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Full Length Research Paper

Epidemiological aspects of bean decline disease caused by *Fusarium* species and evaluation of the bean resistant cultivars to disease in Northwest Iran

H. Saremi[1]*, M. E. Amiri[2] and J. Ashrafi[3]

[1]Department of Plant Pathology, Faculty of Agriculture, Zanjan University, Iran.
[2]Department of Horticulture, Faculty of Agriculture, Zanjan University, Iran.
[3]Department of Plant Protection, College of Agriculture, Ferdowsi University, Mashhad, Iran.

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The national project of Iran aimed to study the etiology of bean decline disease and the induction of bean resistant cultivars to the disease. Samples of 75 infected bean plants (300 in total) were collected from four growing locations in three provinces of Northwest Iran. The isolated pathogens were *Fusarium solani* (41.6%), *Fusarium oxysporum* (32%), *Fusarium sambucinum* Fuckel (18%), *Rhizoctinia solani* (5.4%) and *Pythium debaryanum* (2.7%). Sufficient inoculums of three *Fusarium* species were prepared in chaff medium and added to the natural field soil to obtain 2000 cfu g[1]/soil of each species in treated soils. Ten local and international bean cultivars (Emerson, Dehghan, Joules, Akhtar, Naz, Sayad, Golden, Keshavarz, Daneshkadeh and Soheir L- 90) were sown into 50 infested and 50 non-infested pots; then their growth and yield productions were evaluated. 'Naz' was the most resistant cultivar, which produced the highest dry matter yield (175 g /15 plants) and the lowest yield reduction (9.3%), while 'Joules' had moderate production (110 g/15 plants) and loss (31.7%), and 'Soheir L-90' showed the highest susceptibility with the lowest (60 g/15 plants) and highest yield loss (52%). Thus, the results show the most important bean cultivar resistant to *Fusarium* decline disease, which is significant in favor of yield productions.

Key words: Bean, decline, resistance, *Fusarium.*

INTRODUCTION

Dry bean (*Phaseolus vulgaris* L.) is the most important agricultural crop in Iran found primarily in Northwest Iran. It is being affected by decline diseases which results to major yield loss. Susceptible cultivars show significant losses, especially, if cool conditions prevail during the first few weeks of the seedling stage (Naseri, 2008). Disease incidence and severity often vary greatly in areas with a history of the disease. This disease is an economic problem in most growing locations, which shows up to 70% loss on some areas in Northwest Iran (Saremi et al., 2007; Naseri, 2008).

*Corresponding author. E-mail: saremiho@yahoo.co.uk. Tel: +98 241 515 2266. Fax: +98 241 515 2331.

The most common bean disease is *Fusarium* wilt or *Fusarium* decline, which is associated with *Rhizoctonia* root rot and *Pythium* damping off all over the world (Bowers and Parke, 1993; Bruton and Miller, 1997; Iglesias et al., 2000; Ken and Motoaki, 2006; Aoki et al., 2007; Yangui et al., 2008). *Fusarium* decline is one of the most important complex root rots and vascular diseases affecting dry bean production worldwide (Schwartz et al., 2005; Chaudhary et al., 2006; Cichy et al., 2007; Deeksha et al., 2009). Actually, *Fusarium* species were regularly associated with decline and yield reduction in the bean growing areas. In general, any factor which contributes to a reduced rate of root growth increases the plant's susceptibility to *Fusarium* decline disease. For instance, early planting in cool, moist soil and compactness of soil in the form of tillage or hardpan layers favor

Figure 1. The major bean growing locations in Northwest Iran (green parts), including Qazvin, Zanjan and East Azarbyjan provinces, where infected bean samples were collected.

the disease. Researchers believe that irrigation, soil factors and plant residues affect the main severity of decline disease in bean plants (Horsley et al., 2000; Iglesias et al., 2000; Ogg et al., 2000; Kuruppu et al., 2004). *Fusarium* wilting attacks bean plants which have been subjected to environmental stresses. Severe infections can lead to plant death and crop losses, because the taproot is damaged causing proliferation of a shallow fibrous root system that can only provide inadequate water uptake in dry soils (Bruton and Miller, 1997; Jensen et al., 2004). However, severity of bean root rot disease involves cropping history, plant spacing, soil moisture, temperature stresses and soil compactness (Buruchara and Camacho, 2000; Lozovaya et al., 2004; Nunez et al., 2005).

