

***Fusarium proliferatum* Induces Gum in Xylem Vessels as the Cause of Date Bunch Fading in Iran**

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ABSTRACT

Date bunch fading (DBF) is a serious disease of date palm (*Phoenix dactylifera* L.) in Iran. *Fusarium proliferatum* was isolated from the xylem of fruit bunch samples sent from Kerman and Fars provinces groves to the laboratory. Koch's postulates were completed in the greenhouse by crown injection and root inoculations. Shriveling of the fruits was induced by peduncle inoculations. Symptoms on the seedlings indicated the effects of ethylene. The possible involvement of *F. proliferatum* phytotoxin(s) in the induction of DBF through elicitation of ethylene is discussed. The gas is suggested to be responsible for the gums deposits and, in turn, water stress resulted in shriveling and fading of the fruits.

Keywords: Date palm, Date bunch fading, *Fusarium proliferatum*, *Phoenix dactylifera*.

INTRODUCTION

Date palm (*Phoenix dactylifera* L.) is an economically important plant in Iran. In the last twenty year, a disorder known as date bunch wilt and dry disorder, and, recently, date bunch fading (DBF), consisting of sudden shriveling of the fruits during khalal-rutab stage, has occurred randomly in date palm groves of the southern provinces (Karampour and Pjman, 2006a). Annual losses are estimated to be 20% of the total production. Tests using biological, serological, and electron microscopy determined that agents such as mycoplasma, viruses, and viroids were not found to be associated with the bunch fading (Karampour *et al.*, 2006). Fungi such as *Bipolaris australiensis* and *Nattrassia mangifera*, *Aspergillus* spp., *Trichoderma* sp., *Penicillium* spp., *Thielaviopsis paradoxa*, *Fusarium* sp. and *Rhizoctonia* sp. were isolated from different parts of fruit bunches (Najafinia

and Azadvar, 2002; Karampour and Pjman, 2006a and b; Rahkhodaei, 2006). Although a number of these fungi induced a type of brown streak on the peduncle, none was able to induce fading. Some researchers concluded that hot weather and dry winds were the cause (Pouzesh Shirazi *et al.*, 2006). Parameters such as wind had more effect than humidity and temperature. In some research, treatments such as intercropping, covering fruit bunches, and thinning of fruit bearing branches have reduced the damage (Pjman *et al.*, 2003). A problem known as fruit shrivel (similar to DBF), was prevented in Halawi and Maktoom cultivars in USA by irrigation, thinning, and covering fruit bunches (Nixon, 1942; Sharples and Hilgemen, 1951).

In the groves, spatial distributions of DBF indicate that some type of a pathogen induces DBF. Therefore, the purpose of this research was to study the cause and the mechanism of induction of DBF in date palm groves in southern parts of Iran.

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MATERIALS AND METHODS

Symptoms, Isolation, and Morphology

In 2004, during the visits to the affected groves in Fars and Kerman provinces, symptoms were recorded and samples consisting of the whole date bunches cut from the base of the peduncle (main fruit stalk) were collected. Samples were surface sterilized by rubbing 70% alcohol and sawed vertically at several points. Sawdusts consisting of pieces of xylem ray tissues were collected on a piece of sterile aluminum foil, directly plated onto acidified potato dextrose agar medium (PDA) inside 9 cm Petri plates, which were later incubated at 25° C for 3 to 5 days. Inspecting under a binocular microscope, every colony growing from tissues was sub-cultured onto separate plates. Since all colonies were identified as species of *Fusarium*, single spore colonies were transferred to Carnation Leaf Agar (CLA) inside 9 cm plates and incubated at 25 °C for 12 h in dark: 12 h light photoperiod. After 10 days, all cultures were identified according to the taxonomic system outlined by Nelson *et al.* (1983) and Burgess *et al.* (1994).

Pathogenicity Tests

Injection

In the greenhouse, healthy date palm seedlings cv. Kabkab were grown from seeds. These and Zahedi seedlings, initiated from tissue culture, were transferred into 25 cm plastic pots containing sandy loam soil mixed with green manure (1:1), pH=8.2. Five isolates of *Fusarium* spp. (FPD1-FPD5) were grown on carnation leaf agar (CLA) for 10 days. Inoculums were prepared from each isolate by adding sterile water to petri plates to obtain conidial suspension. Three 1-year-old seedlings of cv. Kabkab and 2-year-old plants of cv. Zahedi were injected above soil line with 0.2 ml of the conidial suspension of the isolates

adjusted to 1×10^4 conidia/ml. Distilled water was used for control. Pots were kept in a greenhouse at 25-30° C with 50-70% RH. Appearances of symptoms were recorded at 2 weeks intervals. Re-isolations were made after 1 month from midribs of the leaves above the place of injection.

