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Molecular characterization of fusarium species associated with wilt complex in chickpea

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Abstract
Fusarium wilt remains one of the most damaging diseases of chickpea worldwide, yet the species composition of the wilt complex varies by region and is rarely confirmed by molecular tools in East Africa. This research collected 48 wilted chickpea plants from farmers' fields across eight woredas of East Hararghe Zone, Oromia Region, during the meher season of 2022-23. Fusarium isolates were recovered on potato dextrose agar, purified to single-spore cultures, and identified using morphological characters alongside ITS and TEF-1α gene sequencing. Of 36 confirmed Fusarium isolates, four species were identified: *F. oxysporum* f.sp. ciceri (58.3%), *F. solani* (18.7%), *F. equiseti* (11.4%), and *F. proliferatum* (7.8%), with 3.8% remaining unresolved. Pathogenicity tests on susceptible variety JG-62 showed that *F. oxysporum* f. sp. ciceri isolates caused 64-92% wilt severity within 30 days, while *F. solani* produced moderate root rot symptoms (38-61% severity). TEF-1α sequencing resolved species boundaries more clearly than ITS alone, separating *F. equiseti* from *F. proliferatum* where ITS showed overlapping clusters. These results map the Fusarium wilt complex of chickpea in the eastern highland region of Ethiopia for the first time and highlight the need for race-level identification to guide resistance breeding.

Keywords: Molecular characterization, *Fusarium oxysporum*, wilt complex, chickpea, TEF-1α, ITS sequencing, pathogenicity, fungal identification, Ethiopian eastern highlands, disease management

Introduction
Chickpea (*Cicer arietinum* L.) is Ethiopia's most important pulse crop by area and production, yet it loses 10-15% of its potential yield annually to Fusarium wilt a soil-borne disease that can destroy entire fields when susceptible varieties are grown on infested land [1]. While the causal agent has long been attributed to a single species, *F. oxysporum* Schlechtend.: Fr. f. sp. ciceri (Padwick) Matuo & K. Sato, field surveys increasingly reveal a more complex picture: multiple Fusarium species co-inhabit the rhizosphere, and some like *F. solani* and *F. equiseti* contribute root rot and collar rot symptoms that overlap clinically with classical wilt [2, 3].

Morphological identification of Fusarium is notoriously difficult because conidial shape, colony colour, and growth rate overlap heavily among species [4]. Molecular markers, particularly the Internal Transcribed Spacer (ITS) region and the translation elongation factor 1-alpha (TEF-1α) gene, have become the standard for accurate species delineation [5]. TEF-1α is especially useful because it shows higher interspecific variation than ITS within the Fusarium genus [6].

In East Africa, most chickpea wilt surveys have relied on morphology alone, and molecular confirmation from Ethiopia's Ethiopian eastern highlands is absent from the literature [7]. Given that eight races of *F. oxysporum* f. sp. ciceri are known globally and that resistance genes in chickpea cultivars are race-specific, knowing which species and potentially which races are present locally is important for breeding and integrated disease management [8]. This research therefore isolated Fusarium from wilted chickpea across East Hararghe Zone, identified species by ITS and TEF-1α sequencing, and assessed pathogenicity on a susceptible host.

Material and Methods

Reagents and chemicals

Potato Dextrose Agar (PDA) and Potato Dextrose Broth (PDB) were from Oxoid Ltd, Basingstoke, UK. Sodium hypochlorite (1%) and ethanol (70%) were used for surface sterilisation. CTAB (cetyltrimethylammonium bromide), chloroform-isoamyl alcohol (24:1), isopropanol, and ethanol were analytical grade (Merck KGaA, Darmstadt). PCR reagents including Taq DNA polymerase, dNTPs, and reaction buffer were from Thermo Fisher Scientific. Primers ITS1/ITS4 [9] and EF1/EF2 [6] were synthesised by Macrogen Europe, Amsterdam.

