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Identification of multi-race Fusarium wilt resistance in chickpea (*Cicer arietinum* L.) using rapid hydroponic phenotyping

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Summary. Fusarium wilt, caused by *Fusarium oxysporum* f. sp. *ciceris* (Padwick) Matuo and K. Sato is a major cause for low productivity of chickpea. Presence of multiple pathogenic races makes it difficult for the breeder to screen for Fusarium wilt resistance. Twenty-two chickpea genotypes were grown in Hoagland solution and inoculated with five different *F. oxysporum* races two isolates of each race), including host and pathogens from the major chickpea growing region of India. The resistant chickpea line "WR 315" showed a "highly resistant" reaction, and the susceptible line "JG 62" showed a "highly susceptible" reaction across all pathogen races and isolates. However, the parent lines "Pusa 372" and "JG 11" showed "susceptible" reactions, while the marker-assisted backcrossing (MABC) lines of "Pusa 372" (IL.11,12,14) and "JG 11" (IL.15,16,17) were superior for assessed characters (lengths of roots and shoots, fresh and dry weights), and were highly resistant to most races. This is the first study to use race specific screening of MABC lines using hydroponic host culture in chickpea.

Keywords. Chickpea introgression lines, marker-assisted backcrossing, phenotyping.

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INTRODUCTION

Chickpea (*Cicer arietinum* L.) is a major cool season grain legume of global importance grown in approx. 57 countries (Merga and Haji, 2019). *Cicer arietinum* is a diploid (2n = 16), annual, self-pollinated plant and with a genome size of approx. 738 Mb (Varshney *et al.*, 2013). The Middle East region between southeast Turkey, northwest Iran and parts of Syria, are the likely the primary centre of origin of this food crop plant. Chickpea is grown in a total of 13.7 million hectares (Mha) each year, with total grain production of world 14.3 million tons (MT) (FAOSTAT, 2020). In India, 8.35 M ha of chickpea are grown each year with 7.17 MT produced (DAFW, 2019). For

self-sufficiency in India, 16 to 17.5 MT production is required from the present 10.5 M ha area, and average required productivity of 1500 to 1700 kg ha^{-1} (Dixit *et al.,* 2019, Hickey *et al*., 2019).

Major constraints of chickpea production and productivity are biotic and abiotic stresses. Within biotic stresses, *Fusarium oxysporum* f. sp. *ciceris* (Padwick) Matuo and K. Sato (Foc), which causes Fusarium wilt, is the most important potential pathogen, which causes yield losses ranging from 10 to 100% (Jimenez-Diaz *et al*., 1989, Sharma *et al*., 2005). High pathogen variability makes development of disease resistant cultivars. Yellowing and wilting pathotypes of Foc have been reported based, on pathogenicity tests with eight physiological races (Haware and Nene, 1982; Trapero-Casas and Jimenez Diaz, 1985). Among them, race 0 and 1B/C cause host yellowing, and 1A, 2, 3, 4, 5, and 6 cause wilting, all the races have distinct geographical distributions (Jiménez-Díaz *et al*., 2015). Foc isolates from chickpea growing in Indian states were highly variable compared to international isolates, with each state having more than one race. Chickpea cultivars tested on a new differential set revealed eight new Foc races in India (Dubey *et al.,* 2012). The soilborne pathogen can survive for many years in the absence of hosts, which poses serious drawbacks for cultivar phenotyping and Fusarium wilt management (Haware *et al.,* 1996). Pathogen variability and mutability result in losses of host resistance, and these remain the main hurdles for plant breeders aiming to develop effective disease resistance (Barve *et al.,* 2001).

Current assessments of host phenotype characteristics for disease resistance in breeding programmes mainly rely on visual scoring of disease under plot or micro plot, artificial inoculation conditions, and screening at disease hotspot locations. These methods are time-consuming, laborious, and expensive, and generate bias in recording of field data. Screening for Fusarium wilt under open field conditions is difficult, complex, inefficient, and unreliable, for screening large numbers of host lines, as results are influenced by environment, host and genetic factors. Effective and reliable phenotyping for *Fusarium* is therefore important to rapidly identify host resistance, and pathogen racial patterns, with limited environmental influences.

Hydroponics techniques have become the most efficient and reliable for screening large numbers of host germplasm lines (Amalraj *et al*., 2019). These methods avoid dependency on soil to provide essential elements for host growth (Sheikh *et al*., 2006). Using hydroponic systems, nutrition, pH, temperature, and dissolved oxygen can be closely controlled (Thompson *et al*., 1998). As well, marker assisted backcross breeding can target specific wilt resistance traits for selection against donor genomes in gene introgression, and can more effectively use molecular markers than conventional backcrossing (Varshney *et al*., 2014; Bharadwaj *et al*., 2021). Marker assisted backcrossing (MABC) is a precise and effective technique to introgress single loci that control traits of interest while retaining other important characteristics of recurrent parents (Collard and Mackill, 2008).

The present study was designed to use the hydroponic techniques for race specific screening of MABC lines against *Fusarium* race isolates, to identify levels of resistance in chickpea hosts at seedling stages.

MATERIALS AND METHODS

Plant material

An *in -planta* infection technique was used to screen chickpea lines for wilt resistance. This was carried out on hydroponically grown seedlings, which were inoculated with virulent Foc conidia. Twenty-two host genotypes were used in this study, including one resistant line "WR 315" (Millan *et al*., 2006; Halila *et al*., 2010), one susceptible line "JG 62" (Haware *et al*., 1992; Ali *et al*., 2002), two parents (JG 11 and Pusa 372) and their MABC introgression lines, along with the three genotypes ILCO (from Latvia), ILCO (from Czechoslovakia) and BGD112, were used in this study (Table 1). All of these introgression lines are advanced BC_3F_3 lines, which contain proposed lines for advanced varietal trial (AVT 1) of the All India Coordinated Research Program (AICRP) on Chickpea. A total of six SSR markers, including TA110, TA37, TR19, GA16, TA27, TA96 reported to be in the cluster containing genes conferring FW resistance on the linkage group CaLG02, were used to identify polymorphic markers for parental polymorphism (Millan *et al*., 2006).

