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Interaction of Different Wheat Genotypes and Nitrification Inhibitor 3,4-Dimethylpyrazole Phosphate Using ^{15}N Isotope Tracing Techniques

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ABSTRACT

To investigate the effect of applying ^{15}N-labeled ammonium sulfate with or without a nitrification inhibitor 3,4-dimethylpyrazole phosphate (DMPP) on fertilizer use efficiency and crop productivity of different wheat genotypes, a field trial was conducted at the Nuclear Agricultural Department's farm of Iran in 2013–2014. The treatments included five wheat genotypes with different 13 C isotope discrimination and three fertilizer treatments, an unfertilized control, ^{15}N-labeled ammonium sulfate, and ^{15}N-labeled ammonium sulfate with DMPP in three replications. Soil samples were taken after 2, 4, and 6 weeks after sowing and also at harvest time. Results from ^{15}N experiment showed that DMPP delayed nitrification of ammonium for 42 days. Genotypes with lower discrimination index had greater uptake of ammonium ions which led to increase crop yield and nitrogen fertilizer use efficiency. The results also suggested that the use of DMPP may not be beneficial in some fast growing wheat genotypes.

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13C isotope; ammonium; fertilizer use efficiency; nitrate; nitrification inhibitor; nitrogen-15

Introduction

Among the essential plant nutrients, nitrogen (N) is the key component of all farm systems because all plants require it in large amounts for protein synthesis and virtually for all aspects of plant growth. Thus, N plays a major role in crop productivity and profitability on farm as well as controlling the diversity, dynamics, and functioning of many terrestrial, freshwater, and marine ecosystems. However, N is highly dynamic and a mobile element, which makes its use efficient and management challenging. Nitrogen inputs under most farm systems come predominantly from chemical fertilizers with the current annual N fertilizer use of 110 million tonnes worldwide; however, less than 40% of fertilizer N is utilized by plants while the remaining N is either lost to the atmosphere and ground water (FAO 2015). Nitrogen fertilizer can thus potentially contribute N to a wider environment as gaseous emissions of nitrous oxide (N_2O), a potent greenhouse gas, and ammonia (NH_3) that damage sensitive aquatic and marine ecosystems, and nitrate (NO_3^-) leaching that damages water quality, ecosystems, and also pose human health risks. In addition, such heavy N losses also have economic implications (FAO 2015).

After surface application, ammonium-based chemical fertilizers undergo quick nitrification within 1–2 weeks (Zaman et al. 2009) by chemolitho-autotrophic bacteria leading to a high

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concentrations of NO_3^- around the fertilizer granule. Nitrate would be repelled from soil exchange sites because of its negative charge and is prone to both leaching losses if drainage occurs and would be emitted to the atmosphere as N_2O via denitrification. Various attempts have been made to enhance fertilizer use efficiency by coating or treating N fertilizers with N process inhibitors including urease and nitrification inhibitors. Nitrification inhibitors such as dicyandiamide (DCD), nitrapyrin,[1] and 3,4-dimethylpyrazole phosphate (DMPP) slow the activity of Nitrosomonas, the genus of nitrifying bacteria responsible for the oxidation of NH_4^+ to NO_2^- and can thereby reduce NO_3^- leaching, N_2O emissions and increase crop productivity (Abbasi and Adams 2000; Acevedo 1991; Merah et al. 2001; Salazar Sosa et al. 1998; Sayre, Acevedo, and Austin 1995). DMPP developed by BASF (Germany) and has several advantages over DCD and nitrapyrin. Lower application rates (0.5–1.0 kg of the active compound ha^{-1}) are needed to achieve optimal nitrification inhibition to reduce N_2O emissions and NO_3^- leaching (Merah et al. 2001; Olson and Swallow 1984). After application, DMPP remains persistent in soil and effective by inhibiting nitrification at 5 °C; however, at 20 °C, the inhibitory effect from DMPP lasts only for 40 days (Merah et al. 2001). In contrast to DCD, DMPP is relatively immobile in the soil, stays close to where the NH_4^+–N is adsorbed, and thus less prone to leaching losses (de Campo et al. 1998; Wahbi and Shaaban 2012).

Extensive research has been done on the application of different nitrification inhibitors worldwide in the past 25 years. However, there is a lack of evidence on inhibition effect of DMPP for increasing yields and N fertilizer use efficiency in different wheat genotypes. In this regard, the most important indicator is selection of wheat genotypes with response to nitrification inhibitors. It seems that "water use efficiency" (WUE) considered as influential variable for separating species. Due to difficulties of measurement of WUE in wide ranges of wheat nursery, the carbon isotope discrimination (CID) index was used as a substitute indicator for selection of genotypes with different ability of yield productivity (instead of WUE). Therefore, the main basis of the hypothesis has been established on high correlation indicator of CID ($\Delta^{13}C$) and these traits.

