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Screening of exotic banana accessions for their resistance to fusarium wilt race 1 and tropical race 4 in India

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Fusarium wilt, caused by the soil-borne pathogen *Fusarium oxysporum* f. sp. *cubense* (*Foc*), is one of the most destructive diseases affecting global banana production. *Foc* Race 1 (*Foc* R1) and Tropical Race 4 (*Foc* TR4) are particularly destructive and lethal, impacting both Cavendish and local commercial cultivars. Deploying resistant varieties is crucial for managing this disease in commercial cultivation. This study evaluated 99 exotic banana accessions from the International Transit Centre (ITC), Belgium, representing diverse genomic groups to identify resistant sources within the banana gene pool. The accessions were assessed against both *Foc* R1 and *Foc* TR4 under glasshouse and hotspot field conditions. Disease severity was assessed using rhizome discoloration at harvest. The results of the study indicated significant variation across genome groups, test conditions, and *Foc* races ($P < 0.001$). Out of 99 accessions, 63 exhibited either an increase or decrease in resistance level in accessions between the test conditions, while 36 remained stable. Similarly, 68 accessions showed shifts between the tested races, while 31 remained stable. In the field evaluation of *Foc* R1, 19 accessions were immune, 13 highly resistant, and 17 were resistant, whereas in the glasshouse, only one accession was immune, nine were highly resistant, and 46 were resistant, indicating a greater resistance response under controlled conditions. For *Foc* TR4, 18 accessions were immune, nine highly resistant, and 19 were resistant in the field, whereas in the glasshouse, one was immune, 30 were highly resistant, and 50 were resistant, demonstrating a notable shift in resistance levels between environments. Some of these banana accessions show potential for direct commercial deployment in Fusarium wilt hot spot areas and/or could be used to identify resistant genes to further improve commercial cultivars through genetic engineering or gene editing techniques.

Keywords Cavendish banana, Fusarium wilt, Grand Nain, VCG 0125, VCG 01213/16, Resistance

Bananas are cultivated in over 130 tropical and subtropical countries, covering 5.33 million ha, with an annual production of 124.98 million tonnes. They are valued for their nutritional content and economic significance, particularly for small-scale and marginal farmers in developing countries. Bananas and plantains are crucial for the food security and livelihoods of over 400 million people. India is the leading global banana producer, with an annual output of 33.06 million tonnes from 0.92 million ha, contributing 26.45% to the global banana production[1]. However, banana production faces significant challenges, notably from biotic and abiotic stresses that vary in impact depending on the agro-ecological and socio-economic conditions. Fusarium wilt, caused by *Fusarium oxysporum* f. sp. *cubense* (*Foc*), poses a major global threat to banana production, particularly in tropical and subtropical regions. While *Foc* R1,and *Foc* R4 affect Cavendish bananas, *Foc* R4, specifically Tropical Race 4 (VCG 01213/16), is considered the most destructive and lethal strain. This is due to its ability to affect not only the Cavendish group, which accounts for about 57% of global production and 99% of export markets[2]but also locally consumed bananas and other banana varieties. *Foc* TR4 was first detected in Taiwan in 1967 and has since spread to 23 countries, with recent outbreaks reported in Venezuela[3] and Grande Comoros Island[4]. In

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2013, financial losses due to Fusarium wilt caused by *Foc* TR4 were estimated to be as high as US$121 million in Indonesia, US$253 million in Taiwan, US$14 million in Malaysia, and over US$7.5 million in northern Mozambique (www.rtb.cgiar.org). Therefore, the spread of *Foc* TR4 could exacerbate poverty and intensify global hunger, particularly in developing nations.

In India, Fusarium wilt of bananas is becoming increasingly severe, where both *Foc* R1 (VCG 0124, 0125, 01220) and *Foc* TR4 (VCG 01213/16) are affecting the Cavendish group of bananas as well as other locally grown commercial cultivars such as Silk (AAB), Pisang Awak (ABB), Pome (AAB), and cooking bananas (ABB). *Foc* R1 has been reported to affect the Cavendish group in the major banana-producing states of India, including Tamil Nadu, Gujarat, Maharashtra, Madhya Pradesh, Uttar Pradesh, Bihar, and West Bengal while *Foc* TR4 was recorded only in Bihar, Uttar Pradesh, West Bengal and recently in Gujarat[5,6]. Disease severity ranges from 5 to 60% and some cases exceeds 90%. This has led farmers in Tamil Nadu to shift to other varieties like Nendran (AAB) and Red banana (AAA) which are showing resistance to *Foc* race 1 (*Foc* TR4 not recorded)[7]. However, in states like Bihar, Uttar Pradesh, West Bengal, and Gujarat, where *Foc* TR4 is a major concern, farmers have shifted to non-remunerative crops, e.g., Maize, resulting in unemployment, particularly among small farmers and landless labourers in rural communities[8].

Various disease management practices, including quarantine, cultural, chemical, can slow the spread of the disease or reduce the pathogen inoculum levels, but these methods are often expensive and less suitable for long-term sustainable management. The deployment of resistant varieties for commercial use and biological control methods are other potential options that may offer comprehensive and sustainable solutions to this significant issue[8]. Currently, there is a lack of available Cavendish cultivars which are completely resistance against *Foc* races. Although genetically modified *Foc* TR4-resistant cultivars are available in Australia[9], their acceptance in many countries remains limited due to regulatory and public concerns. Therefore, it is imperative to promptly identify resistant sources within the banana gene pool and use them in breeding programs to develop resistance against Fusarium wilt. Already, a successful cross between 'SukaliNdizi' and 'TMB2 × 8075-7' has shown complete resistance against *Foc* R1, with the resistance gene named Panama disease 1 (*Pd1*)[10]. Similarly, wild relatives such as *Musa basjoo*, *M. itinerans*, *M. nagensium*, *M. ruiliensis*, *M. velutina*, *M. laterita* and *M. yunnanensis* have been identified as resistant sources to *Foc* TR4 that can be utilised in breeding programmes[26]. In addition, *M. acuminata* sub sp. *malaccensis* has also shown resistance to *Foc* TR4[11].

In the Caribbean and Mozambique, diploids wild species such as Pahang (AA), Calcutta 4 (AA), and *M. itinerans* demonstrated a high degree of resistance to *Foc* TR4[12]. In China, five different *Foc* TR4 resistant/tolerant varieties were developed, of which ZJ9, a triploid accession displayed complete resistance[13]. The Fundación Hondureña de Investigación Agrícola (FHIA), Honduras[14] has also developed several bred hybrids that have tolerance/resistance to *Foc* TR4, including FHIA-01 (Gold Finger), FHIA-18, FHIA-2 (Mona Lisa), FHIA-25, and SH-3640/10 (High Noon)[15]. However, relying heavily on a limited number of resistance sources for *Foc* TR4 and *Foc* R1 poses a significant risk to the durability of host resistance. In *Foc* alone, so far, 24 different VCGs that belong to *Foc* races 1, 2, and 4 have been reported. Besides, many unknown VCGs have also been reported, which indicates that new strains are evolving rapidly, which may break the host resistance.

Given the urgent need for durable resistance, this study screened 99 exotic banana genotypes (diploids, triploids, and tetraploids) from the International Transit Centre (ITC), Belgium against both *Foc* R1 and TR4 under controlled conditions and in designated hotspots. This finding will aid in identifying resistant sources for direct deployment or breeding programmes. The results of this screening are presented and discussed in the following sections.

Results

Screening ITC germplasm

This study employed 99 accessions provided by ITC in Belgium. The accessions belong to ten distinct genome groups, categorised by their ploidy levels. Experiments were conducted under both the glasshouse and hotspot field conditions, to ascertain the resistance levels of ITC accessions against *Foc* R1 and *Foc* TR4. As shown in Table 1, the "AA" genome group is the most frequent, with 29 accessions. Following in abundance are "AAA" (23 accessions), "AAB" (15 accessions), and "ABB" (14 accessions). The remaining groups, including tetraploids (11 accessions), "AB" and "BB" diploids (combined: 5 accessions), and "Rhodo", exhibit substantially lower representation, ranging from 1 to 2 accessions. It is important to note that the AB (3), BB (2), AABB (1) and Rhodochlamys (2) genome group was represented by few accessions in this study. Therefore, the statistical power to detect significant differences within this groups was limited, and these results should be considered preliminary.

Genomic group representation of ITC accessions

The IWDS were analysed for each ITC accession across *Foc* R1 and *Foc* TR4 races in glasshouse and field trials. The distribution and percentage of IWDS are presented in Fig. 1. As data were normally distributed with equal variances, the means were compared using two-way ANOVA and independent samples t-tests to assess accession performance within and between genome groups using the Bonferroni method (Fig. 2). The minimum, maximum, mean, mode and degrees of freedom (*df*) of IWDS values were estimated for all accessions and presented along with pairwise mean comparisons using t-tests and ANOVA in Supplementary Tables 1 and 2 and Figs. 1, 2 and 3. The results for *Foc* R1 show that the genomic groups AA, AAAA, AAAB, AB, and ABB exhibited a significant shift in disease index and internal score. Similarly, for *Foc* TR4, the AA, AAA, and AAB groups also showed a significant shift in both parameters.It was observed that the AA, AAAA, AAAB, AB, and ABB genetic groups are shown to have significant shifts in the disease index and internal score tested against *Foc* R1 in different test conditions. For *Foc* TR4, the shift was noticed only in AA, AAA, and AAB. The pairwise t-test revealed significant shifts in resistance level for 58 out of 99 accessions, regardless of their genome