Pathogen multiplication and conidium production

Two representative isolates from each of five different Foc races, distributed across the major chickpea growing regions of India (Central zone, South zone, Northwest plain zone, and Northeast plain zone) were obtained from the Pulse Pathology laboratory, Division of Plant Pathology, ICAR-IARI New Delhi. These isolates were identified for each race and based on differential responses, and the isolates were characterized into five races based on a new set of differential cultivars (C 104, JG 74, CPS 1, BG 212, WR 315, KWR 108, GPF 2, DCP 92-3, Chaffa and JG 62). A total of 70 isolates of

Table 1. Chickpea genotypes, and introgression line numbers used in this study.

Sr. No.	Genotype	Introgression line No.
1	Pusa 372	-
2	WR 315	-
3	(*3Pusa 372///(Pusa 72/ WR 315)	1
4	(*3Pusa 372///(Pusa 72/ WR 315)	3
5	(*3Pusa 372///(Pusa 72/ WR 315)	5
6	(*3Pusa 372///(Pusa 72/ WR 315)	8
7	(*3Pusa 372///(Pusa 72/ WR 315)	9
8	(*3Pusa 372///(Pusa 72/ WR 315)	15
9	(*3Pusa 372///(Pusa 72/ WR 315)	18
10	(*3Pusa 372///(Pusa 72/ WR 315)	22
11	(*3Pusa 372///(Pusa 72/ WR 315)	25
12	(*3Pusa 372///(Pusa 72/ WR 315)	27
13	(*3Pusa 372///(Pusa 72/ WR 315)	28
14	(*3Pusa 372///(Pusa 72/ WR 315)	34
15	(*3JG 11///(JG 11/ WR 315)	37
16	(*3JG 11///(JG 11/ WR 315)	39
17	(*3JG 11///(JG 11/ WR 315)	42
18	JG 11	-
19	JG-62	-
20	ILCO (Czechoslovakia)	-
21	ILC0 (Latvia)	-
22	BGD 112	-

Foc were characterized, representing the pathogenic and morphological groups of 640 isolates which had been collected from the 13 important 13 chickpea growing states in India. The common cultivars used by Jimenez-Diaz *et al.* (2015) and Dubey *et al.* (2012) were C104, G74, CPS1, BG212, WR315 and JG 62. Earlier studies based on old differentials showed the presence of eight races of the pathogen, of which only races 1A, 2, 3 and 4 were reported in India (Haware and Nene, 1982).

The international differentials developed during 1982, and these needed to be modified with a new set of chickpea cultivars, to keep up with the changed pathogen population (Dubey and Singh, 2008; Dubey *et al.*, 2010). Dubey *et al.* (2012) used a modified set of new differentials that categorised isolates into eight races instead of the four races reported in India by Haware and Nene (1982). Of these eight races, the five most virulent and widely distributed were used to screen genotypes in the present study. Previous research did not study virulence of different isolates to groups of differentials, and utilised molecular characterization which relied on information available in the literature correlated with molecular groups for which virulence information was available. These same sets of isolates were utilised for molecular characterization by four different molecular markers, including random amplified polymorphic DNA, universal rice primers, simple sequence repeats, and intersimple sequence repeats. These four sets of markers exhibited 100% polymorphism, and based on the unweighted paired group method with arithmetic average analysis, the isolates were in eight groups based on genetic similarities from 37 to 40% (Dubey *et al.*, 2012) (Table 2).

Table 2. Races and origins of *Fusarium oxysporum* f. sp. *ciceris* used in this study, including regions of collection (Dubey *et al.*, 2012).

Race	Isolate code	Region (place of collection in India)
R1	118	ICRISAT, Hyderabad, Telangana
	121	Dharwad, Karnataka
R2	119	IIPR, Kanpur, Uttar Pradesh
	129	IIPR, Jhansi, Madhya Pradesh
R3	31	Faridkot, Punjab
	45	Ludhiana, Punjab
R4	153	JNKV, Jabalpur, Madhya Pradesh
	108	IARI, New Delhi
R5	4	RAS, Jaipur, Rajasthan
	6	RAS, Durgapur , Rajasthan

The isolates were each grown on Selective *Fusarium* Agar (SFA) in Petri dishes, and incubated at 28 ± 2°C for 7 d. Four SFA discs of each Foc race isolate were transferred into 20% (v/v) selective *Fusarium* broth for conidium production. Conidium concentration was assessed using a hemocytometer, and average concentrations of 3.2×10^6 conidia per mL^{-1} were used for inoculations.

Hydroponics and host growth conditions

Chickpea seeds were disinfected for 5 min in commercial bleach (0.042% (w/v) sodium hypochlorite) added to deionized water, and were rinsed well in running tap water. The seeds were then imbibed at 4°C for 48 h. Imbibed seeds were then germinated in 10% aerated nutrient solution on mesh in the dark for 3 d. Seven d-old seedlings were then transferred to continuously aerated 25% nutrient solution to grow the seedlings under sterile hydroponic conditions on floats in sterile water containing macro- and micro-nutrients (half-strength Hoagland's nutrient medium) (Hoagland and Arnon, 1950). For the first week, half strength nutrient solution was used, and was then gradually increased to full strength nutrient solution after 10 d. Control sets of seedlings were grown Hoagland's nutrient media to