Based on the theoretical aspect, $^{13}C/^{12}C$ isotope ratio in plant tissues is less than atmospheric carbon dioxide (CO_2). This means that plants discriminate against $^{13}CO_2$ during photosynthesis. It can be used as an indicator for physiological studies on different wheat varieties (Cregg and Zhang 2000; Iqbal et al. 2005; Monneveux et al. 2005; Tokatlidis et al. 2004). In this respect, factors affecting plant photosynthesis and mass movements of different carbon isotopes ($^{13}CO_2/^{12}CO_2$) through leaf pores and its correlation with WUE (and subsequently wheat yield production) are considered as remarkable issues to identify plant physiology, dealing with stress condition in recent decades. The positive correlation between CID such as ^{13}C discrimination index ($\Delta^{13}C$) and wheat yield production help selecting suitable genotype for enhanced crop productivity and WUE under a particular agroecological condition. Many studies in Mediterranean environments reported significant positive correlation between $\Delta^{13}C$ and yield production (Results of various studies in the Mediterranean environments has proved positive correlation between $\Delta^{13}C$ and yield production (Acevedo 1991; Britto and Kronzucker 2002; Merah et al. 2001; Morgan et al. 1993; Nachit 1998; Nachit and Elouafi 2004; Sayre, Acevedo, and Austin 1995; Wahbi and Shaaban 2012). However, some areas also reported a negative correlation between $\Delta^{13}C$ and leaf and grain yield (IAEA 2012)). In accordance with WUE, both positive and negative correlations were reported between grain WUE and leaf and grain Δ, depending on water regime and the plant population under study (IAEA 2012). According to present study, which was carried out over 150 selected genotypes from Dry Land Agricultural Research Institute (DARI), negative correlation ($r = -0.534$) was found between grain production and Delta (statistically significant at 1% level). This means that plants with low CID (index) have physiological characteristics associated with water stress conditions, such as more developed rooting system (to optimize the efficiency of water use, in the present study). This will be effective in absorption of ammonium and nitrate ions (as well as preferential adsorption process).

Delay in the process of converting ammonium to nitrate (through DMPP application) may have two effects. The first effect may lead to more absorption of ammonium by plants. In this regard, genotypes with preferential uptake of ammonium may have greater development in terms of growth

and yield production. This may be due to charge balancing and proton release in the root zone. The result makes rhizosphere more acidic and consequently improves the availability of micronutrients (such as iron, copper, manganese, and zinc) and plant growth is increased. In the second approach, inhibition duration will be considered.

In cold and semi-cold areas of drylands in the North West of Iran, wheat planting takes place in early October. In these regions, N fertilizer applied along with seeds planting (to reduce number of tillage and labor costs). If the conversion of ammonium to nitrate (through DMPP application) delayed for 45 days, due to low temperatures (less than 6 °C) in early December, ammonia absorbed by surface layers of clay (and soil colloids) and consequently remains in the soil. Thus, due to very low requirement for fertilizer in winter, all ammonium ions remain in soil (till the end of winter frost) and by minimizing NO_3^- leaching during the wet winter period, fertilizer use efficiency will be significantly high. Therefore, this study evaluated the effect of DMPP on fertilizer use efficiency and crop productivity of different winter wheat genotypes using different ^{13}C isotope discrimination index under field conditions.

Materials and methods

Experimental soil

A field experiment was conducted during 2013–2014 to evaluate the effect of applying ammonium sulfate $(NH_4)_2SO_4$ with DMPP for five wheat genotypes (with different carbon-13 isotope discrimination or $\Delta^{13}C$ indexes) at Nuclear Science and Technology Research Institute, Karaj, Iran. Study area has a mean annual rainfall and evaporation of about 247 and 2184 mm, respectively. The mean annual air temperature is 14.4 °C and the average relative humidity is 53%. The climate is semiarid with relatively cool winter and summer. Experimental field was not planted in the past few years. The soil is classified as Typic Calcixerepts with sandy clay loam texture (sand = 58.7%, silt = 20.10%, and clay = 21.2%) and samples were analyzed for key soil properties (Table 1).

Experimental design

Experimental design was factorial randomized complete block with three replications. The fertilizers treatment included: control (N), regular $(^{15}NH_4)_2SO_4$ fluoride (F), and $(^{15}NH_4)_2SO_4$ with DMPP (FD). Five wheat genotypes with different CID indexes were selected from genetic resources center of Iranian DARI. The genotypes treatment included G1 (high $\Delta^{13}C$ index), G2 (to some extent high $\Delta^{13}C$ index), G3 (medium $\Delta^{13}C$ index), G4 (to some extent low $\Delta^{13}C$ index), and G5 (low $\Delta^{13}C$ index). Forty-five plots (each plot of 3.6 m^2 area [1.8 m × 2 m] with 1 m border) were established. Three lines in each plot were planted with a spacing of 0.6 m. Seeds were sown at a distance of 5 cm and a density of 120 kg ha^{-1} on October 2014. To measure fertilizer use efficiency, subplots 0.6 m^2 area (0.6 m × 1 m) were established within each main plot. Labeled ammonium sulfate with 4.637 at% ^{15}N enrichment with or without DMPP were applied at rate 120 kgN ha^{-1}. In order to maintain compatibility of soil moisture

Table 1. Key soil properties.

Soil depth	Soil texture %			FC	PWP	SP	pH	EC	TDS	OC	CCE	
cm	Sand	Silt	Clay	%	%	%	*	mS	mg/L	%	%	
0–20	58.7	20.1	21.2	16.8	9.5	26.8	7.8	0.6	375	0.42	16.0	
20–40	61.2	20.0	18.8	16.5	9.2	26.0	7.7	0.7	417	0.25	16.6	
Soil depth	total N	CO_3^-	HCO_3^-	SO_4^-	Ca	Mg	Cl	P	K	Mn	Cu	Zn
cm	%	meq/L							ppm			
0–20	0.04	0.2	1.33	2.88	5.54	4.42	1.5	7.81	125	2.8	0.4	0.8
20–40	0.03	0.2	1.57	3.33	6.10	5.00	1.7	5.90	100	3.1	0.4	0.9

*Saturated paste; FC: field capacity; PWP: permanent wilting point; SP: saturation percent.

Table 2. Key isotopic characteristics and classification of the genotypes base on their water use efficiency traits.