Genome Group	No. of Accessions	Foc R1											Foc TR4										
		Field						Glasshouse					Field						Glasshouse				
		I	HR	*R*	MR	S	HS	I	*R*	MR	S	HS	I	HR	*R*	MR	S	HS	I	*R*	MR	S	HS
AA	29	12	3	7	5	2	-	-	2	18	8	1	5	6	9	3	4	2	-	11	17	1	-
AB	3	-	-	-	-	-	3	-	-	1	1	1	1	-	-	1	1	-	-	1	-	1	1
BB	2	-	1	1	-	-	-	-	-	1	1	-	-	-	-	2	-	-	-	-	2	-	-
AAA	23	4	2	1	4	8	4	-	3	12	7	1	2	-	2	6	10	3	-	9	11	2	1
AAB	15	2	2	4	2	2	3	1	1	6	5	2	6	1	1	3	3	1	1	2	5	4	3
ABB	14	-	-	-	-	2	12	-	-	2	8	4	2	1	2	4	5	-	-	2	7	3	2
AAAA	4	-	2	2	-	-	-	-	1	3	-	-	1	1	1	1	-	-	-	2	2	-	-
AAAB	6	1	2	1	2	-	-	-	2	2	2	-	1	-	2	1	-	2	-	2	4	-	-
AABB	1	-	-	1	-	-	-	-	-	1	-	-	-	-	-	1	-	-	-	-	1	-	-
Rho	2	-	1	-	-	1	-	-	-	-	1	1	-	-	2	-	-	-	-	1	1	-	-
Grand Total	**99**	19	13	17	13	15	22	1	9	46	33	10	18	9	19	22	23	8	1	30	50	11	7

Table 1. Resistance distribution of ITC (International transit centre) accessions across genome groups to *Foc* R1 (VCG 0125) and TR4 (VCG 01213/16) races in field and glasshouse conditions. I-Immune; HR-Highly resistant; R-Resistant; MR-Moderately resistant; S-Susceptible; HS-Highly susceptible and Rho-Rhodochlamys.

group ($P<0.05$, Supplementary Table 1). The remaining 41 genotypes showed no difference in resistance level between glasshouse and field conditions. Similarly, 61 genotypes exhibited a significant resistance shift, while 38 did not when challenged with different *Foc* races ($P<0.05$, Supplementary Table 2). Among the genome groups, accessions belonging to AAB, AB, BB, and Rhodo did not show any significant shifts in resistance levels between races ($P<0.05$, Supplementary Table 3), similarly, AAAB, AABB, and BB genome groups between the test conditions ($P<0.05$, Supplementary Table 3).

Difference in disease severity of accessions across test conditions

Factorial ANOVA showed highly significant variations for IWDSs among the ITC accessions (A: F = 17.54; $df=89$; $P<0.001$), genome (G) groups (F = 111.67; $df=9$; $P<0.001$), races (R: F = 19.70; $df=1$; $P<0.001$), test conditions (T: F = 312.50; $df=1$; $P<0.001$), and their interactions (G×T: F = 37.23; $df=9$; $P<0.001$; T×R: F = 63.24; $df=1$; $P<0.001$; and G×T×R: F = 35.22; $df=9$; $P<0.001$). ITC accessions were classified based on their performance against *Foc* R1 and TR4 in field and glasshouse conditions, using the Plant Disease Index (PDI). It was observed that a great number of accessions were classified as MR-R and S-HS (Table 1) when tested in the glasshouse (55.6% and 46.5%) compared to the field for *Foc* R1 (13.1% and 17.2%). This contrasts with the MR-R category observed for *Foc* TR4, where there were more accessions in the field (41.1%) compared with the glasshouse (80.8%). The number of accessions classified as I and HR varied, ranging from 1.01% (1; glasshouse) to 32.3% (32; field) for *Foc* R1, and from 27.3% (27; field) to 31.3% (31; glasshouse) for *Foc* TR4. Out of the six disease reaction types, most accessions (41.4–80.8%) were classified as MR to R against *Foc* TR4, and 30.3–55.6% were classified as MR to R against *Foc* R1, with the remaining accessions classified as S and HS. Notably, no accessions were classified as HR for both *Foc* R1 and TR4 in the Glasshouse. Overall, resistance was more frequent against *Foc* R1 than *Foc* TR4, especially in glasshouse conditions (46 resistant or moderately resistant accessions for *Foc* R1 vs. 33 for *Foc* TR4). Among the accessions tested, Robusta-2 (ITC-3, AAA) was found susceptible to both the trial conditions and races tested, while Kunnan (ITC-1034; AB) was found to be highly susceptible to both races, except *Foc* TR4 under glasshouse. Following this, Ney Poovan (ITC-459, AB), Pisang Awak (ITC-213; ABB), and Kluai Namwa Khom (ITC-526; ABB) were found to be susceptible to highly susceptible accessions. The ITC accessions, Plantain No.–2 (513; AAB) and KluaiLep Mu Nang (533; AA) were found to be I and R accessions, respectively, in both the trial setup and races tested.

Correlation between field and glasshouse performance

Spearman's rank correlation was applied to the pooled data to determine the association between glasshouse and field experiments. The Spearman rank correlation coefficient based on DI between field and glasshouse for *Foc* R1 ($r=0.42$, $n=99$, $P<0.001$) and *Foc* TR4 ($r=0.23$, $n=99$, $P<0.001$) was highly significant (Fig. 3). The IWDS showed a similar association between field and glasshouse for *Foc* R1 ($r=0.42$, $n=99$, $P<0.001$) and *Foc* TR4 ($r=0.23$, $n=99$, $P<0.001$). The correlation between test conditions for both races within the genome groups was also established and furnished in Supplementary Fig. 4 where there was a significant correlation between the DI of field and glasshouse for the AAB genome for *Foc* R1, while, a significant correlation was observed in the multiple genome groups in *Foc* TR4 such as AA, AAA, and AAAB.

Genome group-specific resistance patterns

A total of 34 diploid ITC accessions were included in the study, with the maximum number of accessions represented by AA (29) followed by AB (3) and BB (2). Twenty-nine AA banana accessions exhibited significant variation in IWDS (F = 8.648, $df=28$, $P<0.001$) in both field (t = −6.122, $P<0.001$) and glasshouse (t = 7.889, $P<0.001$) conditions when challenged with *Foc* R1 (t = −19.574, $P<0.001$) and *Foc* TR4 (t = −5.847, $P<0.001$). AA (29 accessions) displayed significant variation in resistance under field conditions against *Foc* R1, with a high

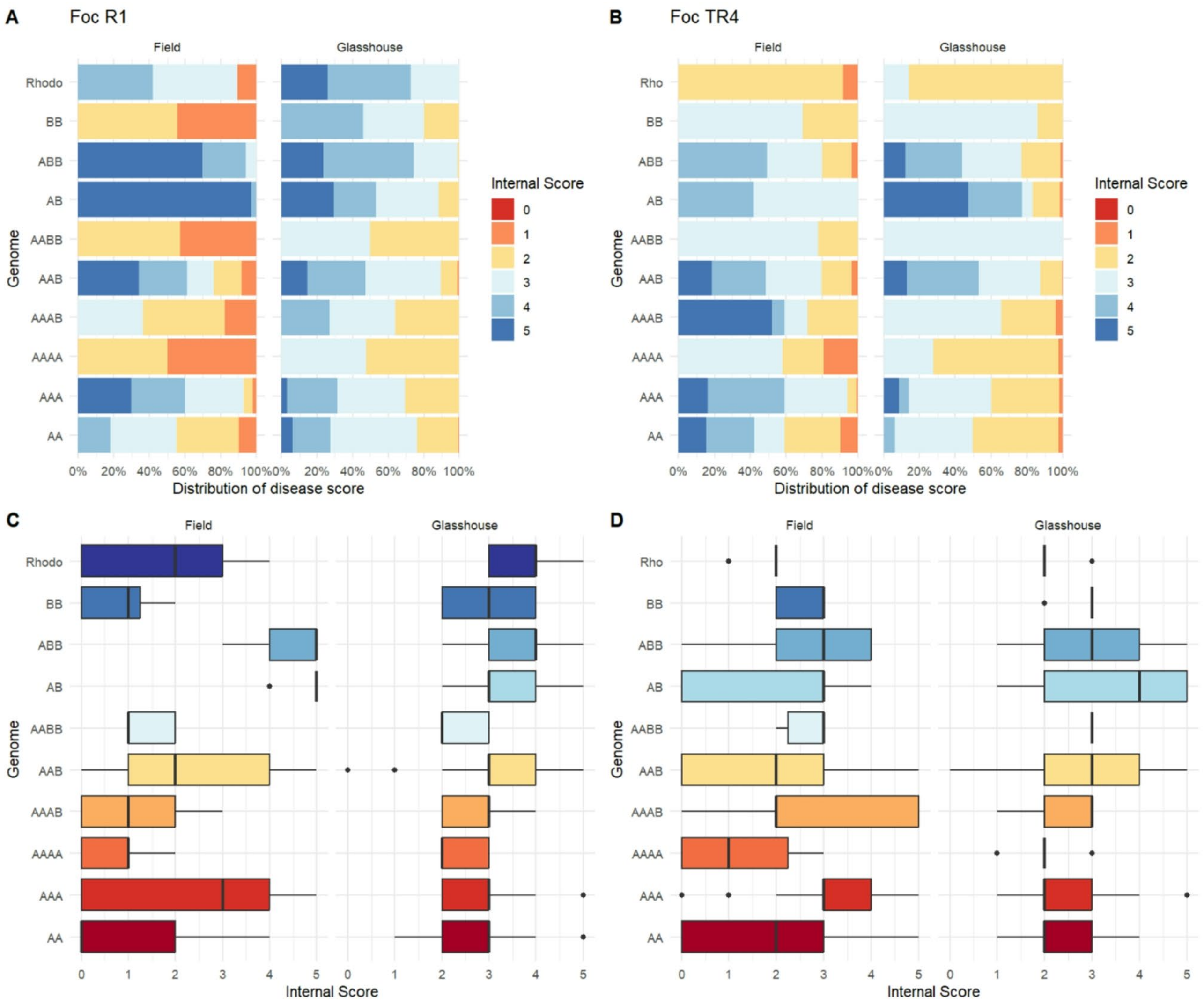

Fig. 1. Distribution (**A&B**) and range (**C&D**) of the internal disease wilting scores under field and glasshouse conditions for *Foc* R1 (**A** & **C**) and *Foc* TR4 (**B** & **D**).(The vertical thick line in the boxes of C & D indicates internal disease wilting score, the lower boundary of the box indicates the standard deviation while the upper boundary of the box indicates 95% percentiles. The lower and upper whiskers representing the minimum and maximum observed value. Filled circles at both below and above the whiskers indicate outliers.).

