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# Diversity of VAM and rhizospheric fungi in wheat (*Triticum aestivum*) grown in mid-hill conditions of Himachal Pradesh

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**Abstract**
In natural environments, plants interact simultaneously with a broad spectrum of both pathogenic and beneficial microorganism that might influence plant performance and survival. Among soil microbes arbuscular mycorrhizal fungi are very common soil micro-organisms that colonize the roots of majority of crop species and establish a symbiosis. They provide direct benefits to the host crop which leads to the increased crop productivity by increasing biomass and grain yield. Notwithstanding the global importance of wheat as an agricultural crop, its responses to AM symbiosis has been poorly investigated. Therefore the present investigation was conducted to study the diversity of arbuscular mycorrhizal fungi (AMF) in wheat crop in mid hill region of Himachal Pradesh. Rhizospheric soils samples along with wheat roots (*Triticum aestivum*) were collected from multiple sites of Chail Chowk areas, near Abhilashi University, Mandi. The analysis revealed the presence of seven diverse species of fungi out of which *Aspergillus* and *Fusarium* were found to be the predominant genera in the fields. Seven species of VAM fungal spores belonging to 3 genera (*Acaulospora*, *Glomus*, and *Gigaspora*) while *Glomus* and *Acaulospora* as were most dominant genus.

**Keywords:** *Triticum aestivum*, mycorrhiza, arbuscular mycorrhizal fungi, vesicular arbuscular mycorrhizal fungi

## Introduction
Wheat (*Triticum aestivum*) is the most essential cereal crop in India, with reference to production, consumption, and cultivated area. Wheat adds about 35.24% to the total food grain basket of the country. In 2019 the estimated yield of wheat in India was approximately 3.5 thousand kilograms per hectare. In India, wheat is second important food crop which is grown on 27 million hectares out of the total 114 million hectares of land under cultivation (Ladha *et al.*, 2000) [8]. Increasing grain yields of food cereal crops is a major goal in future sustainable agriculture. Arbuscular mycorrhizal fungi plays important role in enhancing grain yield of cereal crop like corn, Wheat, rice, barley, oat etc. Mycorrhizae are highly emerged, mutualistic partnership between soil fungi and plants roots. The arbuscular mycorrhizal fungi (AMF) capable of forming symbiotic associations with most of agricultural crops (Wright and Upadhaya, 1998) [22]. VAM (vesicular arbuscular mycorrhizae) ameliorate and advance the tolerance to adverse soil conditions and they increase the plant productivity (Bennett, 2013) [2]. Arbuscular mycorrhizal fungi (AMF) improve the quality of soil like structure, texture, and plant health and these fungi are a promising option in terms of sustainable agriculture and food security as in accordance with Zou *et al*., (2016) [23]; Rilling *et al.* (2016) [13] and Thirkell *et al.* (2017) [18] respectively. Arbuscular mycorrhizal fungi are commonly known as bio-fertilizers. AMF provides tolerance to plants against different stressful conditions like heat, salinity, drought, metals, and extreme temperatures (Rani *et al.*, 2018).
Soil microorganisms show an important role in improvement of waste lands and have important impact on soil fertility and plant health. Mycorrhizal fungi support in nutrient uptake, seed yield, plant growth, and nutritional level in cereal. AM species have powerful effect on root colonization of *Glomus etunicatum* and *G. mosseae* on different wheat cultivars under salinity condition (Daeia *et al.*, 2009) [4]. Wheat occupies most of the northern, western and central region of India (Ladha *et al.*, 2000) [8]. To the best of our knowledge, there is no detailed study on the diversity of AM fungi in mid hill regions. Our study primarily aims at improving the understanding of the broad-scale distribution of AM fungi in different wheat-growing fields in mid hill condition of Himachal Pradesh.

To achieve this, we sampled the wheat soil from multiple sites of Chail chowk, areas, near Abhilashi University Mandi. Present studies were conducted to investigate the biodiversity of VAM and rhizosphere fungi associated with Wheat grown in Mid-hill condition of Himachal Pradesh.

