were identified based on colony morphological specification (McGee, 1986). Seed viability were measured based on 3 methods.

Standard germination test: In Standard Germination test (SG), samples for each lot were used and sown in rolled paper towels. Then, they were moistened with water amount equivalent to 2.5 times the weight of the dry paper substrate. After that they were set to germinate at 25°C. The evaluations were performed on the fifth and eighth days after sowing (ISTA, 1993).

Tetrazolium test: For tetrazolium test (TZ), 2, 3-5 trephines tetrazolium chloride (1% v/v) in phosphate buffer was applied. Fifty seeds of each treatment were soaked in distilled water at 23°C for 12 h. The soaked seeds were dissected on moist filter paper in Petri dish by cutting them longitudinally with a sharp sterile razor blade to expose the main structure of the embryo. Only half of cotyledon of each seed, with attached embryo were used for testing and it were immediately transferred into the tetrazoliom test solution in the dark at 23±2°C for 5 h and then they were briefly washed in distilled water. At the end the percentage of germinated seeds was recorded.

Electrical conductivity test: For Electrical Conductivity (EC), the samples were weighted and placed in plastic cups (200 mL capacity) containing 75 mL Deionized water for 24 h at 25°C. After that the electrical conductivity of the solution was determined by reading on a conductivity meter (Loeffler *et al*., 1988).

All pathology tests were done in plant pathology lab, in plant protection department and the seed viability evaluation were done in seed technology lab located in crop science department area.

All data were analyzed statistically by ANOVA test using SAS software (SAS, 1999). Mean separation was carried out using Duncan's range at p = 5.

RESULTS AND DISCUSSION

Identifying of *Diaporthe phomopsis* complex species: *Phomopsis longicolla* were identified in the most of samples based on colony morphology and spore specification. The formed colonies were floccose, dense and white on PDA after 14 days and they looked colorless with black spreading stomata from the backside of the Petri dish. The Pycnidia were black, stromatic, solitary or aggregated and more unilocular. In some samples, pycnidia were not formed on PDA. All *P. longicolla* isolates produced abundant alpha conidia. The formed alpha conidia were hyaline, oval to fusiform with varying size, 4-10×1.5-3.5 μm. A few beta conidia were observed in some colonies (Fig. 1).

In this study the most species that were caused *Phomopsis* seed decay belonged to *Phomopsis longicolla*. This fungus has a wide distribution across the world and can losses the yield and seed quality in soybean. In earlier studies (Mengistu *et al*., 2009) reported that *Phomopsis longicolla* showed the most frequency in DPC species that they evaluated in six varieties of soybean in 3 years. This fungus is caused primarily seed infection in soybean. Cui *et al*. (2009) also reported *P. longicolla* is one of the most important soybean pathogen causing *Phomopsis* seed decay.

***Phomopsis* incidence and seed viability in three varieties in two seasons:** There were significant differences between varieties for *Phomopsis* infection and seed viability in two seasons (Table 1). AGS 190 variety with 41% had the highest percentage of *Phomopsis* infection but the

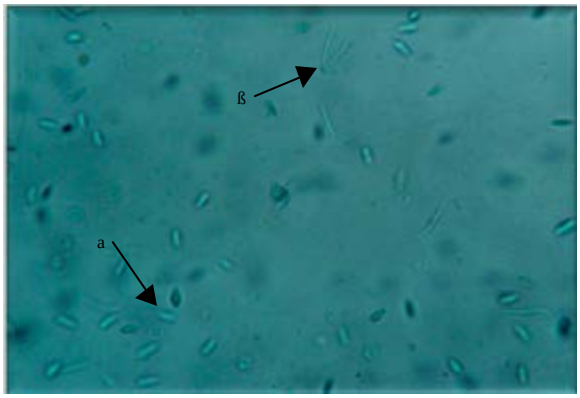

Fig. 1: Alpha and beta conidia in *Phomopsis longicolla* alpha conidia indicated by α, beta conidia indicated by β

Table 1: Mean squares for *Phomopsis* incidence and seed viability on varieties and plant densities in two seasons