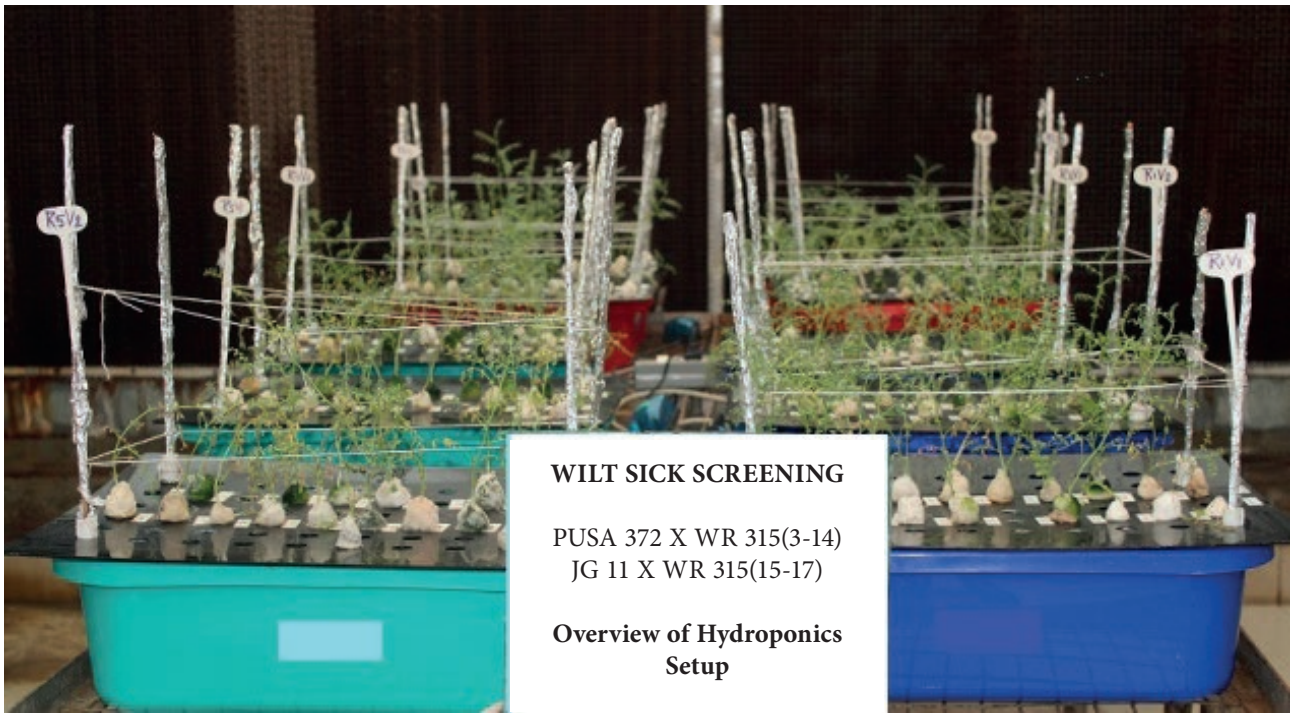

Figure 1. Hydroponics experimental set up used to screen chickpea lines for Fusarium wilt susceptibility.

compare disease incidence with wilt pathogen genotypes. The desired Foc conidium suspensions (3.2×10^6 conidia mL^{-1}) were added to each hydroponic tray, and 12 d-old seedlings were inoculated, and these were examined for wilt reactions (yellowing and wilting) for up to 30 d post inoculation. The nutrient media and conidium suspension of each pathotype/race were replenished at 4 d intervals during this period.

The inoculation experiment was carried out at the ICAR-Indian Agricultural Research Institute, New Delhi, India (Lat. 28.6377° N, Long. 77.1571° E) during 201920, in a temperature-controlled growth facility maintained at 20/14 ± 2°C day/night temperatures, with a daily photoperiod of 16 h and 45% relative humidity at germination, and then maintained 26 ± 2°C for effective infection (Figure 1).

Microscope observations, histopathological studies, disease assessments and disease progression

Microscope examinations of seedlings were carried out at 25 and 30 d after inoculation. The observations Growth and disease progression assessments were made for the resistant ("WR 315") and susceptible (JG 62) controls, and all the MABC lines. Seedling roots were gently washed in tap water to remove adhered particles, and were each hand sectioned into eight to ten 1-2 mm pieces of (cross and longitudinal sections), using a double-sided razor blade. The root pieces were then placed on glass microscope slides in drops of tryptophan blue or water, and then covered with cover slips for microscopic analyses. Single host genotypes were used in three replicates to study microscopic and histopathological characteristics, and for assessments of disease progression. For each assessed seedling, two hand sections were prepared and observed under a compound microscope at ×10 magnification.

After inoculation, the plants were regularly monitored and scored every 3 d for disease symptoms and progression. Disease symptoms were scored using a six point (0-5) scale (modified from that described by Pouralibaba *et al*., 2015), as follows:

0 = "no symptoms"- (0)-Immune,
1 = "tiny initiation on the leaf and yellowing in older leaves"- (0.1–10%) - Highly Resistant,
2 = "leaf showing complete yellowing of older and younger leaves"- (10.1–25%) - Resistant,
3 = "complete yellowing and falling of leaves"- (25.1–50%), Moderately susceptible,
4 = "wilted/curled/dried leaf, or defoliation"- (50–75%), Susceptible,
5 = "dried completely or killed plant" - (75.1-100%), Highly susceptible.

To represent all possible disease patterns in the plants, the disease scores were applied separately to each leaf. For effective comparison of resistance between the host genotypes, the disease score was developed for each complete plant. Considering n. 1, n. 2, n. 3, n. 4, and n. 5 is the number of leaves showing, respectively, symptom types 1, 2, 3, 4 or 5, respectively, the formula used for calculating disease Index (DI)/disease score of each plant was:

$$DI = ((n1 \times 0.10) + (n2 \times 0.25) + (n3 \times 0.5) + (n4 \times 0.75) + (n5 \times 1)/t) \times 100);$$

where 0.1, 0.25, 0.5, 0.75 and 1 are the indices that conform, respectively, to symptom categories y of 1, 2, 3, 4 or 5, and t represents the number of leaves in total including asymptomatic and fallen leaves due to disease. The absence of symptoms was scored as DI = 0 and dead plants as DI = 100 (Srinivasa *et al*., 2019) (Figure 2).

