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Original Research Paper

Screening cultivated and wild genotypes of water melon to identify resistance against *Fusarium oxysporum* f.sp. *niveum* (*Citrullus lanatus* (Thumb) race 2

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ABSTRACT

The present experiment was carried out to screen 341 *Citrullus* sp. accessions for identification of resistant genotypes against *Fon* race 2. The accessions were screened across two consecutive experiments conducted during the late-rainy season of 2017. Spore suspension of *Fon* race 2 was inoculated following the standard pipette method at 14-day seedling stage. An accession showing a mean survival of $\geq$ 67% at 28 days post-inoculation (DPI) was categorized as resistant and the remaining accessions were categorized as susceptible. A total of 30 accessions were found resistant. Of them, eight accessions possessed a mean survival of $\geq$ 90% at 28 DPI and were categorized as highly resistant. All these highly resistant accessions belong to *C. lanatus* with moderate fruit quality traits. Therefore, these genotypes could be used for watermelon improvement programs in the future that aim to incorporate resistance to *Fon* race 2 into the crop's elite and commercial market segments without incurring linkage drag.

Keywords: Fungus, inoculation, management, resistance, screening, soil-borne, watermelon

INTRODUCTION

Watermelon (*Citrullus lanatus* (Thunb.) Matsum and Nakai) is a specialty vegetable grown in tropical and subtropical regions. It belongs to the Cucurbitaceae family and has 22 somatic chromosomes. The crop is known for its nutritious mesocarp and placenta consumed as dessert. Being rich in carotenoids, Vitamin C, flavonoids, minerals, phenolic compounds, and citrulline, it is recognized as a functional food with potential health benefits (Gunn et al., 2015). The crop originated in the areas adjoining the Kalahari desert and was domesticated in North-Eastern Africa and later introduced to Indiaduring the eighth century (Paris, 2015). Cultivation in the different agroecosystems over the centuries and gene flow due to free crossability with the wild species have created plenty of variability for different horticultural characteristics in India. In India, it is being grown on 126.18-thousand-hectare lands with aproduction of 3.63 million tonnes (Anonymous, 2023). However, in recent years, the depletion of genetic diversity arising because of "founder effect" is posing a great threat in watermelon cultivation by making it susceptible to emerging stresses.

Fusarium wilt, caused by *Fusarium oxysporum* f. sp. *niveum*, has become a global constraint for watermelon cultivation, especially under warm and humid climates. The disease exhibits symptoms of loss of turgidity of the leaves and the vines, vascular browning and necrosis of the plants leading to mortality. Production of long-term resting spores, colonization on related plants and rapid development of resistance to various fungicides make the eradication of the pathogen challenging (King et al., 2008). Less effective biocontrol methods and the discontinuation of methyl bromide further complicate the disease management. Over a period, the variability of the genomic constitution of the pathogen had led to the dynamic evolution of four physiological races based on the isolate's capacity to infect a certain set of host differentials. Among them, race 3 is the most virulent and recently reported from Maryland (Zhou et al., 2010) and limited in its occurrence to that region. However, race 2 is most widely distributed throughout the global watermelon growing regions (Bruton et al., 2008) and no commercial variety with *Fon* race 2 resistance is available to the growers. Although resistant accessions like *Citrullus amarus*

PI296341-FR and PI271769 have been identified (Martyn & Netzer, 1991; Wechter et al., 2012), their effective deployment in breeding programmes are hindered by the frequent occurrence of linkage drag of undesirable fruit quality traits (McGregor & Waters, 2013; Meru & McGregor, 2016). Keeping these facts in mind, the present experiment was carried out to artificially screen 341 accessions *Citrullus* sp. to identify potential donors of resistance.

MATERIALS AND METHODS

The experiment was carried out at the ICAR-Indian Institute of Horticultural Research, Hesaraghatta, Bengaluru during the late-*kharif* (rainy) season of 2017.

