

Mycorrhizal inoculation and nitrogen fertilization affect the physiology and growth of spring wheat under two contrasting water regimes

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Abstract

Aims Most plants benefit from mycorrhizal symbiosis through their improved abilities to take up nutrients and water. Information on the interactive effects of fungal inoculation, nitrogen (N) fertilization and drought on water use efficiency (WUE) and productivity of Canadian wheat varieties is scanty.

Methods In this study, we investigated the effects of arbuscular mycorrhizal fungi (AMF) inoculation, N fertilization and water regime (well-watered (WW) vs water-deficit (WD)) on WUE, phosphorus (P) and N

uptake and growth of spring wheat (*Triticum aestivum* var. Superb) in a greenhouse experiment.

Results The specific leaf area (SLA) of flag leaves was significantly increased by AMF inoculation under the two water regimes but was lowered under N fertilization and drought conditions. The AMF inoculation significantly enhanced relative water content under WD. The WUE and instantaneous WUE (WUE_i) were enhanced by N fertilization and AMF inoculation under both water regimes. Combined N fertilization and AMF inoculation significantly increased N concentrations in stem and grain, plant height, biomass and grain yield under WD. The P concentrations in stem and grain were increased under WD, irrespective of other treatments applied. The WUE and grain N, stem N, and P concentrations were positively correlated.

Conclusions Plant physiological characteristics were negatively affected by WD, while N fertilization and AMF inoculation enhanced plant performance under WD, including the increase of N and P concentrations in different components of spring wheat.

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Introduction

Wheat (*Triticum aestivum*) is a major crop grown on the prairie in western Canada, a region characterized by

short growing seasons and frequent occurrence of drought and heat stress. In Alberta, wheat is mostly grown under rainfed conditions, where drought can occur at any time during the growing season. In addition to drought, deficiency of nutrients, especially nitrogen (N) and phosphorus (P), is also a major concern for producers on the prairie.

The symbiosis between mycorrhizal fungi and plant roots can promote plant uptake of water and nutrients and improve plant growth (Ho and Trappe 1975; Martin and Plassard 2001). This symbiosis often results in altered rates of water movement into, through and out of host plants, with consequent effects on tissue hydration and plant physiology (Augé 2001).

Mycorrhizal fungi can modify water relations in host plants (Zhang et al. 2013; Nelsen 1987). Leaf water potential is often higher in mycorrhizal plants under drought conditions due to higher water uptake (Augé et al. 1987; Subramanian et al. 1995), which allows such plants to maintain higher water content than non-mycorrhizal plants. Arbuscular mycorrhizal fungi (AMF) associated plants often show higher photosynthetic rates than their non-mycorrhizal counterparts: stomatal conductance (gs), transpiration (Tr) and photosynthesis (Pn) were stimulated by AMF symbiosis about as frequently under non-stressed as under drought conditions (Tawarayama 2003). Different AMF fungi have different effects on stomatal conductance and rate of photosynthesis during drought (Dixon et al. 1994). The AMF symbiosis has been reported to increase the number of photosynthetic units (Allen et al. 1981), photosynthetic storage and export rates (Wang et al. 1989), and water use efficiency (Simpson and Daft 1990).

Previous studies have mostly been focused on the direct effects of different fungal inoculation on plant growth and yield (Gholamhoseini et al. 2013; Wu et al. 2008). The interactive effects of fungal inoculation, drought and N fertilization on the water use efficiency of wheat grown on the Canadian prairie have not been studied. In this study, we used gas exchange measurement and carbon isotope discrimination to measure WUE (Farquhar et al. 1989) and to evaluate the influence of AMF on WUE_i/WUE and N, P uptake of spring wheat under two levels of N fertilization and two levels of water regime in a greenhouse experiment.

Materials and methods

Plant material and fungal inoculation

Spring wheat variety *Superb*, a semi-dwarf high yielding Canada Western Red Spring Wheat variety with an awny head, short and strong straw, large kernel weight, and late maturing, was used for this study.

The mycorrhizal species used in this study was *Glomus intraradices* (Mix MYKE® PRO PS3). The mycorrhizal species was obtained from Premier Tech Biotechnologies (Québec, Canada). The inoculum (1600 propagules.g⁻¹) was mixed with wheat seed at the rate of 10 mL/kg pre-moistened seeds.