Genotype	Variety/Line	%N	$\delta^{15}N$**	%C	C·N	$\delta^{13}C$**	Δ	Class***
G1	Homa	1.94	−1.74	54.89	28.23	−11.39	3.43	B
G2	Ohadi	2.58	−1.08	59.80	23.21	−9.89	1.91	C
G3	Sabalan	2.21	−1.56	50.99	23.06	−9.29	1.30	C
G4	PYN*	2.54	−2.31	62.98	24.77	−4.62	−0.55	A
G5	Zargana-6	2.03	−0.87	59.55	29.31	−7.46	−3.40	A

*Variety/Line: PYN.BAU.F474S10.1.3.ADMIS.
** Delta values (δ), representing deviations per mil (‰) from standard air (for ^{15}N) or Pee Dee Belemnite (PDB) (for ^{13}C), such that sample = $1000[(R_{sample} \cdot R_{standard}) - 1]$, where R is the $^{15}N \cdot ^{14}N$ or $^{13}C \cdot ^{12}C$ ratio in sample and standard.
***Seeds classification for water use efficiency (A: more efficient).

conditions with dryland areas (in North West of Iran), irrigation was performed by 50 mm only one time (just after sowing) (Moslemi, Roustaii, and Rashidi 2012; Tavakkoli 2013).

Nitrogen fertilizer was applied based on soil testing. In this respect, the soil were treated with a basal dose of P ($CaH_4(PO_4)_2 \cdot H_2O$), K (potassium sulfate (K_2SO_4)), Fe (iron sulfate ($FeSO_4$)), Mn (manganese sulfate ($MnSO_4$)), Zn (zinc sulfate ($ZnSO_4$)), Cu (copper sulfate ($CuSO_4$)) and B (boric acid (H_3BO_3)) at 30, 275, 20, 24, 8, 8, and 6 kg pure element per ha, respectively.

Due to the small dimensions of isotopic subplots (0.6 × 1 m) and possibility of transfer ^{15}N material out of it and ensure uniform application fertilizers, both ^{15}N-labeled and non-labeled fertilizers were dissolved in water and spread over the selected area (by hand sprinkler). In relation to the main plot, all chemicals dissolved in 15 L of water and distributed over non-isotopic plants by hand sprinkler. In relation to the isotopic subplot, all chemicals (included ^{15}N labeled fertilizer) dissolved in 3 L of water and distributed over isotopic plants. After 2 days, irrigation carried out over the areas by the hand sprinkler (to reach 50 mm level). Special attention was down to prevent ^{15}N movement out of the selected area.

The DMPP-coated $(NH_4)_2SO_4$ was supplied by Novatec Solub 21–24 (Compo Company, Germany) and concentration of DMPP was 0.8% of fertilizer. To create labeled DMPP, Novatec Solub mixed with labeled ammonium sulfate (9.274 at% ^{15}N) with an equal division. After mixing, the concentration of DMPP and labeled material were halved (0.4% for DMPP and 4.637 atom% for ^{15}N tracer) and consequently, DMPP concentration was calculated as 0.48 kg ha^{-1}.

Key chemical and isotopic characteristics and classification of the selected genotypes presented in Table 2. All results of the isotope carbon (^{13}C) and ^{15}N (at the natural abundance level) presented as delta value (δ) and standard deviations per thousand (‰) compared to standard marine carbonate fossils (PDB) and air standard.

$$\delta_{\text{sample}} = 1000\left[\left(R_{\text{sample}} \cdot R_{\text{standard}}\right) - 1\right]$$

Craig (1957) presented relation between carbon-13 isotope discrimination ($\Delta^{13}C$) and the delta value ($\delta^{13}C$) as follows:

$$\Delta^{13}C = \left(\delta^{13}C_{PDB} - \delta^{13}C_{\text{plant}}\right)/\left(1 + \delta^{13}C_{\text{plant}}\right)$$

where $\Delta^{13}C$ = carbon-13 isotope discrimination, $\delta^{13}C_{PDB}$ = delta value $\delta^{13}C$ in PDB, and $\delta^{13}C_{\text{plant}}$ = delta value $\delta^{13}C$ in plant sample (in terms of parts per thousand).

Plant tissue and soil sample analysis

After harvesting, plant tissues were separated into grain and straw. Bulk fresh weight harvested from each plot was recorded. To determine plant moisture fraction and N uptake, small plant subsamples were prepared randomly from each plot and transferred to pre-weighed paper bags, and dried at 70 C for 7 days.

After drying, plant tissues were ground in a ball mill for total N and for $^{15}N/^{14}N$ isotopic ratio using emission spectrometer. Calculations of ^{15}N recovery in plants were carried out as described by the IAEA (1990).

$$\text{The percentage nitrogen derived from fertilizer } (\%N_{dff}) = \frac{\%^{15}N \text{ excess in sample}}{\%^{15}N \text{ excess in fertilizer}} \times 100$$

$$\text{Nitrogen Fertilizer Use Efficiency} = \frac{(\%N_{dff} \times \text{yield of N})}{\text{rate of N application}}$$

Soil samples taken at four times (i.e., after 2, 4, and 6 weeks of sowing and at harvest) and analyzed for ammonium and nitrate concentrations by micro-diffusion method (Mousavi Shalmani 2008).

Statistical analysis

Statistical analyses including analysis of variance (ANOVA) and multiple comparisons were performed using GenStat-14. Interactions and main effects factors were extracted and interpreted by GGE Biplot multivariate model (Choukan 2011; Yan et al. 2000).