Root Inoculation

From injection test results, a highly pathogenic isolate (FPD5) was grown on Chaff – Grain Medium (Burgess *et al.* 1988). The basal medium consisted of 50 gr cereal chaff, 10 gr ground cereal grain soaked overnight at 5°C and autoclaved twice at one day interval. The flask was kept on laboratory bench for 2 weeks. The infested medium was kept loose by shaking with hand daily. Potting medium (1 kg) was thoroughly mixed with 60 gr of the inoculums in a separate container to obtain a uniform infested medium. Healthy 2-year-old date palm plants of cv. Zahedi were transferred into 25 cm plastic pots containing inoculating medium in greenhouse as described earlier. After one year, the whole pot was transferred to a 50 cm clay pot. Non-infested Chaff - Grain medium was used to inoculate control plants. Appearances of symptoms were recorded at monthly intervals.

Induction of DBF Symptoms

To induce DBF in adult trees, in April, a healthy grove of date palms cv. Ghasb was selected at Farashband district in Fars province. Three bunches were inoculated at the base of the peduncle (periol) of the fruits bunches after pollination, by placing pieces of PDA culture of the isolate FPD5 in 2 × 3-4 cm and 1 cm sections made after surface sterilization, by rubbing cotton wool soaked in 70% alcohol. Three bunches were also inoculated by PDA media as control. Layers of parafilm tightly covered places of inoculation. Trees were inspected for

appearance of symptoms after July. Re-isolations were made from xylem ray tissues 30 cm above the place of inoculation.

Sections Preparation

Pieces from inner xylem tissues of healthy and inoculated seedlings in the greenhouse and healthy and affected trees in the groves were sectioned longitudinally with a razor blade. Sections were mounted on a glass slide and examined under an Olympus compound microscope 100X for the presence of gums and occlusions in the vascular vessels.

RESULTS

Symptoms

Date palm groves showed symptoms of DBF from late July to September as a sudden shriveling of fruits (Figure 1 a). Early cultivars such as Kabkab, Mazafati, and Shahani, as fresh date cultivars, were the most susceptible. The affected fruits were alternate or the whole bunches were affected. Affected trees were spatially distributed. Trees marked in one year may or may not show the symptoms in the

following years. Beside shriveled fruits, longitudinal necrosis was observed on peduncle of the fruit bunches.

Isolation and Morphology

On PDA, *Fusarium* spp. was isolated from vascular bundles. Single spore colonies from all isolates were creamy white and cottony. On CLA, microconidia were formed in false head or in chain on polyphialides in the aerial mycelium. Macroconidia were thin walled, narrow, and straight with 3-5 septa, and measured $33-89.1 \times 3.3-3.8 \mu\text{m}$. Microconidia were oval, and single celled, flat at one end, $6.6-9.9 \times 1.3-2.5 \mu\text{m}$ (Figure 2a). Chlamydospores were absent. Isolates were identified as *Fusarium proliferatum* (Matsushima) Nirenberg.

Pathogenicity Tests

Necrotic spots appeared on the leaf tissue along the midrib veins 40-50 days after injection of seedlings by the isolates (Figure 2 b). As it is indicated in a 3-month period, severe tip chlorosis was evident following death of the tissue in a number of younger (1-year-old) seedlings

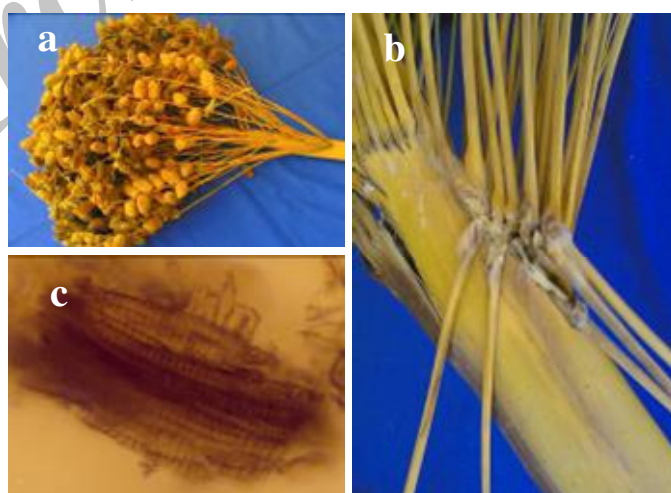


Figure1. a) A single bunch of date palm showing DBF symptoms, b) gum deposition at the point of attachment of fruit strands to peduncle, c) gum deposit in xylem vessels.