Experimental design and growth conditions

The experimental treatments were made up of two levels of N fertilization (0 kg/ha and 180 kg/ha), two soil water regimes (well-watered, WW, where soil water content was maintained at about 95 % field capacity (FC), and water-deficit, WD, where soil water content was maintained at about 40 % FC) and two mycorrhizal inoculations (*Glomus intraradices* inoculation and non-mycorrhizal inoculation). The treatment combinations were arranged in a completely randomized block design with 4 replicates per treatment. Thus, four treatments were set up under WW or WD conditions: control (CK): no N fertilization and non-mycorrhizal inoculation, M: mycorrhizal inoculation with no N fertilization, N: 180 kg/ha N fertilization with no mycorrhizal inoculation, MN: 180 kg/ha N fertilization and mycorrhizal inoculation.

The experiment was conducted in the greenhouse at the Alberta Research Council (now part of Alberta Innovates - Technology Futures) in Vegreville. Plants were grown in pots (21 cm tall and 21 cm diameter) filled with 3.5 kg of soil mix containing field topsoil and peat moss in a 1:3 ratio (v:v). All pots were flushed with 2 L tap water and drained overnight to determine field capacity before seeding.

Eight seeds were sown per pot and were thinned to four seedlings per pot after emergence. After thinning, the surface of each pot was covered with a 2 cm layer of perlite to minimize soil surface evaporation. All pots were then weighed and kept well-watered until the stem elongation stage (BBCH 30, Lancashire et al. 1991) when water-deficit was imposed by withholding watering until the soil moisture content was 40 % FC.

Soil moisture content of pots in the well-watered treatment was kept at 95 % FC. The target soil moisture levels were then maintained for the well-watered and water-deficit treatments until plant maturity.

Pots were weighed every 1–2 days to monitor water use and adjust watering as plant demand increased. Four pots without plants were used to determine the evaporation rate. Fertilizer treatments were carried out after emergence of seedlings by adding N in form of urea at the rate of 180 kg/ha (0.29 g urea per pot) to pots assigned to the N fertilization treatment.

All pots were placed in a greenhouse with a photoperiod of 16 h. Day and night temperatures in the greenhouse were programmed at 25 and 15 °C, respectively. The experiment was seeded at the end of April and harvested at the end of July in 2009.

Physiological measurements

Specific leaf area (SLA) and relative water content (RWC)

The width and length of flag leaves (5 leaves per pot) were measured and used for leaf area calculation at flowering stage. The leaves were then oven dried at 70 °C to a constant weight (about 48 h). The RWC was determined using the flag leaves. Five leaves per pot were cut and weighed immediately and recorded as fresh weight (FW). Then the leaves were placed in a tray lined with moist filter papers and left in the dark in the laboratory over night, they were blotted dry and reweighed to obtain turgid weight (TW). The leaves were then dried overnight at 70 °C and weighed (dry weight, DW). The SLA and RWC were calculated as (Rebetzke and Richards 1999):

$$\begin{aligned} \text{Leaf area} &= 0.8 \times \text{leaf length} \times \text{leaf width} \\ \text{SLA} &= \text{leaf area} / \text{leaf weight} \\ \text{RWC} &= (\text{FW}-\text{DW}) / (\text{TW}-\text{DW}) \times 100 \end{aligned}$$

Instantaneous water use efficiency (WUE_i)

Leaf photosynthesis (P_n) and transpiration (Tr) rates were measured on the flag leaf at the flowering stage using a portable LI-6400 photosynthesis system (Li-Cor, Lincoln, USA) with the following settings: flow rate 500 μmol/s, leaf/block temperature 20 °C, photosynthetically active

radiation (PAR) 1000 μmol/(m² s) and ambient CO₂ concentration 400 mg/kg (ppm). The leaf area was manually entered for each measurement. Six measurements were taken per leaf of all 6 replicates at 15 s intervals after the CO₂/H₂O parameters became stable. The WUE_i was calculated as P_n/Tr, the ratio of photosynthetic rate to transpiration rate (Anyia et al. 2007).