## 2. Materials and methods

Study materials (Rhizospheric soils samples and roots of wheat (*Triticum aestivum*) were collected from selected site of Chail chowk areas, near Abhilashi University Mandi. The collection was made during the month of march and april 2019 from different wheat growing fields at maturity stage. A sterilized trowel (70% alcohol) was used for scrapping the surface soil. Then the soil was dig upto a depth of 10-15 cm around the plant. Soil samples were collected from different depths after removing the top soil i.e 10cm, 15 cm, 20cm below the surface. The samples were collected and transferred to laboratory in a good quality, air tight and clean plastic bags for analysis. These samples were labelled with paper slip for identifying them. Such samples were taken from two different sites. Samples were taken during premonsoon seasons. The soil was cleaned, air dried, crushed, homogenized and sieved and stored in air tight bags for futher study For the isolation of rhizosphere fungi, dilution plate method of Wakesman (1927) [20] and Warcup (1950) [21] was followed. This method includes shaking of a known amount of soil (1gm) taken from region surrounding fresh and active root in 1000ml of distilled water. Then 1ml of this suspension is taken and is added to 100ml of distilled water. From this, 1ml material is taken and various suspensions are made and are dispersed over medium under aseptic conditions. The media used for culturing rhizosphere fungi were Czapeks Dox (Raper and Thom, 1949) [11] and Potato Dextrose Agar (Rawling, 1933) [12]. The sterilized medium was poured into the petriplates under asceptic conditions (laminar flow hood) and allowed to solidify. Samples of each type were taken with sterilized graduated dropper and poured on petriplates containing medium. The samples were uniformly spread on the medium by tilting the petriplates. The inoculated petriplates were placed in incubator and incubated at 22± $^0$C for seven days. These were regularly checked for growth of fungal colonies. For obtaining pure cultures, slants of media were made in test tubes and fungi separated from soil were subcultured in them. For identification, temporary mounts of fungi were made in 1% cotton blue and Lactophenol. An adequate high power microscope was used for observing the slides. The cultures were identified following Barnett and Hunter (1972) [1] and Gilman (1967) [7]. Pure cultures of different fungi were maintained on PDA and Czapeck s-Dox Agar slants which were preserved in refrigerator. Subculturing was done at regular interval in order to maintain cultures. Each fungal species was transferred from parent source to a fresh slant in order to maintain and preserve the cultures.

For isolating VAM spores from soil. "Wet Sieving and Decanting Technique" (Gerdemann and Nicolson, 1963) [6] was used in present study.10g of soil was taken and mixed in a large beaker until all soil aggregates disperse to leave a uniform suspension. 300um, 200um and 100um sieves were arranged in a descending order. 300um sieve is used for removal of large organic matter. The contents of beaker were decanted through the sieve. This process was repeated four and five times. The debris collected on each sieve was carefully collected in a beaker with the help of level pipe separately for each sieve.

The collected debris of each sieve was filtered separately through Whatman No.-1 filter paper. The filter paper was observed under the stereo binocular dissecting microscope with the help of microneedles. Each spore was picked up with the help of a micro needle and was mounted in Lactophenol to make a semi-permanent mount. The VAM fungal spores mounted on slides were observed carefully under high power research microscope for segregation into genera and followed by species identification. The criteria employed for identification were colour, size, shape, wall characteristics, contents and surface ornamentation of spores, nature of spores, the number and arrangement of spores in sporocarp. The identification was done following (Morton, 1988; Schench & Prez, 1988; Trappe, 1982) [9, 15, 19].

## 3. Result and Discussion

This is the first study that has attempted to describe the diversity and distribution of AM fungi in the *Triticum aestivum* in mid hill regions of Himachal Pradesh. Because the sampling effort was standardized, conducted within a relatively short time frame during the wheat growing and harvesting period. 7species of rhizosphere fungi were isolated from the mycorrhizosphere of *Triticum aestivum*. The fungi isolated from field were *Absidia ramose, Aspergillus niger, Aspergillus ustus, Fusarium oxysporium, Rhizoctonia solani, Penicillium funiculosum and Trichoderma viridae* (Table 1). *Aspergillus* was found to be the predominant genera in mycorrhizosphere of *Triticum aestivum* in the field. These fungal isolates were further grouped into subdivisions viz., Ascomycotina, Zygomycotina, and Basidiomycota the Fig.1. In the present study it was observed that maximum genera isolated from the fields belongs to the subdivision Ascomycotina. Sagar and Shivani *et al*. (2013) studied the soil mycoflora of *Triticum aestivum* fields under normal and disturbed conditions in Himachal Pradesh isolated 18 species of fungi belonging to 10 genera (*Absidia* sp., *Absidia ramose, Aspergillus niger, Aspergillus terreus, Aspergillus ustus, Fusarium solani, Fusarium oxysporum, Fusarium* sp., *Gliocladium roseum, Rhizoctonia solani, Trichoderma viridae, Trichoderma* sp., *Cephalosporium* sp., *Cladosporium* sp., *Penicillium funiculosum, Rhizopus oryzae).*

In the present study, Seven species of VAM fungal spores belonging to 3 genera (*Acaulospora, Glomus, Gigaspora*) were isolated from root adhering soils of *Triticum aestivum* from the field. The fungal spores isolated were *Acaulospora bireticulata, Acaulospora denticulata, Acaulospora longula, Glomus aggregatum, Glomus clarum, Glomus rubiforme, Gigaspora gigantean* (Table 2). Sagar and Shivani *et al*. 2013, studied the soil mycoflora of *Triticum aestivum* fields under normal and disturbed conditions in Himachal Pradesh isolated 15 species of VAM fungal spores belonging to 6 genera (*Acaulospora, Glomus, Claroideoglomus, Dentisculata, Scutellospora, Gigaspora*). According to Seng CH *et al*. 2018 various AM fungi species such as *Glomus, Acaulospora, Funneliformis, Rhizophagus* and *Dentiscutata* were isolated from the crop plants and *Glomus* genera was identified as the predominant genera followed by *Acaulospora*. Bouamri *et al*., 2006 [3] isolate different species of AM fungi depending upon plant species and geographical location and *Glomus* was found abundantly and *Acaulospora, Gigaspora* and *Scutellospora* were either absent or found in few numbers. 14 AM fungal species were recorded from the agricultural fields of India (Dalal *et al*.1995 and Singh and Pandya 1995) [5, 17] with only 4-5 species being from the wheat fields (Singh and

Pandya 1995) [17].