Plant materials

The experimental materials of the present experiment were comprised of 341 *Citrullus* sp. accessions belonging to different species *viz*., *C. lanatus* (N=247), *C. mucospermus* (N=47), *C. colocynthis* (N=43), *C. amarus* (N=4) and a hybrid of *C. amarus* × *C. lanatus*. The accessions available at the National Active Germplasm Site, ICAR-IIHR, Bengaluru from different countries *viz*., India (N=215), the United States of America (N=80), Nigeria (N=15), Thailand (N=8), Senegal (N=5), China (N=3), Zimbabwe (N=3), Botswana (N=2), Japan (N=2), Mali (N=2), South Africa (N=2), Ghana (N=1), Moldova (N=1), Taiwan (N=1) and Zambia (N=1). The genotypes are being actively maintained at ICAR-IIHR, Bengaluru. The passport data and the plant morphological character and fruit quality traits of the accessions have been presented in Supplementary Table 1.

Fungal isolate

Plant samples from the wilt-infected watermelon plants were collected from farmers' fields of various parts of the Indo-Gangetic plains in 2016. Fungal isolations were made from the collar regions of the infected plants in full-strength (39-gram media in one liter of double distilled water) potato dextrose agar (PDA) media. It was then morphologically identified as *Fusarium oxysporum* based on conidial morphology (Fig. 1 & 2) (Egel & Martyn, 2007). Subsequently, the culture was used to inoculate 14-day old watermelon seedlings of cv. Sugar Baby which showed typical symptoms of *Fusarium* wilt i.e. vascular browning prior to wilting of plants. Further, the pathogen was confirmed *Fusarium oxysporum* f.sp. *niveum* by sequencing its inter-transcribed spacer

Table 1 : Survival percentage at 28 DPI and magnitudes of AUDPC of the highly resistant accessions identified in the present study in comparison with the susceptible lines

Genotype	Survival per cent at 28 DPI*	AUDPC
IIHR-9	90.83 (72.37)	90.42
IIHR-143	100.00 (90.00)	0.00
IIHR-171	100.00 (90.00)	0.00
IIHR-260	92.26 (73.83)	106.25
IIHR-354	100.00 (90.00)	0.00
IIHR-358	90.71 (72.23)	112.51
IIHR-393	100.00 (90.00)	0.00
IIHR-453	90.00 (76.71)	210.00
IIHR-113 (cv. Sugar Baby)	0.00 (0.00)	1190.00
NS-20 (F_1; Namdhari Seeds Pvt. Ltd.)	0.00 (0.00)	1210.42
IIHR-609 (Suprit (F_1), Known You Seeds Pvt. Ltd.)	8.33 (12.04)	1391.67
IIHR-611 (NS-295 (F_1), Namdhari Seeds Pvt. Ltd.)	36.01 (36.78)	490.63
Critical difference (pd"0.05)	31.49	
Standard error of mean (±)	11.32	
Standard error of difference (±)	16.01	

*Values in the parenthesis are angular transformed

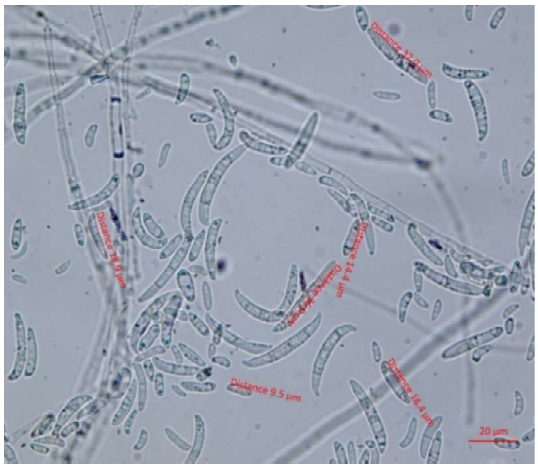

Fig. 1 : Micro and macroconidia of the *Fon* race 2 isolate used to screen the watermelon germplasm