Carbon isotope discrimination

Five leaves (penultimate and flag) were randomly collected per pot and bulked to form a composite sample to measure leaf carbon isotope composition (δ¹³C). All samples were oven dried at 70 °C for 48 h. Dried leaf samples were ground with a Spex Sample Prep 8000D ball mill (Metuchen, NJ, USA) to fine power and analyzed for stable carbon isotope composition using an Iso Prime continuous-flow stable isotope ratio mass spectrometer (GV Instruments Ltd, Manchester, UK) in the Department of Renewable Resources, University of Alberta. Carbon isotope discrimination (Δ¹³C) of leaves was calculated assuming an isotope composition in ambient air of −8.0‰, using the following equation as defined by Farquhar et al. (1989)

$$\Delta^{13}\text{C}(\text{‰}) = [(\delta_a - \delta_p) / (1 + \delta_p)] \times 1000$$

Where δ_a and δ_p are the isotope composition of atmospheric CO₂ and plant samples.

Crop biomass and grain yield

Two plants per pot were harvested at maturity at which time plant height, aerial biomass (biomass) and grain yield were measured, while WUE was determined (WUE was calculated as aerial biomass/total water use from flowering stage to maturity). The grain was also collected and ground for measuring N and P concentrations (described below).

Nitrogen and P concentrations

The grains and stem samples were ground and then digested using the sulfuric acid-hydrogen peroxide digestion procedure. The N and P concentrations in the digest were determined using the Kjeldahl N determination method and phosphomolybdenum blue photometric method, respectively (Perrin 1953).

Statistical analysis of data

All collected data under WW and WD conditions were subjected to analysis of variance using the General Linear Model procedure in SPSS (SPSS software version 16.0, Chicago, Illinois, USA). Differences between means were compared by Tukey's HSD test at $P < 0.05$. The correlations between parameters were analyzed using the Pearson's simple correlation test function in SPSS.

Results

Relative water content and specific leaf area

Nitrogen fertilization and mycorrhizal inoculation significantly increased (12.9 %) the RWC of flag leaves under WW while also significantly increased under WD 17.3 % (Table 1 and Fig. 1a). With regards to the SLA of flag leaf, there were significant effects of N fertilization and mycorrhizal inoculation and water regime (Table 1). The SLA was increased by AMF inoculation while it was lowered under N fertilization and drought (Fig. 1b).

Leaf photosynthesis (Pn), Transpiration (Tr), Water use efficiency (WUE) and carbon isotope discrimination ($\Delta^{13}\text{C}$)

At the flowering stage, the leaf Pn and Tr were higher under WW than under WD treatment (Table 2).

Table 1 Analysis of variance (ANOVA, F values) on the effects of N fertilization and AMF inoculation on relative water content (RWC) and specific leaf area (SLA) of flag leaves at flowering stage of wheat under WW and WD conditions

Water regime	Sources of variation	RWC	SLA
WW	N	25.76**	93.66**
	M	1.55	10.41**
	N*M	0.22	0.30
WD	N	0.19	29.30**
	M	13.87**	11.17*
	N*M	0.38	0.66
ANOVA for water regimes		51.04**	29.47**

N nitrogen regimes, M mycorrhizal inoculation, WW Well-watered, WD Water-deficit

* $P < 0.05$, ** $P < 0.01$

Nitrogen fertilization and mycorrhizal inoculation significantly increased Pn and Tr under WW (Pn increased by 34 % and Tr by 5.1 %, as compared with the control treatments). Similarly, under WD, Pn of leaves was increased by 31 % and Tr by 20.2 % for treatments receiving combined N fertilization and mycorrhizal inoculation as compared with the controls. From flowering to maturity stage, WUE was enhanced under N fertilization and WD with no significant effect from mycorrhizal inoculation (Table 2). Leaf $\Delta^{13}\text{C}$ was slightly lowered under WD, irrespective of mycorrhizal inoculation or N fertilization.

Nitrogen and P concentrations in stem and grain

Stem and grain N concentrations were significantly increased by N fertilization and mycorrhizal inoculation under WW (Table 3). Combined N fertilization and AMF inoculation significantly increased stem and grain N concentrations under WD (Fig. 2a). Nitrogen fertilization significantly increased stem and grain P concentrations under WD while AMF inoculation increased grain P concentration under both water regimes (Table 3). Stem and grain P concentrations were higher under WD than under WW irrespective of other treatments (Fig. 2b).

