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Evaluation of Some Soybean Cultivars for Susceptibility to *Colletotrichum dematium*

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Abstract. In 1998, reddish brown lesions were observed on leaves and stems of soybean plants at the experimental research station of College of Agriculture at Dierab region. The pathogen was identified as *Colletotrichum dematium* based on morphological and cultural characteristics. Twelve soybean cultivars were tested for their response to infection by *C. dematium* under greenhouse conditions. Six seeds of each cultivar were planted in 15 cm. diameter pots with five replicates arranged in completely randomized design on a greenhouse bench. The experiment was conducted twice. Seedlings at growth stage (VII) were inoculated with conidial suspension of $3x10^6$ spores/ml. Disease severity was assessed 7 days after inoculations, using a scale of 0-4. The results indicated that all twelve tested cultivars were susceptible to infection. Vernal, Harkey, Tracy. M and Kansas 5592, were the most susceptible cultivars, where the other tested cultivars varied significantly in their response to infection by the fungus.

Introduction

The most common pathogen associated with soybean *Glycine max* (L.) Merrill anthracnose was *Colletotrichum truncatum* = *C.dematium* (Pers.ex Fr.)Grove var. *truncatum* Arx, (Syn. *C. dematium* var.*truncata* (Shw.) Arx., and *C.glycines*). The disease causes damage in warm humid areas, resulting in reduced stand, seed quality and loss of yields ranged from 26% to 100% in certain areas of Brazil and India [1]. Soybean anthracnose was most serious disease in the tropic, and sub-tropic growing areas of the world. This may be attributable in part, to high temperature optima required for fungal growth and disease development in the field [2]. The optimum temperature for the germination and germ-tube elongation of *C. truncatum* under laboratory conditions was

20°C and 25°C or soybean pod infection. Three hours of light followed by 9 hours dark was best for spore germination and germ-tube elongation [3]. *Colletotrichum dematium* and other fungi were isolated from leaves and seeds of different genotypes of soybean in Argentina, [4].

In the Kingdom of Saudi Arabia, *C. truncatum* was reported in Jeddah, Bisha, and Al-Hassa on *Medicago Sativa* L. (Alfalfa), [5]. Kaushal and Paul [6] showed that 331 soybean cultivars were screened under severe natural infection by *C. dematium* in the Kangra Valley in India. Only 18 soybean cultivars were moderately resistant, 146 moderately susceptible, 150 susceptible, 17 highly susceptible and found also in general, early maturing soybean lines were severely infected than the latter maturing lines. Khan and Sinclair [7] found that Soybean Cultivars, Kansas, Boone, Corsoy 79 and William 82 were differed in resistance to foliar anthracnose caused by *C. truncatum.* In field studies, yield loss estimated due to anthracnose which was 17% for Kansas, 23% for Cosoy 79, and 30% for Williams 82. The objectives of this study were to evaluate twelve soybean cultivars for their reaction to infection by *Colletotrichum dematium* under greenhouse conditions and to evaluate the anthracnose severity on the first true leaf and determine levels of stem resistance to canker development.

Materials and Methods

For isolation of the pathogen from soybean plants, diseased samples were collected from Dierab at the experimental station in Riyadh region. Diseased tissues were cut into 0.5 cm pieces, placed in 0.5% sodium hypochlorite solution for 3 min., rinsed in sterile distilled water, blotted dry and placed on Difco potato dextrose agar (PDA), containing streptomycin sulfate (50 mg/L.) and incubated at room temperature (22c). Fungal colonies from the tissue pieces were then purified using single spore isolation method. The isolated fungi were examined with a light microscope and identified by using the procedure described by [8,9]. The fungus was identified as *C. dematium.*

Inoculum of *Colletotrichum dematium* was prepared by culturing isolate on Potato Dextrose Agar (PDA) in petri dishes for 10 days old at room temperature (22c) under 8 hr fluorescent light. Spore suspensions were prepared by scraping spores from the agar plates flooded with sterile distilled water. The suspension was filtered through two layers of cheesecloth to remove mycelial fragments. Spore concentrations were adjusted to $3X10^6$ spores per milliliter by using a hemacytometer.

Seeds of 12 soybean cultivars (Table 1), were planted in 15-cm diameter pots containing 1:1 steamed sand- soil mix. Six seeds of each cultivars were sown per pot with five replicate arranged in a completely randomized design under greenhouse conditions. The experiment was repeated twice.

Table 1. Evaluation of soybean cultivars to *Colletotrichum dematium* under greenhouse conditions

Cultivars	Disease severity(DS) (percentage of leaf area diseased)	Disease severity rating (DSR) (stem canker)
Dillon	45.00 ***j****	2.00 ***g***
Kansas5592	75.20 ***c***	3.60 ***abcd***
Centennial	52.20 ***h***	3.00 ***f***
Epps	47.40 ***I***	1.96 ***g***
Leflare	56.20 ***g***	3.32 ***e***
Hill	67.40 ***e***	3.54 ***cde***
Lee	64.00 ***f***	3.50 ***de***
Tracy. M	78.20 ***b***	3.76 ***abc***
Prolina	70.38 ***d***	3.54 ***cde***
Vernal	82.80 ***a***	3.84 ***a***
Ransom	64.10 ***f***	3.56 ***bcde***
Sharkey	76.oo ***c***	3.80 ***ab***

*Means followed by the same letter within column are not significantly different (*P= 0.05*).
**Values represent the means of 60 seedlings of each soybean cultivar in five repli cates (6 seedlings per replicate) in two combined experiments.