Source of variation	df	Phom (%)	SG (%)	TZ (%)	EC (μS cm^{-1} g^{-1})
Season	1	156.00	294.00**	266.77*	96.96
S×R	2	27.08	2.19	1.02	1.36
Rep	2	167.00	130.00	30.36	57.02
Variety	2	1419.00**	384.00*	562.00**	4888.00**
Density	1	434.00*	476.00**	1272.00**	1806.00**
V×D	2	52.77	1.44	73.52	53.08
S×V	2	108.00	23.11	21.19	21.52
S×D	1	84.00	17.36	13.44	0.25
S×V×D	2	36.11	1.44	3.86	3.25

*, **Significant at p≤0.05, 0. 01, respectively, S: Season, R: Replication, V: Variety, D: Density, Phom: *Phomopsis*, SG: Standard germination, TZ: Tetrazolium test, EC: Electrical conductivity

lowest percentage of infection belonged to Pershing variety (19%). This higher infection can be resulted due to some reasons. Although, AGS190 variety may genetically be sensitive to PSD, because it had been shown high infection in earlier study (Gutema, 2006). AGS190 variety also is a late maturity group and the period of seed filling to maturity stage is long. This variety needs to more days for harvesting, so it is exposed to more moisture and pathogen inoculums than other varieties and could be infected more. Similar results reported by Mengistu and Heatherly (2006); they observed that the late maturity group of soybean that faced with higher moisture had higher infection. Higher infection to *Phomopsis* was caused lower Seed viability. The viability based on Standard Germination (SG) test was 77% for Pershing and AGS190 had 66% germination in two seasons. Tetrazolium test (TZ) also followed standard germination test and it was 70 and 58% for Pershing and AGS190 varieties, respectively. Normally the higher infection to *Phomopsis* causes lower percent of seed germination. Koning *et al.* (2001) reported that the soybean variety with higher infection to *Phomopsis* had lower percent of seed viability in terms of seed germination and vigor test. Mengistu *et al.* (2009) also indicated that the lower seed germination that happened for soybean in irrigated system due to higher infection to *Phomopsis* but not in non irrigated system. Electrical conductivity showed, the lowest amount of EC belonged to Pershing variety

(63 μS cm^{-1} g^{-1}) and it showed the highest value (98 μS cm^{-1} g^{-1}) for AGS190 variety. Electrical conductivity shows the electrolyte leachates from the imbibed seed and the more leachates shows the lower quality of seed, so the highest value of EC belonged to AGS190 variety that had the highest infection to PSD. Gutema (2006) also reported that the electrical conductivity was more in the varieties that infection of PSD was higher like AGS190.

***Phomopsis* incidence and seed viability in two plant densities in two seasons:** Two plant densities showed significant differences for infection of *Phomopsis* and seed viability in two seasons (Table 1). Higher percentage of *Phomopsis* infection was occurred in higher plant density; it was 34% and the lower percentage of infection was 27% in lower plant density (Fig. 2). The results for seed viability based on SG and TZ were inverse and higher percentage of seed viability was showed in lower plant density. SG was 76 and 68% in lower and higher plant density, respectively (Fig. 3). For TZ it was 72% in lower plant density, whereas it was 60% in higher plant density (Fig. 4).

Electrical conductivity was recorded less in lower plant density. It was 68 μS cm^{-1} g^{-1} whereas it was higher in higher plant density (Fig. 5).

The earlier studies were showed higher incidence of DPC in higher plant density. Feritas *et al.* (2002) found higher infection to PSD in some evaluated varieties of soybean not all of them. Normally there was a unique microclimate that caused canopy had been closure sooner in higher plant density, so the relative humidity was higher in these microclimates. According to Wiebold and