Stem N, grain N and grain P concentrations were significantly correlated under different N, water and mycorrhizal inoculation treatments (data not shown). There were positive correlations between grain P and N concentrations ($r = 0.50$, $P < 0.05$) and between grain P and stem N concentrations ($r = 0.68$, $P < 0.01$).

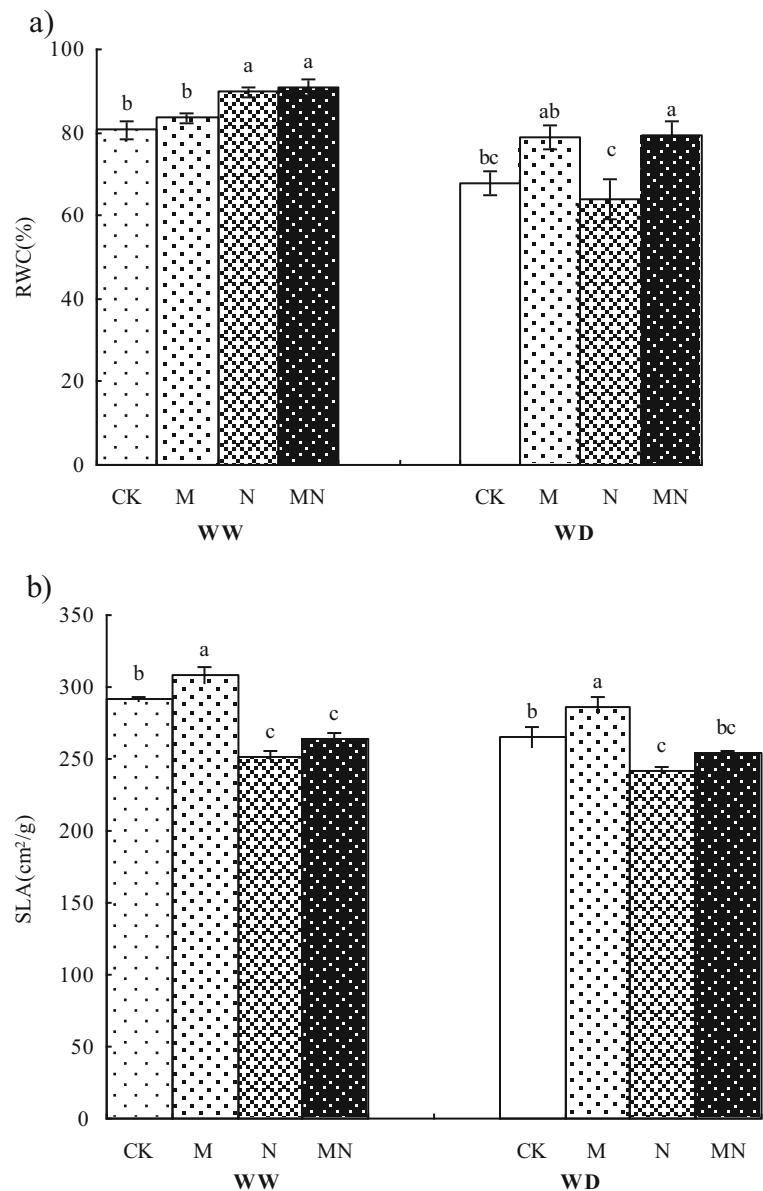
Plant height, biomass and grain yield

Nitrogen fertilization and mycorrhizal inoculation all significantly increased plant height under both water regimes with no interaction among the factors (Table 4). Combined N fertilization and AMF inoculation increased plant height, biomass and grain yield under two water regimes (Fig. 3).