proportion of HR (12 accessions; 41.38%) and MR (7 accessions; 24.14%) accessions. However, in glasshouse conditions, the majority were S (18 accessions; 62.07%) and HS (8 accessions; 27.59%). AA showed a similar resistance pattern to *Foc* TR4, with a notable proportion being R (6 accessions; 20.69%) and MR (9 accessions; 31.03%) in the field. However, glasshouse conditions revealed higher S (17 accessions; 58.62%) and HS (8 accessions; 27.59%) accessions. Twelve accessions, that are cv. Rose, Gu Nin Chio, Long Tavoy, M.ac.11 – 9/02, Pa (Rayong), Pahang, Pisang Berlin, Pisang Cici Alas, Pu-te-La-Bum, Selangor, TjauLagada and *M. acuminate* ssp. *zebrina*, showed no disease symptoms in the field which was infested with *Foc* R1, but had a DI of 2.1 to 3.7 in the glasshouse. Similarly, five accessions, namely Bie Yeng, M.ac.11 – 9/02, Pisang Jari Buaya, Pisang Tongat, and Pitu showed no disease symptoms in the glasshouse when inoculated with *Foc* TR4 inoculum, but had a DI of 2 to 3 in the field. Based on t-tests conducted across various races and trial conditions, it was observed that four accessions, namely M.ac.11 – 9/02, *M. acuminata* ssp. zebrina, Pisang Mulik, and Bie Yeng, have displayed the lowest disease score. These accessions have consistently performed well against both *Foc* R1 and TR4 under field conditions. As a result, they can be the candidate accessions among AA genome groups for further improvement.

The diploid AB genome group, represented by three accessions, showed significant differences among the accessions ($F = 41.63$, $df = 2$, $P < 0.001$), race ($F = 35.217$, $df = 1$, $P < 0.001$), test conditions ($F = 0.370$, $df = 1$, $P = ns$) and its interaction (R × T: $F = 42.811$, $df = 1$, $P < 0.001$) according to analysis of variance. Comparison of accessions against *Foc* R1 ($t = 8.147$, $P < 0.001$) and TR4 ($t = -3.302$, $P < 0.001$) in field ($t = 8.995$, $P < 0.001$) and glasshouse conditions ($t = -0.422$, $P < 0.001$) were highly significant. Overall, the accession performance is HS in the field and HS to MR in glasshouse conditions. Notably, the ITC accession, Safet Velchi, exhibits an MR

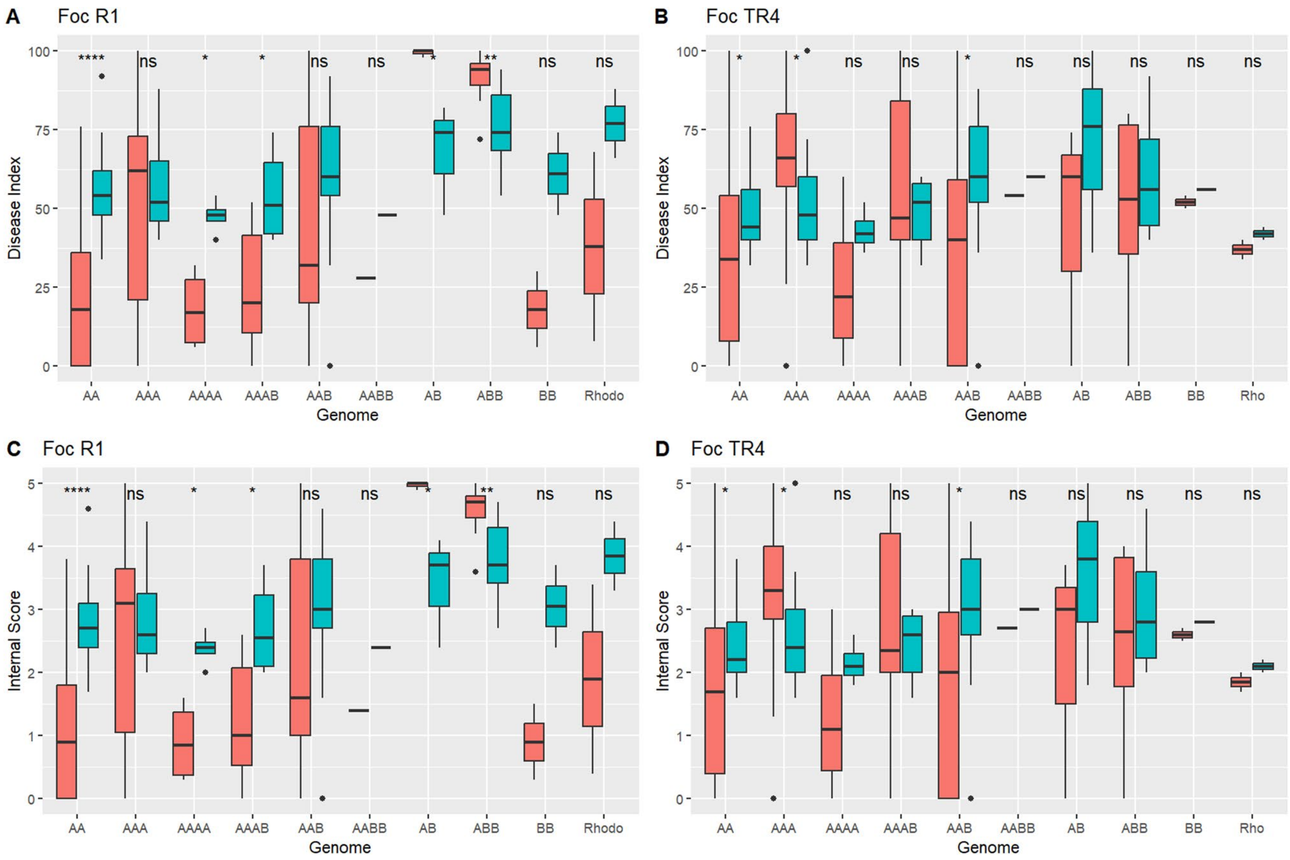

Fig. 2. Mean comparison of disease index (**A** & **B**) and internal disease wilting score (**C** & **D**) within the genome between the experimental conditions. (The horizontal line in the boxes indicates mean disease index or internal disease wilting score, the lower boundary of the box indicates the standard deviation (STD) while the upper boundary of the box indicates 95% percentiles. The lower and upper whiskers representing the minimum and maximum observed value. Filled circles at both below and above the whiskers indicate outliers. Levels of significance was denoted in the top of each genome group is data subjected to ANOVA, where * is significant at the 0.05 level; ** is significant at the 0.01 level; *** is significant at the 0.001 level; and ns: not significant at the 0.05 and 0.01 levels.).

reaction in the glasshouse against *Foc* R1, but displayed I and R reactions under field and glasshouse conditions against *Foc* TR4.

The diploid BB genome group comprised two accessions, Singapuri and Tani, which exhibited resistance to highly resistant (HR) or moderately resistant (MR) reactions under field and glasshouse conditions against *Foc* R1, respectively. However, both accessions demonstrated MR reactions under both field and glasshouse conditions against *Foc* TR4.

Of the 99 accessions, 52 accessions represent the triploid genome group. Among these, the AAA genotype accounted for the highest number of accessions, with a total of 23, followed by AAB with 15 accessions and ABB with 14 accessions. A total of 23 Cavendish triploid accessions in the AAA genome groups that exhibit a significant variation (F = 14.093, *df* = 22, $P < 0.001$) between the races (F = 6.132, *df* = 1, $P < 0.001$) and trial conditions (F = 1.874, *df* = 1, $P < 0.001$). The comparison of DI between races, *Foc* R1 (t = −2.663, $P < 0.001$) and *Foc* TR4 (t = 5.326, $P < 0.001$) and trial conditions, Field (t = −4.257, $P < 0.001$) and Glasshouse (t = 3.520, $P < 0.001$) showed considerable variations. The AAA genome group registered a median disease score of three for both field and glasshouse conditions of *Foc* R1 (Fig. 1), while for *Foc* TR4, it is two and three, respectively, in both field and glasshouse conditions. A total of four ITC accessions, viz., Guineo, Red Dacca, Williams, and Biu Ketip, scored zero in the field conditions against *Foc* R1, while they registered 2 to 4 IWDS under Glasshouse conditions. In contrast, two ITC accessions, Pisang Berangan, and GCTCV-215, registered zero scores in Glasshouse while they had 1.6 to 2 scores in the field. Of the tested ITC accessions, Dwarf Cavendish and GCTCV-119 registered HR against *Foc* R1 under field conditions, while they are MR with *Foc* TR4 in field performance.