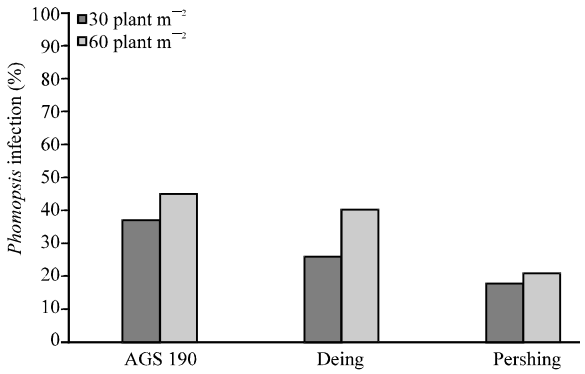

Fig. 2: *Phomopsis* incidence in varieties and densities in two seasons

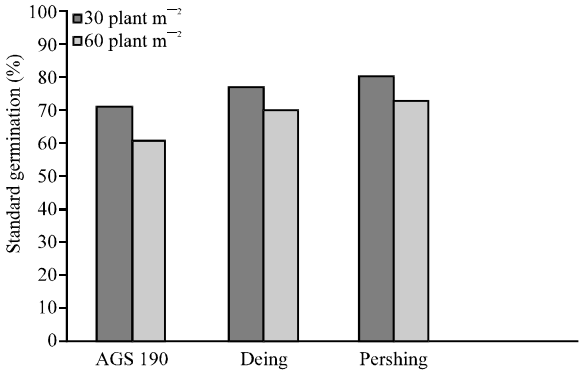

Fig. 3: Standard germination test in varieties and densities in two seasons

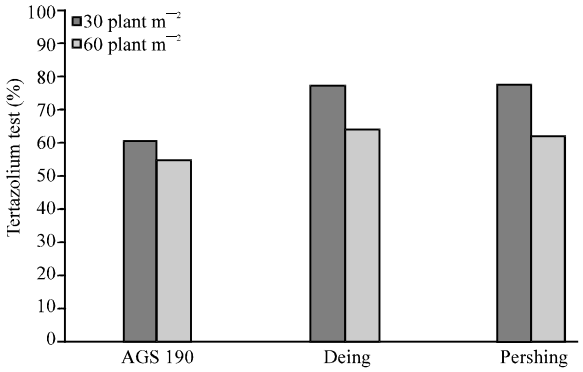

Fig. 4: Tetrazolium test in varieties and densities in two seasons

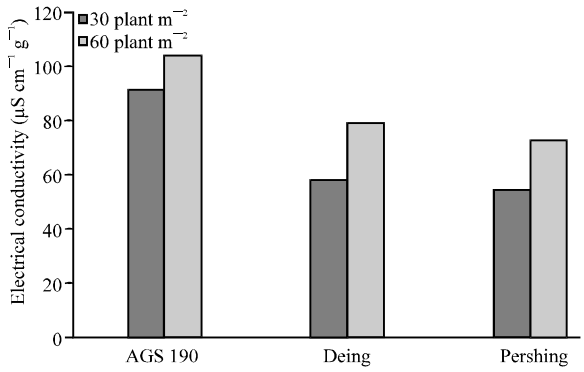

Fig. 5: Electrical conductivity in varieties and densities in two seasons

Belt (2006) the canopy in higher population of plants has more relative humidity than a lower plant population. The higher relative humidity can be a reason for more infection of PSD. Wet and humid condition is favorable for *Phomopsis* incidence and if the harvest associated with delay in this condition, the infection of PSD will be more (Tenuta, 2010).

The responses of varieties in two plant density (interaction effects of varieties and plant density) for percentage of *Phomopsis* incidence, standard germination, tetrazolium test and electrical conductivity were showed in Fig. 2-5. The highest *Phomopsis* infection was recorded in AGS190 variety in higher plant density (Fig. 2). Seed germination was higher in Pershing variety in lower plant density based on standard germination test (Fig. 3) while based on tetrazolium test it was higher in Pershing and Deing varieties in lower plant density (Fig. 4). Electrical conductivity was higher in AGS190 variety in higher plant density (Fig. 5), that resulted to lower quality of seed in this variety.

Relationship between *Phomopsis* seed decay and seed viability tests: The high levels of *Phomopsis* incidence was inversely related to standard germination test (Fig. 6). This negative relationship indicates higher PSD infection will reduce soybean seed quality. PSD infection>30% will reduce percentage germination to<60%.

Similar negative relationship between *Phomopsis* incidence and percentage TZ was observed (Fig. 7).