**Table 1:** Fungi isolated from rhizosphere soil samples of wheat (*Triticum aestivum*) from multiple sites of Chail Chowk areas in mid hill region of Himachal Pradesh

| Sr. No. | Name of fungus isolated |
|---|---|
| 1 | *Absidia ramose* |
| 2 | *Aspergillus niger* |
| 3 | *Aspergillus ustus* |
| 4 | *Fusarium oxysporium* |
| 5 | *Rhizoctonia solani* |
| 6 | *Penicillium funiculosum* |
| 7 | *Trichoderma viridae* |

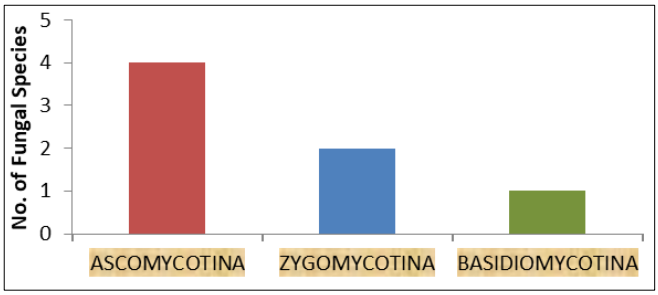
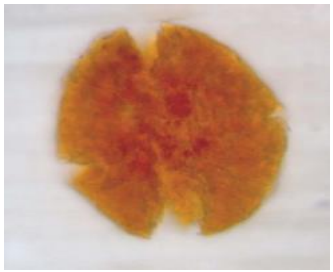



**Fig 1:** Distribution of fungal species (in different fungal subdivisions) isolated from the rhizosphere soil samples of wheat (*Triticum aestivum*) from multiple sites of Chail Chowk areas in mid hill region of Himachal

**Table 2:** Occurrence of different VAM fungal spores isolated from the rhizosphere soil of wheat (*Triticum aestivum*) from multiple sites of Chail Chowk areas in mid hill region of Himachal

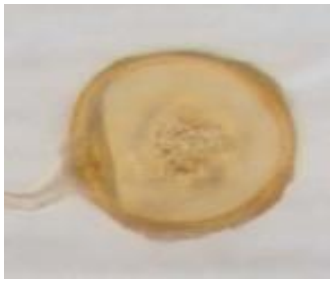
| Sr. No. | VAM Fungal spore isolated |
|---|---|
| 1 | *Acaulospora bireticulata* |
| 2 | *Acaulospora denticulata* |
| 3 | *Acaulospora longula* |
| 4 | *Glomus aggregatum* |
| 5 | *Glomus clarum* |
| 6 | *Glomus rubiforme* |
| 7 | *Gigaspora gigantea* |



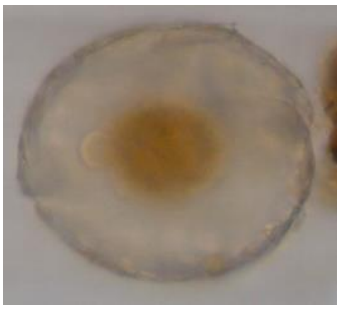
a) *Acaulospora bireticulata*

b) *Acaulospora denticulate*

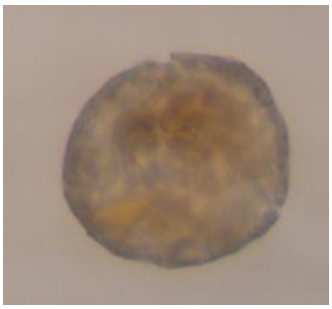
c) *Acaulospora longula*



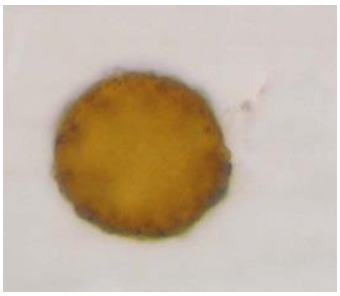
d) *Glomus aggregatum*



e) *Glomus clarum*



f) *Glomus rubiforme*



g) *Gigaspora gigantea*

**Plate 1:** VAM spores isolate from multiple sites of Chail Chowk areas in mid hill region of Himachal

**4. Acknowledgements**
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