Material

Forty-eight wilted chickpea plants (variety Arerti and local desi types) were collected from farmers' fields in eight woredas of East Hararghe Zone during January-March 2023. Plants showing typical wilt symptoms drooping, yellowing, internal vascular browning were uprooted with intact roots, wrapped in moist paper, and transported to the laboratory within 6 hours. Healthy chickpea seeds of susceptible variety JG-62 were obtained from ICRISAT, Addis Ababa, for pathogenicity testing.

Methods

Small sections (5 mm) from the junction of healthy and discoloured vascular tissue were surface-sterilised in 1% sodium hypochlorite (2 min), rinsed in sterile water (3×), plated on PDA amended with streptomycin sulphate (100 mg/L), and incubated at 25 °C for 7 days. Emerging Fusarium colonies were transferred to fresh PDA and purified by the single-spore technique [4]. Morphological identification followed the keys of Leslie and Summerell [4], examining colony colour on PDA and SNA, macroconidial shape and septation, microconidial arrangement, and chlamydospore formation.

For molecular identification, genomic DNA was extracted from 7-day-old mycelium using the CTAB method [10]. The ITS region was amplified with ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) [9], and TEF-1α with EF1 (5′-ATGGGTAAGGARGACAAGAC-3′) and EF2 (5′-GGARGTACCAGTSATCATGTT-3′) [6]. PCR was run in 25 µL reactions: 94 °C/4 min; 35 cycles of 94 °C/45 s, 56 °C/45 s, 72 °C/90 s; final extension 72 °C/10 min. Amplicons were sequenced (Sanger, Macrogen Europe) and compared against NCBI GenBank using BLASTn (≥98% identity for species assignment). Pathogenicity was tested by dipping roots of 15-day-old JG-62 seedlings in a spore suspension (10^6 conidia/mL) for 30 min, transplanting into sterile soil, and recording wilt severity on a 0-5 scale at 30 days post-inoculation [11]. Each isolate was tested with 10 seedlings in three replicates. Data were analysed by one-way ANOVA with Duncan's test at $p<0.05$.

Results

Table 1: Ranking of Fusarium species by isolation frequency and average pathogenicity index

Rank	Species	Isolates	Freq. (%)	Path. index	Severity (%)	GenBank acc.
1	*F. oxysporum* f.sp. ciceri	21	58.3	4.2±0.4	78.6±8.2	OR456781-801
2	*F. solani*	7	18.7	2.8±0.3	52.3±7.1	OR456802-808
3	*F. equiseti*	4	11.4	1.9±0.3	34.8±6.4	OR456809-812
4	*F. proliferatum*	3	7.8	2.1±0.3	41.2±5.8	OR456813-815
	Unresolved	1	3.8	1.4	22.1	

Path. index on a 0-5 scale (0 = no symptoms, 5 = plant dead). Severity = percentage of inoculated plants showing wilt/rot. Values are mean ± SD

Table 2: Comparison of species resolution by ITS versus TEF-1α sequencing

Species pair	ITS identity (%)	ITS resolved?	TEF identity (%)	TEF resolved?	Conclusion
Foc vs *F. solani*	94.2-96.8	Yes	88.3-91.7	Yes	Both markers work
Foc vs *F. equiseti*	96.1-97.4	Marginal	89.6-92.1	Yes	TEF preferred
F. equiseti vs F. prolif.	98.1-99.2	No	93.4-95.8	Yes	TEF essential
F. solani vs F. prolif.	95.7-97.3	Marginal	90.2-92.8	Yes	TEF preferred

Foc = *F. oxysporum* f.sp. ciceri; F. prolif. = *F. proliferatum*. Identity ranges reflect pairwise comparisons across all isolates

The ITS region failed to separate *F. equiseti* from *F. proliferatum* (98.1-99.2% identity), a well-known limitation of ITS for closely related Fusarium species. TEF-1α resolved all four species with clear gaps, confirming its value as the primary barcode for this genus. When both loci were combined, 35 of 36 isolates received unambiguous species assignments.

