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ตำแหน่งและการถ่ายทอดของเชื้อรา *Macrophomina phaseolina* ในเมล็ดพันธุ์ถั่วเขียวผิวดำ

Location and Transmission of *Macrophomina phaseolina* in Blackgram Seeds

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Abstract : Experiments were undertaken on location detection in seed components and seed to seedling transmission of *Macrophomina phaseolina* in blackgram seeds. *Macrophomina phaseolina* was detected only in the seed coat of blackgram while other seed components like cotyledon, radicle and plumule did not show the presence of this pathogen. In seed transmission study, according to blotter and test tube agar method, restricted germination and production of blemished radicle were found. Soil and sand methods showed remarkable pre-emergence mortality. In some cases, seeds produced seedlings with infected stem and cotyledon, which led to the death of whole plant in the form of damping-off and collar rot eventually. Few seeds were able to emerge giving rise to apparently healthy seedlings, but within about three weeks, they ultimately died producing numerous sclerotia and pycnidia on the dead plants.

บทคัดย่อ : ทำการศึกษาหาตำแหน่งของเชื้อรา *Macrophomina phasiolina* ในส่วนประกอบของเมล็ด และการถ่ายทอดเชื้อรา จากเมล็ดสู่ต้นกล้าในเมล็ดพันธุ์ถั่วเขียวผิวดำ เชื้อรา *Macrophomina phasiolina* พบเฉพาะบนส่วนของเปลือกเมล็ด โดยไม่พบบนส่วนอื่นๆ เช่น ใบเลี้ยง รากอ่อนและยอดอ่อน จากการศึกษาการถ่ายทอดเชื้อโดยวิธีเพาะบนกระดาษชื้นและหลอดอาหาร พบว่าเชื้อรานี้ทำให้ความงอกของเมล็ดลดลง

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และเกิดแผลสีน้ำตาลที่รากอ่อน และจากการศึกษาโดยวิธีเพาะในดินและทราย เชื้อราทำให้ต้นกล้าตายก่อนงอก ในกรณีที่ต้นกล้าสามารถเจริญได้ เชื้อราเข้าทำลายลำต้นและใบเลี้ยง แต่ต่อมาเกิดการตายทั้งต้น ในลักษณะของโรคเน่าคอดินและโคนเน่า เมล็ดบางส่วนสามารถงอกเป็นต้นกล้าปกติได้ แต่หลังจากนั้น 3 สัปดาห์ต้นเหล่านี้จะตาย และมีการสร้างเม็ด sclerotium และโครงสร้าง pycnidium มากมายเจริญอยู่

Index words : ถั่วเขียวผิวดำ โรคหลังเก็บเกี่ยว โรคเมล็ดพันธุ์ เมล็ดพันธุ์
Blackgram, Postharvest discase, seed pathogen

Introduction

Macrophomina phaseolina has been considered as an important fungal pathogen of blackgram in Thailand. It reduces the germination and vigour of the seed and also produces blackish lesions on the edible sprouts (Grewal, 1988; Nath, 1970). Moreover, *M. phaseolina* causes stem and root rot, leaf blight and leaf spot in mungbean which is related species of blackgram (Grewal, 1988). Plenty of works have been carried out on this fungus as a seed-borne pathogen on various crops, albeit the works especially focused on seed transmission on blackgram are still lacking. Therefore, the present investigation was undertaken to know the mode of transmission of *M. phaseolina* from seed to seedling in blackgram and to ascertain the exact location of this pathogen in the seed parts.

Materials and Methods

Seed samples of blackgram variety Uthong 2 was taken for location detection and transmission study.

Location of the pathogen in seed:

The seed sample was carrying 24.00 percent *M. phaeolina* infection as revealed by blotter test recommended by ISTA (1976). One hundred seeds were soaked in distilled water for 2 hours and then they were aseptically dissected into seed coat, cotyledon and radicle and plumule. These dissected components were surface-sterilized with 1.0 percent sodium hypochlorite solution for three minutes. They were then washed in sterile distilled water for three times, and dried on sterile blotter sheet. The four components of each seed were placed side by side on moist blotter paper (Whatman no.1) in 9 cm petridishes. The boundary between adjacent seed components was marked clearly. The plates were incubated under 12 hours alternate cycles of near ultraviolet (NUV) light and darkness for seven days as recommended by ISTA (1976). The presence of *M. phaseolina* was examined using stereo-binocular microscope in each component of seed.