A total of 15 ITC accessions in the AAB genome group, showed a significant difference among them (F = 26.505, *df* = 14, $P < 0.001$), and with race (F = 5.1221, *df* = 1, $P < 0.001$) and trial conditions (F = 1.8.679, *df* = 1, $P < 0.001$). The differences within accessions of the *Foc* R1 (t = −4.351, $P < 0.001$) and TR4 (t = −7.366, $P < 0.001$) are highly significant as same as the field (t = 2.350, $P < 0.001$) and glasshouse conditions (t = −0.238.187, $P < 0.05$). Of the tested accessions, a maximum of 26.7% are R accessions followed by 20.0% of HS, and all other reaction types are S, MR, I, and HR, which registered 13.3% each against *Foc* R1 in field conditions. However, the reaction

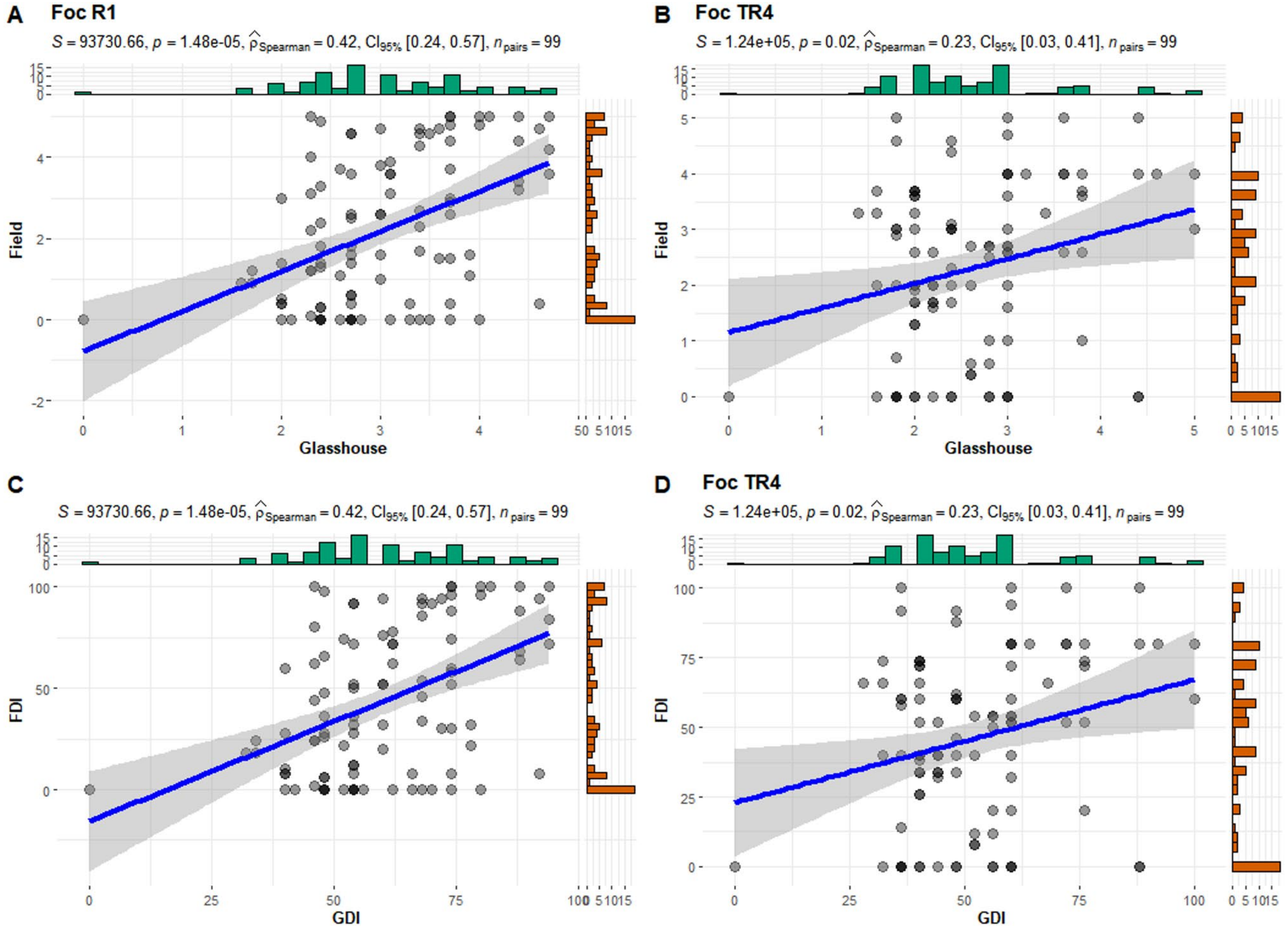

Fig. 3. Pearson correlation analysis based on the disease index (**A** & **B**) and internal vascular discolouration score (**C** & **D**) for *Foc* R1 (**A** & **C**) and *Foc* TR4 (**B** & **D**) of the ITC (International transit centre) banana accessions between glasshouse and field conditions.

types range between S and R against *Foc* R1 under glasshouse conditions, where MR is a maximum of 40.0%, followed by 33.3% of S, 13.3% of HS, and I and R of 6.7% each. In contrast, a maximum number of accessions 33.3 to 40.0% registered MR to I reactions to *Foc* TR4, respectively, under field and glasshouse conditions. A total of three AAB triploid accessions registered HR reactions, in which Akpakpak and ObubitNtnga green mutant against *Foc* R1, and Pisang Ceylan against *Foc* TR4 both under field conditions. Only the Popoulu accession was found to be resistant against both *Foc* R1 and TR4, despite seven accessions in total showing an R response which are Akpakpak, Foconah, Maiden Plantain, Rajapuri India, Pisang Nangka, and Pisang Keling.

The ABB genome group consists of 14 accessions exhibiting significant variations among the accessions ($F = 8.318$, $df = 13$, $P < 0.001$) in terms of race ($F = 323.856$, $df = 1$, $P < 0.001$) and experimental conditions ($F = 2.271$, $df = 1$, $P < 0.001$). When comparing the accessions to *Foc* R1 ($t = 8.741$, $P < 0.001$) and TR4 ($t = -2.669$, $P < 0.001$) under field ($t = 15.902$, $P < 0.001$) and glasshouse conditions ($t = 8.009$, $P < 0.001$), a higher level of significance was observed. Under field conditions, 12 out of 14 accessions exhibited HS to *Foc* R1, while four to eight accessions were S to HS and two were MR under glasshouse conditions. In contrast, the glasshouse environment showed a higher prevalence of intermediate (I) and moderately resistant (MR) accessions. Similarly, field conditions comprised more susceptible accessions (HS and S) for *Foc* TR4. However, the glasshouse environment displayed a more diverse response, with more accessions falling into the R and MR categories. Three triploid accessions such as Blue Torres Strait Island, Dole and Cachaco were able to elicit a reaction against *Foc* TR4. Among the R accessions, Blue Torres Strait Island exhibited a comparable response in the glasshouse environment. Nonetheless, all other accessions demonstrated varying reactions.

A total of 11 tetraploid ITC accessions were included in the study, which were classified into distinct genome subgroups that comprising four AAAA, six AAAB, and one AABB. Both *Foc* R1 and *Foc* TR4 induced varying levels of disease severity in the evaluated tetraploid banana accessions. The field IWDS values for the AAAA genome group range from 0 to 2, whereas for the glasshouse, the values are 2 and 3 against *Foc* R1. In the case of *Foc* TR4, the IWDS values ranged from 1 to 3 in the field and from 0 to 3 in the glasshouse. For both *Foc* races, a higher proportion of accessions exhibited resistance (R, MR) under glasshouse conditions compared to field conditions. This genome group shows significant variation between the accessions ($F = 7.214$, $df = 4$, $P < 0.001$), screening method ($F = 4.747$, $df = 1$, $P < 0.001$) and test organism ($F = 1.458$, $df = 1$, $P < 0.001$). Comparison of

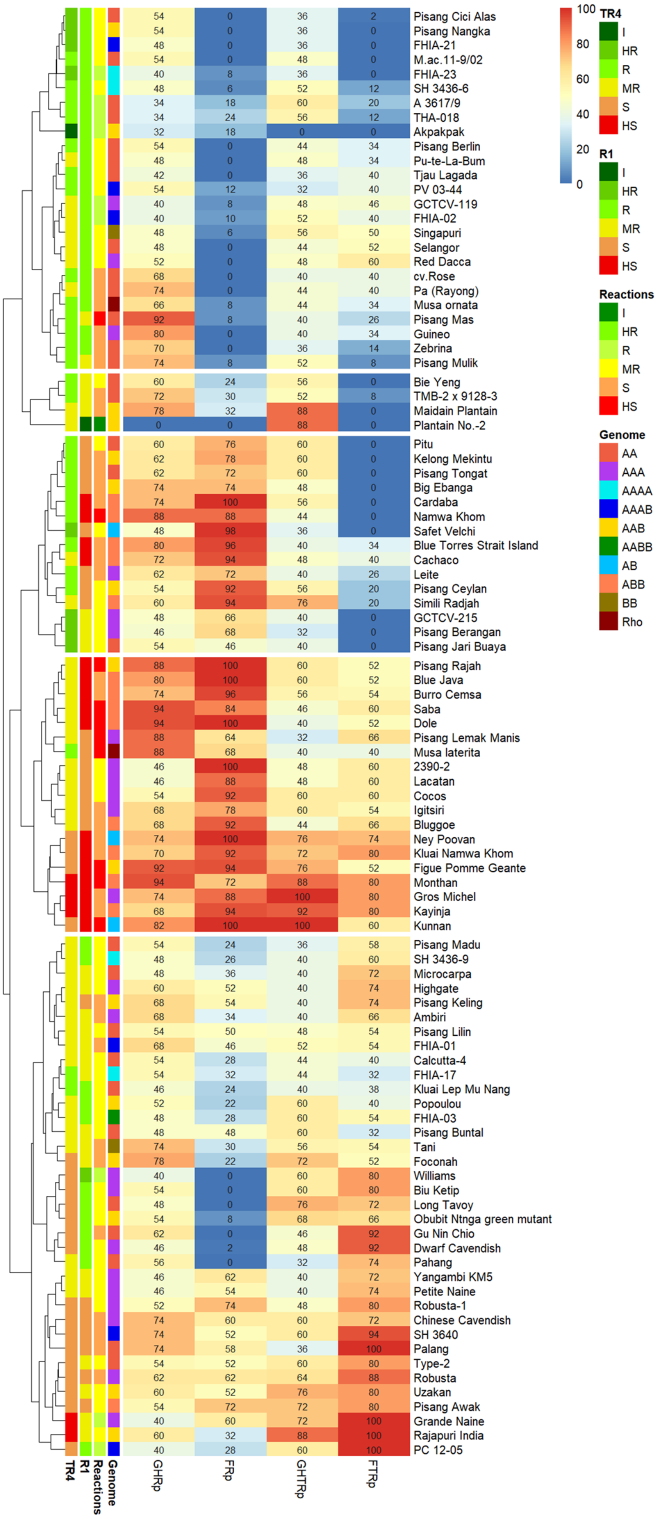

Fig. 4. Hierarchical clustering heatmap analysis of the disease index of banana measured under glasshouse (GDI) and field (FDI) conditions against *Foc* R1 and *Foc* TR4. (GHRp: Glasshouse *Foc* R1; FRp: Field *Foc* R1; GHTRp: Glasshouse *Foc* TR4; FTRp; Field *Foc* TR4) A.

