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Short Article

Investigating Vegetative Compatibility and Fusion Reactions among different isolates of *Fusarium* spp

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Abstract

The study was conducted to investigate the vegetative compatibility between different isolate of *Fusarium* to enable appropriate identification and characterization of individual isolates during July, 2024 to September, 2024, in the Department of Plant Pathology, CSKHPKV, Palampur. Microscopic examination identified four isolates as *Fusarium oxysporum*. Vegetative compatibility testing among the four isolates of *Fusarium oxysporum* using dual culture plate method and hyphal fusion assays revealed that F3 and F1 were compatible, forming heterokaryons without zone of inhibition. In contrast, other combinations exhibited zones of inhibition or coiling of hyphae, indicating incompatibility. These findings suggest that F3 and F1 belong to the same vegetative compatibility group (VCG), while others belong to different VCGs. This study provides insights into genetic relationships among *Fusarium* isolates and demonstrates the reliability of vegetative compatibility grouping for identifying physiological races.

Keywords: Vegetative compatibility, *Fusarium*, Hyphal fusion, dual culture method

Vegetative compatibility refers to the ability of the hyphae of two individual fungal isolates to fuse together and form viable heterokaryons. In fungi lacking sexual stages, vegetative compatibility may serve as an important means of genetic exchange generating genetic diversity (Leslie1993). Isolates that are vegetatively compatible with each other are said to be members of the same vegetative compatibility group (VCG) (Joaquim and Rowe 1991). Conversely, vegetatively incompatible isolates are incapable of establishing stable heterokaryosis.The vegetative compatibility assay has been used to measure population diversity of fungi and to enable appropriate identification and characterization of individual isolates. Vegetative compatibility assays have been used to determine the population structures of many plant-pathogenic fungi, including *Fusarium* spp. (Katan and Katan 1988). The conventional method of determining VCGs involves the detection of a darkly pigmented lytic area, or barrage zone, where mycelium from two incompatible isolates meet. According to the conventional method VCG determination, if the barrage zone is absent, isolates are said to be vegetatively compatible. Vegetatively compatible isolates of a fungal species are placed in the same VCG (Burgess*et al*. 2009)

An *in vitro* experiment was conducted in the Laboratory of Plant Pathology Department, CSKHPKV University, Palampur, India to assess the compatibility among different isolates of *Fusarium*. Soil samples was collected from the experimental farm of Plant Pathology, Vegetable science, Seed science and Organic farming. For the isolation of *Fusarium oxysporum*, serial dilution method was used. Samples were collected from top five cm of soil. Collected soil samples were kept in polythene bags and carried out to laboratory for further study. The rhizosphere soil samples were semi dried in laboratory and then 10 grams soil were mixed in 100 ml of sterile distilled water and the flask were shaken for 10 minutes. The resultant solution were considered as stock solution. From the stock solution serial dilution series were

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made up to the dilution of 10^{-5}. An aliquot (0.1 ml) of diluted suspension were spread over the plates containing Potato Dextrose Agar (PDA) and incubated at 25+ 1°C for 7 days. After incubation, fungal colonies were observed under microscope for identification of *Fusarium* isolate. Selected isolates were then kept in duplicates in PDA slants.

Vegetative compatibility was determined by observing the formation of a heterokaryon between complementary species. *In vitro* compatibility test between different isolates of *Fusarium oxysporum* using dual culture method described by Siddiqui and Shaukat (2003) was employed. Accordingly, 5mm disc of one isolate of *Fusarium* sp (9 days old) was placed on one side of a Petri dish containing PDA and in the same way the other side of the petri-dish was inoculated with another isolate of *Fusarium.* The plates were then incubated at 25±1°C and zone of inhibition (if any) was observed. The test was performed in triplicates. The complementation test was considered incompatible, when there was presence of zone of inhibition and compatible, when absence of zone of inhibition.

Hyphal fusion between different *Fusarium oxysporum* isolates was examined microscopically. For this purpose, *Fusarium* isolates were grown 4cm apart over a thin layer of 2 per cent water-agar on a slide. Observations were made with a compound microscope.

