FUSARIUM NYGAMAI A CAUSAL AGENT OF ROOT ROT OF *VICIA FABA* L. IN THE SUDAN

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SUMMARY

Wilted and rotted plants of *Vicia faba* were received from different localities in the Sudan. Among several *Fusarium* spp., *Fusarium nygamai* was recovered. Conspicuous symptoms were among others black root rot, associated with rot and death of the lateral root system. Severely infected plants showed black neck canker at soil level. These symptoms were usually accompanied by loss of the leaves' turgor, these then turned brown and died. Death of intact leaves also occurred. Most of the strains proved to be pathogenic to *Vicia faba*. Disease intensity varied between 28-100 %. This is the first report of *Fusarium nygamai* as a pathogen of *Vicia faba*.

INTRODUCTION

Vicia faba, is an annual grain legume which originated in the Near East. There are three seed types: broad bean or Windsor bean which is a large seed type, horse bean, a medium seed type, and a small seeded type (about the size of a pea) known as faba bean or tick bean. *Vicia faba* is grown in semi-tropic countries and at higher elevations or in the cool season in the tropic parts of Asia, and Africa as well as in Mediterranean regions and in the temperate zone. The area of faba bean production in the Sudan extends from the north around Dongola area (19° 10' N, 30° 28' E) to Khartoum State (15° 36' N, 32° 31' E) and south to Gezira, Elrahad and New Halfa (Salih, 1994). Faba bean is one of the most important legume crops in the Sudan and it is grown under irrigation as a winter crop. The total faba bean area for the season 2000/2001 in the Sudan was approximated at 60900 ha (Suliman, personal communication). However, the area of production fluctuates yearly according to farmers preference for broad beans over French beans as influenced by market prices. The average yields vary from 2857.14 kg/ha under research condition to about 1428.57 kg/ha under farmers condition. The yield prediction is also erratic due to the prevalence of wilt and/or root rot which is caused by *Fusarium* spp. and other damping off pathogens. This disease complex became serious in recent years and it is considered one of the main constraints for faba bean production in the Sudan, especially in years when relative high temperatures prevail. Several pathogenic *Fusarium* spp., such as *Fusarium oxysporum* Schl. f.sp. *fabae* Yu & Fang, *F. solani* (Mart.) Sacc. f. sp. *fabae* Yu & Fang, *F. avenaceum* (Fr.) Sacc., *F. inflexum* Schneider and *F. equiseti* (Corda) Sacc. were reported on faba bean (Yu 1944; Yu and Fang 1948; Joshi and Saksena

1983; Ivanovic *et al.* 1987; Lenti 1991). The main pathogens thought to be associated with wilt and root rot disease of faba bean in the Sudan are *Fusarium acutatum* Nirenberg and O'Donnell, *Fusarium oxsporum*, *Fusarium solani*, *F. subglutinans* as *Fusarium moniliforme*, *F. equiseti*, *Pythium* spp., *Rhizoctonia solani*, and *Sclerotium rolfsii* (Ibrahim and Hussien 1974; Ibrahim and Owen 1981; El Hilu Omer and Mukhtar 1986; Kurmut *et al.* 2000). In addition to the effect on crop yield, the disease significantly reduces seed quality particularly the protein content. Frequent isolation of *Fusarium nygamai* from infected plants initiated this work to study its pathogencity towards *Vicia faba* plants.

MATERIALS AND METHODS

Source of Infected Plant Samples: 15 samples of wilted and/or rotted plants of *Vicia faba* were received from Hudeiba Research Station and Shambat Research Station, Sudan, respectively, each sample consisting of 5 plants.