IWDS between races, *Foc* R1 (t = −10.467, $P < 0.001$) and *Foc* TR4 (t = 4.148, $P < 0.001$) and trial conditions, Field (t = −1.79, P = ns) and Glasshouse (t = 2.068, P = ns) also showed significant variations. The tetraploid FHIA-23 displayed the most minimal score in both the field and glasshouse environments when compared to the other members in the genome group, with *Foc* R1 and TR4. Among the four accessions in the AAAA genome group, FHIA-23 and SH 3436-6 exhibited HR in the field, whereas SH 3436-9 showcased HS in the Glasshouse.

Regarding genomic group AAAB, which consisted of FHIA-01, FHIA-02, FHIA-21, PC 12 – 05, PV 03–44, and SH 3640, there was a highly significant variation observed among the different accessions ($F = 32.276$, $df = 5$, $P < 0.001$), tested race ($F = 27.255$, $df = 1$, $P < 0.001$), and test conditions ($F = 24.388$, $df = 1$, $P < 0.001$). There was a significant difference among the races, *Foc* R1 ($t = -8.502$, $P < 0.001$) and *Foc* TR4 ($t = 1.242$, P = ns) and trial conditions, Field ($t = -5.639$, $P < 0.001$) and Glasshouse ($t = 2.025$, P = ns) based on the DI of the accessions. Of the tested accessions, FHIA-02 and PV 03–44 against *Foc* R1 in the field and SH 3640 against *Foc* TR4 in the Glasshouse conditions registered HR, while FHIA-21 registered I in both *Foc* R1 and TR4 in the field and the Glasshouse conditions, respectively. A single AABB tetraploid accession, FHIA-03, in the study showed MR to R against *Foc* R1, and MR against *Foc* TR4.

In the case of the Rhodochlamys genome group, there were two species, *Musa laterita* and *Musa ornata*, which displayed MR to R reactions under field conditions against *Foc* TR4. In contrast, *M. ornata* exhibited HR reactions to *Foc* R1 under field conditions, however, it exhibited susceptible (S) reactions under glasshouse conditions.

Multivariate classification of accessions

As there was a prominent shift in the IWDS and DI within ITC accessions, prompting the utilization of multivariate techniques such as hierarchical clustering heat-maps (HCHs) and Principal coordinate analysis (PCoA). These methods were employed to classify and visually identify the superior accessions based on the disease reaction categories, which include Immune-I, Highly resistant-HR, Resistant-R, Moderately resistant-MR, Susceptible-S, and Highly susceptible-HS. These disease reaction categories were derived from glasshouse (GDI) and field (FDI) observations of both tested races.

Hierarchical clustering heatmap (HCH)

Based on the HCH (Fig. 4), the evaluated ITC banana accessions were grouped into two major clades. Major clade 1 (C1, Fig. 4) included 52 accessions, while major clade 2 (C2, Fig. 4) contained 47 accessions. Major clade 1 was further divided into three subclades: subclade 1 comprised 13 accessions, subclade 2 contained 21 accessions, and subclade 3 included 18 accessions. Major clade 2 was divided into two subclades: subclade 4, with 20 accessions, and subclade 5, with 27 accessions. Subclade 1 consisted of 13 accessions that exhibited I to R reactions under field conditions against *Foc* TR4, including two diploids (AA & AB) and two triploids (AAA & AAB). The ABB genome group accounted for the majority of the triploid accessions in this clade. Subclade 2 included 21 accessions that displayed MR to S reactions against both *Foc* races. This subclade comprised 18 accessions dominated by three triploid genome groups of AAA (8), AAB (2), and ABB (8), with the remaining three accessions belonging to the AB (2) and Rhodochlamys (1) genome groups. Subclade 3 included 18 accessions that showed R and S reactions, mainly represented by three triploids of AAA (6), AAB (3), and ABB (1), two tetraploids of AAAA (1), and AAAB (3), and one diploid AA (4) genome groups. Specifically, these accessions were susceptible (S) to highly susceptible (HS) to *Foc* TR4 under field conditions but exhibited resistant (R) to moderately resistant (MR) responses to *Foc* R1. Subclade 4 included 20 accessions that exhibited complete I to R reactions to both *Foc* R1 and TR4 under field conditions. This subclade was majorly represented by 11 diploid AA accessions, five triploid accessions of AAA (1) and AAB (4), and four tetraploid accessions of AAAA (3) and AAAB (1) genome group. Subclade 5 comprised 27 accessions that exhibited I to R reactions to *Foc* R1 under field conditions. These accessions also demonstrated a certain degree of resistance to *Foc* TR4, particularly accessions in the minor subclades 1 and 2, which were R and MR, respectively. This clade included 14 diploid accessions (12 AA and 2 BB), nine triploid accessions (six AAA and three AAB), three tetraploid accessions (two AAAB and one AABB), and one Rhodochlamys accession.

Principal coordinate analysis (PCoA)

A principal coordinate analysis was conducted to visually categorize the disease reactions of the 99 ITC banana accessions. The data for each accession were centred at zero and then normalized to achieve unitary variance. The eigenvalues for each *Foc* race were analyzed separately to determine the variation explained by the principal coordinates, where FDI was represented by PC1, and GDI was represented by PC2. The PCoA for *Foc* R1 revealed that PC1 accounted for 72.07% of the total variation, while PC2 explained 27.93% of the variation (Fig. 5). As shown in Fig. 5a, distinct clusters separate the banana accessions into I, HR, R, MR, S, and HS categories based on their positions along PC1 and PC2. Accessions categorized as I, HR, MR, and R are located on the right side of the graph, with PC1 loadings ranging from − 0.05 to 0.28 and PC2 loadings from − 0.18 to 0.23. In contrast, accessions classified as S and HS are positioned in the centre to the right side of the graph, with PC1 loadings from 0.0 to −0.31 and PC2 loadings from − 0.21 to 0.22. Green points, labeled with “I,” represent accessions with an immune reaction (2 accessions), while red points, labeled with “HR,” indicate accessions with a highly resistant reaction (1 accession). Blue points, labeled with “R,” correspond to resistant accessions (25 accessions), copper-blue points, labeled with “MR,” represent moderately resistant accessions (20 accessions), pink-blue points, labeled with “S,” denote susceptible accessions (20 accessions), and brown points represent accessions with a highly susceptible reaction (15 accessions).

The PCoA for *Foc* TR4 showed that PC1 accounted for 62.49% of the variation, while PC2 explained 35.51% of the variation. Figure 5b illustrates distinct clusters that categorise banana accessions into I, HR, R, MR, S, and HS groups based on their positions along PC1 and PC2. Accessions classified as I, HR, and R are located on the left side of the graph, with PC1 loadings ranging from − 0.02 to −0.28 and PC2 loadings from − 0.18 to 0.18. Accessions showing an MR reaction are positioned toward the centre to the right side of the graph, with PC1 loadings ranging from 0.05 to −0.05 and PC2 loadings from − 0.18 to 0.28. The green points represent accessions with an I (1), the red points indicate accessions with HR (5), and the blue points correspond to R (18). Copper-blue points signify MR (27), pink points denote S (17), and brown points indicate HS (5) accessions. Overall,

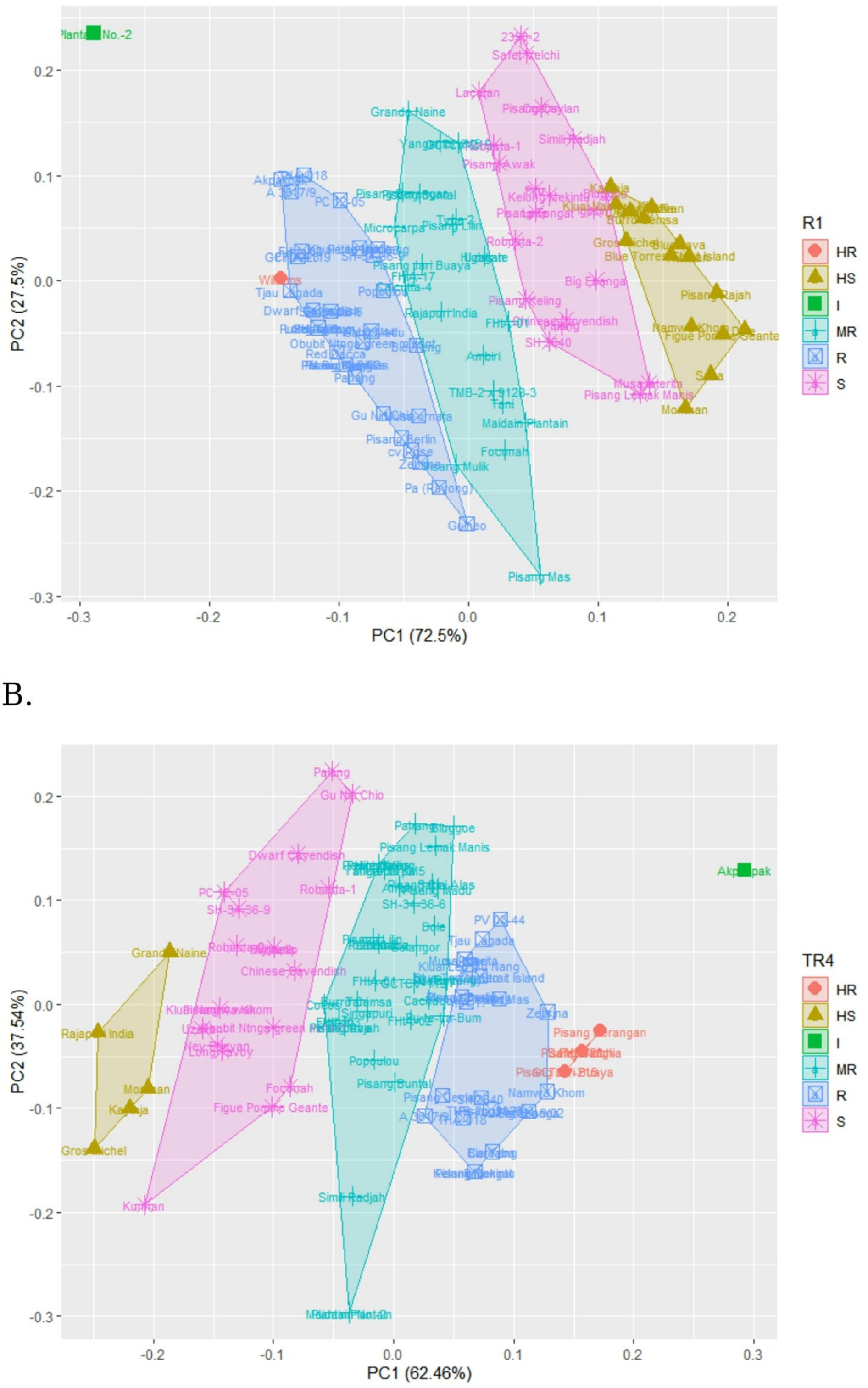

Fig. 5. Principal coordinate analysis of the 99 ITC (International transit centre) banana accessions based on the internal vascular discolouration score and disease index for *Foc* R1 (**A**) and *Foc* TR4 (**B**).

the PCoA analysis further validated the findings observed in the dendrogram, providing additional evidence for categorising the disease reactions of the tested banana genotypes.