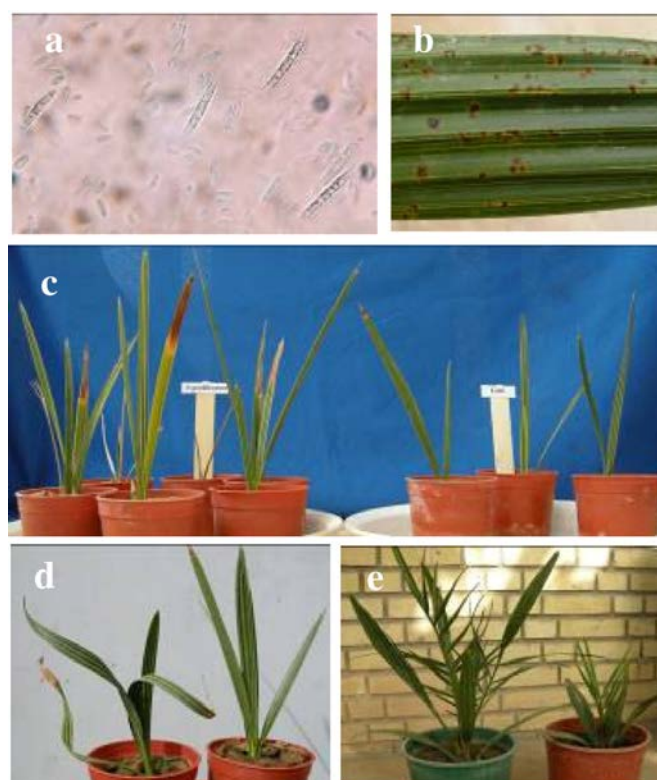


Figure 2. a) *F. proliferatum* macro and microconidia, b, c) necrotic spots and chlorosis and necrosis of leaf tips, d) distortion of leaves, e) stunting of the whole plant.

(Figure 2 c) (Table 1). No symptoms developed on the control plants. Re-isolation of the pathogen was made from midribs of the leaves. The most conspicuous symptoms of root inoculations were stunting of the whole plants and curling of lower leaves (Figure 2 d and e). No other symptoms developed on

the young seedlings.

Induction of DBF Symptoms

Inoculation of peduncle of date palm in a grove resulted in typical shriveling of the fruits on the strands of the fruit bunches. No

Table 1. Pathogenicity of *Fusarium proliferatum* isolates in the date palm seedlings cv. Kabkab.

Type of symptoms	Isolates				
	FPD1	FPD2	FPD3	FPD4	FPD5
Leaf tip necrosis and chlorosis	+	+	+	+	+
Necrotic spots	+	+	—	+	+
Death of seedlings	+	—	—	—	+

Inoculations were made by injection of 0.2 ml conidial suspension adjusted to 1×10^4 conidia/ml above the crown. Assessments were made during 3-month period.

symptoms developed on the control plants. Re-isolation of the *F. proliferatum* used to inoculate the peduncle was successfully made from midribs above the place of inoculations.

Sections Preparation

Microscope examination of longitudinal sections indicated heavy gum deposition in the xylem vessels of all types of tissues prepared from diseased seedlings and trees. No gum deposition was observed in healthy tissues (Figure1 c).

DISCUSSION

F. proliferatum was isolated on PDA from all samples collected from date palm trees with date bunch fading disorder in the southern groves of Iran. The fungus is reported to induce severe diseases in several plants such as asparagus, maize, mango and sorghum adapted to warm climate (Summerell *et al.*, 2003; Lima *et al.*, 2009). The fungus is reported to cause death of date palms, a disease similar to bayoud induced by *Fusarium oxysporum* f.sp. *albedinis* (Killian and Maire) Gordon in Saudi Arabia (Abdalla *et al.*, 2000). However, DBF was not part of the symptoms of the infected trees (Moriatti, personal communication).

A number of soil- and air-borne plant pathogens invade the xylem system of angiosperms and cause a variety of symptoms. In susceptible plants, before development of the symptoms, the pathogen must gain access to the xylem and continue to colonize xylem vessels more or less extensively (Beckman and Talboys, 1981). The extent of fungal growth within the xylem appears to be the result of a number of factors including the presence of tyloses, gums and gels induced by vascular pathogens (Bishop and Cooper, 1984). Tjamos and Smith (1975) indicated gels produced in the xylem of monogenically resistant tomato plants act as a barrier to

Verticillium albo-atrum growth, conferring resistance to the pathogen. Beside internal symptoms, externally systemic infections generally induce wilting, leaf chlorosis, and necrosis, stunting, epinasty and formation of adventitious roots (Dimond, 1972). In pathogenicity tests, all these symptoms were induced, indicating DBF is the result of a systemic disease.