The Foc inoculum was first used on seedlings that were 15 d-old, and relative progression of Fusarium wilt was observed at 10, 15, 18, 21, 24, 27, 30 d after inoculation to calculate the areas under the disease severity curves (AUDSC), using the formula Y = Pn–1 i = 1 [(Xi + Xi + 1)/2] (ti + 1 − ti), where Y is the AUDPC, Xi is the disease incidence of the ith evaluation, and Xi + 1 is the disease incidence in next observation, and (ti + 1 –ti) is the number of days between two observations (Gupta *et al*., 2021). Final assessments were made 45-d-old seedlings.

Plant phenotypic characters

Sampling was carried out for each plant in all the three replications, and growth parameters were assessed, including root and shoot lengths, and root and shoot fresh and dry weights, to determine effects of Fusarium wilt (Foc) on plant phenotypes. Harvesting of roots and shoots were carried out separately, and to remove surface contamination, the roots were rinsed in distilled water for about 20s, followed by blotting to remove excess moisture. The dry weight of roots and shoots were determined by drying the plant parts at 80°C for 72 h. Genotypes with the lowest and highest disease scores were considered, respectively, to be highly resistant and susceptible to wilt. A completely randomized design was used in the experiment, and for phenotyping, single genotypes were used in each replication. These data were combined to determine means, descriptive analysis, correlations of root and shoot lengths, root and shoot dry and fresh weights, and mean wilt scores for each genotype.

Figure 2. Disease Indices (0–5) used for assessments of chickpea plants for severity of Fusarium wilt.

Kruskal-Wallis rank-sum test for comparison of disease scores

The chickpea genotypes showing the lowest disease scores were considered to be resistant to Fusarium wilt, and those with high values were classified as susceptible. Disease scores and Kruskal Wallis tests gave comprehensive assessments of resistant and susceptible genotypes in this study (Supplementary Tables 3 and 4). The Kruskal Wallis test is non-parametric that does not assume that the data come from a particular distribution. Here ranks of the data values were used in the test rather than the actual data points.

Statistical analyses

Results were determined for different means, standard errors, standard deviations, and coefficients of variation. Statistically significant values were tested at $P = 0.05$, and Tukey's test was applied at this probability assess significant differences between means. All the data including t tests were analysed using the STAR programme (Statistical Tool for Agricultural Research Version 2.0.1, 2014). A heat map was created using the R software 'stats' package, and correlation analyses were carried out using the "corrplot" package (version 0.84).

RESULTS

Histopathological observations

Presence of fungal mycelium was observed in the susceptible and resistant control plants. In the resist-

Table 3. Mean AUDPCs (± standard errors) for different chickpea genotypes (Sr. No.) inoculated with different races of *Fusarium oxysporum* f. sp. *ciceris*.

Sr. No.	Race 1	Race 2	Race 3	Race 4	Race 5
1	264.72 ± 8.92	155.68±8.36	385.08±10.58	459.065±12.79	390.83±7.08
2	11.39±1.41	5.72±0.68	14.34±1.17	7.44±0.91	11.66±1.0
3	161.85±8.00	31.14±1.61	49.17±3.16	33.95±3.01	128.25±5.42
4	131.13±6.86	62.64±3.08	64.76±3.79	149.07±6.33	129.91±5.75
5	146.88±8.94	123.30±6.01	86.22±4.17	82.73±3.98	86.71±4.23
6	20.82±1.73	121.08±5.43	1.92±0.32	159.73±7.11	99.14±4.21
7	57.26±4.28	25.72±1.97	8.26±0.66	216.76±7.29	118.76±4.95
8	165.13±7.29	99.93±5.54	74.42±3.60	110.37±5.53	228.51±5.51
9	82.86±4.15	12.15±0.74	79.24±3.67	129.82±5.5	147.87±7.03
10	91.77±6.66	41.64±1.89	81.45±4.24	235.41±6.8	181.45±6.25
11	101.43±5.19	11.0±0.71	121.03±5.72	67.44±3.5	148.15±6.80
12	61.61±3.85	1.36±0.23	13.87±1.12	131.62±5.86	27.70±1.42
13	159.21±8.62	90.27±4.10	60.19±4.01	29.79±1.82	59.33±2.60
14	18.73±2.32	17.51±1.34	71.75±4.43	16.03±1.36	63.58±2.51
15	89.90±4.92	69.02±3.29	57.44±2.97	51.90±3.28	65.42±2.88
16	36.25±3.26	32.92±1.54	71.73±3.41	73.33±3.46	112.04±4.56
17	92.12±3.96	52.80±2.6	85.07±3.63	123.70±5.1	65.31±4.43
18	197.74±9.53	360.62±8.04	456.50±11.14	389.54±10.44	373.2±9.99
19	865.07±23.26	877.81±19.8	1053.49±16.06	1161.10±17.27	778.21±17.85
20	512.94±16.10	281.69±14.34	1200.07±19.11	493.61±13.71	159.68±8.23
21	109.92±5.05	123.75±6.09	68.06±2.73	170.06±7.65	311.16±9.85
22	202.22±4.44	110.11±5.23	133.95±5.81	51.56±3.32	77.32±3.59
t value	4.014***	3.0292***	2.8013***	3.6371***	4.7276***

Table 4. Descriptive statistics for seven chickpea plant traits analysed in this study.

Trait*	Min	Max	Range	Mean	SD	CV
SL	11.9	30.3	18.4	20.46	3.52	10.59
RL	8.9	29.3	20.4	16.07	3.82	4.97
SFW	0.47	1.64	1.17	0.87	0.23	7.61
SDW	0.044	0.16	0.0116	0.0873	0.02	6.71
RFW	0.35	1.56	1.21	0.79	0.267	4.92
RDW	0.036	0.172	0.136	0.0744	0.02	10.11
DS	0	100	100	15.6	18.4	13.3

*SL, shoot length; RL, root length; SFW, shoot fresh weight; SDW, shoot dry weight; RFW,-root fresh weight, SDW, shoot dry weight. DS = Disease Score;

ant controls (WR 315) the xylem vessel discoloration was not prominent compared to the susceptible controls (JG 62), where xylem vessel discolorations and complete disruption were observed at the early stages of infection (Figure 3).