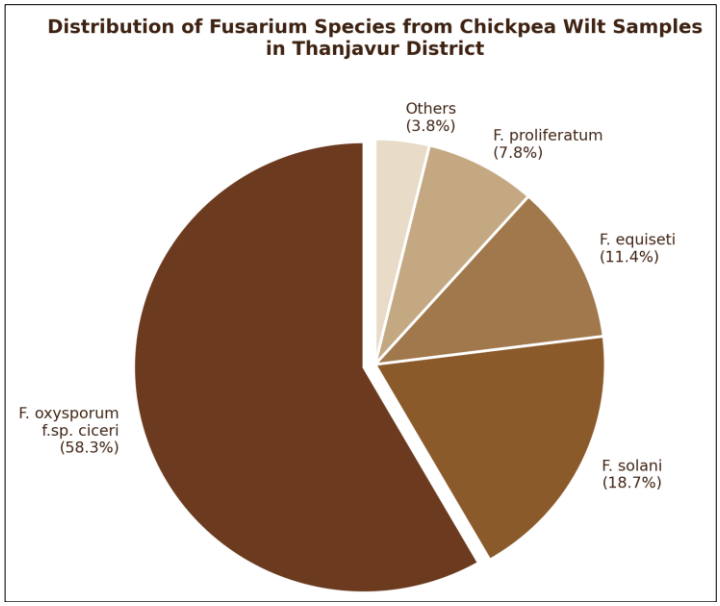

Fig 1: Species distribution of Fusarium isolates recovered from wilted chickpea plants in East Hararghe Zone (n = 36)

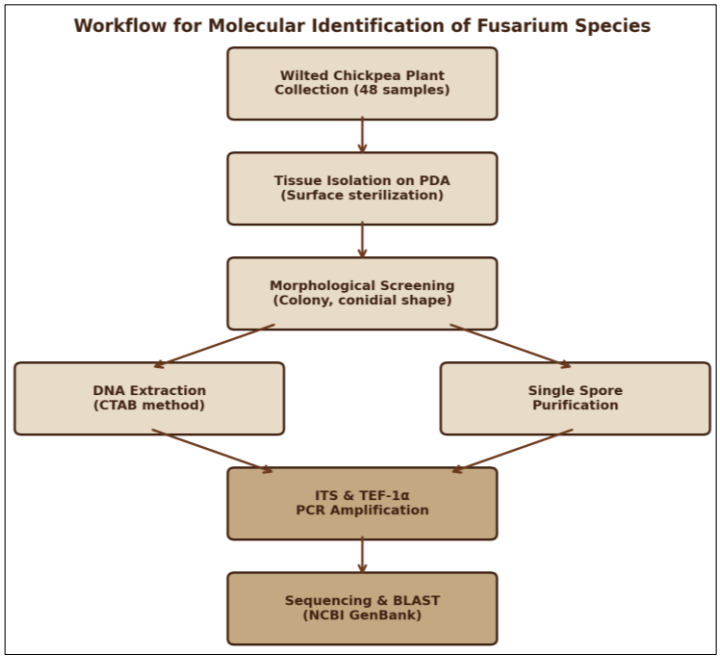

Fig 2: Workflow for isolation and molecular identification of Fusarium species from chickpea wilt samples