Relationships between WUE and stem and grain N and P concentrations

The WUE and grain N, stem N and P concentrations were significantly positively correlated ($r = 0.43$, 0.59 , 0.49 , respectively; $P < 0.05$) (Table 5). Leaf $\Delta^{13}\text{C}$ was significantly negatively correlated with grain and stem N and P concentrations ($r = -0.60$, -0.51 , -0.49 , -0.46 ,

Fig. 1 Effects of N fertilization and mycorrhizal inoculation under Well-watered (*WW*) and Water-deficit (*WD*) conditions on **a**) relative water content (*RWC*); **b**) specific leaf area (*SLA*) of flag leaves at flowering stage. CK: no N fertilization and non-mycorrhizal inoculation; M: mycorrhizal inoculation with no N fertilization; N: 180 kg/ha N fertilization with no mycorrhizal inoculation; MN: 180 kg/ha N fertilization and mycorrhizal inoculation. Error bars were standard errors



respectively; $P < 0.05$), while WUE_i was only significant correlated with grain P concentration ($r = 0.53$, $P < 0.05$) (Table 5).

Discussion

Plant growth and production

Under drought condition, the effect of AMF inoculation and N fertilization on plant growth and biomass

production was linked with plant morphology. Plant growth and crop yield are particularly dependent on water availability (Omirou et al. 2013), and as expected WD had a detrimental effects on wheat growth and yield in this study (Tables 1 and 3, Figs. 1 and 3). The WD treatment reduced biomass production and grain yield through affecting several physiological parameters. The canopy absorption of photosynthetically active radiation (PAR) may be reduced by a drought-induced limitation of the leaf area

Table 2 Analysis of variance (ANOVA, F values) on the effects of N fertilization and AMF inoculation on leaf photosynthesis (P_n), transpiration (T_r), instantaneous WUE (WUE_i), carbon isotope discrimination (CID) of wheat at flowering stage, and water use efficiency (WUE) from stem elongation stage to flowering stage under WW and WD conditions

Factors		P_n $\mu\text{mol CO}_2/(\text{m}^2 \text{ s})$	T_r ($\text{mmol}/(\text{m}^2 \text{ s})$)	WUE_i ($\mu\text{mol CO}_2/\text{mmol H}_2\text{O}$)	WUE (g/kg)	CID (%)
WW	CK	20.28±0.97	7.21±0.99	2.81±0.15	2.75±0.17	21.76±0.36
	M	21.48±0.85	7.28±0.24	2.95±0.74	3.10±0.10	21.46±0.18
	N	24.87±1.52	7.97±0.80	3.11±0.21	6.61±0.44	22.24±0.39
	MN	27.10±0.62	7.58±0.24	3.56±0.32	6.50±0.53	22.62±0.34
WD	CK	17.27±0.22	5.05±1.09	3.42±0.10	3.13±0.33	20.21±0.40
	M	19.78±0.93	5.08±0.24	3.90±0.12	3.32±0.36	20.20±0.41
	N	19.94±0.99	6.26±0.79	3.30±0.36	6.51±0.80	20.68±0.20
	MN	22.65±3.38	6.01±0.57	3.77±0.39	7.07±0.81	21.36±0.40
Analysis of variance (F Value)						
WW	N	2.06	4.45*	4.75*	101.51**	6.27*
	M	2.31	0.18	2.02	0.12	0.02
	N*M	0.03	0.03	0.55	0.40	1.07
WD	N	20.00**	18.46**	0.01	32.97**	5.55*
	M	6.18**	0.04	0.99	0.37	0.83
	N*M	0.25	0.24	0.03	0.09	0.90
ANOVA for water regimes (F value)		13.51**	9.16**	7.15**	28.03**	30.98**

CK: no N fertilization and non-mycorrhizal inoculation; M: mycorrhizal inoculation with no N fertilization; N: 180 kg/ha N fertilization with no mycorrhizal inoculation; MN: 180 kg/ha N fertilization and mycorrhizal inoculation; WW: Well-watered, WD: Water-deficit. The values presented in the first part of the table are mean±standard errors. The last part of table means the F value

* $P < 0.05$, ** $P < 0.01$

expansion (Guo et al. 1998), which in turn reduces new dry matter production and limit the yield (Stone et al. 2001). The decreased in SLA

and RWC by WD (Fig. 1) in this study is consistent with other research on wheat (Zhang et al. 2010). The principal reason for reduced