Results and discussion

Effect of DMPP on ammonium–N concentration in the soil

The concentration of ammonium and N in the soil derived from different sources [fertilizers (ANDFF) and soil (ANDFS)] during sampling period presented in Figure 1. When regular ammonium sulfate applied to all genotypes, almost all ammonium ions derived from fertilizer source converted to nitrate (Figure 1a). This process contributes to successful conversion of ammonium to nitrate by nitrification process. The results were quite different when the ammonium sulfate applied to all genotypes with DMPP. By adding DMPP, ANDFF was at its maximum level until 42 days and then decreased to zero level (Figure 1b). The results also indicated that nitrification process was more effective on ANDFF than ANDFS (Figure 1). The results also indicated that inhibitory effect of DMPP for protecting ammonium is just good for 42 days and then soil's exchange sites serve as a source of resupply for ammonium ions. It seems that ammonium ions on the exchangeable sites of soil have been less affected by the nitrification process and therefore remain in the soil for a long period of time (180 days). The availability of ammonium ions derived from soil for plant absorption declined after complete consumption of all ammonium ions derived from fertilizer source in 180 days. This may be due to interaction of soil particles and colloids with ammonium ions or release of these ions beyond the nutrition demand period of plant (as a result of ammonium ion accumulation in the soil). This is in accordance with Chen et al.'s (2010) report that ammonium ions were disappeared from the soil after 28 days and nitrification process reached to its final stage over 4 weeks. Li et al. (2008) reported that ammonium concentration derived from fertilizer increased at a rate of 19.8% and 23.2% for rice and oilseed rape, respectively, after application of DMPP in rice–oilseed rape cropping system.

In comparison of different genotypes, nitrification process was almost the same in all genotypes (except G1 treatment in early growing season) when regular ammonium sulfate was applied to soil (Figure 1a). But after addition of DMPP, the results were quite different. G3 and G5 genotypes absorbed more ammonium ions (Figure 1b). It is worth mentioning that high level of ammonium ions absorption was recorded in G5 genotype compared to other genotypes (Figure 1b).

Effect of DMPP on nitrate–N concentration in the soil

The concentration of nitrate in the soil samples derived from different sources (ANDFF) and (ANDFS) during sampling periods presented in Figure 2. The results showed that high levels of nitrate concentrations verified during 2 and 4 weeks (8.2 and 7.8 kgN ha^{-1}, respectively). Then, it was increased to 16.6 kgN ha^{-1} and after 180 days, it was decreased to an average level of 6.6 kgN ha^{-1} (Figure 2a). Lower concentrations of

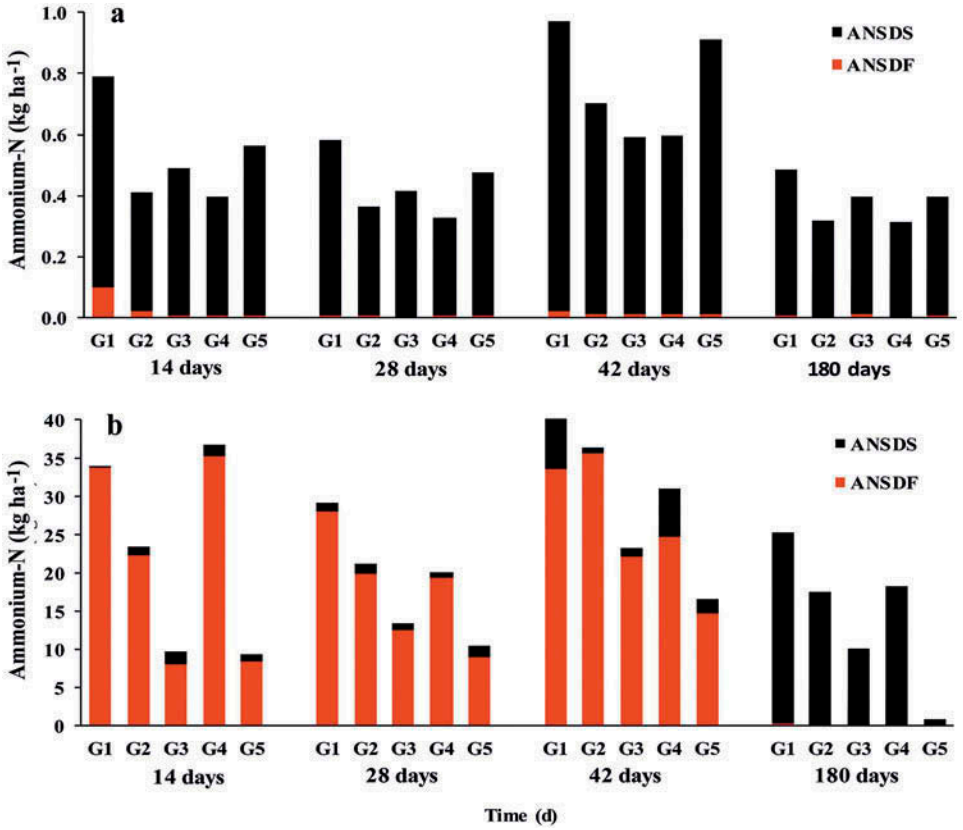

Figure 1. The concentration of ammonium-15N in the soil, derived from fertilizer (ANDFF) and soil (ANDFS) during the growing period in different wheat genotypes, a: regular ammonium sulphate, b: ammonium sulphate with DMPP.

nitrate during the first month of vegetative growth can be attributed to ion absorption by plant or net immobilization. When DMPP was applied to the soil samples, nitrate concentration (derived from fertilizer) at 2 and 4 weeks was 1.6 and 2.2 kgN ha^{-1}, respectively. Then, the nitrate concentration reached to 6.8 kgN ha^{-1} and finally to an average level of 6.4 kgN ha^{-1} (Figure 2b). These results confirmed the inhibitory effect of DMPP and blocking conversion of ammonium to nitrate. The results also showed that after adding DMPP, G3 and G4 genotypes absorbed more nitrate from fertilizer (Figure 2b).