Seed to seedling transmission:

To determine how *M. phaseolina* transmitted from the seed to seedling, several variations of the seedling symptom tests were

employed. The different types of growing on tests are as follows:

I. Blotter method:

In this method, blackgram seeds were plated in glass Petri plates (9 cm diameter) on moistened triple layer of filter paper. Four hundred seeds were taken randomly from the seed sample and were placed at the rate of 10 seeds per Petri plate. All Petri plates were incubated at alternating conditions of 12 hours near ultraviolet light (NUV) and darkness. Observations were made on transmission of *M. phaseolina* after 7 days of incubation.

II. Test tube agar method:

One hundred surface disinfected seeds with 1.0 percent sodium hypochlorite solution for 3 minutes were transferred singly on Pyrex glass test tube (20cm x 2.5cm) under aseptic condition. The test tubes contained 20 ml 2 percent water agar, which was priory autoclaved and solidified. All the test tubes were incubated under alternating NUV light and darkness conditions as described in (I). Data were recorded after 7 days.

III. Sand and soil method:

Macrophomina phaseolina infected seed selection: seed samples were tested by blotter method to find out the *M. phaseolina* infected seeds. After incubation for 3 days, the infected seeds with symptom of *M. phaseolina* infection were separated and used for sand and soil method.

In the plastic pot (10cm x 16cm) containing sterilized sand, *M. phaseolina* infected seeds were planted at the rate of 5 seeds per pot. Total 100 seeds of blackgram were sowed. In the same way, healthy seeds without infection were also planted as control.

M. phaseolina infected seeds were transferred in plastic pots (10cm x 16cm) with sterilized soil, at the rate of 5 seeds per pot. Total 100 seeds were planted. Healthy seeds were also planted as control in the same manner.

In all the methods, data on germination, symptoms appeared on seeds and seedlings were recorded. Isolations were made from root to tips in the infected plants subject to confirmation of *M. phaseolina* infection.

Results and Discussion

Macrophomina phaseolina was detected only in the seed coat of blackgram seeds up to 23 percent (Table 1). The fungus formed both sclerotia and pycnidia on the seed coat (Fig. 1). In other components like cotyledon, radicle and plumule no any presence of *M. phaseolina* was observed. Gangopadhyay *et al.* (1970) also observed the site of *M. phaseolina* only in the seed coat in soybean seeds. Moreover, Raut (1983) and Sadashivaiah *et al.* (1980) also found the highest incidence of *M. phaseolina* in seed coat and pericarp in sunflower seeds, although Raut (1983)

detected this fungus in endosperm and to a lesser extent in the cotyledon in sunflower seeds.

It is evident from the transmission study that *M. phaseolina* gets transmitted from seed to seedlings. Percentage of transmission in blotter and test tube agar method was found 24.00 and 22.00 percent respectively (Table 2). In most of the infected seeds, blemished radicle containing profuse sclerotia and pycnidia of *M. phaseolina* were observed after 3 to 4 days of incubation (Figure 2). Besides, some of the infected seeds did not germinate at all while in others only radicle was initiated which turned into blemishes and died later on; no plumule was developed (Figure 3). Due to this reason, edible sprouts of blackgram very often regarded as dirty and unfit for consumption. Owing to contamination of *M. phaseolina*, blackgram of Thailand is always complained by the importers (Chainuvati *et al*, 1987; Pichitporn and Thavarasook, 1990; Putasamai and Surin, 1988). In sunflower seeds, similar observation was found due to *M. phaseolina* by Fakir *et al.*, (1976) and Raut (1983).

Table 1 **Percentage of *Macrophomina phaseolina* incidence in different seed components of blackgram by blotter method (based on 100 seeds).**

Seed components	Percent incidence of *M. phaseolina*
Seed coat	23
Cotyledon	0.0
Radicle	0.0
Plumule	0.0

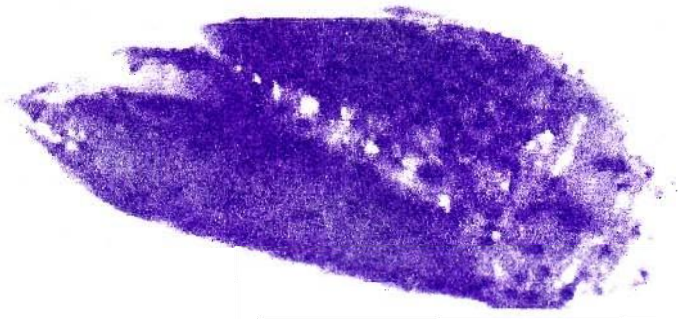

Figure 1 **Sclerotia and pycnidia of *Macrophomina phaseolina* on the seed coat of blackgram seed.**

Table2 Types of infection resulting from *M. phaseolina* infected blackgram seeds in blotter and test tube agar methods.