Fusarium root rot is usually more apparent during blossoming and in the beginning of pod set, which is the most critical stage of bean productivity. Many of the *Fusarium* species are capable of surviving long periods in soil as thick-walled resting spores or chlamydospores, so the disease may occur even in the absence of bean planting (Saremi, 2000). Generally, bean plants can be affected at any stage from seedling until harvest, that is, the majority of plant death appears to occur when plants are in the seedling stage to the four-leaf stage of growth (Schwartz et al., 2005). Although infected bean plants may be scattered throughout the field, most of them occur in localized areas and are stunted, in that they show severe decline symptoms. As a result, certified seeds, tolerant to *Fusarium* wilting or resistant varieties, are recommended to the bean growers since it can be the best way for increasing yield production. Usually, different soil-borne fungal pathogens are widespread in dry bean and snap bean growing areas of Northwest Iran; generally, *Fusarium* species are cosmopolitan soil borne fungi and their populations fluctuate in terms of geographical distribution all over the world (Saremi et al., 1999).

Management of *Fusarium* decline disease would be significant in most bean growing areas, even though chemical control and crop rotation are not effective in infested fields (Chowdbury et al., 2002). The biological control process has also been reported as a way of controlling *Fusarium* diseases on crops, but it showed some limitations on bean disease management (Mojibur et al., 2006; Modupe et al., 2007). On the other hand, resistant cultivars to *Fusarium* diseases have recently become available (Fall et al., 2001; Schwartz et al., 2005; Roman-Aviles and Kelly, 2004; Sudhamoy et al., 2009). Therefore, the main aim of this study was to determine the resistance or susceptibility of some bean cultivars against bean decline diseases in Northwest Iran.

MATERIALS AND METHODS

Collection of plant samples and culturing

Four main bean growing areas located in three provinces (Gazvin, Zanjan and Azerbaijan), in Northwest Iran, were visited during the three growth seasons (Figure 1). The specific climatic characteristics of these areas are as follow: the latitude north is 32 to 33°;

the above sea level (altitude) is 340 to 750 m; the average temperatures are 16.2 to 18.4°C; the average maximum temperatures are 33.4 to 36.2°C (July); the average minimum temperatures are 2.5 to 2.8°C (January) and the average annual rainfalls are 360 to 410 mm. The physical and chemical characteristics of the soils grown under bean were clay (15 to 21%), silt (18 to 23%) and sand (61 to 65%), while limestone was <4.5 to 5.1%, pH = 7.5 to 7.9, electric conductivity (EC_e) was 5.4 to 5.6 dm m^{-1} and organic matter was less than 1%. The infected plant samples were collected from bean fields that showed decline, damping off, stunted growth and wilting. In each area, 75 infected plants were randomly collected to give a total of 300 infected plant samples. Three root pieces of infected root from each sample were cultured on potato dextrose agar (PDA), as a common medium after surface sterilization was done for isolation of the pathogens.

Isolation and identification of the pathogens

Infected root samples were cut into 0.5 to 1.0 cm pieces, and then dipped into sodium hypochlorite (0.5%) for 2 min. The pieces were rinsed in sterile distilled water, dried on sterile filter paper and plated on the medium in Petri dishes. The samples were cultured in PDA, in The Plant Pathology Lab, Zanjan University, to isolate causal agents. All cultures were kept in a room that had light (25 ± 2°C, RH 72 ± 5%, day and night with a 12 h photoperiod) to allow the growth of colonies. Morphological characteristics of isolates including colony morphology, shape of conidia and conidiophores were examined in order to identify the pathogens (Burgess et al., 1994; Yurika et al., 2007; Yangui et al., 2008).