In relation to the inhibitory effect of DMPP on nitrate derived from soil, the situation is like fertilizer source (but with a lower slope) (Figure 2b). In other words, in F treatment, increasing trend continued till 6 weeks (Figure 2a), but in D treatment, N-nitrate concentration (derived from soil) was at its minimum level (1.5 kgN ha^{-1}) till 4 weeks. Then, by "increasing trend" reached to 6.0 kgN ha^{-1} and finally reached to an average level of 3.5 kgN ha^{-1}. It can be concluded that N-nitrate derived from soil has been at its minimal level in the first 4 weeks (due to DMPP application). Then, with slight slope, it goes up and finally, it returns to its initial level. These findings are in agreement with those reported by Yu et al. (2007) to evaluate impact of DMPP on N leaching. They concluded that by DMPP application, nitrate leaching was at its minimum level till 40 days. Then gradually increased and eventually reached to 4 mg L^{-1}. Li et al. (2008) also reported that after 10 days, nitrate leaching of DMPP treatment was about 2 mgN kg^{-1} less than urea treatment (in the field experiment). This reduction continued until 80 days, but the difference gradually decreased and after 100 days, almost no significant difference has been observed between DMPP treatments and urea. They also reported that DMPP could reduce nitrate N derived from fertilizer (N_{dff}) source in rice by 46.1%.

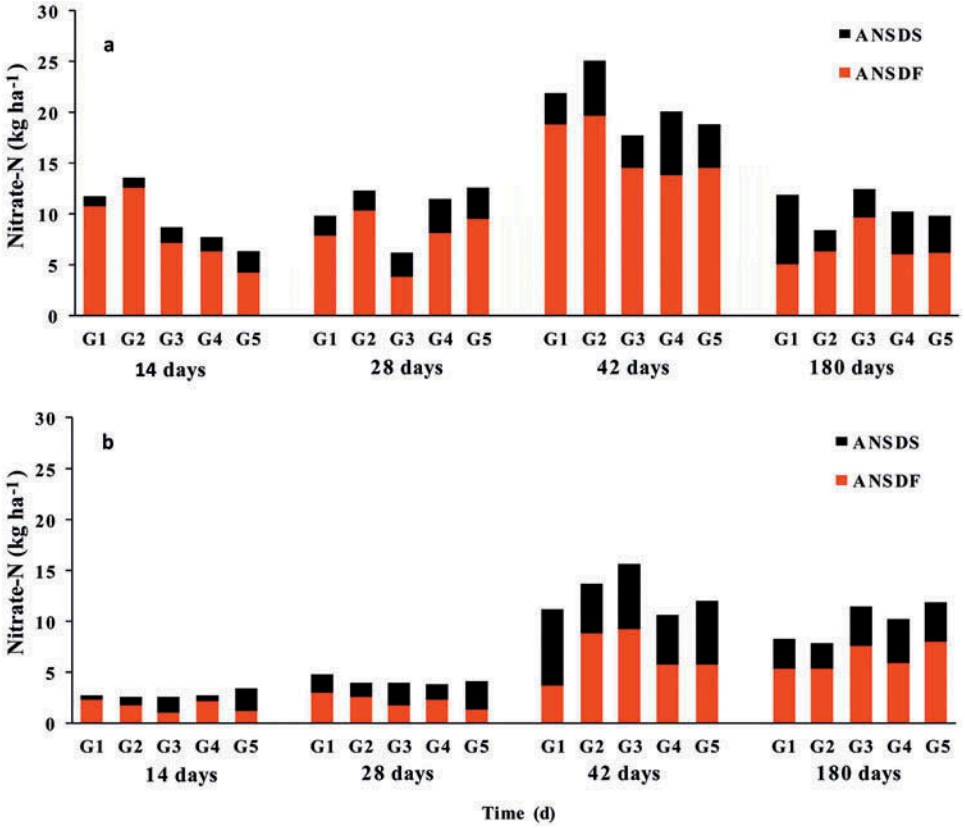

Figure 2. The concentration of nitrate-15N in the soil, derived from fertilizer (ANDFF) and soil (ANDFS) during the growing period in different wheat genotypes, a: regular ammonium sulphate, b: ammonium sulphate with DMPP.

Effect of DMPP on nitrogen fertilizer use efficiency

ANOVA (mean square and significance level) for yield, protein content, N_{dff} and N use efficiency (NUE) in grain and straw of wheat presented in Table 3. Results showed that fertilizer treatments had significant effect on yield, protein content, $\%N_{dff}$, and NUE of grain at the level of 1% ($p > 0.01$). In relation to straw, significant differences were observed in yield and protein ($p > 0.01$) and for NUE ($p > 0.05$).

Data of wheat yield (grain and straw) and its protein content, N_{dff}, and NUE are presented in Table 4. The Duncan's multiple range test ($p \leq 0.01$) showed that the genotypes with lower $\Delta^{13}C$ index (G5, G4, and G3) with an average grain production of 3264 kg ha^{-1} and straw 9394 kg ha^{-1}

Table 3. Analysis of variance for yield, protein content, N_{dff}, and nitrogen use efficiency in wheat (grain and straw).