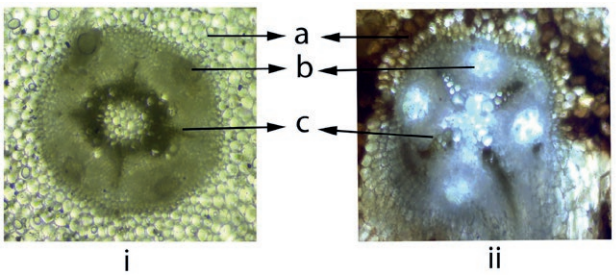

Figure 3. Example micrographs of chickpea root cross sections used in histopathological studies of Fusarium wilt. (i) resistant control host line WR 315, and (ii) susceptible control host JG 62. a, cortex; b, phloem; and c, xylem.

Identification of resistance against Fusarium wilt under a hydroponic system, based on in planta infection

Wilt symptoms were observed in the susceptible cultivar (JG 62) at 8 d after inoculation, but no symptoms were observed in the resistant genotype (WR 315). Parent Pusa 372 showed moderately susceptible responses against the Foc races 1, 3, 4, and 5, and showed resistant phenotype for race 2. JG 11 showed moderate susceptibility for

races 2, 3, 4 and 5, and showed resistant phenotype for race 1. Eight host genotypes showed highly resistant reactions against five races of Foc. Among the 22 genotypes tested, four showed highly resistant responses to race 1, four were resistant to race 2, ten were resistant to race 3, seven were resistant to race 4, and seven genotypes were resistance to race 5. Fifteen MABC introgression lines of Pusa372 and JG 11 were tested against all five races s among which nine showed highly resistant response under hydroponic conditions. MABC introgression lines of Pusa 372 (IL.11,12,14) and JG 11 (IL.15,16,17) showed high resistance reactions to Fusarium wilt (Figures 4 and 5). ILC (CZ) showed varied reactions, with highly susceptible phenotypes for race 3, susceptible reaction to races 1 and 4, and moderately susceptible phenotypes for races 2 and 5. ILC (Lat) was highly resistant to races 1, 2 and 3, and moderately susceptible for races 4 and 5. The variety Pusa Green 112 (BGD 112) was resistant to races 2, 3, 4, and 5. Control plants grown in nutrient solution not show any disease symptoms during the experimental period.

Also, AUDPCs were calculated for all the host genotypes and with respect to all five Foc races under consideration. For race 1, AUDPC was greatest (865.07) for the susceptible control JG 62, was next greatest for ILCO (CZ), followed by parent Pusa 372 (264.72) and JG 11 (197.74). For MABC lines for race 1, the lowest AUDPC (18.73) was recorded for IL14, followed by IL6 (20.82) and IL16 (36.25). The resistant control WR 315 gave the lowest AUDPC value of 11.39. For race 2, AUDPC was greatest (877.81) for the susceptible control JG 62, next greatest (360.62) for JG11, followed by ILCO (CZ) (281.69 and parent Pusa 372 (155.68). For the MABC lines for race 2, the lowest AUDPC (1.36) was recorded for IL12, followed by IL11 (11.0) and IL9 (12.15). The resistant control WR 315 gave an AUDPC of 5.72. For race 3, AUDPC was greatest for ILCO (CZ) ((1053.49, followed by susceptible control JG 62 (1200.07), JG 11 (456.50) and parent Pusa 372 (385.08). For the MABC lines and race 3, the lowest AUDPC (1.92) was recorded for IL 6, followed by IL 7 (8.26) and IL12 (13.87). The resistant check WR 315 gave the lowest AUDPC of 14.34. Against race 4, AUDPC was greatest (1161.10) for the susceptible control JG 62, followed by ILCO (CZ) (493.61), and parent Pusa 372 (459.06) and JG11 (389.54). For MABC lines against race 3, the lowest AUDPC (16.03) was recorded for IL 14, followed by IL 13 (29.79) and IL3 (33.95). The resistant control WR 315 gave the lowest AUDPC value of 7.44. For race 5, AUDPC was greatest (778.21) for the susceptible control JG 62, followed by parent Pusa 372 (390.83), and JG11 (373.2). For the MABC lines against race 5, the lowest AUDPC (27.70) was recorded for IL 12, followed by IL 13 (59.33) and IL14 (63.58). The resistant control WR 315 gave the lowest AUDPC value of 11.66. For AUDPCs, the susceptible check JG 64 gave the greatest value followed by parent Pusa 372, and the lowest AUDPC was recorded for the resistant control WR 315 (Table 3, Supplementary table 1). Results for AUDPC values for selected host genotypes are presented in Figure 6.

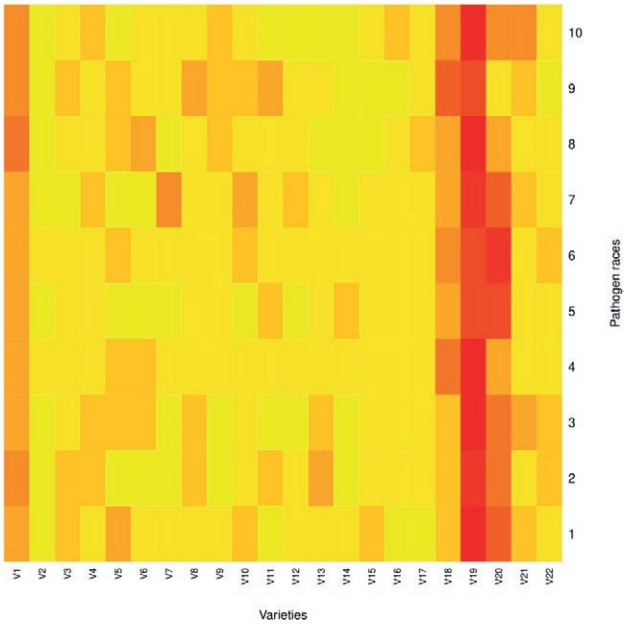

Figure 4. Heat map indicating Fusarium wilt severity scores for chickpea genotypes. from highly susceptible (red) to highly resistant (yellow) lines.