Methods	Ungerminated seeds seeds (%)	Seedlings with only blemished radicle (%)	Seedlings with infected cotyledon (%)	Total infected seeds (%)
Blotter method [1/]	9.00	13.00	2.00	24.00
Test tube agar method[2/]	7.00	12.00	3.00	22.00

[1/] Based on 400 seeds

[2/] Based on 100 seeds

Figure 2 Germinating dead blackgram seed with numerous sclerotia and pycnidia of *M. phaseolina*.

Figure 3 *Macrophomina phaseolina* infected seedlings of blackgram.

In sand and soil methods, almost similar results were found (Table 3). After transferring of infected seeds in pots, within 7 days, 55 and 63 percent seeds showed pre-emergence mortality in sand and soil methods respectively. When the seeds dug up, numerous pycnidia and scerotia were observed on that. Nineteen and 18 percent seedlings in sand and soil methods respectively showed brown spot on the cotyledonary leaves due to *M. phaseolina* infection. These infections gradually spread to whole stems and leaves, and turned into yellowish colour, which eventually caused death of seedling (Fig. 4). Of course, few symptoms were yielded in cotyledonary leaves in blotter and agar method. Twenty-five and 13 percent seeds in sand and soil methods respectively produced apparently healthy seedlings, but within 21 days all of them died. The different parts of the infected seedlings were cultured in PDA and in every cases the presence of *M. phaseolina* were found. After 23 days of sowing, numerous pycnidia and sclerotia of *M. phaseolina* were formed on all dead seedlings. No any absolutely healthy seedlings stemmed from the infected seeds. But non-infected or healthy seeds produced cent percent robust seedlings in all cases. A similar role of *M. phaseolina* in sunflower seeds was reported by Raut (1983).

Table 3 Percentage of seed to seedling transmission of *Macrophomina phaseolina* by sand and soil method (based on 100 seeds).

Methods	Nature of seeds	Pre-emergence mortality	Pos-emergence Mortality (seedlings with symptom)	Post-emergence mortality (apparently healthy emerged seedlings which died within 21 days	Absolute healthy Seedlings
Sand	Healthy	0	0	0	100
	Diseased	55	19	26	0
Soil	Healthy	0	0	0	100
	Diseased	63	18	19	0

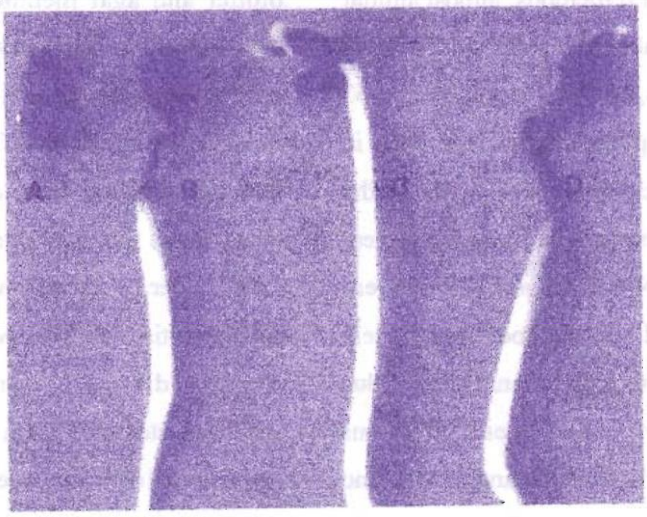

Figure 4 ***M. phaseolina*** **infected seed and seedlings. A- ungerminated seed; B,C & D- spreading symptoms from infected cotyledon to stem.**

Conclusion

From this study it is revealed that seed coat is exclusively the location of *M. phaseolina* in blackgram seeds. It suggests that for controlling this pathogen, surface seed treatment like coating of seeds with chemical fungicides or with biological antagonists in addition to hot water treatment might be effective. The transmission study warns that this fungus gets transmitted to different plant parts and to the soil through infected debris thereby leading to the proliferation of *M. phaseolina* in soil as well as becomes the source of inoculum for further infection cycles.

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