# *First report on the occurrence of races 1 and 2 of* Fusarium oxysporum *f. sp.* niveum *infecting watermelon in India*

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SHORT COMMUNICATION

# First report on the occurrence of races 1 and 2 of *Fusarium oxysporum* f. sp. *niveum* infecting watermelon in India

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## Abstract

Watermelon is an important cucurbit vegetable crop throughout the globe as well as in India. Around 74% of watermelon comes from the major growing belts viz., Indo-Gangetic plains and the Deccan Plateau regions of the country. With increasing demand, its cultivation is also being expanded to non-traditional areas and is being grown in different seasons. In this context, wilt disease caused by *Fusarium oxysporum* f. sp. *niveum* (*Fon*), is emerging as a major yield-limiting factor. To date, four races of the pathogen have been reported globally. However, no such reports on the prevalence of pathogen races were available from India. In this study, we have characterized two virulent Indian isolates into race 1 and race 2 of *Fon* by studying their ability to infect specific set of host differentials. To our knowledge, this is the first report on the occurrence of races 1 and 2 of *F. oxysporum* f. sp. *niveum* infecting watermelon in India.

**Keywords** Watermelon · Fusarium wilt · *Fusarium oxysporum* f. sp. *niveum* · Races · India

Watermelon, a major cucurbit crop of India, is cultivated in two major belts viz., Indo-Gangetic plains and the Deccan Plateau regions accounting for 73.9% of total production. With increasing demand, its cultivation is also being expanded to non-traditional areas and seasons. *Fusarium* wilt, caused by *Fusarium oxysporum* f. sp. *niveum* (*Fon*) is a major yield-limiting disease throughout the country. The pathogen may cause complete crop loss in a warm and humid climate. *Fon* produces resilient chlamydospores that can survive long periods in soil, making it difficult to manage this disease. To date, four races of *Fon* (0, 1, 2 and 3) have been reported globally based on the ability of the isolate to infect specific set of host differentials (Martyn and Bruton 1989; Martyn and Netzar 1991; Zhou et al. 2010). Though *Fon* is widely distributed in the watermelon growing areas of both Indo-Gangetic plains and the Deccan Plateau regions, no report on the prevalence of specific races in India is available. This research was focused to begin to understand the *Fon* race scenario prevailing in India.

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## Fusarium isolates

During 2016, several plant samples were collected from wilt-infected watermelon plants in farmer fields of different regions of the Haryana-Uttar Pradesh belt (Indo-Gangetic plains) and Karnataka (Deccan plateau). Fungal isolates were collected from the collar region of the infected plants onto full-strength PDA media and were identified morphologically (Egel and Martyn 2007) as *Fusarium oxysporum*. Sixteen pure cultures thus obtained were used to inoculate 14-day old watermelon seedlings (cv. Sugar Baby) to determine their pathogenicity. All the isolates were found to be pathogenic, causing typical vascular browning and wilting of the plants. From these, the two most aggressive isolates, which could cause more than 75% mortality of the susceptible cultivar Sugar Baby plants within 14 days post-inoculation, were selected and used further. The first isolate, hereafter referred to as KNL isolate, was collected from Chamrajanagar, Karnataka and the second isolate, hereafter referred to as N-isolate, was collected from Karnal, Haryana. These two isolates also were characterized by sequencing their Inter-Transcribed Spacer region (ITS) using specific primers (ITS1: 5′-TCCGTAGGTGAACCTGCGG-3′ and ITS4: 5′-TCCTCCGCTTATTGATATGC-3′). The ITS sequence of the KNL isolate (KY786126) had 100% similarity with accession KJ806518 (Yao 2014) while the

ITS sequence of the N-isolate (KY786127) showed 99.8% similarity with accession KY798172 (Wen and Guo 2017) in NCBI-BLASTn. Both these sequences (KJ806518 and KY798172) are of ITS region of *Fusarium oxysporum* isolates, collected from infected watermelon plants in China.

## Preparation of inoculum and screening methodology

A single spore derived culture of the pathogens was used for preparing the inoculum on to full-strength potato dextrose agar (PDA) medium in Petri-plates. The plates were maintained at 16/8 h light/dark conditions and 28 ± 2 °C temperature for next 12 days. The spores were harvested in sterile distilled water by scraping the mycelia with a loop and the concentration was adjusted to $1 \times 10^6$ conidia per ml using a haemocytometer.

