Response of Arbuscular Mycorrhizal Fungi on Wheat (*Triticum aestivum* L.) Grown Conventionally and on Beds in a Sandy Loam Soil

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Abstract The present study was undertaken to assess the benefit and compare the functioning of AM fungi on wheat grown conventionally and on beds. Ten treatment combinations were used, treatments 1 and 2: no fertilizers with and without arbuscular mycorrhizal (AM) fungi (In vitro produced *Glomus intraradices*); 3:100% of recommended NPK: (120 kg ha⁻¹ N; 60 kg ha⁻¹ P; 50 kg ha⁻¹ K), and 4 and 5: 75% of recommended NPK dose with and without AM inoculation in a 5 × 2 split-plot design on wheat using conventional/flat system and elevated/raised bed system. The maximum grain yield (3.84 t ha⁻¹) was obtained in AM fungi inoculated plots of raised bed system applied with 75% NPK and was found higher (although non-significant) than the conventional (3.73 t ha⁻¹) system. The AM inoculation at 75% fertilizer application can save 8.47, 5.38 kg P and 16.95, 10.75 kg N ha⁻¹, respectively, in bed and conventional system. While comparing the yield response with 100% fertilizer application alone, AM inoculation was found to save 20.30, 15.79 kg P and 40.60, 31.59 kg N ha⁻¹, respectively, in beds and conventional system. Mycorrhizal inoculation at 75% NPK application particularly in raised bed system seems to be more efficient in saving fertilizer inputs and utilizing P for producing

higher yield and growth unlike non-mycorrhizal plants of 100% P. Besides the yield, mycorrhizal plants grown on beds had higher AM root colonization, soil dehydrogenases activity, and P-uptake. The present study indicates that the inoculation of AM fungi to wheat under raised beds is better response (although non-significantly higher) to conventional system and could be adopted for achieving higher yield of wheat at reduced fertilizer inputs after field validation.

Keywords Arbuscular mycorrhiza · Cultivation systems · *Triticum aestivum* · Field response

In India, wheat (*Triticum aestivum* L. emend. Fiori and Paol.), under rice cropping system alone contributes about 35.24% to the total food grain basket of the country. Despite having great potential, India's average productivity of wheat is 2802 kg ha⁻¹, which is almost of world average and less than the yield obtained in European countries (2007/08, Ministry of Agriculture, Government of India). In India wheat is continuously grown in flat system and upland well drained soils having good tilth. But wheat is undergoing stagnation in productivity despite the continuous use of chemical fertilizers. The declining trend in crop and soil productivity may be due to the adoption of conventional practices and has been showing negative yield trend from long-term field trials on rice–wheat [1].

Hence, there is need to increase the yield of wheat in rice wheat rotation by adopting alternative practices to improve on the input use efficiency, soil productivity and soil biological health without compromising the plant productivity [2]. Bed planting is extremely used and has been found promising for irrigated wheat [3, 4]. Moreover, the integrated use of beneficial microbes and their

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optimization under the raised bed planting with minimum tillage practices will further improve the soil and crop productivity, which has not been studied so far.

It is known that the adoption of minimum tillage and zero tillage practices can help in restoration of rhizosphere microorganisms [5], higher microbial biomass carbon [6] and higher microbial community necessary to maintain the soil quality [7]. The use of arbuscular mycorrhizal fungi (AMF) capable of forming symbiotic associations with most agricultural crops and has potential under such systems due to its higher binding capabilities [8] and mineral nutrition [9]. Field responses of wheat to AMF inoculation was determined and found higher biomass and grain yields of wheat plots inoculated with AM fungi when compared to non-mycorrhizal plots irrespective of soil moisture regimes [10]. Several studies have assessed differences between AM colonization levels and spore populations in differently managed agricultural systems [11, 12].

Farming system significantly affected the populations of AMF [11]. In addition, the tillage operations also affect the AMF populations [13]. The symbiotic interaction between AMF and host plants grown under reduced tillage and raised bed system applied with different doses of fertilizers need to be evaluated in order to optimize beneficial effects of AMF.