Seedlings of each cultivar at the first true leaf stage (at the V2 growth stage) were sprayed to runoff with Spore suspensions of *C. dematium* using atomizer. Anthracnose severity on the first true leaf was rated 7 days after inoculation on the following scale 0= no disease, 1= 1-10%, 2=11-30% 3=31-60, 4= 61-9o% of leaf area affected (Fig1), [10,11]. Stem canker phase rated 15 days after inoculation on the following scale 0= no disease, 1= 1-20%, 2=21-40 % 3=41-60, 4= 61-85% of stem area affected.

The percentage of disease severity was calculated as % DS= $\sum$ (n X r) X 100 / 4N, Where n = number of leaves of a given disease rating, r = Disease severity rating, and N Total number of stem rating [12,13]. Data were analyzed by the SAS ANOVA procedure [14]. Means were separated using least significance difference (LSD) (*P=0.05*) [15].

Results and Discussion

Soybean anthracnose symptoms were observed 7 days after inoculation on the first true leaf (Trifolioate leaf) and unifoliolate leaf as dark brown or reddish – brown areas and necrosis of lamina viens. These symptoms resembling those observed in the field were apparent. Stem symptoms began as tan brown discolorations, that later speared, becoming dark purplish-brown. Sunken, necrotic lesions and small black acervuli also were visible on the stems. Symptoms of stem canker phase developed 15 days after inoculation as irregularly shaped brown areas covering the surface of the infected stems (Fig 2), and showing that considerable variation in symptoms on soybean cultivars.

Fig.1. Disease rating scale on the first true leaf of soybean 0= Healthy (No symptoms), 1= 1-10%, 2=11-30%, 3= 31-60%, 4= 61-90% of leaf area affected.

The combined data of two greenhouse experiments showed that significant differences were observed between tested soybean cultivars for disease assessment, *(P=0.05*) of this study (Table 1). Vernal, Tracy M, and Sharkey cultivars were found highly susceptible to *C. dematium* with average disease severity (DS) of 82.80%, 78.20%, and 76.00%, respectively and average disease severity rating (DSR) of stem canker phase (DSR) of 3.84, 3.76, and 3.80, respectively. Dillon and Epps cultivars were less susceptible with average disease severity (DS) of 45.00% and 47.40 respectively and average disease severity rating (DSR) of stem canker phase (DSR) of 2.00 and 1.96, respectively. Generally, our results support those obtained by other investigators [16] who found that forty-eight soybean cultivars were evaluated in the field after inoculation at the pod initiation stage by *C. truncatum.* None of soybean cultivars were immune, however the degree of susceptibility varied among tested cultivars. Roy [17] found that C. *truncatum* was extremely virulent on soybean seedlings and caused considerable pre- and post - emergence seedling death. Yoshide and Shirata, (1999) suggested that *C. dematium* can overwinter in infected or latently infected leaves and that these leaves can be a source of primary inoculum the following year. Non of the tested soybean cultivars in this study were resistant to *C. dematium.*

Fig. 2. Considerable variation among soybean cultivars (Vernal, Sharkey, and Dillon), in their response to infection by *C. dematium*.

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تقييم بعض أصناف فول الصويا لمدي استجابتها للفطر كوليتوتريم ديماتيم

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ملخص البحث: في عام ١٩٩٨م شوهدت بقع بنية حمراء اللون على أوراق وسيقان نباتات فول الصويا بمحطة الأبحاث الزراعية بكلية الزراعة في منطقة ديراب. عرف المسبب المرضي على أنه كوليتوتريكم ديماتيم اعتماداً على الصفات المورفولوجية والمزرعية المميزة للفطر . تم اختبار ١٢ صنفاً من فول الصويا لمدى استجابتهما للإصابة بالفطر تحت ظروف البيت المحمي بكلية الزراعة. تم زراعة ٦ بذور من كل صنف في أصيص قطره ١٥سم في خمس مكررات وتم تصميم التجربة في قطاعات عشوائية وكررت مرتين. تم تلقيح البادرات في مرحلة تكشف الورقة الحقيقة الثانية بمعلق من جراثيم الفطر عند تركيز ٣ × ١٠ ٦ جرثومة / مل . قدرت شدة الإصابة بعد ٧ أيام من التلقيح باستخدام مقياس شدة الإصابة تراوح بين صفر و ٤ درجات. أوضحت النتائج أن جميع الأصناف المختبرة كانت قابلة للإصابة. الأصناف فيرنل و هاركي ٥وتراسي وكانسس ٥٥٩٢ كانت من أكثر الأصناف قابلية للإصابة بالفطر ، أما بقية الأصناف الأخرى أظهرت اختلافات معنوية في مدي استجابتها للفطر.