Isolation of *Fusarium* isolates

Four (F1, F2, F3 and F4) isolates of *Fusarium oxysporum* species were obtained from soil samples, one each from experimental farm of department of Plant Pathology, Vegetable science, Seed science and Organic farming. Upon microscopic examination, these isolates revealed characteristic morphological features indicative of *Fusarium oxysporum*. Notably, the hyphae exhibited septate, hyaline, and branched structures, while the conidia displayed multiseptate morphology, often produced in chains (Plate A). These morphologically distinctive features collectively confirm that the isolated species belongs to *Fusarium oxysporum*. Sood *et al*. (2024) also isolated different isolates of *Fusarium* spp. from pea plant defined their morphological characters as conidia with 1-5 septa having fusiform shape with slightly cylindrical apical points. Similar work was done by Kumari *et al*. (2016)

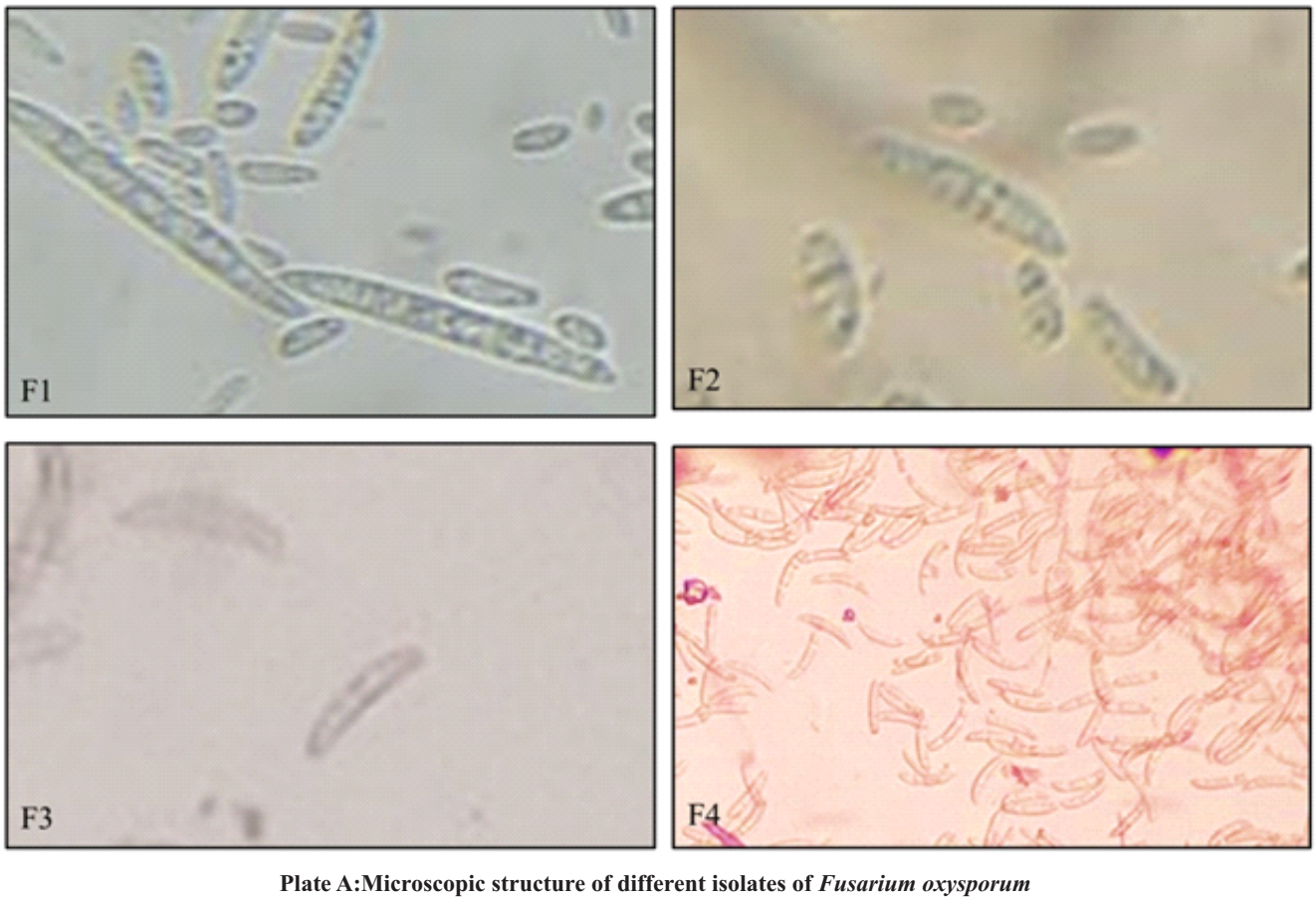

Plate A:Microscopic structure of different isolates of *Fusarium oxysporum*

and identified different *Fusraium* isolates as *Fusarium solani* f.sp. *pisi*. Kushwaha *et al*. (2024) also isolated *Fusarium* spp. and characterized them as *Fusarium oxysporum.*

Vegetative Compatibility test