Figure 5. Differential responses of susceptible, resistant MABC lines, and resistance controls under Fusarium wilt stress (WS), and comparison with a very susceptible line (Control).

Host plant phenotypic traits measured under hydroponic conditions

The descriptive statistics for all seven host traits showed significant variations under wilt stress condi-

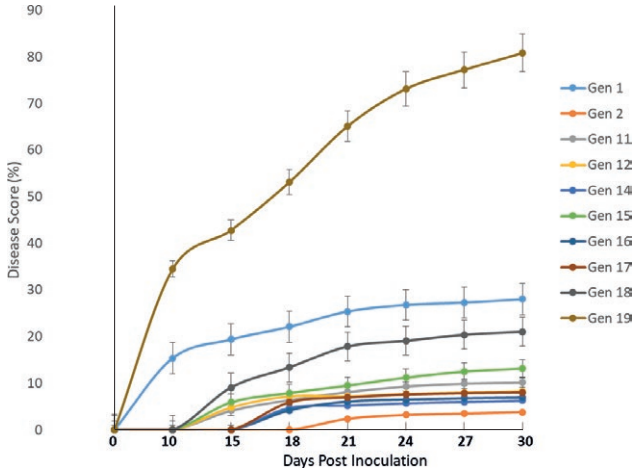

Figure 6. Disease progress curves for selected chickpea genotypes grown in hydroponic culture inoculated with *Fusarium oxysporum* f. sp. *ciceris* race 1.

tions, indicating considerable variation among the host genotypes (Table 4). Mean plant height under wilt stress was 20.5 cm, with a minimum of 11.9 cm (JG 62) and a maximum of 30.3 cm (IL.14). Mean root length under wilt stress was 20.0 cm, with a minimum of 8.9 cm (JG 62) and a maximum of 29.3 cm (IL.14). For mean shoot fresh and dry weights under wilt stress conditions were 0.87 g and 0.87 g, with respective minima of 0.47 g (JG 62) and 0.044 g (JG 62), and maxima of 1.64 g (IL.4) and 0.16 g (IL.6). Mean root fresh and dry weights were, respectively, maxima 1.56 g (IL.12) and 0.172 g (IL.13), and minima 0.79 and 0.07 and 0.35 (IL.20) and 0.036 (JG 62).

Correlations between host plant parameters

Correlation analyses were carried out for seven host plant traits (Figure 7). Correlations were statistically significant and strongly positive between the traits root fresh and dry weights (0.88), shoot fresh and dry weights (0.86), and root fresh weights and root lengths (0.53). Root and shoot weights were significantly and positively associated (0.44). Significant and negative associations occurred between disease scores and root lengths (-0.37) and root fresh weights (-0.33). Root fresh weights and shoot fresh weights (0.44) were positively related.

Reductions in host phenotypic traits

The greatest mean reductions in host plant parameters were observed for susceptible control plants of JG 62, with reductions of 47% in shoot length, 91% in root length, 34% in shoot fresh weight, 50% in shoot dry weight, 75% in root fresh weight, 56% in root dry weight, and an overall average 59% reduction across traits. Mean reductions for parent Pusa 372 were 35% for shoot length, 31% for root length, 31% for root length, 22% for shoot fresh weight, 19% for shoot dry weight, 54% for root fresh weight, 48% for root dry weight, and an overall average reduction across all traits of 35%. Mean proportional reductions for IL3 were 13% in shoot length, 38% in root length, 19% in shoot fresh weight, 11% in root dry weight, 23% in root fresh weight, 44% in root dry weight, with an overall average reduction across all traits of 25%. Mean reductions for IL11 were; 13% in shoot length, 21% in root length, 24% in shoot fresh weight, 37% in shoot dry weight, 40% in root fresh weight, 18% in root dry weight, with a 25% overall average reduction across all traits. Mean reductions for IL12 were; 10% in shoot length, 27% in root length, 24% in shoot fresh weight, 7% in shoot dry weight, 37% in root fresh weight, 17% in root dry weight, with a 20% overall reduction across all traits.

Greatest proportional reductions considering all host characters was measured for JG 62 (59%), then for Pusa 372 (35%) and JG 11 (32%), and the least reduction was for the resistant control WR 315 (11%) (Table 5, Supplementary Table 2).

Host parameters were affected differently by the different pathogen races. For shoot lengths, greatest reduction (21%) was measured for race 5, followed by race 3 (20%), race 2 (18%), race 1 (17%), and race 4 (12%) respectively. For root lengths, the greatest reduction was measured from race 5 (32%), followed by race 3 (32%), race 1 (23%), race 4 (20%), and race 2 (19%). For shoot fresh weights, the greatest reduction was from race 5 (47%), followed by race 1 (32%), race 3 (31%), race 2 (30%), and race 4 (22%). For shoot dry weights, the greatest reduction was measured from in race 5 (47%), followed by race 3(26%), race 1 (24%), race 2 (23%), and race 4 (17%). For root fresh weights, the greatest reduction was from race 3 (40%), followed by race 5 (38%), race 4 (37%), race 1 (37%), and race 2 (33%). For root dry weights, the greatest reduction was from race 5 (43%), followed by race 1 (36%), race 2 (34%), race 3 (24%), and race 4 (14%). The results for the different races when subjected to "t" tests showed statistically significant differences among the host genotypes in responses host parameters assessed (Tables 5 and 6).