Isolation and characterization of fungi from diseased plants: Isolations were made from rotted and wilted roots of naturally infected plants of faba bean. The roots were thoroughly washed under running water to remove adhering soil particles and left to dry for 10-20 minutes. Infected plant roots were passed through flame for surface sterilization, then they were cut into small pieces of 1mm and placed into plastic Petri dishes of 9 cm in diameter containing SNA (synthetic low nutrient agar: 1 l distilled water, 1 g KH_2PO_4, 1 g KNO_3, 0.5 g $MgSO_4.7H_2O$, 0.5 g KCl, 0.2 g dextrose, 0.2 g sucrose, 0.6 ml 1N NaOH, 23 g techn. agar No 3 by Oxoid; Nirenberg 1990). Bacterial growth was suppressed by a combination of antibiotics (chlortetracycline 10 mg/l, dihydrostreptomycin-sulphate 50 mg/l, penicillin G 100 mg/l). Plates were incubated in the laboratory at room temperature. After 2-4 days colonies were typified on the basis of conidiophores and conidia on the aerial mycelium using a Zeiss stereomicroscope. Pure cultures of the strains were made by streaking the conidia from the aerial mycelia on SNA containing antibiotics in Petri dishes. For microscopic studies isolates were transferred to SNA with a ca. 1 x 2 cm piece of sterile filter paper placed on top of the cooled agar. Cultures were incubated for 10-14 days at 20 °C under permanent black light (Philips TDL 18 w/08; four light bulbs spaced 12 cm apart and 25 cm above the Petri dishes; Nirenberg, 1990) as well as in complete darkness. Petri dishes were examined under the low power of a Zeiss microscope (x100-200) to study features of the aerial mycelium. The sporulation area was marked on the reverse of the Petri dish, cut out (1×1 cm pieces), mounted on a microscopic slide with a drop of sterile water as well as a cover slip on the top of it for further investigation with a Zeiss Axiomat photomicroscope. At x 400 and x 800 magnifications the measurements and the characteristic morphological features of the isolates

were taken, noted and photographed. Representative cultures were preserved in tubes containing 10 g sterilized soil, at 5±°C.

Pathogenicity

To produce inoculum of the isolates for the infection test, a small amount of the stored soil in which the fungi were preserved, was spread on SNA Petri dishes. After 2 days the growing colonies were inspected for purity. Pure cultures were immediately transferred onto potato dextrose agar (Difco) plates. The plates were incubated for 7 days at room temperature. Inoculum was prepared by transferring agar pieces cut from the periphery of the cultures into 250 ml Erlenmeyer flasks filled with 100 g sterilized sand and maize meal (9:1). Control flasks were inoculated with sterile agar plugs. Inoculated flasks were incubated for 14 days at 22°C ± 2°C. Hundred grams of this inoculum were mixed with 2 l steam-sterilized sandy loam and filled in 15 cm disinfected pot. Each pot was planted with 4 surface sterilized seeds of *Vicia faba*. Seeds were surface sterilized by immersion in 0.5 % solution of sodium hypochlorite for 2-3 min., then the seeds were removed, washed four times in distilled sterilized water, and air dried on the top layer of three sterilized filter paper sheets before sowing. The treatments were replicated 4 times and randomly distributed on the glasshouse benches at 25-30 °C and under a 12/12 h light and dark cycle. Appearance and development of disease symptoms were observed daily, the final infection count was taken 4 weeks after inoculation. Infection response is expressed by the disease level (%) based on the incidence of infected plants and severity of disease symptoms (Ibrahim and Nirenberg 1993). Disease severity was calculated as percentage based on a numerical scale (0-4): 0 = no symptoms; 1 = symptoms appear only on the cotyledons or first leaf of plant; 2 = symptoms appear above the first true leaf of the plant; 3 = whole plant starts to shrivel and turns dark brown showing foot rot symptoms; 4 = death of plant. Reisolations from infected plants were made.

$$\text{Disease level \%} = \frac{\Sigma_i^k (n_i i)}{n \times k} \times 100$$

i= 1; k= 4

RESULTS

More than one *Fusarium* species was recovered consistently from infected roots. Isolates were identified as *Fusarium acutatum, Fusarium compactum, Fusarium nygamai, Fusarium oxysporum, Fusarium proliferatum, Fusarium semitectum, Fusarium solani*. Other Fusarium species which were also isolated but not so consistently were *Fusarium equiseti, Fusarium incarnatum*; and a few other species of *Fusarium* section Gibbosum. Fungi other than

Fusarium were also detected: *Macrophomina phaseolina*, *Rhizoctonia* sp. and *Verticillium* sp. The most prevalent species recovered in almost all samples were *Fusarium solani*, *Fusarium nygamai*, *Fusarium acutatum* and *Fusarium oxysporum* respectively (Table 1 and 2).

