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Arbuscular Mycorrhizal Fungi Alleviate Ozone Stress on Nitrogen Nutrition of Field Wheat

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ABSTRACT

The nitrogen (N) nutrition, crop yield, and responses of wheat to arbuscular mycorrhizal fungi (AMF) were tested in an experimental field under free-air ozone concentration [O_3] enrichment (FACE) conditions. The experiment included three treatments: ambient [O_3] (Ambient), elevated [O_3] (FACE, targeted at ambient [O_3]×1.5), and elevated [O_3] inoculated with an AMF consortium consisting of several *Glomus* species (FACE+AMF). AMF inoculation responsiveness of wheat was estimated by comparing plants grown in unsterilized soil inoculated with the exogenous AMF and in untreated soil containing indigenous AMF. Compared with the Ambient, relatively higher N contents but lower shoot biomasses of wheat plants were observed in the FACE treatment without AMF inoculation from the tillering stage in February and heading stage in April, respectively, which significantly (P< 0.05) decreased grain yield by 28% at harvest in June. Under the FACE condition, compared with the non-inoculated treatment, AMF inoculation significantly (P< 0.05) increased root colonization rates both at the tillering stage and heading stage, and also significantly (P< 0.05) increased shoot biomass at the heading stage and, hence, significantly (P< 0.05) increased grain yield by 40% at harvest. However, AMF inoculation significantly (P< 0.05) decreased total N content in wheat shoots at the tillering stage, suggesting that AMF consortia may enhance plant tolerance to elevated [O_3] by elevating root colonization rate rather than plant total N content at early growing stages.

Keywords: AMF consortia, Crop yield, Free-air ozone concentration enrichment (FACE), Nitrogen content, Soil urease activity.

INTRODUCTION

Surface ozone (O_3) is a secondary pollutant formed by photochemical reactions involving nitrogen oxides, volatile organic compounds, and carbon monoxide. Surface O_3 concentrations ([O_3]) have increased during the last decades and will continue to rise in the coming years (Chameides *et al.*, 1994). Being toxic to plants at concentrations as low as 30 nl L^{-1} (Karlsson *et al.*, 1995), high [O_3] have long been known to affect physiological and biochemical processes as well as biomass growth and yield of agricultural crops (Shi *et al.*, 2009). It is well documented that chronic exposure to elevated [O_3] causes a range of adverse effects on plants including

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reduced photosynthetic activity, altered carbon allocation, diminished biomass accumulation, reduced yield and accelerated senescence, with or without visible injury (Feng *et al.*, 2011). In agricultural ecosystems, wheat (*Triticum aestivum* L.) is the world's most important cereal crop in terms of both cultivated area and total production, and the demand for wheat will continue to increase in the coming decades due to the population growth and cropland losses. However, a meta-analysis of 53 peer-reviewed studies showed that elevated [O_3] (averaged 73 nl L^{-1}) decreases leaf photosynthetic rate by 20% and grain yield by 29% in wheat plants compared with those grown in carbon-filtered air (Feng *et al.*, 2008), suggesting that decreased photosynthesis was a key factor driving yield loss in wheat exposed to elevated [O_3]. Enhancing the resistance of wheat plants to high surface [O_3] is, therefore, of crucial importance for food security of the world in the near future.

Arbuscular mycorrhizal fungi (AMF), as ubiquitous mutualists found in both natural and agricultural ecosystems, provide a direct link between soil and roots, and are renowned for their ability to increase plant mineral nutrients (Smith and Read, 2008; Lakshmipathy *et al.*, 2012) and enhance plant resistance and tolerance against biotic and abiotic stresses, such as salinity stress, desert condition, heavy metal contamination, and soil-borne pathogens (Hu *et al.*, 2010). Responsiveness of host plants to mycorrhizal colonization, in terms of improved biomass growth and/or plant nutrition, has been widely reported. However, few experiments have studied relationships among plant, AMF, and O_3 stress (McCool and Menge, 1984; Duckmanton and Widden, 1994). Recently, Wang *et al.* (2006b) found that AMF could mitigate the damage of elevated [O_3] on horsebean (*Vicia faba* L.), suggesting the use of AMF for mitigating O_3 pollution. In a previous wheat study with O_3 fumigation in the field, the elevated [O_3] significantly decreased grain yield with a relatively higher content of total N, which was not significantly affected by inoculating a single AMF strain (Hu *et al.*, 2009). As AMF occur as communities in soil and in roots, they are likely to collectively contribute to plant growth (Jakobsen *et al.*, 2001); thus, the use of AMF consortia rather than single AMF species should be considered in the future investigation under elevated [O_3]. In addition, it should be helpful to explore the under-ground mechanisms for alterations of N supply capability in the soils. As an important extracellular induced enzyme, urease drives N cycling by catalyzing the hydrolysis of urea-type substrates (Bremner and Mulvaney, 1978) thus, testing soil urease activity may be useful for evaluating soil capability of both converting organic N into available forms and supplying mineral N that can be assimilated by plants.