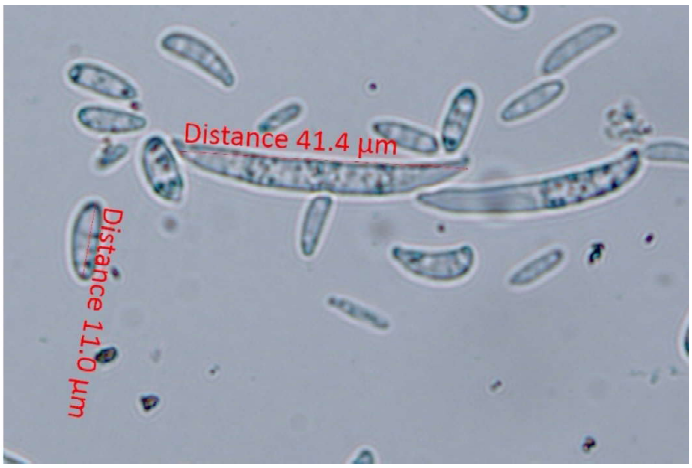

Fig. 2: Magnified photo of micro and macroconidia of the *Fon* race 2 isolate

(ITS) region using ITS1 (5'-TCCGTAGGTGAACCT GCGG-3') and ITS4 (5'-TCCTCCGCTTA TTGATA TGC-3') primers. The sequence (deposited to NCBI database with accession ID: KY786127) was found 99.8% similar to that of *Fon* isolate collected from infected watermelon plants in China (Wen & Guo, 2017). Further, a standard set of host differentials *viz*., Sugar Baby, Charleston Gray, Calhoun Gray and PI296341-FR were utilized to designate the isolate as per globally reported physiological race classification. The differentials *viz*., Sugar Baby, Charleston Gray and Calhoun Gray was found sensitive to this isolate while only PI296341-FR was found resistant, thus designating the isolate as *Fon* race 2 (Pal et al., 2020).

Inoculum preparation

The pure isolate, derived from a single spore, was first multiplied in PDA medium for 12 days at 16+8 hours of light and dark period, respectively at 28±2°C temperature (Fig. 3). The fungus was then mass-multiplied on sterile sorghum (*Sorghum bicolor* L., family Poaceae) seeds as per Kamdi et al. (2012) at the same photoperiod and temperature conditions for next 14 days. On the day of the inoculation of the seedlings, the inoculated sorghum seeds (Fig. 4) were washed by rubbing with hand in water to collect the mycelial mat with the spores adhering to the surface of the seeds. The suspension was then sieved with two layers of cheese cloth. The concentration of spore suspension was then adjusted to 1×10^6 conidia/mL by using a haemocytometer. A surfactant 'Tween-20' was added to the final spore suspension @ 2 mL per litre to ensure uniform distribution of conidia.

Screening methodology

Two screening experiments were carried out at in a polyhouse located at the ICAR-Indian Institute of Horticultural Research, Bengaluru at 890 m above mean sea level at 13°082 26.63 N latitude and 77°302 2.23 E longitude, during the late-*kharif* (rainy) season of 2017 *i.e.* 22nd August to 3rd October for the first screening assay and from 25th October to 15th November for the second assay. The seeds of the germplasm accession were sown in pro-trays following randomized complete block design with two replications. The pro-trays were filled with autoclaved-cocopeat. The trays were kept in dark for next two days to ensure uniform germination. After germination and emergence of the seedlings, the trays were irrigated daily up to 13th day. On 14th day (at one to two true leaf stage of the seedlings, Fig. 5) five mL of the spore suspension (containing 1×10^6 conidia/mL) was delivered to each plant plug with the help of a pipette (Eppendorf, USA). To ensure complete absorption of the applied inoculum by avoiding leaching, the trays were not irrigated on the day of inoculation. After inoculation, the trays were maintained in a naturally ventilated polyhouse with ambient temperature ranging at 28±5°C. Irrigation was provided daily thereafter. Misting was done through overhead foggers for one minute in every two hours starting from 11 am to 4 pm to maintain favourable humid conditions for the

