A technique for obtaining monokaryotic haploid hyphae of
\textit{Ustilago tritici}, causal agent of wheat loose smut

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\textbf{Key words:} Wheat, loose smut, Monokaryotic haplonts, \textit{Ustilago tritici}

Loose smut of wheat, which is caused by \textit{Ustilago tritici} (Pers.) Rostr. is a serious disease of wheat and causes heavy losses when susceptible varieties with infected seed are grown under monocropping. It occurs in all the wheat growing areas of the world and is more common in regions with a cool, moist climate during flowering of the host (4-5).

Teliospores of loose smut of wheat germinate and produce promycelium without sporidia and each of the four cells grow into a short hypha that may fuse with one another to form two dikaryons. Molecular phylogenetic analysis requires the production of monokaryotic haploid hyphae, which is difficult to obtain. Christensen (1) reported a technique in which he had cut the promycelium with a microknife. However, the survival rate was very low. Subsequently, various research workers used different methods for obtaining promycelium so that haploid cells from it can easily be obtained and separated by using microknife. Lange De la Camp (2) germinated teliospores on beer-wort agar at room temperature and after formation of promycelium, transferred the cultures to 4°C. In a few days, constriction developed between cells of the promycelium and single cells could be isolated by microsurgery. Nielsen (3) germinated teliospores on a medium containing benzoic acid for isolation of four monokaryotic haplonts. Wilcoxson and Saari (5) reported isolation of monokaryotic hyphae by using special medium containing aspartic acid.

In the present investigations twenty isolates of \textit{U. tritici} were collected from various parts of northern India and used for monokaryotic haplonts production (Table 1).

Teliospores from the infected plant samples were surface sterilized by filtering teliospore suspension through cheese cloth to remove debris and centrifuged at 1000 rpm for 2-3 minutes to get the teliospore pellet. The pellet obtained was washed with sodium hypochlo-

\begin{table}
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\caption{Isolates of \textit{U. tritici} collected from various parts of India during 1999}
\begin{tabular}{|l|l|l|}
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Locations & Isolates & \\
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Haryana & UT1 (Gurgaon), UT2 (Bathinda), UT3 (HAU, Hisar), UT4 (Thaska, Hisar), UT5 (Kaimri, Hisar), UT6 (Karnal) & \\
Himachal Pradesh & UT7 (HPKV, Palampur), UT8 (Palampur), UT9 (DWR, Shimla), UT10 (IARI, Shimla) & \\
Uttar Pradesh & UT11 (Faizabad), UT12 (Kanpur), UT13 (Varanasi) & \\
Bihar & UT14, UT15 (Pusa), UT16, (RAU, Pusa), UT17 (Patna) & \\
Punjab & UT18, UT19 (PAU, Ludhiana) & \\
Delhi & UT20 (IARI, New Delhi) & \\
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\end{tabular}
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rite (0.25%) for 45-60 seconds followed by rinsing twice in sterile distilled water. The pellet was again subjected to centrifugation for 2 minutes at 1000 rpm at room temperature to remove surfactant and water. Sterilized teliospores were resuspended in sterile distilled water and 150-250 μl of sterilized teliospore suspension was evenly spread on petri dishes containing 1.5-2 mm thick, 1.5% water agar and DL-aspartic acid (0.147 mg/ml water). The petri dishes were incubated at 20°C for about 30 hours. The dikaryon formation was observed and subsequently 1 square cm blocks of medium from these plates were transferred to plates with 1.5-2 mm thick, 1/5 of normal nutrient concentration of PDA (potato dextrose agar) and incubated in refrigerator overnight. The squares were then transferred to another 1.5-2 mm thick layer of 1/5 PDA pre-warmed to 25°C and kept at this temperature for 4-6 hours.

Monokaryotic haploid hyphae were obtained and isolated by microsurgery with very thin pasteur pipettes and transferred to a thick layer of 1/5 PDA and kept at 20°C (Fig. 1). Growth of haplonts was relatively slow and was visible only after 4-5 days. After 2 weeks, the
Surface sterilization of teliospores

Plating on 1.5% Water agar+Aspartic acid and incubation at 20°C for about 30 hours.

Transfer to 1/5 PDA and incubation in refrigerator overnight

Transfer to 1/5 PDA pre-warmed to 25°C and incubation at 25°C for 4-6 hours.

Isolation of monokaryotic haploid hyphae with thin pasture pipettes

Transfer to 1/5 PDA and maintain at 20°C

Fig 1. Steps involved in obtaining monokaryotic haploid hyphae of *U. tritici.*

Verrucose colonies of fungus which were dense, cream to pinkish cream could be seen. The mycelial mass production of the mycelium of haploid hyphae can be obtained by inoculation in PSB (potato sucrose broth) in shaker incubator at 130 rpm, 20°C for 14-20 days.

Pasteur pipette technique is more convenient to use for isolating the monokaryotic haploid hyphae as compared to microknife. Addition of DL-aspartic acid to the medium ensures uniform development of dikaryons and mitosis does not occur. When blocks are transferred to PDA which does not contain aspartic acid, mitosis occurs and from each dikaryon, two monokaryotic haploid cells of opposite mating type can be obtained. The present technique proved to be effective in obtaining the monokaryotic haploid hyphae of *U. tritici* in case of all the twenty isolates used in the present investigation.

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REFERENCES


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