Discussion

Screening of Fusarium wilt resistant germplasms may provide a strong potential for an efficient application of resistant plant materials and/or genes to improve resistance of cultivars[26]. Similarly, some of the exotic cultivars if found resistant, could be directly exploited for commercial cultivation under Indian conditions. In this direction, an attempt was made to screen 99 exotic accessions imported from ITC, Belgium against *Foc* R1 and TR4 for the aforesaid activities. In the present study under hotspot conditions, out of 99 accessions evaluated, 19 were found to be immune, 13 were highly resistant, and 17 were resistant to *Foc* R1. For *Foc* TR4, 18 accessions were immune, 9 were highly resistant, and 19 were resistant. However, under glasshouse conditions, only one accession was immune and nine were resistant to *Foc* R1, while one was immune and 30 were resistant to *Foc* TR4. Furthermore, it was observed that the number of common accessions falling under the broad category of resistance (including immune, highly resistant, and resistant types) under both glasshouse and field conditions was 19 for *Foc* TR4 and 9 for *Foc* R1 (Supplementary Tables 4 and 5). Similarly, the number of common accessions categorized as resistant under hotspot conditions for both *Foc* TR4 and *Foc* R1 was 29 (Supplementary Table 6), while under glasshouse conditions, only 2 accessions showed resistance to both the races (Supplementary Table 7). Among all the 99 accessions, only two accessions viz. FHIA-23 (1265-AAAA) and Akpakpak (0217-AAB plantain) were found falling under resistant category against both *Foc* R1 and *Foc* TR4 races in both glass house and field conditions.

Interestingly out of 99 accessions evaluated under field conditions, 19 and 18 ITC accessions exhibited immune reaction to *Foc* R1 and TR4 respectively. Of which, one wild type (*M.ac.*11 – 9/02), and three plantain types (Pisang Nangka, Plantain No.2 and FHIA – 21) showed immune reaction to both the *Foc* races (Supplementary Table 6). Among four accessions, *M.ac.*11 – 9/02 with green yellow male flower bud is highly polleniferous which makes it a suitable male parent for resistant gene introgression into cultivated varieties. Owing to its female fertility, it could also be used as female parent in gene pyramiding programmes towards the development of synthetic hybrids with multiple resistances. Among the three plantain types, except Pisang Nangka which is a low yielding type with smaller and less sweeter fruits, the remaining two accessions namely FHIA 21 and Plantain No.2 could be promoted for commercial cultivation. FHIA 21 is a promising cultivar with high yield and good quality fruits besides its resistance to TR4[12] and it is similar to Nendran in terms of texture and sweet but heavy yielder (20–25 kg/plant) than Nendran (12–15 kg/plant). Plantain No.2 is another French plantain type with fruit characteristics similar to normal Nendran (grown under Indian conditions). Hence, they can be used as a substitute in TR4 infested areas.

A total of 29 ITC accessions belonging to AA diploids were screened in the hot spot region. Out of these, five accessions, namely Pisang Tongat, *M. acuminata* (11 – 9/02), Bie Yeng, Pitu, and Pisang Jari Buaya were found to be immune, and six accessions namely *M. acuminata* ssp. *zebrina*, TMB2 × 9128-3, Pisang Cici Alas, Pisang Mulik, A3617/9, and THA-018 exhibited a high level of resistance, scoring < 1 under hotspot conditions for *Foc* TR4 (Supplementary Table 8). Specifically, the wild diploids such as *M. acuminata* (11 – 9/02; I), *M. acuminata* ssp. *zebrina*, A3617/9, and Calcutta 4 could be used as potential male and female parents in breeding programs. These could also be used as one of the parents in the development of mapping population that is very much essential to gain insights into the inheritance pattern of *Foc* TR4 resistance. Interbreeding among the wild diploids might also result in synthetic diploids with improved resistance due to gene stacking which could be further utilised in breeding for development of resistance in the high value susceptible cultivars. *M.a.* ssp. *zebrina* which belongs to the Eumusa section besides its ornamental value was found resistant to *Foc* TR4. It's male and female fertile nature has been well proven by its successful use in the development of ornamental hybrids by crossing with the members of the section Rhodochlamys (Anonymous, 2021).

In the present study, cv. Rose exhibited immune and resistant reactions to *Foc* R1 and TR4, respectively, while Pisang lilin displays a MR reaction to both races under field conditions (Supplementary Tables 8 and 9). The results are in confirmation with those of earlier workers[12] which could be the reason that they are universally utilized as male parents in the breeding program to enhance Fusarium wilt resistance. Pisang Jari Buaya possesses multiple resistances to various biotic stresses including *Foc* TR4 as reported by Zuo et al.[12]. However, its limited pollen production prevents it from being utilized as a male parent in resistant gene introgression. Nevertheless, these accessions can be valuable resources for identification of resistance genes or to understand the molecular mechanism underlying *Foc* TR4 resistance, so that they can be used to improve commercial cultivars through genetic engineering or gene editing techniques.

Though Pisang Berlin (R) and Pisang Mulik (HR) are both male and female sterile, they are rich in Provitamin A content and hence they could be directly promoted for commercial cultivation in *Foc* TR4 hotspots of India. KluaiLep Mu Nang displayed resistance under field conditions for both races (Supplementary Tables 8 and 9). Their pollen fertility makes them suitable for use as potential male parents in the breeding programmes.

Among the three AB accessions, Safed Velchi was found immune to *Foc* TR4 (Supplementary Table 8), whereas Ney Poovan, which is very similar to Safed Velchi, was susceptible. In India, these AB Ney Poovan clones are cultivated under various local names such as Ney Poovan, Elakki bale, Ezharasi and Nijalipoovan in a large scale. Similarly, it is known as Ranel in Sri Lanka, Kisubi in Uganda, Apple in the West Indies and Farine in France[27]. Therefore, in Ney Poovan growing areas of India, Sri Lanka, Uganda, and the West Indies, it would be wise to replace the susceptible Ney Poovan with the resistant Safed Velchi, which is a sought-after commercial cultivar. Moreover, Safed Velchi has a crop duration of 13–14 months, an average bunch weight of 12 kg/plant, yields around 150 fruits/plant, and has a TSS of 31° Brix. These characteristics are comparable to those of Ney Poovan, which has a bunch weight of 15–20 kg/plant, a crop duration of 12–14 months, 200–220 fruits/plant, and a TSS of 29–30° Brix. Besides it was observed that within the BB group no germplasm was identified as resistant against both the races (Supplementary Tables 4 to 7) and these data probably indicate that the key resistance genes might not exists in the BB genome.

Out of the 23 AAA accessions, none of them exhibited an immune response to both races, while four accessions, namely Williams, Red Dacca, Guineo, and Biu Ketip, demonstrated immune reaction to *Foc* race 1 under hotspot (Supplementary Table 9). Among these, only Williams displayed a resistant reaction, while Red Dacca and Biu Ketip exhibited a moderate reaction under pot culture. Williams, a high yielding Cavendish type, has the potential to replace Grand Nain in the hot spot area of *Foc* R1 infecting Cavendish. However, like Grand Nain, Williams is also highly vulnerable to *Mycosphaerella fijiensis*, the causative agent of the black leaf Sigatoka[28] and therefore requires appropriate management practices to prevent yield losses due to leaf spot diseases.

Interestingly, Red Dacca exhibited MR reaction to *Foc* TR4 in both field and glasshouse conditions, thereby providing reassurance that this could be cultivated in *Foc* TR4-infested regions as part of the integrated disease management strategies. But Guineo though it showed resistant reaction to *Foc* TR4 both under field and glass house conditions, it could not be promoted for commercial cultivation because of the lesser yield and poor-quality pulp.

It has been observed that GCTCV-215 and Pisang Berangan accessions exhibited immune and resistant reactions against *Foc* TR4 under both field and glasshouse conditions respectively. However, they exhibited susceptible reactions for *Foc* R1 under field conditions. GCTCV-215, which is a tissue culture variant often known as Thai-Chiao No.1, is a Giant Cavendish type exploited commercially[29] due to its resistance to *Foc* TR4 both under pot and field conditions. On the other hand, Pisang Berangan is a highly popular variety in Malaysia with an average bunch weight of 15 kg and excellent fruit quality such as yellow pulp with a delightful aroma and taste. Therefore, these two accessions could be recommended as a substitute for Grand Nain in *Foc* TR4-affected regions of India. Besides India, Pisang Berangan could be introduced and promoted for commercial cultivation in the *Foc* TR4-affected regions across the world.