Fusarium species isolation

Fusarium species were isolated, as the main causal agents of root rots and wilting were cultured in selective medium, peptone PCNB agar (PPA), natural medium and carnation leaf agar (CLA). The cultures were incubated under fluorescent lights with a 12 h photoperiod and intermittent black UV light tube at 25°C during the day and 20°C at nights, and then identified according to the key for taxonomy of *Fusarium* species (Burgess et al., 1994; Saremi, 2005).

Status of decline disease in the bean fields

Bean decline disease in the infected field was classified into four groups based on percentage of the symptom. Practically, healthy plants showed 1 to 25%, mid to intermediate showed 25 to 50%, intermediate showed 50 to 75% and sever showed 75 to 100% of decline symptom. However, foliage wilting was associated with the quantity of yield production of bean cultivars as the resistance index (RI).

Fusarium cultures in chaff medium

Chopped wheat chaff was soaked overnight in water and drained, after which 50 g was placed in polyester oven bags, which were then stopped with cotton wool and autoclaved twice on successive days. Each bag was inoculated with 2 ml conidial suspension (10^6 ml^{-1}) of a single species and mixed by shaking. *Fusarium* cultures were incubated in intermittent black UV light room, as previously described, and shaken regularly to enhance colonization. The cultures were air dried after four weeks in a laminar flow cabinet and then milled individually using an electric mill which was swabbed with ethanol before and after milling inoculums of each species. The milled material was sieved (710 µm mash) and mixed thoroughly with 4 to 5 volumes of autoclaved sieved soil. The concentration of colony forming units (cfu) in the chaff-soil inoculums of each species was determined by dilution plating on PPA, according to Burgess et al. (1994) and Saremi et al. (1999).

Natural infected soil preparation and soil dilution technique

Five soil samples were randomly collected from bean growing fields in Abhar, as a major natural infected location in Zanjan province. They were air dried and sieved to remove stones and large particles, after which they were mixed thoroughly. Soils were diluted and plated twice to determine the propagule density of *Fusarium* species resident in common grown fields. Inoculums' density of *Fusarium* species occurring in naturally-infested fields was assessed using soil dilution technique. Specifically, 0.5 g of agar was poured in 1 L of water, after which 100 ml of the solution was poured in a special glass bottle and was then autoclaved. 1 g of contaminated soil was added to the sterilized water agar (WA) to prepare soil dilution; then 1 ml of the prepared soil suspension was taken by pipette and dispersed on the selective medium (PPA) of the Petri dishes uniformly and incubated for three days. The numeral of the colony forming unit (cfu) of *Fusarium* species was assessed to determine their propagule density in the field soil (Saremi et al., 1999).

Planting bean cultivars in pots

Natural infested soil

An experiment was conducted to distinguish the resistance of ten local and international bean cultivars (Emerson, Dehghan, Joules, Akhtar, Naz, Sayad, Golden, Keshavarz, Daneshkadeh and Soheir L- 90) on three major *Fusarium* species, considered as the main agents of bean wilt. Hundred uniform white plastic pots (30 cm in diameter and 30 cm in depth) were lined with a plastic bag to prevent drainage of water and 50 pots were filled with 7 kg of natural infested soil from the bean field. The isolated (500 cfu/g^{-1}) *F. oxysporum* Schlecht emend Snyder and Hansen (700 cfu/g^{-1}), and *F. sambucinum* Fuckel (500 cfu/g^{-1}) were observed in the collected soil.

Artificial infested soil

Artificial infested soil was provided by mixing the required inoculums of *F. solani, F. oxysporum* and *F. sambucinum* obtained from the chaff-soil mixture, with the natural collected soil. The procedure was followed to provide appropriate population density of *Fusarium* species in soil and each pot was filled uniformly with 7 kg artificial infested soil. The final propagule density of each *Fusarium* species in the infested soil was determined as 2000 cfu g^{-1} in the regular population that has been already reported (Saremi et al., 1999). The statistical design was based on a complete block design with five replicates and each replicate contained three seedlings. For each cultivar, characteristics of vegetative growth, yield production (g/ 15 plants) and yield loss (%) were monitored during the experiment.