In 2007, as a part of the project to estimate O_3 impacts on crop production in East Asia, a free-air [O_3] enrichment (FACE) system was set up in a rice (*Oryza sativa* L.) and wheat rotation field in Jiangsu Province, China. The study was conducted in a fully open-air field without altering microclimatic or biotic environment, to represent our best simulation of the future world under higher [O_3] (Shi *et al.*, 2009). The primary objective of this system was to analyze wheat and rice in their yield responses to elevated [O_3], and the results obtained here should provide the critical information for the impact prediction as well as adaptation strategies of crop production systems under high [O_3] in the near future. In the present study, therefore, we investigated AMF inoculation impacts on plant growth, crop yield, and N-uptake of wheat, as well as mycorrhizal colonization rate and soil urease activity under FACE condition. This work may contribute to our understanding of the impacts of elevated [O_3] on AMF and plant N nutrition, and to developing AMF application schemes based on specific functions in the future.

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MATERIALS AND METHODS

Experimental Site and FACE System

The FACE system was established in Xiaoji Town, Jiangdu County, Jiangsu Province, China (119º42′E, 32º35′N). The soil is classified as Shajiang Aquic Cambosols with a sandy–loamy texture. Relevant soil properties in 2007 prior to inception of the FACE experiment are shown in Table 1. The treatments were ambient [O_3] and elevated [O_3] (targeted at ambient [O_3]×1.5) with three replicate plots of each. Each plot had an area of ca. 240 m^2. The experimental design comprised of completely randomized plots allocated to either ambient or FACE. The FACE plots were separated by at least 70 m from any other plot to avoid cross-contamination. The O_3 fumigation ran from 9:00 am to sunset from March to wheat harvest and from July to rice harvest, except when raining or when the background [O_3] was lower than 20 ppb or higher than 170 ppb. Standard cultivation practices as performed in the region were followed in all experimental plots. For more details of fumigation and fertilization, see Pang *et al.* (2009). Each plot has received the same [O_3] management every year since 2007.

AMF Inoculum Preparation

The tested AMF inocula consisted of several *Glomus* species, including *G. caledonium* (Nicol. and Gerd.) Trappe and Gerdemann, *G. mosseae* (Nicol. and Gerd.) Gerdemann and Trappe, and other *G.* spp., isolated from a fluvo-aquic soil in Hennan Province, China, and were deposited at the Institute of Soil Science, Chinese Academy of Sciences, Nanjing, China. As a mixture of rhizospheric soil containing spores, hyphae, and mycorrhizal root fragments, the AMF inocula were propagated on Sudan grass [*Sorghum sudanese* (Piper) Stapf.] grown in an autoclaved (121°C for 1 hour on 3 successive days) sandy soil in pots for two successive propagation cycles (2 month each). At the same time, the non-mycorrhizal inoculum was also prepared with the same sterilized substratum on which Sudan grass was cultivated under the same conditions. All these inocula were air-dried and sieved (< 2 mm) before inoculation.

Field Experiment and Wheat Harvest

On 20 November 2009, winter wheat seeds were sown with a basic seeding density of 210 plants m^{-2} and a row space of 25 cm in all experimental plots. A micro-plot method was used to determine AMF inoculation impacts on wheat *cv*. Yangmai 16. In each replicate plot of the FACE treatment, two polyvinyl chloride (PVC) pots (24 cm diameter×22 cm depth, without bottom) were inserted ca. 20 cm below the surface of the soil (pot rim was 2 cm above ground). Subsequently, one pot was inoculated with 250 g of air-dried and sieved AMF inocula (+AMF), while the other was inoculated with an equivalent amount of non-mycorrhizal inoculum. Besides, one PVC pot was inserted into the soil in each replicate plot of the ambient treatment, and was also inoculated with an equivalent amount of non-mycorrhizal inoculum. Thus, the experiment included three treatments with three replicates: Ambient, FACE, and FACE+AMF. Fifty wheat seeds were sown into each pot. Fumigation began on 5 March 2010 at tillering stage of wheat and