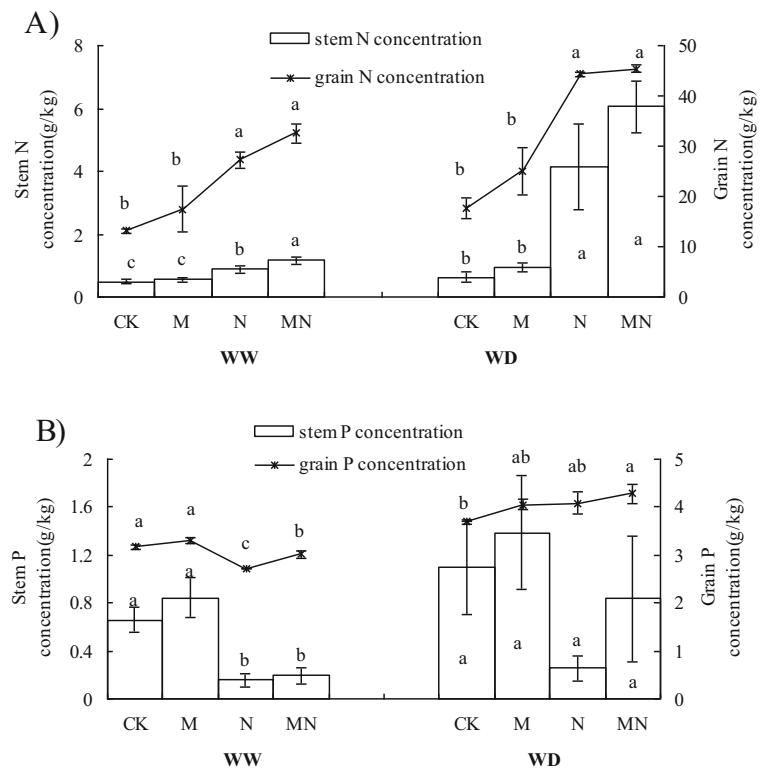
Table 3 Analysis of variance (ANOVA, F values) on the effects of N fertilization and AMF inoculation on N and P concentrations in stem and grain of wheat at maturity stage under WW and WD conditions

Water regime	Source of variation	N concentration		P concentration	
		Stem	Grain	Stem	Grain
WW	N	30.47**	31.97**	28.93**	44.05**
	M	4.61*	4.57*	1.14	15.15**
	N*M	1.40	0.04	0.51	2.41
WD	N	28.88**	81.49**	2.91	3.75
	M	1.92	2.62	1.16	4.82*
	N*M	1.03	1.52	0.13	0.22
ANOVA for water regime		50.49**	40.67**	7.39*	228.05**

N nitrogen regimes, M mycorrhizal inoculation, WW Well-watered, WD Water-deficit

* $P < 0.05$, ** $P < 0.01$

Fig. 2 Effect of N fertilization and mycorrhizal inoculation under Well-watered (*WW*) and Water-deficit (*WD*) conditions on **a)** stem and grain N concentration; **b)** stem and grain P concentration at maturity stage. CK: no N fertilization and non-mycorrhizal inoculation; M: mycorrhizal inoculation with no N fertilization; N: 180 kg/ha N fertilization with no mycorrhizal inoculation; MN: 180 kg/ha N fertilization and mycorrhizal inoculation. Error bars were standard errors



leaf expansion under drought stress is the reduction in leaf cell hydraulic conductivity, which, in turn, decreases water transport and hinders both cell division and enlargement, and leaf development (Gholamhoseini et al. 2013).

Subramanian and Charest (1995) showed that leaf area of corn under AMF inoculation was

higher than that without inoculation, consistent with the higher SLA under AMF inoculation in this study (Fig. 1b). Plant water content can be increased by AMF inoculation (Morte et al. 2000). In this study, AMF inoculation resulted in a 10 % increase in leaf RWC (Fig. 1a). We also observed positive effects of AMF inoculation