		Mean square							
		Grain				Straw			
		Yield	Protein	N_{dff}	NUE	Yield	Protein	N_{dff}	NUE
Source of variation	df		%	%	%		%	%	%
Block	2	202733	0.04	8.37	48.18	5.49	0.03	274329	29.87
Fertilizer treatments	2	545106**	0.07**	9.74**	97.44**	0.00^{ns}	0.19**	5813631**	169.85*
Genotypes	4	3178541**	0.83**	41.99**	728.25**	85.49**	0.04^{ns}	21404515**	145.48**
Genotypes × Fertilizer	8	88508*	0.06**	8.17**	19.7^{ns}	81.81**	0.01^{ns}	1194284**	70.37^{ns}
Error	28	39180	0.01	1.40	11.08	3.84	0.03	224429	29.5
CV%	–	7	3.9	2.3	11.3	4.9	20.5	5.7	25

ns, *, ** were nonsignificant, significant at 5% and 1%, respectively.

were more efficient than genotypes with higher $\Delta^{13}C$ index (G1 and G2) with an average grain production of 2205 kg ha^{-1} and straw 6795 kg ha^{-1}. The interaction between genotypes and fertilizer treatment showed that application of DMPP had significant effect on grain and straw yield in different genotypes compared with regular ammonium sulfate. In G5 treatment, application of regular ammonium sulfate increased grain yield by 8.8% but by adding DMPP, grain production decreased by 4.8% (Table 4).

The interaction effect (genotypes and fertilizer treatments) of yield production in grain and straw is shown in Figure 3 using multivariate statistical model GGE bi-plot. Regarding grain yield (Figure 3-left), two components (PC1 and PC2) could justify 99.16% of total variations of main factors interactions (the first and second components 90.81% and 8.35%, respectively). Genotypes made a pentagon's shape (Figure 3). Perpendicular lines drawn on each side of polygon (passed from center of Biplot) divided the test environment into five parts. This kind of environmental grouping is one of the special advantages of GGE Biplot modeling. It provides the possibility of significant environmental grouping, suggested in an area (or sub region). Then according to some special characteristics, differences and similarities between these groups can be interpreted. Fertilizer treatments engrossed in three groups (of five regions). Thus, G5 and F treatments placed in the first group (I) and G3, G4, and D treatment placed in the third group (III). Treatment N placed in the second group (II) and did not have any correlation with any genotype. It seems that genotypes G3, G4 are the most compatible treatments with DMPP in grain yield production. In relation to the wheat straw production, genotypes made a tetrahedron shape and perpendicular lines drawn on each side of polygon, divided the test environment into four parts (Figure 3-right). As can be seen, genotypes with lower CID (G3, G4, and G5) have been established the most similarity with all fertilizer treatments.

The interaction effect of genotypes and fertilizer treatments in grain and straw fertilizer use efficiency shown in Figure 4. Regarding grain fertilizer use efficiency (Figure 4-left), two components (PC1 and PC2) justified 99.87% of total variations of main factors interactions (the first and second components 64.69% and 35.18%, respectively). As can be seen, genotype made a pentagon's shape. Perpendicular lines drawn on each side of polygon (passed from center of Biplot) divided the test environment into five parts. Fertilizer treatments focused in two groups. Thus, G3, G4, and D, F treatments placed in the first group (I) and G5 and N treatment placed in the fifth group (V). It seems that genotypes G3, G4 are the most compatible treatments with DMPP in grain fertilizer use efficiency (Figure 4-left). In relation to the wheat straw fertilizer use efficiency, genotypes composed a tetrahedron shape and perpendicular lines drawn on each side of polygon, divided the test environment into four parts (Figure 4-right). As can be seen, genotypes G3, G4, and D, F treatments placed in first group (I) and established maximum similarity together.

Comparing different wheat genotypes with different carbon-13 isotope discrimination indexes, and its relation with DMPP, it depends on cultural media and plant growth stage. Wheat generally preferred nitrate uptake instead of ammonium (Roosta and Schjoerring 2008) but when environmental conditions are suitable for increasing the intensity of photosynthesis and subsequently rapid growth (e.g., stem elongation stage), wheat prefers ammonium and its relationship with nitrate decreased (Baligar et al. 1993). In similar areas like dryland in West and North West of Iran also, of which cold stress is limiting factor for ion absorption and growth of wheat, the plants prefer absorption of ammonium (instead of nitrate). This is because of lower temperature and roots of wheat (and barley) absorb ammonium better than nitrate (Breteler and Smith 1974). It seems that genotypes with lower $\Delta^{13}C$ because of higher vigor at the beginning of the growing season prefer absorption of ammonia (instead of nitrate). This caused increase in yield and NUE by the means of DMPP. In other words, application of DMPP retains N in ammonium form and thus provides this opportunity to plants. It may be concluded that the N nutritional status of different genotypes was due to differences in genetic and root structure. Van Sanford and MacKown (1986) concluded that difference in wheat genotypes in terms of absorption of N from fertilizers, more related to their N fertilizer use efficiency (Britto and Kronzucker 2002). In other words, N uptake and NUE had high correlation with different wheat genotypes.

Table 4. Comparison of experimental factors (fertilizer treatments, genotypes, and their interactions) on yield, protein content, nitrogen derived from labeled fertilizer and nitrogen use efficiency.