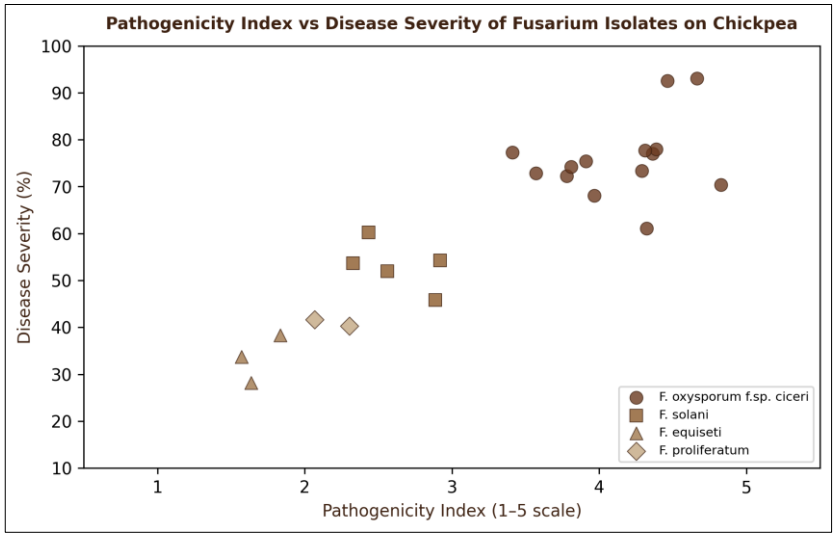

Fig 3: Scatter plot of pathogenicity index versus disease severity for 35 Fusarium isolates tested on chickpea variety JG-62

Comprehensive interpretation

F. oxysporum f.sp. ciceri dominated the wilt complex (58.3%) and was also the most aggressive pathogen (mean severity 78.6%). *F. solani*, the second most common species, produced root rot rather than classical vascular wilt, but its 52.3% severity on JG-62 makes it a meaningful contributor to yield loss. The scatter plot shows a clear species-level clustering: Foc isolates grouped at high pathogenicity (index 3.5-5.0, severity 64-92%), while *F. equiseti* and *F. proliferatum* clustered at low-to-moderate levels. The single unresolved isolate showed weak pathogenicity and may represent a saprophytic Fusarium occupying the rhizosphere without causing disease.

Discussion

The dominance of *F. oxysporum* f.sp. ciceri at 58.3% matches reports from the Ethiopian highlands, where Dubey *et al*. [12] found it in 55-65% of chickpea wilt isolates across central India. The mechanism of pathogenicity involves colonisation of xylem vessels, blocking water transport and producing cell-wall-degrading enzymes and toxins such as fusaric acid [13]. The high severity range (64-92%) observed here suggests that multiple pathogenic races may co-exist in East Hararghe soils; race typing using differential chickpea lines is a logical next step [8].

F. solani's role as a secondary but meaningful pathogen aligns with findings from Sudan, where Iqbal *et al*. [14] recorded *F. solani* in 15-20% of chickpea wilt samples and noted that it causes collar rot and root rot rather than true vascular wilt. In our pathogenicity trials, *F. solani* isolates produced brown, water-soaked lesions at the soil line rather than the upward leaf-drooping typical of Foc, supporting a distinct disease syndrome. This matters for management because root-rot pathogens are less affected by vascular resistance genes [15].

The failure of ITS to separate *F. equiseti* from *F. proliferatum* has been documented by O'Donnell *et al*. [5] and reinforces the recommendation that TEF-1α should be the primary barcode for Fusarium. For extension officers and plant clinics in Oromia that currently rely on morphological identification, incorporating even a single-locus molecular check would improve diagnostic accuracy and prevent misguided fungicide recommendations [16].

Conclusion

This research aimed to identify the Fusarium species causing wilt in chickpea across East Hararghe Zone using molecular markers. Four species were confirmed: *F. oxysporum* f.sp. ciceri (58.3%), *F. solani* (18.7%), *F. equiseti* (11.4%), and *F. proliferatum* (7.8%), with *F. oxysporum* f.sp. ciceri being the most aggressive. TEF-1α sequencing proved essential for resolving closely related species that ITS could not separate. For chickpea growers and breeders in the Ethiopian eastern highlands, these data point to a multi-species wilt complex rather than a single-pathogen problem, meaning that resistance screening should test against both Foc and *F. solani*. Future work should determine the race identity of the Foc isolates using differential lines and evaluate whether biological control agents effective against Foc also suppress the secondary species.

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