Out of 15 AAB accessions which included various subgroups namely six Plantain types, three Pome types, two Nendrapadathi, one Mysore type, and three unique types. Obubit Ntanga green mutant (Unique) demonstrated HR reaction for *Foc* R1 under hot spot area while being susceptible to *Foc* TR4. Foconah, a Pome type, showed resistant and moderate reaction (MR) for *Foc* R1 and *Foc* TR4 respectively under field conditions but it showed susceptible reaction under pot culture screening. This is contradicting the results of Zuo et al.[12] who found it to be resistant to *Foc* TR4 both under glass house and field conditions. The variation in the degree of resistance of the same genotypes grown in different locations might be due to age of the plant[30,31] and environmental factors like temperature, nutrient application and utilization efficiency and also variation in the *Foc* strain. The Mysore sub-group accessions showed HR reaction to *Foc* TR4 but showed HS reaction to *Foc* R1 under field conditions. Figue Pomme Geante, belonging to the Silk group, exhibited a highly susceptible (HS) reaction to *Foc* R1 and a moderately resistant (MR) reaction to *Foc* TR4 under field conditions contradicting the findings of Zhan et al. (2022)[32]. In totum, it can be concluded that among the AAB genomic group except Plantains all other subgroups are susceptible to both races.

Out of the seven Plantain types, which consist of six triploids (AAB) and one tetraploid hybrid (AAAB), the variety Akpakpak (AAB) exhibited an immune reaction to *Foc* TR4 and resistant reaction to *Foc*R1 under both field and glasshouse conditions (Supplementary Tables 8 and 9). Similar results were reported by Zuo et al.[12], and Li et al.[33], while Plantain type 2 demonstrated an immune reaction to both races under field conditions, but an immune reaction for *Foc* R1, HS reaction to *Foc* TR4 under glasshouse conditions. Consequently, among the various plantain types, FHIA-21 is highly recommended for cultivation in hotspot areas affected by Fusarium wilt, irrespective of the races, owing to its high yield potential. Besides all, FHIA-21 is capable of withstanding *Foc* R1 and R2 as well as a Black leaf streak (*M. fijiensis*)[34]. Two accessions, namely Big Ebanga and Kelong Mekintu, which exhibit immune reaction to *Foc* TR 4 under field conditions[32] exhibited susceptibility to *Foc* R1. Conversely, Uzakan did not display a resistant response to either races. Interestingly, the unique Obubit Ntanga green mutant, which was found to be susceptible to *Foc* TR4 in this investigation, has been categorised as resistant by Zuo et al.[12], and Li et al.[33], for the same race in pot culture studies. Besides the accession Popoulou (AAB), which has large-sized fingers and is thus suitable for chip making, it only showed field resistance to *Foc* TR4. Unfortunately, it lacked both pollen and female fertility, making it unsuitable for use in breeding programs; however, it can be deployed directly for farmers' use. The degree of resistance to *Foc* TR4 varied even within the same subgroup which might be due to the presence of diverse genes in different accessions of the same subgroup[32]. Further the resistance could be quantitative and regulated by combination of multiple genes[12].

Out of 14 accessions, which fall under the ABB genomic group, four accessions belong to Pisang Awak subgroup, while the remaining accessions are of the cooking type. Specifically, within the Pisang Awak subgroup, Namwa Khom exhibited immune and moderately resistant reactions in field and pot culture against *Foc* TR4 respectively. Additionally, the cooking type known as Cardaba also displayed similar reactions. These aforementioned subgroups showing resistance against *Foc* TR4 could be deployed in states like Bihar, Uttar Pradesh, and West Bengal, Gujarat and Maharashtra where *Foc* TR4 is emerging as the major problem. The Blue Torres Strait Island accession demonstrated resistant reaction against *Foc* TR4 but as the finger size is very small compared to other cooking types and the market acceptability would be very less. However, all the 14 accessions exhibited susceptible reactions against *Foc* R1.

Among 11 tetraploid hybrids, four of them belonged to the Cavendish type (AAAA genomic group). Among these Cavendish hybrids, three hybrids, namely FHIA-17, FHIA-23, and SH3436-6, exhibited resistant reaction in the field for both *Foc* race 1 and TR4. Orjeda et al.[35]. and Rivera and Dueñas[36]have previously reported that FHIA-23, is a well-accepted and highly productive tetraploid hybrid (AAAA) with a cylindrical bunch and weight ranging from 25 to 30 kg possessing partial resistance against Fusarium wilt and black leaf streak disease. Furthermore, Nowakunda et al.[37], have provided evidence that FHIA-17 demonstrated greater productivity compared to the EAHBs. Consequently, it is recommended for polyclonal cultivation alongside various Cavendish types that exhibit varying degrees of resistance to Fusarium. Despite its limited market acceptability for direct consumption, it can be effectively utilized for processing purposes. Moreover, FHIA-17 is gaining popularity in Kenya due to its high yield (25 kg) and resistance to various banana diseases and pests (https://www.aciar.gov.au/sites/default/files/2022-08/HORT2005136-final-report.pdf).

Another hybrid, FHIA-21 is a high yielding plantain type with good quality fruits falls under the plantain-type (AAAB), showed resistance against both races under field and pot culture conditions. In addition to its wilt resistance, FHIA-21 also displayed resistance against black leaf streak, as reported by Ploetz et al.[27],. Consequently, it is recommended for cultivation in areas with a higher infestation of *Mycosphaerella* pathogen.

Among the five synthetic tetraploid hybrids of the Pome subgroup (AAAB), FHIA-02 and PV 03–44, exhibited HR and R against *Foc* R1 and *Foc* TR4 hotspot areas, respectively. Conversely, FHIA-02 was found to be susceptible to both races, as reported by Smith et al.[38],. Though PV 03–44 is resistant to Fusarium wilt it cannot be promoted for commercial cultivation because of its poor-quality fruits with sour taste.

In previous work[12,33]129 banana accessions were screened to identify genotypes resistant to *Foc* TR4 under greenhouse conditions, and 100 of these were also evaluated under field conditions. The results indicated that accessions such as Igitsiri (AAA), Obubit Ntanga green mutant (AAB), Pisang Ceylan (AAB), Pisang Rajah (AAB), Phang (AA), Pisang Lilin (AA), Tani (BB), Foconah (AAB), and Blue Java (ABB) exhibited resistance to *Foc* TR4. However, in our current study, these same genotypes demonstrated a response ranging from moderate resistance to susceptibility against *Foc* TR4. This variation in disease reaction could be attributed to differences in *Foc* TR4 strains[9] and environmental conditions[12].

In conclusion, the present study evaluated 99 exotic banana accessions from diverse genomic groups and identified nine accessions resistant to *Fusarium oxysporum* f. sp. *cubense* (*Foc*) race 1 and 19 accessions resistant to tropical race 4 (TR4) under both glasshouse and field conditions. As *Foc* R1 and TR4 pose a significant threat to banana cultivation in India, these resistant accessions hold strong potential for direct deployment in affected regions or incorporation into conventional breeding programs to enhance resistance in commercial varieties.

Specifically, AA diploids such as M.ac.11 – 9/02, *M. ac.* ssp. *zebrina*, A3617/9, and Calcutta 4 are promising genotypes which can be used in breeding programmes. Among plantain types, FHIA-21 (AAAB), a high-yielding cultivar, and Plantain No. 2 (AAB), a French plantain which are akin to Nendran, exhibited field resistance to *Foc* TR4 and that could be used directly for commercial cultivation. Similarly, Pisang Berlin (AA) and Pisang Mulik (AA) which are rich in provitamin A but male and female sterile also could be directly promoted for cultivation in Foc TR4 affected regions of India.

Safed Velchi (AB), an ecotype of Ney Poovan, offers a potential substitute for the *Foc*-susceptible Ney Poovan in Fusarium wilt affected areas. Furthermore, resistant AAA Cavendish types such as GCTCV-215 and Pisang Berangan could serve as alternatives to the Grand Nain cultivar in *Foc* TR4-affected regions globally. Finally, the AA genotype cultivar cv. Rose, which is immune to *Foc* R1 and resistant to TR4, represents a valuable genetic resource for resistance gene discovery and could support genetic engineering efforts aimed at improving disease resistance in susceptible banana cultivars.

Methods

Plant material

A total of 99 exotic accessions, randomly selected from different genome groups in the Musa Germplasm Information System (MGIS) database, were received as tissue culture plantlets from the International Transit Centre (ITC), Belgium, through the ICAR- National Bureau of Plant Genetic Resources (NBPGR), New Delhi. These accessions were chosen based on yield, resistance to biotic stresses and fruit quality (MUSA database; www.alliancebioversityciat.org). These banana accessions pertain to distinct genomic groups (classified based on morphological traits) such as AA (29), AB (3), BB (2), AAA (23), AAB (15), ABB (14), AAAA (4), AAAB (6), AABB (1) and Rhodochlamys (2) and the details are shown in Table 1. The plantlets were then sub-cultured and multiplied at the tissue culture facility of the Crop Improvement Division, ICAR-National Research Centre for Banana (NRCB), Tiruchirappalli, Tamil Nadu, India, and then maintained in the field gene bank at ICAR-NRCB farm. The suckers extracted from the mother plants of ITC accessions were employed for both glasshouse and field screening against Fusarium wilt.

Pathogen isolation

The Fusarium wilt pathogens, both *Foc* R1 (VCG 0125) and *Foc* TR4 (VCG 01213/16), have been isolated from dried vascular strands of wilt-infected Cavendish banana (cv. Grand Nain-AAA), using 25% strength potato dextrose agar medium[5]. The single spore culture obtained was maintained on carnation leaf agar medium[16] for long-term use, the culture was stored as dried filter paper cultures at 4 °C[17]. The pathogenicity/virulence of the *Foc* races was assessed in a controlled environment using the pot culture method employing Grand Nain tissue-cultured plants which are susceptible to both *Foc* R1 and TR4. The pathogen was mass-cultured in a 19:1 sand maize medium for 10 days and subsequently employed to infect the plants. A necessary negative control (without pathogen inoculation) was also maintained to confirm that the wilt symptoms were caused by pathogen inoculation.