Table 1. Selected properties of the tested soil.

pH	Organic C content	Total N content	Total P content	Available P content
6.8	1.5%	1.59 g kg^{-1}	1.23 g kg^{-1}	10.4 mg kg^{-1}

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continued to the harvest. On 27th February and 23rd April, respectively, three random samples of wheat plant per pot were collected for the analysis of mycorrhizal colonization, shoot biomass, and N content. On 7th June, all other mature wheat plants were harvested. At each sampling time, soil samples were also collected at a depth of 0–15 cm, and were sieved (< 2 mm), removing plant materials and stones, for the analysis of urease activity.

Mycorrhizal Colonization and Plant/Soil Analysis

Wheat plants were divided into grains (if available), shoots, and roots. Fresh roots were thoroughly rinsed with tap water, and were used for mycorrhizal colonization assessment by the grid-line intersect method (Giovannetti and Mosse, 1980) after clearing with 10% KOH and staining with acid fuchsin (Phillips and Hayman, 1970). Shoots and grains were weighed after oven drying at 70°C for 48 hours. Subsamples of dried and ground samples were taken for immediate sulphuric acid/hydrogen peroxide digestion followed by Kjeldahl digestion to measure tissue N concentration (Lu, 1999). Then, the total N uptake by wheat plants were calculated by multiplying the corresponding biomass (shoots plus grains, if available). Soil urease activity was determined by the method proposed by Kandeler and Gerber (1988) with slight modification, and expressed in units of mg NH_4^+-N produced g^{-1} soil d^{-1} and on an oven-dried soil weight basis by correcting for water content in the soil (105°C, 24 hours).

Statistical Analysis

The means and standard deviations of three replicates were computed. An analysis of variance was carried out using the One-ANOVA procedure with SPSS software while the comparison of mean effects was based on least significant difference (LSD) multiple-comparison tests ($P< 0.05$).

RESULTS

Mycorrhizal Colonization Rate

Without AMF inoculation, all wheat plants were colonized by indigenous AMF, and the average mycorrhizal colonization rate varied within 14–25% (Figure 1-a). Compared with

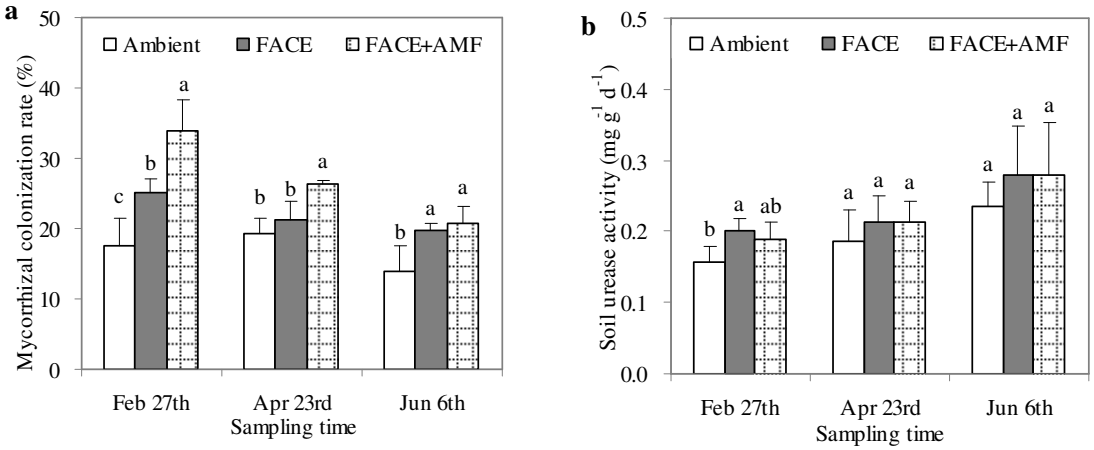

Figure 1. Mycorrhizal colonization rate (a) and soil urease activity (b) with or without arbuscular mycorrhizal fungi (AMF) inoculation under elevated surface [O_3]. Vertical *T* bars indicate standard deviations. Bars not topped by the same letter within the same sampling time indicate a significant difference in values ($P< 0.05$).