		Straw				Grain			
		DMY	Protein	N_{dff}	NUE	DMY	Protein	N_{dff}	NUE
Experimental factors		kg/ha	%	%	%	kg/ha	%	%	%
Fertilizer treatments	N	$7941^{b} \pm 509$	$3.8^{b} \pm 0.2$	–	–	$2646^{b} \pm 192$	$13.9^{b} \pm 0.5$	–	–
	F	$8052^{b} \pm 361$	$4.6^{ab} \pm 0.6$	$49.0^{a} \pm 1.4$	$24.3^{b} \pm 3.1$	$2847^{a} \pm 191$	$14.3^{ab} \pm 0.3$	$66.7^{b} \pm 0.7$	$36.2^{b} \pm 3.1$
	D	$9070^{a} \pm 449$	$5.1^{a} \pm 0.9$	$49.0^{a} \pm 1.2$	$29.0^{a} \pm 2.3$	$3027^{a} \pm 153$	$14.7^{a} \pm 0.5$	$67.8^{a} \pm 0.8$	$39.8^{a} \pm 2.2$
Genotypes	G1	$6021^{c} \pm 354$	$4.2^{a} \pm 0.5$	$45.2^{d} \pm 1.7$	$18.2^{b} \pm 2.2$	$2082^{b} \pm 172$	$11.4^{c} \pm 0.7$	$63.4^{c} \pm 0.4$	$21.4^{c} \pm 3.6$
	G2	$7569^{b} \pm 400$	$5.0^{a} \pm 0.2$	$48.9^{bc} \pm 1.8$	$27.7^{ab} \pm 0.7$	$2328^{b} \pm 113$	$14.0^{b} \pm 0.2$	$67.0^{b} \pm 0.6$	$32.3^{b} \pm 2.3$
	G3	$9085^{a} \pm 353$	$4.2^{a} \pm 0.6$	$50.6^{b} \pm 0.9$	$27.1^{ab} \pm 3.0$	$3108^{a} \pm 185$	$15.2^{a} \pm 0.2$	$67.6^{b} \pm 0.4$	$43.9^{a} \pm 2.2$
	G4	$9371^{a} \pm 429$	$4.9^{a} \pm 0.7$	$46.0^{cd} \pm 1.4$	$29.9^{a} \pm 4.1$	$3354^{a} \pm 127$	$15.4^{a} \pm 0.6$	$67.4^{b} \pm 0.6$	$44.7^{a} \pm 1.5$
	G5	$9726^{a} \pm 339$	$4.2^{a} \pm 0.1$	$54.6^{a} \pm 1.6$	$30.4^{a} \pm 3.3$	$3330^{a} \pm 162$	$15.5^{a} \pm 0.8$	$70.8^{a} \pm 0.5$	$47.9^{a} \pm 1.6$
Interactions	NG1	$5460^{de} \pm 242$	$3.2^{a} \pm 0.2$	–	–	$1924^{c} \pm 58$	$10.3^{d} \pm 0.6$	–	–
	FG1	$4834^{e} \pm 210$	$4.2^{a} \pm 0.5$	$45.2^{bc} \pm 0.0$	$12.0^{b} \pm 1.5$	$2042^{c} \pm 39$	$12.0^{cd} \pm 0.2$	$61.9^{e} \pm 0.3$	$19.7^{d} \pm 0.8$
	DG1	$7769^{bc} \pm 44$	$5.4^{a} \pm 0.7$	$45.2^{bc} \pm 0.0$	$24.5^{ab} \pm 3.5$	$2280^{c} \pm 152$	$12.0^{cd} \pm 0.2$	$64.9^{de} \pm 0.7$	$23.1^{d} \pm 2.1$
	NG2	$6803^{cd} \pm 36$	$4.2^{a} \pm 0.4$	–	–	$1969^{c} \pm 146$	$12.5^{bc} \pm 0.5$	–	–
	FG2	$7484^{bc} \pm 342$	$5.1^{a} \pm 0.9$	$44.7^{bc} \pm 1.6$	$22.7^{ab} \pm 4.6$	$2326^{bcd} \pm 59$	$14.3^{ab} \pm 0.5$	$65.6^{cd} \pm 0.1$	$28.3^{cd} \pm 1.6$
	DG2	$8418^{ab} \pm 246$	$5.6^{a} \pm 0.6$	$53.1^{a} \pm 1.8$	$32.7^{a} \pm 4.8$	$2689^{bc} \pm 183$	$15.1^{a} \pm 0.3$	$68.4^{abcd} \pm 0.1$	$36.2^{bc} \pm 3.2$
	NG3	$8844^{ab} \pm 170$	$3.7^{a} \pm 0.4$	–	–	$3368^{ab} \pm 78$	$15.2^{a} \pm 0.2$	–	–
	FG3	$9562^{a} \pm 224$	$4.6^{a} \pm 0.3$	$54.0^{a} \pm 1.3$	$33.6^{a} \pm 0.4$	$3285^{abc} \pm 154$	$15.3^{a} \pm 0.6$	$68.8^{abc} \pm 0.8$	$42.8^{ab} \pm 1.9$
	DG3	$9708^{a} \pm 374$	$4.3^{a} \pm 0.5$	$55.1^{a} \pm 1.5$	$38.0^{a} \pm 3.7$	$3410^{a} \pm 178$	$15.1^{a} \pm 0.3$	$68.3^{abcd} \pm 0.8$	$46.5^{ab} \pm 3.9$
	NG4	$9697^{a} \pm 547$	$4.2^{a} \pm 0.6$	–	–	$3198^{abc} \pm 83$	$15.8^{a} \pm 0.2$	–	–
	FG4	$9789^{a} \pm 407$	$5.0^{a} \pm 0.5$	$40.0^{c} \pm 0.6$	$23.4^{ab} \pm 3.6$	$3312^{abc} \pm 34$	$14.6^{a} \pm 0.2$	$71.5^{a} \pm 1.4$	$46.9^{a} \pm 1.0$
	DG4	$9691^{a} \pm 178$	$5.4^{a} \pm 0.2$	$51.9^{a} \pm 0.7$	$27.7^{ab} \pm 0.8$	$3479^{a} \pm 41$	$15.9^{a} \pm 0.1$	$70.2^{ab} \pm 1.3$	$48.9^{a} \pm 1.1$
	NG5	$8687^{ab} \pm 55$	$3.9^{a} \pm 0.7$	–	–	$3106^{abc} \pm 186$	$15.7^{a} \pm 0.5$	–	–
	FG5	$9668^{a} \pm 79$	$4.2^{a} \pm 0.5$	$49.4^{ab} \pm 0.8$	$25.9^{ab} \pm 2.6$	$3446^{a} \pm 64$	$15.4^{a} \pm 0.2$	$66.8^{bcd} \pm 0.3$	$41.3^{ab} \pm 1.6$
	DG5	$8901^{ab} \pm 357$	$4.6^{a} \pm 0.3$	$51.7^{a} \pm 1.5$	$28.3^{ab} \pm 0.1$	$2773^{bc} \pm 234$	$15.3^{a} \pm 0.3$	$66.0^{cd} \pm 1.2$	$46.4^{ab} \pm 2.7$