Maintenance of pots in glasshouse

All pots were maintained under glasshouse, and the main environmental factors including soil water, relative humidity, daily temperature and light intensity were as same as the field conditions. During bean plant growth, all pots were watered uniformly twice a week, in equal amount. Different aspects of plant

Table 1. Percentage of isolated pathogens from the 300 collected samples from four bean growing locations in Takeatan, Abhar, Mahneshan and Mianeh, in Northwest Iran.

Isolated species	Takeatan	Abhar	Mahneshan	Mianeh	Infected rate[a] (%)
	Number of isolate / 75 sample				
F. solani	32	25	37	31	41.6**
F. oxysporum	26	27	20	23	32.0**
F. sambucinum	10	19	14	12	18.3*
Rhizoctonia solani	5	2	3	6	5.4[n.s.]
Pythium debaryanum	2	2	1	31	2.7[n.s.]

[a]Infected percentage for each species was calculated based on (total isolated species) /300 X100.

Figure 2. Root rot (a) and vascular reddish-brown lesions (b) on infected bean caused by *Fusarium* species grown in Zanjan province.

growth, such as plant appearance and yield productions (seeds) were calculated for each cultivar to assess the effects of pathogens on bean growth and the function of decline disease.

Yield production

Three months after planting, yield (seed) was harvested and then weighted. Since each treatment (cultivar) contained 15 plants, the yield was therefore calculated as g/15 plants. Final yield production was judged as the Resistance Index (RI) and then the yield loss was calculated as X non-infested yield/ 100 (non-infested yield minus infested yield). Data were evaluated by analysis of variance with SPSS. When the F test was significant, means were separated by Duncan's Multiple Range Test ($P \leq 0.05$).

RESULTS

Isolated causal agents

Several fungal species were isolated and identified from the samples collected in the fields. The isolated pathogens that were frequently recovered were *Fusarium* species, as well as *F. solani*, *F. oxysporum* and *F. sambucinum*. The study showed that *F. solani* was the most often recovered pathogen (41.6%), followed by *F. oxysporum* (32%) and *F. sambucinum* (18.3%) in all the four locations. Other soil borne fungal pathogens such as *Rhizoctonia solani* (5.4%) and *Pythium debaryanum* (2.7%) were also occasionally shared with the decline disease (Table 1).

Infected plant symptoms

The appearance of the entire bean cultivar plants was not similar, in that the infected bean plants exhibited variable sizes (stunting and wilting). The infected roots were marked by red-brown, streaking crown and vascular system in the fields (Figure 2). The pathogen caused death and decay of lateral roots which was accompanied by plant wilting in advanced stages. Affected plants survived by proliferating new fibrous roots above the

a b

Figure 3. The symptoms of pre emergence (a) post emergence, (b) root rot and wilting which resulted to bean decline, infected by *Fusarium* species, in Zanjan province.

a b c

Figure 4. Susceptibility to the decline disease among different bean cultivars, like 'Naz', which is a healthy cultivar. (a) Akhtar was mid to intermediate; (b) Daneshkadeh as sever wilting symptom grown in Zanjan location field.

infected root area in some cases. The survey used in this study showed that continuous cropping of dry beans in the same location (Zanjan province) resulted in increase of the root rot pathogens in field soils. In some areas, infected seeds failed to germinate, in that there was pre- or post-emergence of wilting plants (Figure 3a and b). Roots or stems of some emerged seedlings were also attacked at the soil line and were wilted. In other words, infected roots were generally discolored or rotten and showed reddish-brown lesions in some parts (Figure 4b).