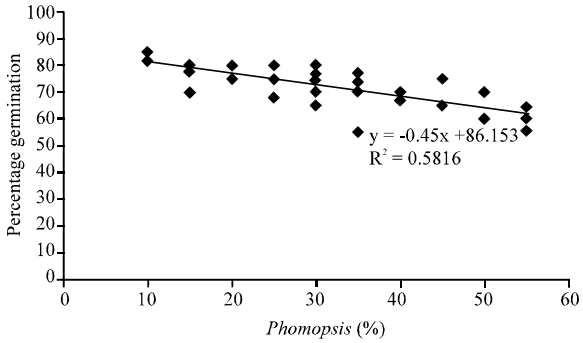

Fig. 6: Relationship between *Phomopsis* incidence and standard germination in two varieties

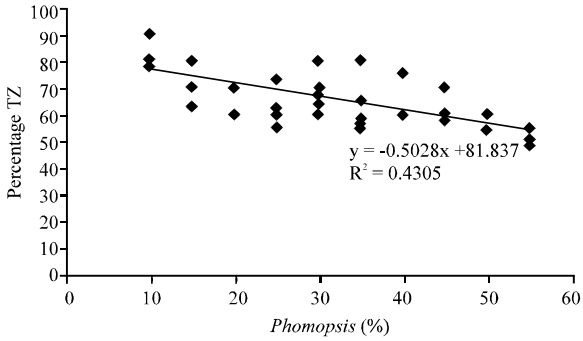

Fig. 7: Relationship between *Phomopsis* incidence and tetrazolium test in two seasons

Seed viability showed negative relationship with PSD incidence for seed germination and tetrazolium test. Many of research emphasized to this relationship. Incidence of *Phomopsis longicolla* was negatively correlated with seed health rate that demonstrated in seed germination, The correlation determined for soybean in 1995, 1996 and 2001 with -0.77, -0.90 and -0.87 respectively (Mengistu and Heatherly, 2006). They demonstrated that seed germination reduced, when seed infection by *p. longicolla* increased. In another study high negative correlation reported between incidence *Phomopsis* and seed germination and vigour of soybean for two years. It was -96 and-86 for 1996 and 1997 respectively (Koninga *et al.*, 2001). This high relationship is the reason of that the seeds are the last part of plant that to exposed the infection and so the infected seeds show this problem in germination processing.

Electrical conductivity showed positive correlation with *Phomopsis* seed decay in soybean varieties (Fig. 8). This result is agreed with Koninga *et al.* (2001) which found a positive relationship between *Phomopsis* incidence and bulk conductivity.

Although the electrical conductivity showed a positive relationship but this test shows the leachates of electrolyte from the seed and less leachated from the imbibed seed indicated higher quality of seed. According to Vieira *et al.* (1994), conductivity values are positively correlated with seed deterioration, so more values of EC shows lower quality of seed. In this study electrical conductivity was the most in AGS190 variety and also was more in higher plant density, due to higher infection to PSD.

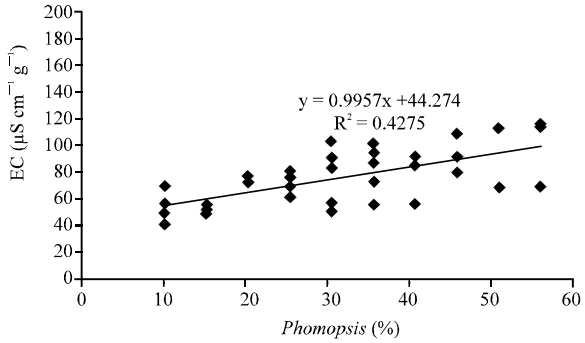

Fig. 8: Relationship between *Phomopsis* incidence and electrical conductivity in two season

This study indicated that the PSD incidence depends on environment and variety and some agronomic practices that effect on canopy microclimate like plant density. The results of this study are similar with what Mengistu *et al.* (2009) obtained. He stated that seed infection by *Phomopsis longicolla* is moisture and variety dependent.

CONCLUSION

This study indicates, soybean can be produced in Malaysia but for producing of soybean especially for seed production, the field environment is very important and the variety that need more days for harvesting must be planted in proper condition with less rainfall and relative humidity. Plant density also can impress on infection to *Phomopsis*, so it must be arrange based on different variety and also in different environment and plant densities varieties may vary in terms of infection to *Phomopsis* seed decay. So the proper variety must be selected for each environment in a suitable plant density.

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