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the ambient one, significantly (P< 0.05) higher colonization rates were observed in the FACE treatment on both 27th February and 6th June. Under the FACE condition, compared with the non-inoculated treatment, AMF inoculation increased colonization rate significantly (P< 0.05) on both 27th February and 23rd April.

Soil Urease Activity

Without AMF inoculation, the average soil urease activity varied within 0.158–0.230 mg NH_4^+-N g^{-1} soil d^{-1} (Figure 1-b). Compared with the ambient one, a significantly (P< 0.05) higher soil urease activity was observed with the FACE treatment on 27th February, and a trend towards higher soil urease activity was also observed with the FACE treatment during the whole growing season. Under the FACE condition, compared with the non-inoculated treatment, AMF inoculation had no significant effects on soil urease activity, except for a decreasing trend on 27th February.

Wheat Shoot Biomass, Grain Yield and N Uptake

Without AMF inoculation, FACE treatment had relatively lower shoot biomass of wheat plants on both 23rd April and 6th June, and the grain yield was significantly

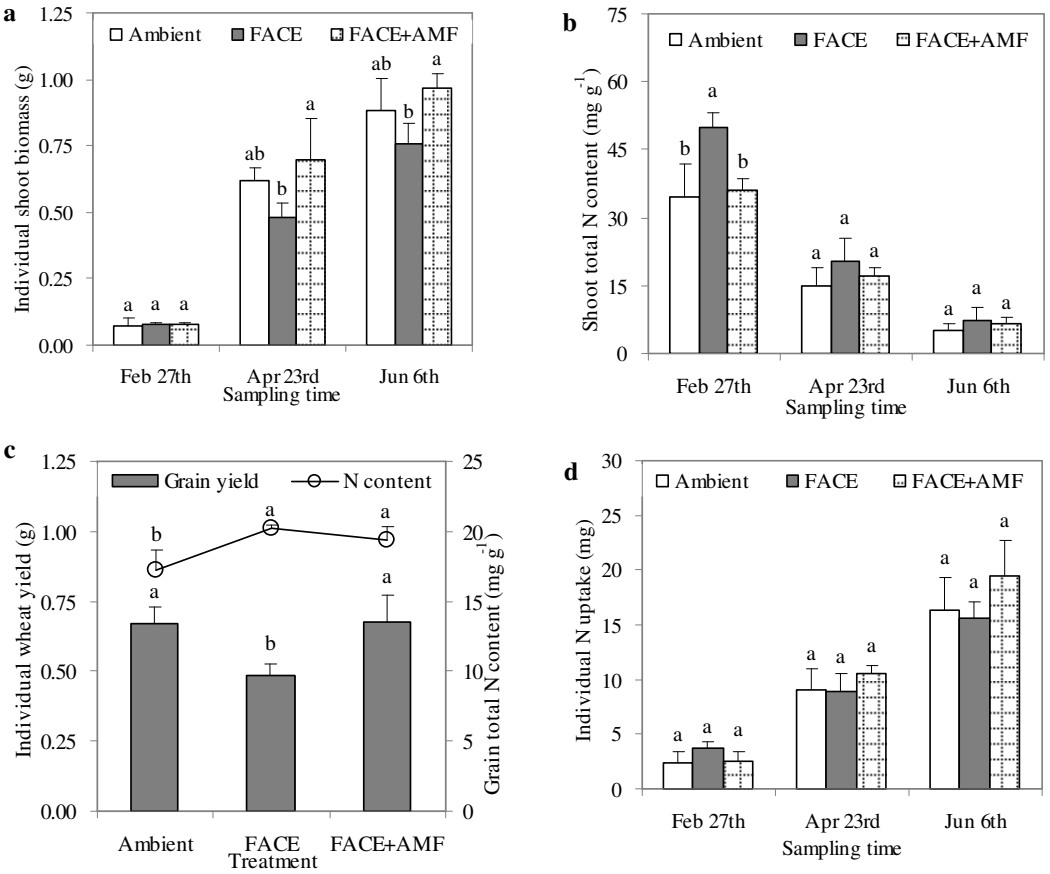

Figure 2. Shoot biomass (a) and total N content (b), grain yield and total N content (c), and total N uptake of wheat plant (d) with or without arbuscular mycorrhizal fungi (AMF) inoculation under elevated surface [O_3]. Vertical *T* bars indicate standard deviations. Bars not topped by the same letter within the same sampling time indicate a significant difference in values (P< 0.05).