The proposed study envisaged to test the hypothesis that growing mycorrhizal wheat under raised bed system would result in higher AMF response compared to mycorrhizal wheat growing conventionally at reduced doses of fertilizers.

Materials and Methods

Experimental Site and Soil Preparation

The field trial site is in Uttranchal State, upper Gangetic plain region and situated at the bank of Ganga River. The annual mean precipitation is 1000–1200 mm. The site was under forestry for about 6 years where *Populus deltoides* (a deciduous agroforestry tree) was planted and followed one rotation of rice and wheat prior to the field trial. The soil type was sandy loam.

Soil samples were collected from a depth of 0–30 cm, air-dried and passed through a 3 mm sieve. The chemical

characteristics of the soil of the site are given in Table 1. Soil pH and electrical conductivity were measured (1:2.5, soil to water ratio) using a digital pH and EC meter. Available phosphorus (Olsen's P) in the soil was determined colorimetrically on spectrophotometer at 882 nm by extraction with sodium bicarbonate for 30 min [14]. Organic carbon was estimated colorimetrically [15]. Mycorrhizal spore population was determined by using wet sieving and decanting method [16]. Exchangeable potassium was determined by the ammonium acetate method [17]. Nitrogen was determined by modified kjeldahl distillation method [18]. The total micronutrients in soil samples was determined by digesting the samples using microwave digestion system (MARS, Unichem Corp., USA) in acid with hydrofluoric acid (HF) and measured over AAS (Analytik Gena).

Mycorrhizal Inoculum and Planting Germplasm

Sheared root based AM fungal inoculum; *Glomus intraradices* produced through *invitro* method was obtained from Centre for Mycorrhizal Research, TERI New Delhi and was used for inoculation. Inoculum potential was tested prior to the experiment and expressed as the number of propagules per gram of substrate using a bioassay [19, 20]. Seeds of wheat (*Triticum aestivum* L.) cultivar UP-2338 were obtained from GB Pant University of Agriculture and technology, Pantnagar, Uttranchal, India.

Experimental Setup and Design

A field area of 75 × 66 m was used in the experiment, with ten treatments allocated randomly in 5 × 2 split plot design. Raised beds were prepared with the help of tractor (35 HP) drawn bed planter cum zero tiller machine provided by Rice–Wheat Consortium, CIMMYT, New Delhi, India.

The treatments consisted of five fertilizer and inoculation treatments each in flat/conventional and raised beds. Each treatment plot (5 × 33 m) was replicated in three blocks.

Fertilisers doses viz., 0 (no fertilisers), 75% of recommended NPK (Nitrogen 90 kg ha⁻¹; Phosphorus 45 kg ha⁻¹; Potassium 37.5 kg ha⁻¹) with and without AMF (*Glomus intraradices*) application was used. 100% of recommended NPK dose (Nitrogen 120 kg ha⁻¹;

Table 1 Biochemical and soil nutrient characteristics (0–30 depth) of experimental soil at the time of initiation of field trial

pH (1:2.5 H ₂ O)	EC (dS/m)	Organic C (%)	AMF spore density (no/10 g soil)	Olsen P (ppm)	Total N (%)	Exchangeable K (ppm)	Zn (ppm)	Mn (ppm)	Fe (mg/100 g soil)	Cu (ppm)
7.1	0.58	0.36	3.2	6.54	0.052	117.5	15.45	49.66	2,067.34	12.65

EC electrical conductivity

Phosphorus 60 kg ha⁻¹; Potassium 50 kg ha⁻¹) without AMF was used for comparison in both the systems.

The diammonium phosphate (DAP) fertiliser was used as P and partly N source and potassium was applied in the form of muriate of potash (MOP) before sowing. Nitrogen was applied in two split doses; half of the dose was applied at the time of planting in the form of DAP and urea and remaining half was applied as top dressing in the form of urea after first irrigation.