DISCUSSION

Screening of chickpea genotypes for susceptibility to Foc is usually carried out in wilt "sick" plots or

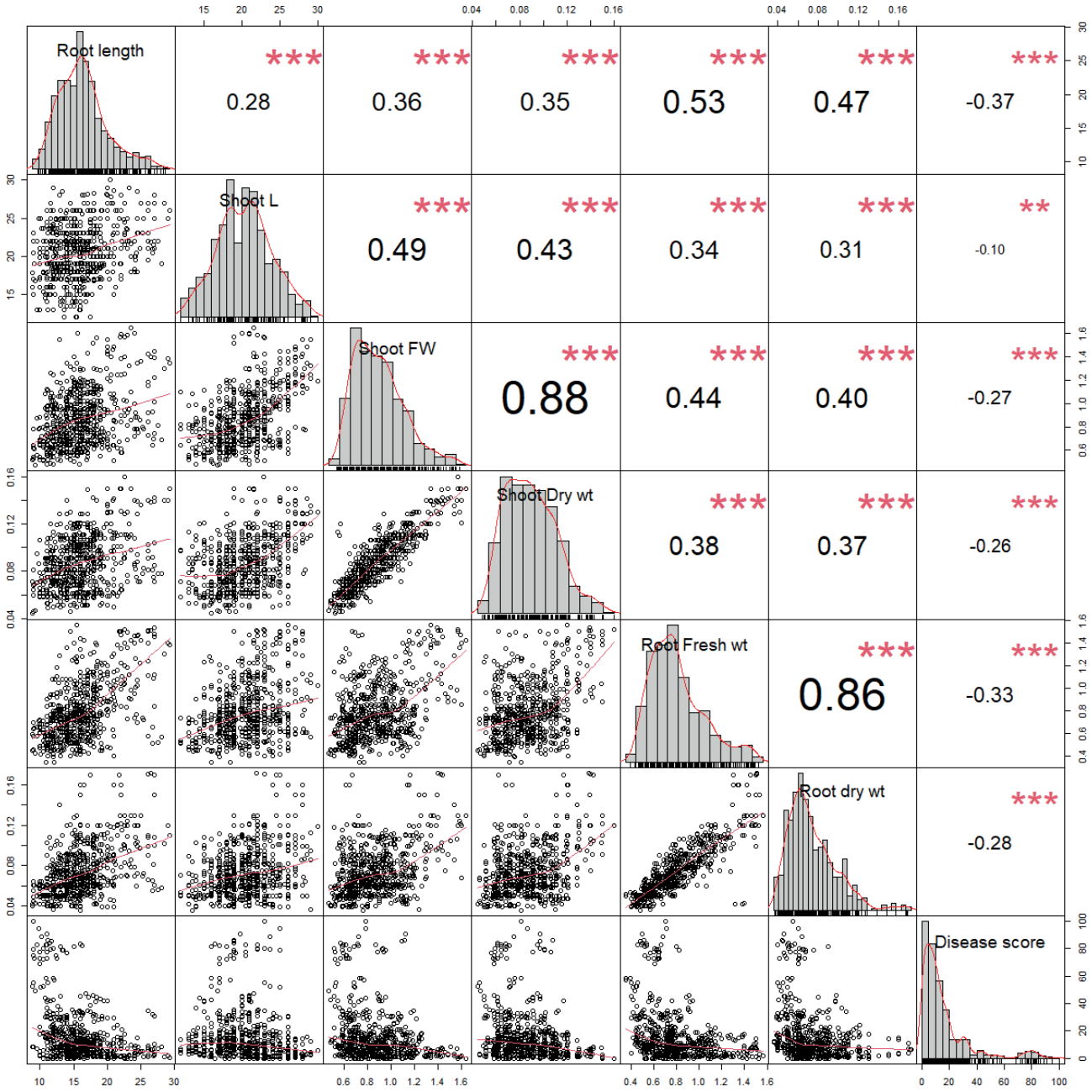

Figure 7. Correlations and distributions of six mean chickpea root parameters and mean Fusarium wilt scores, measured after hydroponic culture with *Fusarium oxysporum* f. sp. *ciceris*.

using pot culture techniques, which are environmentally sensitive, time consuming, involve complex inoculation methods, and where it is difficult to screen large numbers of host genotypes (Belaidi, 2016). Hydroponic screening for disease resistance has been reported for bean root rot (Anderson and Guerra, 1985), *Fusarium* sp. in banana (Zheng *et al*., 2018), in *Medicago sativa* (Cong *et al*., 2018), and screening for Phytophthora root rot resistance in chickpea (Amalraj *et al*., 2019). The present study is the first successful use of hydroponic culture for screening race-specific Fusarium wilt resistance in chickpea.

Histological distortions of vascular tissues in host roots and shoots in resistant and susceptible controls were observed. Formation of cavities were observed between phloem and xylem tissues, medulla and xylem,

Table 5. Average percentage reductions for different chickpea host lines (Sr. No.) for traits after inoculations with different races (R1 to R5) of *Fusarium oxysporum* f. sp. *ciceris*.

Sr. No	R1	R2	R3	R4	R5	Average
1	31	66	56	19	51	35
2	15	13	9	9	24	14
3	15	12	13	7	13	12
4	32	23	21	13	10	19
5	7	18	2	6	1	7
6	13	6	13	5	7	9
7	1	5	36	2	22	11
8	33	15	3	1	3	10
9	13	6.	18	10	8	11
10	18	7	36	12	22	18
11	10	17	16	10	9	12
12	12	0.8	14	11	12	10
13	9	11	8	6	0.9	7
14	29	12	5	10	16	14
15	18	19	10	4	24	15
16	3	21	38	7	41	20
17	0.3	54	21	14	24	20
18	31	19	36	29	31	29
19	30	40	50	35	91	46
20	16	9	11	9	1	9
21	2	21	8	24	12	13
22	17	2	14	2	33	12
t values	7.2896***	5.3361***	6.2446***	6.2377***	4.8384***	

Table 6. Percentage reduction of SL, RL, SFW, SDW, RFW and RDW with respect to five different races of *Fusarium oxysporum* f. sp. *ciceris*.