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(P< 0.05) decreased by 28% compared with the ambient one (Figure 2). On the contrary, compared with the ambient one, FACE treatment had significantly (P< 0.05) higher total N contents in wheat shoots on 27th February and in wheat grains on 6th June, and there were no significant difference in total N uptake of wheat plants between FACE and the ambient treatments during the whole growing season. Under the FACE condition, compared with the non-inoculated treatment, AMF inoculation significantly (P< 0.05) decreased total N content in wheat shoots on 27th February, but significantly (P< 0.05) increased shoot biomasses on both 23rd April and 6th June, and also significantly (P< 0.05) increased grain yield by 40% on 6 June. Both the total N content in wheat grains and the total N uptake by wheat plants were not significantly affected by AMF inoculation.

DISCUSSION

The direct effects of elevated [O_3] on plant biomass was first observed at the heading stage, and average crop yield was decreased by 28% at harvest, while N content in grains was greatly increased by the elevated [O_3]. It seemed that elevated [O_3] led to physiological regulation such as increasing N nutrition of wheat plants to enhance their tolerance to high [O_3]. Although the elevated [O_3] contributed to higher contents of amino acid and protein in wheat grains, it resulted in a significant decrease of starch content (Bueeker, 1994; Guo *et al.*, 2001), decreasing wheat grain quality. Such requirement may be due in part to the increases of respiration costs in plant leaves during detoxification and remediation of stresses caused by elevated [O_3] (Polle and Pell, 1999). The increased soil urease activity under elevated [O_3] was consistent with the hypothesis of higher N uptake. However, before the seasonal fumigation of O_3, total N content in wheat shoots was significantly higher in the FACE treatment than in the ambient one at the tillering stage. In other words, soil biochemical properties had been affected negatively after three years of O_3 fumigations, and below-ground changes could have important feed-back effects on plant growth. Although the above-ground biomass was removed from the field after every growing season, the responses of below-ground processes to elevated [O_3] were likely to be continued.

Since O_3 does not penetrate into soil (Turner *et al.*, 1973), direct effects of elevated [O_3] on the components of soil ecosystems are unlikely, therefore, the effects of elevated [O_3] on the below-ground system are likely to be mediated indirectly through altering plant processes. Elevated [O_3], in turn, is known to decrease net photosynthesis, dry matter production and C allocation to roots (Andersen, 2003). Due to these changes, both rhizodeposition and soil microbial processes could be altered or modified (McCrady and Andersen, 2000; Andersen, 2003). Along with changes in biomass production, C allocation and rhizodeposition, elevated [O_3] may lead to significant alterations in nutrient cycling and, therefore, the whole N cycling process may be affected (Kanerva *et al.*, 2006). Although the rate of N cycling was observed to increase, there were no significant difference in total N uptake of wheat plants between FACE and the ambient treatments during the whole growing season, except a trend towards higher total N uptake which was observed with FACE at the tillering stage. It follows that under the same application level of N, the supply capability of N in soils under FACE condition was more limited than that in the ambient fields since the heading stage of wheat. Thus, with the current trend for crop yield improvement and yield sustainability, new approaches beside increasing N content are highly needed to enhance plant tolerance to elevated [O_3].

In order to enhance the tolerance to high [O_3] and/or N uptake of wheat plant, increased mycorrhizal colonization rates were observed in wheat roots with the FACE treatment. However, from our previous

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investigation in the same field in June, 2008, AMF spore density decreased from 486 to 304 per 25 g soil, and total number of AMF species found at the field sites decreased from 20 to 18 (data not published). It follows that inoculation of exogenous AMF may play a great role in enhancing plant tolerance and promoting plant growth by accelerating root colonization. In this study, it is worth noting that AMF inoculation with the FACE treatment significantly decreased shoot N content at the tillering stage, significantly increased shoot biomass at the heading stage, and, hence, significantly increased grain yield by 40% at harvest. It demonstrated that the application of AMF consortia could enhance plant tolerance to elevated [O_3] by increasing root colonization rate rather than elevating plant N content at early growing stages. To the best of our knowledge, this observation has not been reported before. The coexistence of AMF species in the consortia might be facilitated by fine-scale spatial structure of fungal communities (Sharma *et al.*, 2009). Various AMF species may collectively contribute to the transportation or translocation of nutrients, or to host tolerance and resistance (Hu *et al.*, 2010). Therefore, an inoculum rich in AMF diversity is more ecologically beneficial.