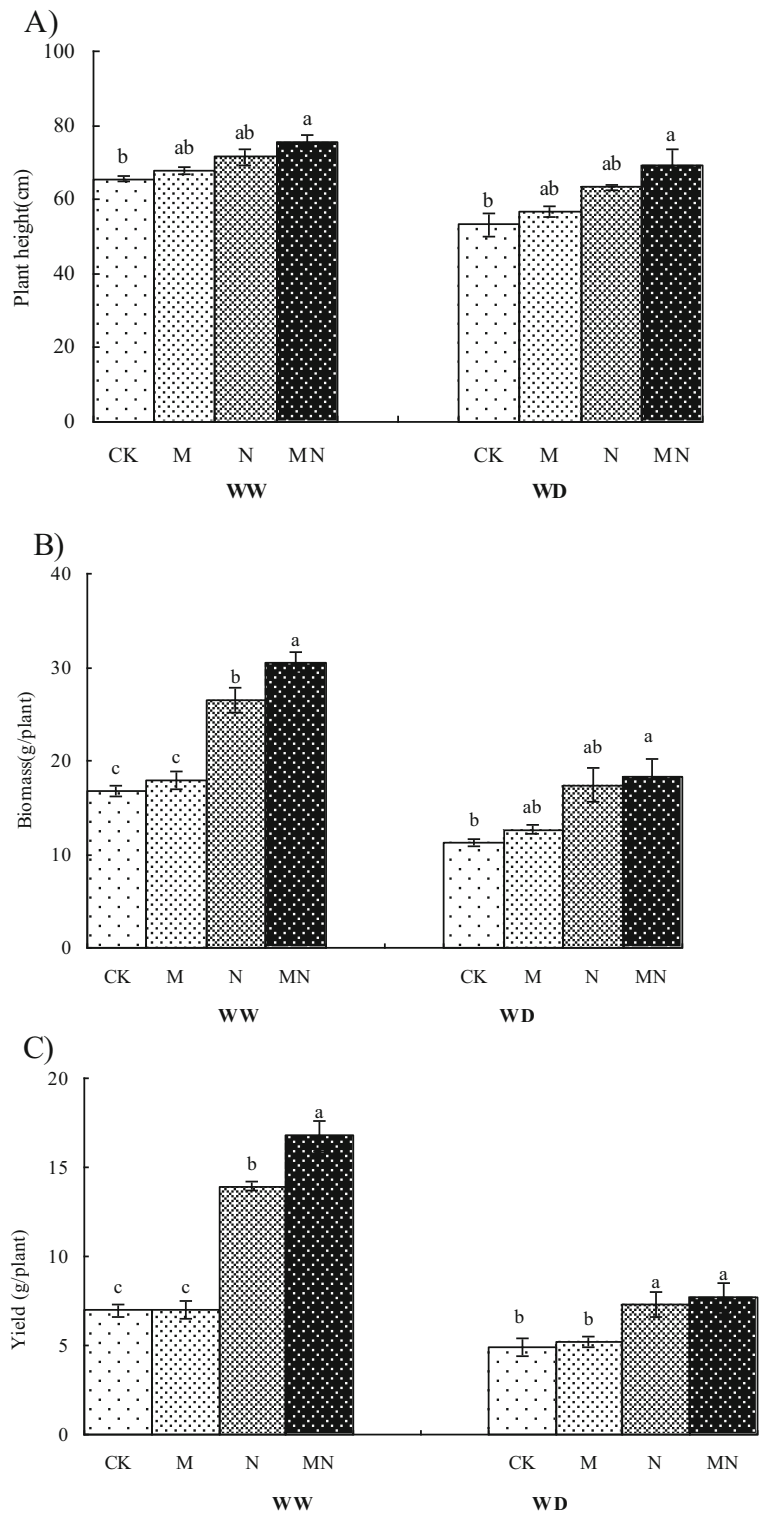
Table 4 Analysis of variance (ANOVA, F values) on the effect of N fertilization and AMF inoculation on plant growth, biomass and yield of wheat at maturity stage under WW and WD conditions

Water regime	Sources of variation	Plant height	Biomass	Yield
WW	N	19.98**	117.23**	254.47**
	M	4.44*	6.44*	7.38*
	N*M	0.52	1.78	7.19*
WD	N	16.40**	15.28**	12.14**
	M	4.99*	5.68*	5.32*
	N*M	0.18	0.04	0.01
ANOVA for water regime		51.07**	124.81**	207.62**