The well-rotten FYM (farmyard manure) was applied at the rate of 5 t ha⁻¹ as basal dose during the field preparation. Seeds of wheat at 125 kg ha⁻¹ was sown through 35 HP tractor drawn zero tiller in the respective treatment plot.

Mycorrhizal (*Glomus intraradices*) inoculation was done through seed encapsulation. One thousand infective propagules in the form of sheared root powder were mixed with 100-g seed (dried in shade). Based on this, the amount of inoculum required for seeds to be sown in one treatment plot (165 m²) was extrapolated accordingly.

After Care, Harvest and Analysis

The crop was irrigated five times covering all the critical stages of growth and harvested after 140 days of sowing. Dry shoots weight (dried at 70°C for 90 h), harvest index, shoot height, grain weight per spike and 1000 seed weight were recorded. Plant P analysis was done by colorimetrically [21]. Root samples were used for clearing staining [22] and assessing the colonization [23]. Plants were harvested from each plot at 8–10 cm above the ground level and threshed through power-operated thresher and grain yield was recorded. The grain yield of AM plants per unit externally applied nitrogen and phosphorus was calculated mathematically for fertilizer savings by AM application. Soil samples were drawn at tillering stage and immediately processed for soil dehydrogenases activity and was determined colorimetrically (485 nm) using 3% 2,3,5-Tri phenyl tetrazolium chloride (TTC) and incubated at 28°C for 24 h [24].

Statistical Analysis of Data

The data was analyzed using the analysis of variance [25]. The standard error of mean was calculated from the values of each block using Sigmastat (Jandel Scientific Software). The least significant differences (LSD) were used to separate the treatment means using Costat statistical software (Cohort Berkeley, Calif.).

Results and Discussion

Mycorrhizal inoculation by *Glomus intraradices* and inorganic fertilizers application to wheat grown

conventionally (farmers practice) and in beds (raised beds) was observed (Table 2; Fig. 1). The analysis of variance indicated that all plant growth and agronomic parameters on wheat grown under beds and conventional system responded positively to mycorrhizal inoculation. AM inoculation in unfertilized plots under both elevated and flat system produced significantly higher grain yield when compared to uninoculated plots.

The grain yield of AM plants grown at 75% of recommended dose of NPK under beds was found higher (3.84 t ha⁻¹) than in conventional/flat system (100% of recommended NPK) (3.73 t ha⁻¹) although found statistically at par. The results are in concurrent to earlier study conducted under Rice–Wheat Consortium for Indo-Gangetic Plains (CIMMYT-India) showed that wheat planted on raised beds produced 6.4% higher yield when compared to flat planting [4]. Enhanced net returns on wheat planted on beds were also reported [26].

The bed planting for wheat in various parts of world including India (under CIMMYT programme) was found to enhance grain yield by 8.32% (average increase in yield from various trials across the countries) over flat system in addition to savings on irrigation [2]. However, in those trials mycorrhizal fungi were not included.

In the present study, the grain yield and harvest index of mycorrhizal inoculated plants grown in beds at 75% of recommended dose fertilizer application was higher than the yield responses achieved in rest of treatments. However, under flat system the yield of mycorrhizal plants grown at 75% of recommended dose fertilizer was at par with 100% recommended fertilizers applied plots (Fig. 1; Table 2). It is indicated that AM fungi application to wheat planted on beds with 75% recommended NPK fertilizers seems to be beneficial in terms of increasing yield and saving fertilizer inputs. The savings on external application of nitrogen (N) and phosphorus (P) due to mycorrhiza to produce maximum yield seems to be more in beds than in conventional system. It was found that plants grown on beds at 75% fertilizer application can save 8.47 kg P and 16.95 kg N ha⁻¹. Whereas, while comparing the yield response with 100% fertilizer application alone, AM inoculation at 75% fertilizer application was found to save 20.30 kg P and 40.60 kg N ha⁻¹ without sacrificing the yields. On the other hand, the correspondence saving on N and P inputs due to AM inoculation in conventional system was found to have 5.38 kg P, 10.75 kg N ha⁻¹ at 75% fertilizer application. Whereas to produce similar yield (3.73 t ha⁻¹) plants grown at 100% fertilizers requires additional 15.79 kg P and 31.59 kg N ha⁻¹ application. The higher response of AMF in beds could be due to developing higher AMF hyphal net work and better exploitation of soil pool resources [27]. In a 2 year wheat–oat rotation, mycorrhizal root colonization was found to be