Percentage of decline in the fields

The findings of this study show that decline or wilting was the main disease on bean plants cultivars in the fields during the growing season. The study indicate that there was diverse susceptibility to the wilting disease among different bean cultivars. Beside, the 'Naz' was an almost healthy cultivar with less wilting symptom (0 to 25%) in most locations (Figure 4a), whereas 'Soheir L-90' and Daneshkadeh demonstrated the highest susceptibility with sever wilting symptom (75 to 100%) in the field (Figure 4c). Some other cultivars, such as Joules and Emerson, showed mid to intermediate (25 to 50%), while Golden and Akhtar indicated intermediate (50 to 75%) wilting symptoms in the field (Figure 4b). However, the percentage of wilting could affect the vegetative growth and yield production of each cultivar in the growing field.

Resistant cultivars and rate of yield losses

Cultivation of bean cultivars on infested soils caused root rot and wilt disease that resulted to significant yield losses (P = 0.05) on some susceptible cultivars (Table 2).

Table 2. Yield losses in the ten bean cultivars (15 plants for each cultivar) grown in glasshouse, Zanjan province, Iran[a].

Cultivar	Yield production (g/15 plants)		Yield loss (%)
	Non-infested plant	Infested plant	
Naz	193^{d}	175^{i}	9.3^{a}
Sayad	172^{c}	128^{f}	25.6^{b}
Joules	161^{c}	110^{df}	31.7^{b}
Emerson	149^{b}	91^{d}	38.9^{c}
Golden	148^{b}	88^{c}	40.5^{c}
Dehghan	139^{b}	83^{c}	42.2^{c}
Akhtar	150^{cb}	84^{c}	44.5^{cd}
Keshavarz	128^{a}	5.0^{a}	49.2^{d}
Daneshkadeh	130^{a}	64^{b}	50.8^{d}
Soheir L-90	125^{a}	60^{b}	52.0^{d}

[a]Means within each column with the same letter are not significantly different using LSD = $P \leq 0.05$.

Yield productions of bean cultivars grown on non infested soil pots were compared with dry matter production on infested soil pots. The results show that there were significant differences between yield productions in various cultivars (Table 2). Among the 10 different cultivars, 'Naz' had the less wilting and produced the greatest yield (193 g/15 plants) (Table 2), whereas 'Soheir L-90' was the highest susceptible cultivar to *Fusarium* wilt, and resulted in a 52% reduction in yield (Table 2). Other cultivars, including Joules, Emerson, Golden and Akhtar, also showed significant susceptibility to *Fusarium* infection, which resulted in a considerable yield reduction (Table 2). The average traditional yield of 'Naz' in the bean fields of the studied areas was higher than that of the other planted cultivars (Saremi et al., 2007). However, other soil borne fungi including *R solani* and *P. debaryanum* were infrequently associated with the decline disease in some growing areas.

DISCUSSION

This study reports the results of an initial screening of ten bean cultivars in order to select the bean cultivar that is most resistant to *Fusarium* wilt disease. Most cultivars (for instance, Sayad, Golden and Soheir L- 90) showed visible symptoms of injury and yield reduction in the range of 25 to 75% wilting and 31 to 52% production, whereas 'Naz', which is the best resistant cultivar, showed a significant yield production, but there were no visual wilting symptoms in the infested pot soil. This cultivar also demonstrated seed quality, which is an economical factor in marketing conditions. In addition, it seems that plant growth based on shoot and pod number and indicators of symbiosis effectiveness such as nodule number and nodule mass were satisfactory.

Generally, it was reported that the severity of root disease of bean plant significantly depended on environmental factors such as moisture, temperature and constant culturing of crop cultivation. These soil and environmental factors promoted long-term survival of *Fusarium* species as thick-walled resting spores, by considering that the environmental conditions in Northwest Iran favored the development of *Fusarium* root rot disease. For instance, the average density of root rots propagules reached a height of 1100, 700 and 500 cfu for *F. solani, F. oxysporum* and *F. sambucinum* respectively, which led to root rot disease.