N nitrogen regime, M mycorrhizal inoculation, WW Well-watered, WD Water-deficit

* $P < 0.05$, ** $P < 0.01$

Fig. 3 Effect of fertilization and mycorrhizal inoculation under Well-watered (*WW*) and Water-deficit (*WD*) conditions on **a)** plant height; **b)** biomass; **c)** yield at maturity stage. CK: no N fertilization and non-mycorrhizal inoculation; M: mycorrhizal inoculation with no N fertilization; N: 180 kg/ha N fertilization with no mycorrhizal inoculation; MN: 180 kg/ha N fertilization and mycorrhizal inoculation. Error bars were standard errors



on the biomass and yield of wheat under WW and WD conditions. The positive effects are likely due

to improved N nutrition (Bethlenfalvay et al. 1988; Sweatt and Davies 1984), increased uptake of water by

Table 5 Correlation coefficients (r) among water use efficiency (WUE), instantaneous WUE (WUEi), carbon isotope discrimination (CID), and N and P concentrations in stem and grain

	WUE	WUEi	CID	Grain N	Stem N	Grain P	Stem P
WUE	1						
WUEi	0.22	1					
CID	-0.71**	-0.04	1				
Grain N	0.43*	0.19	-0.60**	1			
Stem N	0.35	0.31	-0.51**	0.78**	1		
Grain P	0.59**	0.53**	-0.49**	0.50**	0.68**	1	
Stem P	0.49**	0.14	-0.46*	-0.28	-0.26	0.28	1

* $P < 0.05$, ** $P < 0.01$

hyphae (Faber et al. 1991) and overall enhancement of plant growth (Bryla and Duniway 1997).

Water use efficiency

The WUE and WUEi of spring wheat were greater under WD than under WW (Table 2), consistent with results on cowpea (Anyia and Herzog 2004) and barley (Anyia et al. 2007; Chen et al. 2013). Under WD, gs and leaf Tr were markedly reduced but leaf Pn was slightly decreased and WUE increased.

Under WD, WUE was significantly higher in inoculated plants, indicating that AMF inoculation was effective in improving plant performance under a drought condition. Mycorrhizal inoculation of plants has been previously reported to increase the ability of crops to mitigate the negative effects of water stress by increasing WUE (Kaya et al. 2003). Similarly, in the current study, WUE increased ($P < 0.05$) by 10 % in inoculated than in non-inoculated plants grown under water stress (Table 2). AMF inoculation helps to improve water uptake by roots through the formation of extensive network of hyphae on roots.

Drought stress decreased leaf $\Delta^{13}\text{C}$ while AMF inoculation increased leaf $\Delta^{13}\text{C}$ (Table 2). Yasira et al. (2013) also reported a decrease in $\Delta^{13}\text{C}$ under limited irrigation in 49 different wheat varieties. Significant negative association between $\Delta^{13}\text{C}$ and WUE was observed in our experiment (Table 5), consistent with the theory of Farquhar et al. (1982) that stated that $\Delta^{13}\text{C}$ and WUE are negatively correlated in C_3 plants.

Plant uptake of N and P

The AMF inoculation increased stem and grain N and P concentrations, consistent with the results of Attia and Nemat (2003). In this study, inoculation of plants with AMF under WD increased N and P concentrations in stem and grain (Fig. 2). Increased transport of N and P by external AM fungal hyphae to maize has been reported under drought condition (Subramanian and Charest 1999). Similar to past studies, N and P acquisition and assimilation were affected after AMF inoculation regardless of N addition in our research. The significant positive correlation between P concentration in stem and N concentration in stem and grain likely reflected the synergistic effect of AMF on N and P uptakes (Tawaraya et al. 2007).

Conclusions

As water increasingly becomes a limiting factor in crop production in many regions, AMF inoculation is a promising strategy to improve wheat production under rainfed conditions. In this study, AMF inoculation enhanced N and P uptake and increased WUE and yield of wheat under WD in a greenhouse experiment. The WUE of plants grown under WD was significantly higher in inoculated plants, indicating that AMF inoculation was effective in improving plant performance under WD. The positive correlations between WUE and grain N, stem N and stem P concentrations suggests that these traits are closely related and yield enhancement by N fertilization and AMF inoculation was likely a combination of positive effects on WUE and nutrient uptake. However, further research under field conditions

and with more genotypes will be needed to evaluate and extend the results.

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