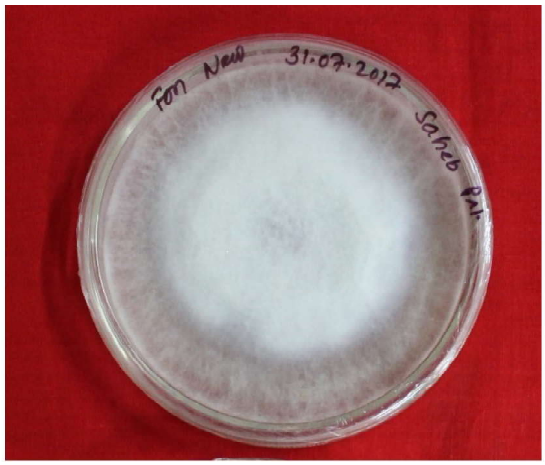

Fig. 3 : Growth of the *Fon* race 2 isolate on full-strength potato dextrose agar medium

Fig. 4 : Mass multiplication of *Fon* race 2 isolate on sterilized sorghum seeds

Fig. 5 : Stage of the watermelon plants at the time of inoculation of Fon race 2

Fig. 6 : Reaction of the resistant and susceptible accessions at 28 DPI of Fon race 2

pathogen to colonize the root zone and enable quick expression of symptoms. The inoculated trays were maintained up to 28 days post inoculation (DPI) to record final survival (Fig. 6).

Recording of observations and statistical analysis

For each accession and in each replication, the number of healthy plants were counted before inoculation and was notes as initial count. After inoculation, the number of healthy, symptom free and surviving plants in each entry was counted on 7th, 14th, 21st and 28 days post-inoculation. The survival percentage was calculated using the following formula:

$$\text{Survival percentage} = \frac{\text{Initial count-final count} \times 100}{\text{Initial count}}$$

After completion of each screening experiment, the plants were removed from the trays and the used media was collected for disposal by incineration for preventing contamination of the polyhouse and adjoining fields.

The data obtained from two screening experiments were pooled before statistical analysis as the assays were conducted in the same season and under similar conditions of naturally ventilated polyhouse where congenial temperature and humidity were maintained during both the screening experiments. The area under disease progress curve (AUDPC) was calculated for each entry using the following formula of Campbell & Madden (1990):

$$\text{AUDPC} = \sum_{i=1}^{n=1} [\{\frac{x_i + x_{i+1}}{2}\} \times \{t_{i+1} - t_i\}]$$

where, X_i = survival percentage at ith observation, X_{i+1} = survival percentage at i+1st observation, $t_{i+1} - t_i$ = number of days between two observations and

n= total number of observations. Genotypes with mean survival of e" 90% at 28 DPI were categorized as highly resistant. Further, a genotype with mean survival e"67% at 28 DPI was categorized as resistant and the remaining accessions were categorized as susceptible following the disease scale of Zhou et al. (2010). The final survival percentages and the magnitude of AUDPC of the accessions were analyzed using IBM-SPSS v. 20.0 software packages.