On the other hand, crop rotation can reduce residual populations of root rot pathogen, with crop sequences of beans planted after a rotational crop every fourth year (Rupe et al., 1997). Potatoes and sugar beets may increase fungus inoculums in soil and should not be included in the rotation if a field has a history of *Rhizoctonia* root rot. Previous crop stubbles should be well incorporated into the soil and allowed to decompose before bean planting. This prevents temporary nitrogen deficiency and plant stress early in the season. It can be inferred that not only the used resistance cultivars (that is, Naz), but also the traditional cropping pattern in all studied areas needed to be corrected. However, the increasing quantity and quality of yield requires an application of adequate water and fertilizer, but not an excessive application. Shortened rotation intervals, reduced tillage, use of susceptible cultivars and soil compaction are all factors that may be contributing to a more favorable environment for the development of root rots caused by the pathogens (Bruton and Miller, 1997; Cross et al., 2000; Doohan et al., 2003).

This work was designed to study the causal agent of bean wilt and the induction of bean cultivars resistant to wilt disease. Therefore, detection of resistant cultivar was emphasized due to ineffectiveness of chemical, biological and cultural methods in thev control management. Use of resistant cultivars (that is, Naz) would enable farmers achieve high yield production. Nonetheless, the obtained results determined that bean cultivars possess different mechanisms for resistance to wilt diseases. Generic variation in root architecture existed among common bean classes and was evident under field conditions; however, the variation in root traits was minimal in environmentally controlled glasshouse conditions (Fall et al., 2001; Nimbalkar et al., 2006; Ramos et al., 2007).

Planting susceptible cultivars in the fields continuously in multiple growing seasons without rotation with non-host crops increased inoculums density resulting in increased severity of *Fusarium* wilt. It was reported that inoculums density of *Fusarium* species decreased in soils where dry beans were produced (Rupe et al., 1997; Naef and Defago, 2006; Fernandez et al., 2008). Edaphic factors, including coarse-textured and poorly fertilized soils also contribute to disease development in these areas (Yergeau et al., 2006). Environmental conditions, such as high soil moisture, low soil temperature and continuous cropping also contribute to increased severity of root rots and wilts (Doohan et al., 2003). However, shoot and pod growth and yield productions of different cultivars were affected differently as a result of differing resistance to *Fusarium*, which resulted in different harvest incidences. Consequently, significant differences were observed in the comparison of the yield production in the infested and non-infested pots in glasshouse, especially the rational production in treated soils with high propagule density of *Fusarium* species (2000 cfu) which can make sever decline or wilting disease on bean (Saremi et al., 1999). On the other hand, suitable conditions and normal environmental characters were prepared for bean biomass growth or yield production. In fact, field evaluation of bean plants for *Fusarium* wilt disease has not been effective, while the infection period is slow and other variables such as distribution of inoculum concentration is difficult to control. Therefore, a study was carried out to develop a semi-qualitative bioassay for early simple evaluation of resistance to *Fusarium* wilt disease in bean cultivars. The biomass productivity in infected roots and finally the yield production of bean plants were determined to relate the levels of tolerance or susceptibility to *Fusarium* wilt disease.

Cultivars were tested for resistance to *Fusarium* wilt in uniform conditions after inoculation with *Fusarium* propagules. The study's results indicate that the use of these genetic parameters have the positional ability to predict tolerance or susceptibility to *Fusarium* wilt disease in other crop cultivars (Salgado et al., 1995; Schneide et al., 2001; Ramos et al., 2007). This study show that *Fusarium* species were the main soil borne wilting pathogens on bean with wide distribution that have been known for a long time as important plant pathogens. Other soil borne fungal pathogens, such as *Rhizoctonia solani* and *Pythium debaryanum* appeared to contribute little to bean wilt diseases.

Accordingly, a trial was only made to improve the understanding of bean infection by the major *Fusarium* pathogens in growing soils with high propagule densities. The detection of resistance is important, although other factors that increase root rot and wilt will also contribute to sustainable yield production. It can be concluded that the introduction of 'Naz' intended for use by all bean growers would also contribute to sustainable dry bean production because of its resistance to common *Fusarium* species and due to its high yield and quality.

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