However, the mechanisms causing increased plant tolerance upon AMF inoculation in this field experiment are not fully understood, and the applying strategy still needs an in-depth study. Usually, AMF appear to enhance host tolerance by improving root growth and function. Azcón-Aguilar and Barea (1996) have pointed out that the prophylactic ability of AMF could be exploited in cooperation with other rhizospheric microbial antagonists to improve plant growth and health. Thus, it is needed to elucidate the processes involved in interactions among AMF colonization, N acquisition, and O_3 stress. Since mycorrhizal growth responsiveness correlates with AMF genotypes, plants, soil, microorganisms and many other biotic or abiotic factors, optimal conditions under which AMF can play maximum roles must be considered (Wang *et al.*, 2006a). Furthermore, an evaluation of the role of inoculated AMF for plant growth needs to take into account the identification of the colonized AMF in root systems since the root colonization rate may not reflect the mutualistic efficiency of an inoculated AMF in terms of enhancement of host growth and tolerance, especially in unsterilized soils containing indigenous AMF.

CONCLUSIONS

Elevated surface [O_3] significantly decreased vegetative growth and crop yield of wheat plants, but greatly increased root mycorrhizal colonization rate and plant total N content to enhance their tolerance to high [O_3]. In that case, the inoculation of an AMF consortium consisting of several *Glomus* species significantly decreased plant total N content at the tillering stage and, hence, significantly increased plant biomass at the heading stage and also grain yield at harvest. It is suggested that the application of AMF consortia could enhance plant tolerance to elevated [O_3] by increasing root colonization rate rather than elevating plant total N content.

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تاثیر قارچهای میکوریز آربسکولار در کاهش اثر تنش ناشی از اوزن روی تغذیه نیتروژن گندم در مزرعه

ژ. س. چویی، ج. ل. هو، ژ. ج. لین، ف. ی. وانگ، ر. ر. چن، ج. ه. وانگ، و ج. گ. ژو

چکیده

به منظور بررسی تغذیه نیتروژن، عملکرد محصول، و پاسخ گندم به قارچهای میکوریز آربسکولار (AMF)آزمونی مزرعه ای در شرایط غلظت اوزن در هوای آزاد [O_3]و غلظت تغلیظ شده (FACE) انجام شد. این پژوهش سه تیمار داشت که عبارت بودند از: غلظت اوزن در هوای آزاد[O_3]، غلظت افزون شده اوزن (FACE) که در ان غلظت اوزن یک ونیم برابر هوای آزاد بود، و بالاخره تیمار تغلیظ شده اوزن همراه با تلقیح با مخلوطی از گونه های (FACE+AMF) *Glomus*. پاسخ دهی گندم به قارچهای آربسکولار AMFبا مقایسه گیاهان کاشت شده در خاک غیر سترون شده و تلقیح شده با AMF خارجی و خاک تیمار نشده حاوی قارچ های میکوریز آربسکولار موجود در آن بر آورد شد. در مقایسه با شرایط هوای آزاد، در تیمار FACE بدون تلقیح، در مرحله پنجه زنی در فوریه (بهمن–اسفند) مقدار نیتروژن گیاهان نسبتا بیشتر و در مرحله خوشه در آوریل(فروردین–

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اردیبهشت) مقدار زیست توده ساقه(قسمت هوایی) گندم کمتر بود و این شرایط منجر به کاهش معنی دار ($P<0.05$) تولید دانه به میزان ۲۸٪ در مرحله برداشت در ژوئن (خرداد-تیر) شد. در شرایط تغلیظ اوزن و در مقایسه با تیمار تلقیح نشده، در تیمار تلقیح شده مقدار کلونیزاسیون ریشه در مراحل پنجه زنی و خوشه رفتن به طور معنی داری($P<0.05$) بیشتر بود و نیز زیست توده ساقه در مرحله خوشه رفتن بیشتر شد ودر نتیجه در مرحله برداشت، عملکرد دانه به میزان ۴۰٪ افزایش یافت. با این همه، در تیمار تلقیح AMF مقدار نیتروژن کل در ساقه گندم به طور معنی داری کاهش یافت . این نتایج اشاره به آن دارد که تلقیح با مخلوطی از ریسه های AMFمی تواند مقاومت گیاه به افزایش اوزن [O_3]را با افزایش کلونیزاسیون ریشه و نه با افزایش کل نیتروژن در مراحل اولیه رشد زیاد کند.

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