Fig. 2 Effect of arbuscular mycorrhizal fungi and fertilizers on shoot P concentration (ppm) of wheat grown under raised bed and conventional/flat system in a sandy loam Alfisol (analyzed at harvest). The bars of treatment followed by *same letter* did not differ significantly by Duncan's multiple range test (DMRT; $P = 0.05$); *LSD* least significant difference by ANOVA

Fig. 3 Soil dehydrogenases (TPF $\mu\text{g/g soil/24 h}$) of various plots of wheat as influenced by application of arbuscular mycorrhizal fungi and fertilizers under raised bed and conventional/flat system in a sandy loam Alfisol (analyzed at 45 DAS). The bars of treatment followed by *same letter* did not differ significantly by Duncan's multiple range test (DMRT; $P = 0.05$); *LSD* least significant difference by ANOVA

mycorrhizal plants grown at 75% fertilizers which means that efficient uptake due to AMF was obtained at 75% fertilizers. The higher P uptake of plants without appreciating higher yield could be attributed to low P utilization efficiency by which plants utilizes the luxury consumption of P. To explain the ability of AM fungi to enhance P in plants grown in beds at 75% fertilizers could be due to

extension of external hyphae which decreases the distance of diffusion for P ions to mycorrhizal roots [30]. The soil dehydrogenase enzymatic activity recorded at tillering stage (45 days after germination) showed higher activity in mycorrhizal plants (Fig. 3). The soil dehydrogenase activity did not influence due to fertilization. However, in general AMF plants grown in elevated beds did show higher activity. Another study where in a long-term tillage system under sorghum cultivation observed that no tilled soil had higher activity of soil dehydrogenase enzyme besides water soluble C, aggregate stability and other enzymes than tilled soils [31]. However, mycorrhiza was not included in their study. The higher activity in mycorrhizal plots of beds observed in the current study could be due to formation of soil aggregates as glycoprotein, glomalin formed due to AM's extra radical mycelium [8], which contributes to higher microbial activity.

In general irrespective of P-fertilization, AM colonization increased over non-inoculated plants in both the systems it could be due to since the sites are not under long-term fertilization. However the plants grown on beds irrespective of inoculation and fertilization showed significantly higher mycorrhizal root colonization than the plants grown in conventional system (Table 2). On the other hand, under raised system due to minimum tillage (no-tillage; NT), the AMF filament network left intact from the previous growing season can readily serve as AMF inoculum and colonize roots of germinating seedlings. In another study, while working on wheat cultivars to mycorrhizal responsiveness has found that mycorrhizal colonization is not an appropriate indicator of mycorrhizal responsiveness [32]. No relationship between root colonization and growth and dry matter in grasses was also observed [33]. Very recently a relationship between soil P and indigenous AMF colonization was established and found a threshold level of soil P for beneficial AMF colonization [34]. They suggested that the optimum level of soil P should be considered to manage indigenous AMF for getting higher wheat yield.

The enhanced growth, yield and P uptake in mycorrhizal wheat plants observed here demonstrates the potential of mycorrhizal inoculation and reduce external application of fertilizers on wheat grown under raised beds. However, the AMF response beyond 75% of recommended fertilizers under both the systems need to be evaluated rigorously since there is moderate benefit derived from the symbiosis beyond 75% of recommended dose of specially nitrogen and phosphorus under present set of conditions.

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