The results indicated that only F3 and F1 were vegetatively compatible, as they formed a heterokaryon without any zone of inhibition. In contrast, combinations involving F1&F2; C: F3&F2; D: F4&F1; E: F2&F4; F: F4&F3 exhibited zones of inhibition, indicating incompatibility (Plate B). Merzoug and Belabid (2017) conducted a vegetative compatibility study on 21 isolates of *Fusarium oxysporum* f. sp. *phaseoli* (FOP), representing four races, and one non-pathogenic *F. oxysporum* isolate in Algeria. The analysis revealed that the FOP isolates could be classified into four main vegetative compatibility groups (VCGs), which corresponded to races 1, 2A, 2B, and 5. Notably, the race 6 isolate fell into the race 1 VCG. This study provides valuable insights into the genetic structure of the FOP population in Algeria and demonstrates the reliability of vegetative compatibility grouping for identifying physiological races of FOP.

Microscopical observations

The hyphal fusion test revealed that F3 and F1 were compatible, exhibiting hyphal fusion and thick mycelium formation. In contrast, combinations

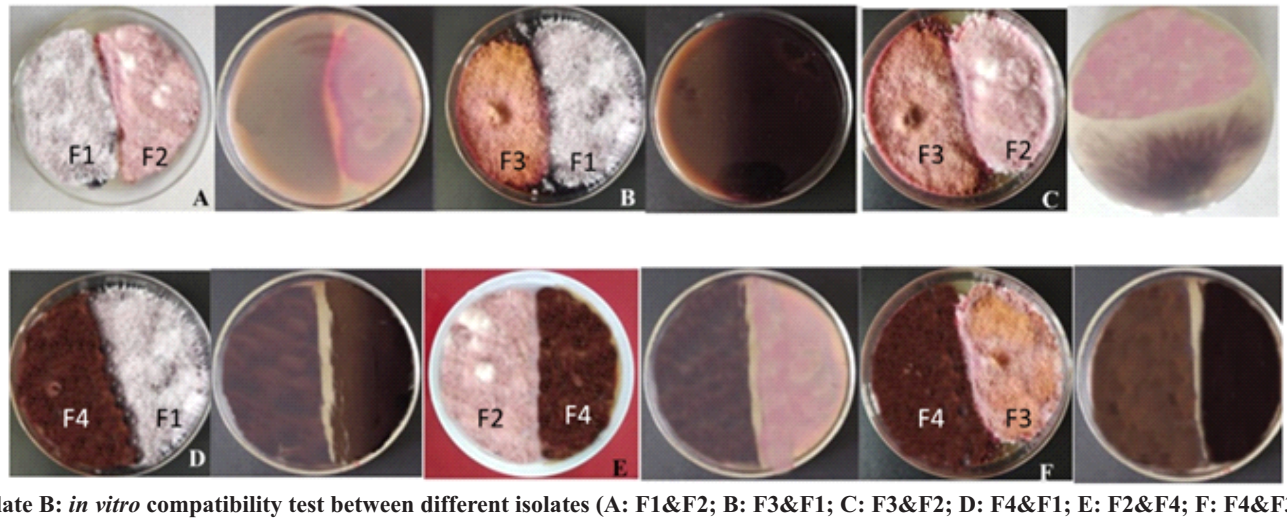

Plate B: *in vitro* compatibility test between different isolates (A: F1&F2; B: F3&F1; C: F3&F2; D: F4&F1; E: F2&F4; F: F4&F3) of *Fusarium* using dual culture plate method