The characteristic feature of vascular wilt syndrome is the induction of water stress (Hall and Machardy, 1981). Vascular gels are a major cause of water stress and their formation precedes wilting (Street and Cooper, 1984). In transverse sections, the presence of gums in xylem bundles was evident. Heavy gums deposits at peduncle-strands joints on the fruit bunch with DBF (Figure1 b) completely block passage of water to the fruits.

All of the factors that stimulate gum exudation also promote ethylene production in plant tissues (Saniewski *et al.*, 2006). Vascular gels was considered a direct consequence of ethylene action (Van der Molen *et al.*, 1977; Gedalovich and Fahn, 1985). Wilting can be considered as an indirect effect of the elicited ethylene imposing water stress. Stunting of the whole plants is also among distinct effects of ethylene. This symptom was observed on date palm seedlings during pathogenicity tests.

Several authors have suggested that low molecular weight phytotoxins are responsible for interveinal necrotic spots and leaf chlorosis and necrosis in a number of hosts (Talboys, 1958; Stoddart *et al.*, 1966; Mussell, 1972; Mansoori *et al.* 1995). In pathogenicity tests, appearance of such symptoms on the seedling leaves suggest the effect of such a toxin(s). *F. proliferatum* produces well-known mycotoxins such as fumonisin B1 (FB1), moniliformin (MON), beauvercin (BEA), fusaric acid (FA) and fusaproliferin (FUP). Among them FA, MON, FB1 have well-known phytotoxic activity (Abdalla *et al.* 2000; Gaumann, 1957; Cole *et al.* 1973; Lamprecht *et al.* 1994). Indeed, one of the factors, which



elicit production of ethylene, is phytotoxins (Kenyon and Turner, 1992; Moussatos *et al.*, 1994; Greulich *et al.*, 1995; Moore *et al.*, 1999; Asai *et al.*, 2000; Mansoori and Smith, 2005).

To describe the mechanism of fading, in nature date palm trees are systemically infected by *F. proliferatum*, which, in turn, induce formation of gums through release of ethylene. Gums block the vessels that induce water stress. Trees cannot compensate for the amount of water lost in hot weather accompanied by dry winds that prevail in the late July to September in the south of Iran, when fruit berries are at khelal-rutab stage period. Thus, the fruits shrivel and desiccate more completely.

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قارچ *Fusarium proliferatum* القاء تجمع صمغ درون آوند موثر در بروز عارضه خشکیدگی خوشه های خرما در ایران می کند

ب. منصوری

چکیده

عارضه چروکیدگی و خشکیدگی خوشه های خرما در اغلب مناطق خرما کاری جنوب ایران شایع می باشد. طی بررسی های انجام شده از قسمت های آوند نمونه های خوشه ارسال شده از تعدادی باغ آلوده از استان های فارس و کرمان قارچ *Fusarium proliferatum* جدا گردید. بیماریزائی این قارچ با انجام مراحل فرضیه کخ تحت شرائط گلخانه شامل تزریق اسپورهای این قارچ نزدیک به محل طوقه و آلودگی ریشه به اثبات رسید. تلقیح در محل پهن دم خوشه (Periol) بعد از عمل گرده افشانی توسط یکی از سویه ها باعث بروز علائم چروکیدگی در تعدادی از حبه های خرما گشت. در نمونه برش های نازک تهیه شده از آوند چوبی بدست آمده از قسمت های مختلف گیاهچه های آلوده صمغ زیادی مشاهده شد. وجود صمغ در ناحیه اتصال بند ها به دم میوه اصلی باعث تغییر رنگ شدید آن قسمت شده بود. در آزمایشات بیماریزائی بروز برخی از علائم منجمله تولید صمغ موید تولید و تاثیر گاز اتیلن تشخیص داده شد. تولید گاز اتیلن در اثر تحریک گیاه توسط زهرابه های قارچ و بنوبه ترشح صمغ به عنوان نتیجه یکی از این تاثیرات مورد بحث قرار گرفته است. تجمع صمغ در آوند های چوبی باعث انسداد و نرسیدن آب کافی به میوه های خرما در نتیجه چروکیدگی و خشکیدگی آنها به هنگام وزیدن بادهای گرم شود.