The pathogencity test with *Fusarium nygamai* isolates showed 28.58-100 % disease incidence. All 6 strains isolated from infected plants and tested were pathogenic to *Vicia faba*. The highest percentage of infection was produced by isolate BBA 71178, which caused complete death of all plants within 2 weeks, followed closely by isolates BBA 71181, BBA 71180, and BBA 71561, respectively (Table 3). Infection sometimes gave rise to pre-emergence seed rot. Usually the seeds were completely covered by mycelial growth of the fungus. Infected emerging seedling showed root rot within 15-21 days. Aerial symptoms of root rot infected plants were flaccidity of the lower leaves, later the margin of the leaves started to turn brown and finally the leaves withered and dropped. After 5 weeks infected plants having survived exhibited reduced plant height and fewer leaves per plant compared to the control. The rot started at soil level as a necrosis extending downwards along the main root; affected tissue was black and dry associated with rot and death of the lateral root system. The cortex and vascular tissue of the root were invaded by the fungus and completely decayed, turning dark brown. Severely infected plants showed black neck canker at soil level. Isolation from infected root sections yielded *Fusarium nygamai*.

Table 1: Frequency of *Fusarium* spp. and infected plant samples at Hudeiba (1170 recovered isolates)

Isolates	Frequency of recovered isolates (%)	Infected plant samples (%)
F. acutatum	11.54	66.67
F. compactum	2.99	40.00
F. nygamai	17.95	66.67
F.oxysporum	14.79	60.00
F. proliferatum	2.31	26.67
F. smitecticum	1.28	20.00
F. solani	21.79	80.00
Other species	27.35	-

Table 2: Frequency of *Fusarium* spp. and infected plant samples at Shambat (1393 recovered isolates).

Isolates	Frequency of recovered isolates (%)	Infected plant samples (%)
F. acutatum	11.56	73.33
F. compactum	5.17	40.00
F. nygamai	15.43	80.00
F.oxysporum	13.28	66.67.
F. proliferatum	2.30	40.00
F. smitecticum	3.80	33.33
F. solani	21.54	86.67
Other species	26.92	-

Table 3: Response of *Vicia faba* to different isolates of *F. nygamai* (Mean of 4 replications)

Isolate No. /BBA	Disease Level (%)
Control	0
BBA 71561	78.8
BBA 71177	28.6
BBA 71178	100
BBA 71179	64.3
BBA 71180	78.6
BBA 71181	85.5

DISCUSSION

The results of the infection test on faba bean with strains isolated from infected roots have shown that *Fusarium nygamai* is able to cause root rot as well as stunting, retarded growth, and shriveled leaves. The pathogenicity of the isolates was confirmed and the stability of the pathogen was demonstrated.

This is the first report of *Fusarium nygamai* as a causal pathogen of a root rot on faba bean. Isolation of *Fusarium nygamai* from roots, seeds and soil was reported. In their description of *F. nygamai* Burgess & Trimpoli (1986) wrote that they initially isolated the fungus from the roots of sorghum and subsequently from head of grain sorghum, bean roots and from soil. Onyike *et al.* (1991) and Onyike and Nelson (1992) were able to isolate *F. nygamai* from millet and sorghum from Nigeria, Zimbabwe and Lesotho. Although *Fusarium nygamai* is commonly isolated from the roots, seeds, and soil, no one has described it as pathogenic to any crop before. This shows that *F. nygamai* had been considered as a none pathogen to sorghum, millet, and bean by plant pathologists. If identified on the basis of the symptoms only, *F. nygamai* can be confused with *F. acutatum*, *F. oxysporum*, and *F. solani* which were already described as root rot pathogens on faba bean (Ibrahim & Hussien 1975, Ibrahim and Owen 1981, Kurmut *et al.* 2000). Now it is evident that *F. nygamai* is a root rot pathogen of *Vicia faba* in the

Sudan and it can be added to the already known *Fusarium* spp. causing root rot of *Vicia faba*.

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