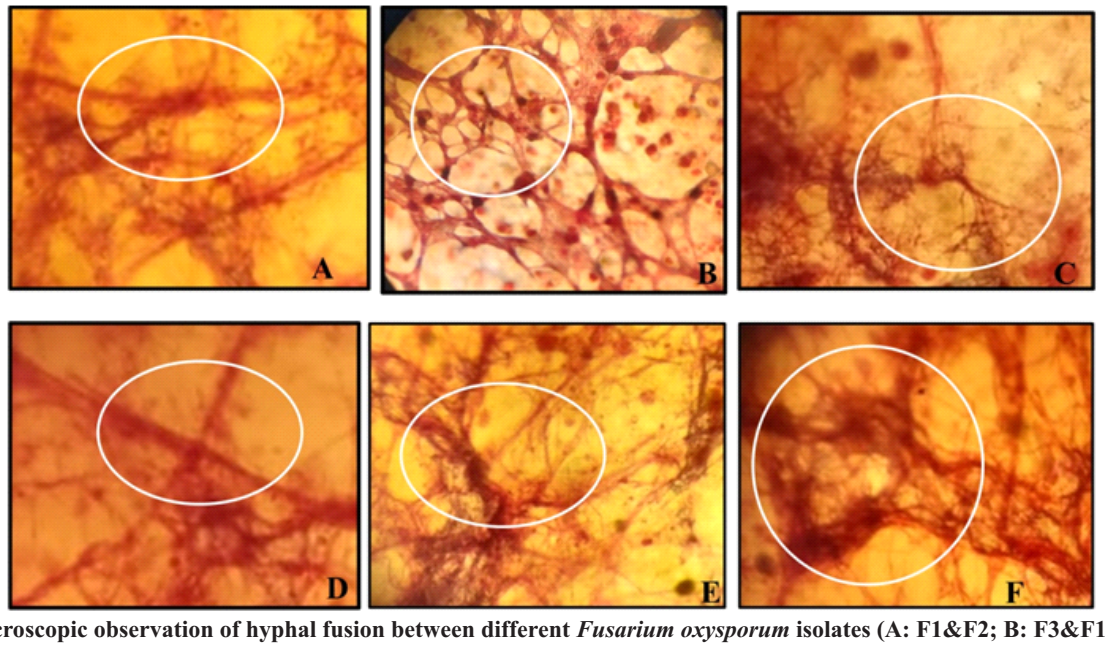

Plate C: Microscopic observation of hyphal fusion between different *Fusarium oxysporum* isolates (A: F1&F2; B: F3&F1; C: F3&F2; D: F4&F1; E: F2&F4; F: F4&F3)

involving F1&F2; C: F3&F2; D: F4&F1; E: F2&F4; F: F4&F3 showed coiling of hyphae, indicating incompatibility (Plate C).These findings suggest that F3 and F1 belong to the same vegetative compatibility group (VCG), while F1&F2; C: F3&F2; D: F4&F1; E: F2&F4; F: F4&F3 belong to a different VCG. This information is crucial for understanding the genetic relationships among these *Fusarium* isolates. Ishikawa *et al*. (2012) examined hyphal fusion in *Colletotrichum lindemuthianum*, labeling nuclei with fluorescent proteins to track compatibility. Results showed that while mature colony fusion led to cell death, early-stage fusion via conidial anastomosis tubes tolerated incompatibility, forming heterokaryotic colonies with novel phenotypes.

Conclusion

This study investigated the vegetative compatibility among four *Fusarium* oxysporum isolates (F1, F2, F3, and F4) obtained from soil samples in Palampur, India. The results revealed that F3 and F1 belonged to the same vegetative compatibility group (VCG), while the other combinations exhibited incompatibility. Microscopic observations confirmed hyphal fusion and thick mycelium formation in compatible isolates. These findings demonstrate the reliability of vegetative compatibility grouping for identifying genetic relationships among *Fusarium oxysporum* isolates.

Conflict of interest: The authors declare that there is no conflict of interest among the authors in this research paper.

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