Two disease screenings were conducted from April to July 2019. In the first experiment, the pipette inoculation method was followed (Latin and Snell 1986). Seeds of the standard differential genotypes (Table 1) were sown in autoclaved cocopeat in 98-celled pro-trays with each cell volume of 30 $cm^3$. After 14 days, at first, the true leaf stage of the seedlings, 5 ml conidial suspension ($1 \times 10^6$ spores/ml; > 80% microconidia) (Nisini et al. 2002) was delivered to each plug around the seedlings (n = at least 18 plants in each differential) via a pipette. In the second experiment, the standard root-dip method of inoculation (Latin and Snell 1986; Martyn 1987) was followed. At the first true leaf stage of the seedlings (14 days after sowing), 30 uniform seedlings for each differential were selected, slowly uprooted and the adhering cocopeat was thoroughly washed in running tap water. The washed roots of 20 plants of each cultivar differential were dipped in the conidial suspension ($1 \times 10^6$ spores/ml; > 80% microconidia) for 15 min and were transplanted in pro-trays filled with sterilized cocopeat. The remaining 10 plants of each differential were dipped in sterile distilled water for 15 min and were transplanted as un-inoculated controls. The experiment was laid out in randomized complete block design with two replications (10 plants in each). The protrays were kept under 75% shade for 24 h to aid the easy establishment of the plants by minimizing the planting shock. The following day, the trays were moved to a polyhouse, fitted with a fan-pad cooling system to maintain temperature and relative humidity of 29 ± 1 °C and 65 ± 5%, respectively. The plugs were lightly irrigated daily with a squeeze bottle to avoid over-irrigation.

## Grouping of the isolates into races

Plants were observed individually for the typical symptoms of *Fusarium* wilt (i.e. drooping, yellowing followed by wilting) at weekly intervals up to 28 days post-inoculation (DPI). Percent survival of plants were calculated based on the final reading (28 DPI). An accession showing more than 33% mortality was considered to be susceptible and accession with less than 33% mortality was considered to be resistant as suggested by Zhou et al. (2010).

The survival percentage of the differentials inoculated with each of the isolates by pipette and root-dip methods are presented in Table 2. For the KNL isolate, Sugar Baby and Charleston Gray were highly susceptible with 0% survival in pipette method; and 20% and 0% survival, respectively with the root-dip method. The other two differentials viz. Calhoun Gray had a survival of 78.04% and 85% in the pipette and root-dip methods respectively. The line PI-296341-FR had 100% survival in both methods. Hence these two accessions were categorized as resistant.

The host-differentials responded differently to the N-isolate as Sugar Baby, Charleston Gray and Calhoun Gray were found to be susceptible with 100%, 95.45% and 61.76% mortality respectively in the pipette inoculation method and 90%, 100% and 70% mortality respectively in the root-dip inoculation method. However, PI-296341-FR recorded a resistant reaction with a mean survival of 96.15% and 100% with a pipette and root-dip inoculation methods respectively.

Based on these results, the KNL isolate is designated to be *Fon* race 1 and the N-isolate as *Fon* race 2. The first occurrence of *Fon* race 1 was reported from South Carolina and Georgia, USA (Smith 1894). *Fon* race 2 was first reported from Greece (Netzar 1976), Texas (Martyn 1987), Oklahoma (Bruton et al. 1988), Florida (Martyn and Bruton 1989), Cyprus (Ioannou and Paullis 1991), Spain (Gonzalez-Torrens et al. 1993), Maryland and Delaware (Zhou and

**Table 1** Host differentials for *Fusarium oxysporum* f. sp. *niveum* used in the current study and their expected reaction to different races

| Differentials | Race 0 | Race 1 | Race 2 | Race 3 | References |
|---|---|---|---|---|---|
| Sugar Baby | S | S | S | S | Martyn and Netzar (1991); Dane et al. (1998); Wechter et al. (2012) |
| Charleston Gray | R | S | S | S | |
| Calhoun Gray | R | R | S | S | |
| PI-296341-FR | R | R | R | S | |

*S* susceptible, *R* resistant

**Table 2** Survival percentage at 28 days post-inoculation of the host differentials with the pipette and root-dip methods of inoculation for two isolates of *Fusarium oxysporum* f. sp. *niveum*

| Differentials | KNL isolate | | | | | N-isolate | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Pipette method | | Root-dip method | | Disease reaction | Pipette method | | Root-dip method | | Disease reaction |
| | Total number of plants screened | Survival percentage | Total number of plants screened | Survival percentage | | Total number of plants screened | Survival percentage | Total number of plants screened | Survival percentage | |
| Sugar Baby | 29 | 0 | 20 | 20.00 | S | 31 | 0 | 20 | 10.00 | S |
| Charleston Gray | 19 | 0 | 20 | 0 | S | 22 | 4.55 | 20 | 0 | S |
| Calhoun Gray | 41 | 78.04 | 20 | 85.00 | R | 34 | 38.24 | 20 | 30.00 | S |
| PI-296341-FR | 18 | 100.00 | 20 | 100.00 | R | 26 | 96.15 | 20 | 100.00 | R |

*S* susceptible, *R* resistant

Everts 2001), Indiana (Egel et al. 2005), Georgia (Bruton et al. 2008) and China (Duan et al. 2007). To our knowledge, our result is the first confirmed report of the occurrence of both races 1 and 2 of *Fusarium oxysporum* f. sp. *niveum* from India. This information will help in region-specific deployment of globally available *Fon* race 1 and race 2 resistant accessions for future watermelon breeding programmes in India.

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**Author contributions** R. N. Thontadarya collected the infected plant samples with guidance from Subbaraman Sriram and Eguru Sreenivasa Rao; Neethu K. Chandran collected the fungal isolates and maintained them. Saheb Pal conducted the mass multiplication and performed the screening experiments with guidance from Eguru Sreenivasa Rao. Saheb Pal and Eguru Sreenivasa Rao wrote the manuscript.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that there is no conflict of interest.

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