RESULTS AND DISCUSSION

Fusarium wilt is a devastating soil borne disease of watermelon. The gradual increase in susceptibility is mainly due to its narrow genetic base of the varieties/ hybrids derived from a few founder genotypes in breeding programme (Guo et al., 2013) or loss of desirable alleles during domestication (Pal et al., 2022). Thus, the disease can cause up to complete yield loss if the crop is grown as monoculture (Branham et al., 2019). The need for identifying and deploying resistant sources in breeding programs is essential. In the present experiment, significant ($p \leq 0.05$) differences were recorded for the mean survival percentage and AUDPC at 28 DPI which ranged from 0.00% to 100% and 0.00 to 1837.50, respectively (Supplementary Table 2). A total of eight accessions *viz*., IIHR-9, IIHR-17, IIHR-143b, IIHR-260, IIHR-354, IIHR-358, IIHR-393 and IIHR-453 were identified as highly resistant against *Fon* race 2 (Table 1). In addition to these, 22 other genotypes *viz*., IIHR-30, IIHR-38, IIHR-271, IIHR-442, IIHR-392, IIHR-395, IIHR-397, IIHR-398, IIHR-399, IIHR-404, IIHR-407, IIHR-440, IIHR-570, IIHR-351, IIHR-353, IIHR-357, IIHR-366, IIHR-368, IIHR-369, IIHR-370, IIHR-382 and IIHR-357 were found as resistant. Further, in the present study susceptibility of the majority (N=311/341, 91.20%) of the genotypes confirms the narrow genetic base and loss of resistant alleles against the dynamically evolving pathogen. To date, complete immune reaction of *Citrullus* sp. accessions towards *Fon* race 2 has not been made. However, in the present experiment, only four genotypes *viz*., IIHR-143, IIHR-171, IIHR-354 and IIHR-393 showed complete survival after 28 days post-inoculation (Supplementary Table 2), necessitating further confirmation with a greater number of plants and beyond 28 DPI. In the previous studies, resistant sources against *Fon* race 2 were identified (Dane et al., 1998; Huh et al., 2002; Boyhan et al., 2003; Wechter et al., 2012). However, the majority of these identified accessions belong to *C. amarus*, involvement of which may result in linkage drag of undesirable fruit quality traits, necessitating other tools like disruptive selection as well as foreground and background selection. All these highly resistant accessions identified in the present study belong to *C. lanatus* with moderate fruit quality traits (Supplementary Table 1), making them suitable donors for future breeding programs. The accession IIHR-9 is an inbred derived from Hybrid-313 variety from the USA. It has round fruits with pink flesh with an average Total soluble solids (TSS) of 7.05°B. IIHR-17 is an indigenous collection from Pali, Rajasthan, having round fruits that showed segregation between pink and salmon yellow with an average TSS of 6.30°B. IIHR-143b is a derivative from a hybrid variety of Nunhems Seeds Pvt. Ltd. It has round fruits with pink flesh and an average TSS of 9.20°B. IIHR-260 (IC0523047) is an Indian accession with round fruits which showed segregation of flesh colour between red and canary yellow with an average TSS of 6.06°B. IIHR-354 (EC759805) is an exotic collection from the USA with pyriform fruits with red flesh and an average TSS of 5.30°B. IIHR-358 (EC794421) is an exotic collection from the USA with round fruits with red flesh and an average TSS of 5.10°B. IIHR-393 (EC794456) is an exotic collection from USA with round fruits, pink flesh and 8.00°B average TSS. IIHR-453 (EC797223) is also an exotic collection from the USA and has round fruits with pink flesh and an average TSS of 7.00°B. The fruit of some of the resistant accessions have been presented in Fig. 7.

Fig. 7 : Fruit of some of the highly resistant accessions identified

The magnitude of AUDPC serves as an indicator of the progression of the disease over time. Thus, an accession with early mortality tends to have a greater magnitude of AUDPC and vice-versa (Pal et al., 2022). In the present study, the AUDPC ranged from 0.00 in IIHR-9, IIHR-143b, IIHR-354 and IIHR-393 to 210.00 in IIHR-453, while, it was 90.42 in IIHR-9, 106.25 in IIHR-260 and 112.51 in IIHR-358. Thus, in future watermelon improvement programmes, the four genotypes *viz*., IIHR-9, IIHR-143b, IIHR-354 and IIHR-393 would be preferable.

CONCLUSION

The present study identified 30 novel *Citrullus lanatus* accessions possessing resistance (survival $\geq$ 67% at 28 days post-inoculation) to *Fon* race 2, and the remaining 90.12% of genotypes were found as susceptible. This confirms the loss of resistant loci from these genotypes during domestication. Of the resistant accessions, eight were found highly resistant. Thus, these genotypes can be deployed in future breeding programmes for incorporation of resistance to *Fon* race 2 into different elite and commercial market segments of watermelon without incurring linkage drag of undesirable fruit-quality traits.

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