Sr.No.	Race 1	Race 2	Race 3	Race 4	Race 5	Average	t value
SL	16.56	18.49	20.32	11.70	21.15	16.49	6.9***
RL	22.87	19.35	31.99	20.11	32.16	23.75	6.79***
SFW	32.42	29.77	30.58	22.03	46.59	22.59	16.14***
SDW	24.31	22.67	26.47	16.86	46.98	24.52	6.50***
RFW	36.91	33.23	39.52	37.30	38.43	33.20	10.589***
RDW	36.31	34.03	24.12	14.64	43.04	27.83	7.62***

phloem and cells of cortical parenchyma, and proliferation of cells in vascular cambium was also observed. Stem cross sections revealed xylem colonization by the pathogen, while resistant plants showed normal development. The hydroponic system allowed effective assessments of compatible and incompatible interactions between chickpea races and resistant and susceptible cultivars (Jiménez-Fernández *et al*., 2013). Similar results of xylem colonisation between resistant and susceptible cultivars were observed by Caballo *et al.* (2019) for race 5 of *Fusarium oxysporum* f. sp. *ciceris*. Obstruction of host water conduction systems affect photosynthesis due to closure of stomata induced by water deficit, and also affect functioning of RuBisCO (Pedrosa *et al*., 2011). Fusarium wilt affects three crucial photosynthesis processes, including thylakoid electron transport, carbon reduction cycles, and CO_2 supply for stomata (Allen *et al*., 1997).

Compatible interactions (susceptibility) of Foc infections inhibit plant growth through water stress and pathogen action. Therefore, increased root length in resistant plant genotypes is an efficient host defence mechanism against root-invading pathogens (Caballo *et al*., 2019). Host leaves also lose turgidity, which leads decreased shoot and root dry weights (Jalali and Chand, 1992; Jimenez-Díaz *et al*., 2015). Increased root length and fresh weight could be used as selection criteria for host resistance using hydroponic techniques. In the present study, negative and low correlations were observed between disease scores, shoot lengths, and shoot and root fresh and dry weights, indicating low variation among MABC lines for disease resistance. These are in advanced selection generations (AVT lines), and are near-isogenic for disease resistance.

Disease observations were taken by observing individual leaves, which increased accuracy of disease score calculation. These measurements showed that parent host lines (Pusa 372 and JG 11) were moderately susceptible to most of the assessed Foc races. The susceptible control (JG 62) was highly susceptible to all of the Foc races, and the resistant control (WR 315) was highly resistant to these races (Sharma *et al*., 2005; Milan *et al*., 2006). For all of the seven measured host physical characters and disease score, the parent Pusa 372 introgression lines (11, 12, 14) and JG 11 introgression line (17, 18) were generally superior for all characters, and were highly resistant to disease. Line ILC (CZ) showed varied reactions for different Foc races, and was moderately susceptible only to Foc races 5 and 2. ILC (Lat.) showed was highly resistant against all the tested Foc races, showing the potential for utilization of this landrace for diversification of resistant controls to other than WR 315. The variety Pusa Green 112 (BGD112) was resistant to all assessed races except race 1, and is therefore a potent donor source for Fusarium wilt resistance (Yadav *et al*., 2004). Analysis of AUDPCs also showed that MABC lines had the lowest disease progression.

Reductions due to Foc were detected in growth traits of the resistant chickpea susceptible control, MABC lines, and the other varieties studied. Average plant parameter reductions were greatest after inocula-

tion with Foc race 5 (for shoot and root lengths, shoot fresh and weights, and root dry weight) and race 5 (for root fresh weight). For all the host phenotypic characters assessed, the greatest reductions were observed in root fresh weight (Table 5).

Average percentage reductions were 55% for MABC line 3, 25% for line 11, and 20% for line 12, and 35% for parent Pusa 372, 12% for donor parent WR 315, 31% for the national control JG 11, and 59% for the susceptible control JG 62. This indicates that in the case of MABC lines and a resistant check showed a lower percent reduction in the growth traits suggesting resistant response as compared to parents and susceptible check (Maitlo *et al*., 2014). Punja and Rodriguez (2018) studied *Fusarium* species infecting roots of hydroponically grown marijuana plants, and observed reductions in root lengths and volumes, which were similar to reductions measured in the present study. This emphasizes how Foc can affect different host landraces, parents and MABC lines, and indicates the superiority of MABC lines over other genotypes.

MABC is regarded as a rapid method for developing host varieties that are resistant to Fusarium wilt (Varshney *et al.,* 2014). MABC development in chickpea has relied mostly on field or "wilt sick" plot screening for variety development (Mannur *et al.,* 2019; Roorkiwal *et al*., 2020). Nevertheless, some of these varieties have become susceptible to Fusarium wilt, possibly due to changes in wilt incidence, pathogen genotype differences, and genotype/environment interactions (Neupane *et al.,* 2007; Sharma *et al*., 2012). In the present study, race purity of Foc inocula was maintained under hydroponic culture, and the inoculated races were reisolated. This gave accurate, reproducible and reliable disease quantification, at an early stage of host infection. Screening using hydroponics based screening will increase accuracy and be useful for future MABC development programmes.

Hydroponic systems can be costly compared to traditional "sick plot" method, requiring specific skills, and host plants that are readily infected by pathogens. Many hosts require similar nutrient media, and hydroponic systems allow rapid inoculum spread (Pandey *et al*., 2009; Sardare and Admane, 2013). Use of this method will aid current and future efforts in breeding for Fusarium wilt resistance in chickpea, as for genetic studies.

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AUTHOR CONTRIBUTIONS

CB and NS conceived this study. JJ and AR performed the experiments. JJ, ST and NK analysed the data. The manuscript was written by JJ, CB, KRS and BSP, with help from all the co-authors.

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