Glasshouse evaluation

Disease-free suckers, weighing approximately 1.5 to 2.0 kg and extracted from healthy mother plants raised in a confined disease-free field were collected for all ITC banana accessions. These suckers were then subjected to paring and pralinage treatments (dipping the pared suckers in Triazophos-40% EC at 0.5% for 30 min) to protect them from nematodes and insect pests after planting. These treated suckers were planted individually in mud pots (30 × 30 × 15 cm) filled with a sterilized potting mixture containing red earth, sand, and farmyard manure in equal proportions. Suckers grew for 30 days with regular watering to establish them in to plants. Thereafter, about 30 g of sand maize *Foc* inoculums (*Foc* R1 VCG 0125 and *Foc* TR4 VCG 01213/16) containing approximately 10^6 CFU g^{-1}(quantified by serial dilution plating) was applied to the soil around the plants in each pot. The experiment was conducted in a completely randomised design and with ten plants per accession. Five months after *Foc* inoculation, the plants were pulled out, and internal wilt disease score (IWDS) was carried out based on the percentage of discoloured area in the rhizome on a 0–5 scale, where 0 = corm completely clean, no vascular discolouration; 1 = 1–5%, 2 = 6–25%, 3 = 26–50%, 4 = 51–75%, and 5 = over 75% of discoloured rhizome[11,12]. Based on these observations, the wilt index was calculated as follows: Disease Index (DI) = (average of disease score of each plant/the highest disease index value) × 100. The disease index was calculated, and the average value was presented. Based on the disease index, the germplasm lines were classified into six categories as described by Zuo et al. 2018[7,12] with minor modifications. The reactions were immune (I) (PDI: 0), highly resistant (HR) (PDI: >0–20), resistant (R) (PDI: >20–40), moderately resistant (MR) (PDI: >40–60), susceptible (S) (PDI: >60–80), and highly susceptible (HS) (PDI: >80–100).

Field evaluation

All the ITC accessions were evaluated during 2021 and 2022 at Muthalapuram village of Theni district of Tamil Nadu for *Foc* R1 and Falka village of Katihar district of Bihar for *Foc* TR4, which are considered as hot spot areas for Fusarium wilt. These selected sites were once commercial plantations under banana cultivation and then abandoned due to severe wilt incidence (> 90%) in the Grand Nain cultivar. Further the pathogen presence in soil was confirmed by qRT-PCR method and there was up to 1100 ng of *Foc* DNA per 2.5 g of soil. The soil is red loamy at the *Foc* R1 site at Muthalapuram with a pH of 6.2. The average annual rainfall was 1,000 mm with an ambient temperature of 20.3–38.6 °C and an RH of 37–80%. In the case of the site for *Foc* TR4, the soil is alluvial with a pH of 7.1. The average annual rainfall was 1,281 mm with an ambient temperature of 8–42 °C and an RH of 45–83%. The banana suckers were planted at a spacing of 2×2 m. The experiments were conducted in a completely randomised block design, with each genotype represented by ten replications per cycle and each plant was considered as one replication. Timely application of fertilizers, water (through drip irrigation), and other cultural operations was followed according to standard production practice[18]. The observation on the IWDS was taken based on the percentage of discoloured areas in the rhizome at the time of harvest and the germplasm lines were categorized based on disease index as mentioned in the glasshouse evaluation. Summary of whole work flow in the current study has been given in Fig. 6.

Statistical analysis

All analyses and graph/plot generation took place in R, version 4.3.1[19] using "dplyr"[20], and "ggplot2"[21] packages. To describe the difference in the IWDS of the banana accessions against *Foc* R1 and *Foc* TR4, the distribution of the IWDS (0–5 scale) under both glasshouse and field experiments was analysed separately. To confirm the assumption of normality and variance in the disease scale within a genotype, the Shapiro-Wilk test was used with untransformed data using the R function "shapiro.test()". The homogeneity of the variance across experiments and genomic groups was assessed using Bartlett's test, as implemented in the R function "var.test()". The mean IWDS of accession was assumed if an accession has a significantly ($P < 0.05$) higher frequency of occurrence as analyzed by the χ^2 (Chi^2) test using the "chisq.test()" R function. In the case of field experiments, the data from the first and ratoon crops were pooled and analyzed in every instance. Further, the difference between the accessions within the genome group was elucidated by comparing the mean IWDS and DI of the genotypes by one-way analysis of variance (ANOVA) using the R function "aov()" where replications were included as a random variable to account for the non-independence of successive counts from the same genotype, followed by DMRT using the "agricolae" package[22] with the "duncan.test" function. The difference in the mean IWDS of banana accessions versus experimental conditions (i.e., glasshouse and field) was tested with a two-sample paired Tukey's HSD test using the R function "TukeyHSD()", where $P > 0.05$ is considered as significant, which indicates a shift in the resistant reaction. The concurrence between the glasshouse and field in terms of IWDS and DI was established separately by Spearman's rank correlation coefficient (r) using the "ggstatsplot"[23] package with the "grouped gghistostats" function since the data are non-normally distributed according to the Shapiro-Wilk test for normality. The multivariate techniques, hierarchical clustering heatmap (HCH) and principal coordinate analysis (PCoA) are used to classify and visually select the genotypes based on the color scale of the disease reaction category in a three-dimensional space (x, y, and z axes), where DI measured under both the glasshouse (GDI) and field (FDI) are centered and scaled before performing the PCoA. A HCH was drawn using the complete linkage method in the "heatmap.2" function with a Minkowski distance metric that includes Euclidean distance ($p = 2$) of the "gplots"[24] package. PCoA was drawn using the "ape::pcoa()" followed by the "autoplot" function of the "ggfortify"[25] packages, based on the distance matrix of the genotypes.

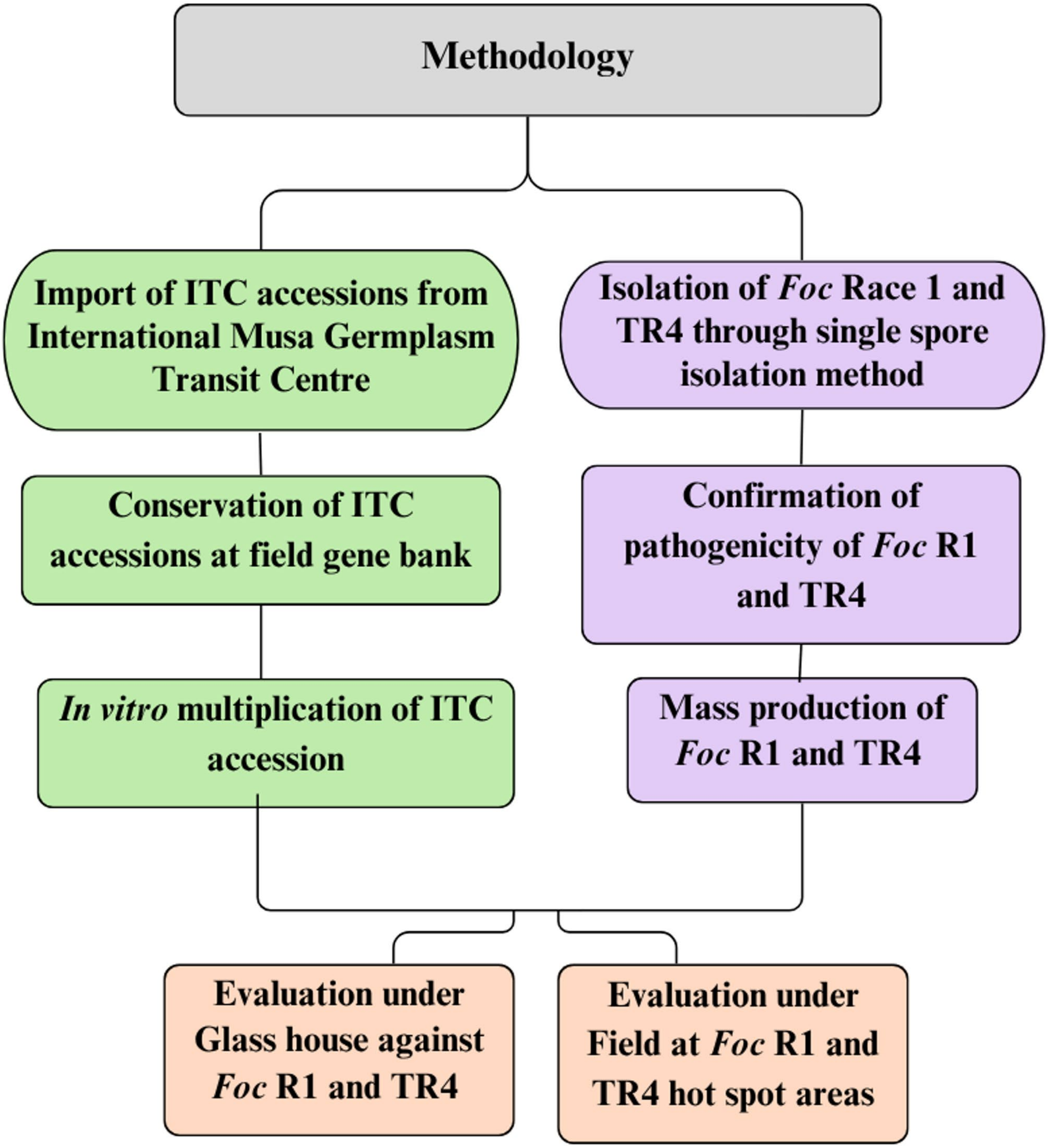

Fig. 6. Flowchart illustrating the research methodology used in the study.

Data availability

"All data supporting the findings of this study are available within the paper".

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Author contributions

RT, ML and SU designed the research, performed the experiments, compiled data, and wrote the manuscript SB, PD, MSS supply of Germplasm accessions and performed the experiments and compiled data EER performed statistical analyses and drafted the paper; and wrote the paper. RT, ML and SU contributed equally BN editing of Manuscript. NR and RS correction of Manuscript all authors read and approved the final manuscript.

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Declarations

Competing interests

The authors declare no competing interests.

Conflict of interest

The authors declare that the research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

Additional information

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