Similar letters in each column (and each experimental factors) indicate no significant difference at the 5% level (Duncan test);Fertilizer treatments: N: no fertilizer; F: labeled ammonium sulfate; D: labeled ammonium sulfate plus DMPP inhibitors;DMY: dry mater yield; N_{dff}: nitrogen derived from labeled fertilizer; NUE: nitrogen use efficiency.

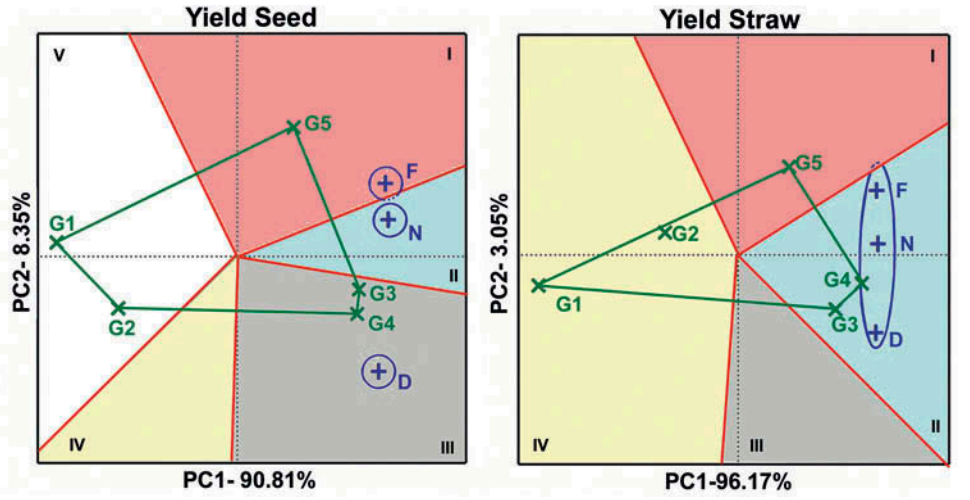

Figure 3. Interaction effect of different wheat genotypes (G1 to G5) and fertilizer treatments (N, F and D) with GGE Biplot multivariate statistical model, left: DMY grain, right: DMY straw.

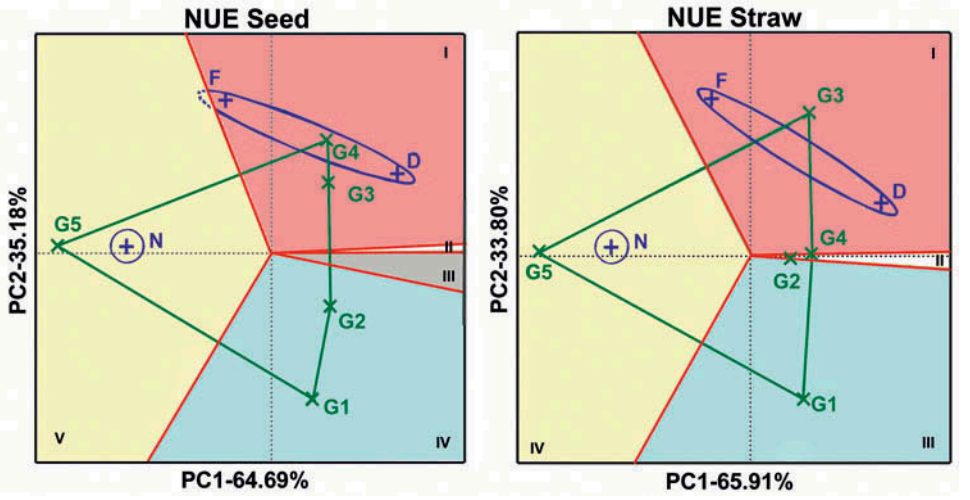

Figure 4. Interaction effect of different wheat genotypes (G1 to G5) and fertilizer treatments (N, F and D) with GGE Biplot multivariate statistical model, left: NUE grain, right: NUE straw.

Regarding negative correlation between grain yield and fertilizer use efficiency in the G5 treatment (Zargana-6) with nitrification inhibitor DMPP, this wheat genotype was introduced to Iranian institute for Dry land through CYMMYT[2] and ICARDA[3] international tests. In initial evaluation, this genotype showed relative response in cold and temperate regions (in comparison with common control treatments). The genotype G5 was resistant to lodging and its thousand kernel weight was less than control lines. In some regions and years, it showed greater production capacity (yield) than control lines. This line is one of the fast growing lines, with desirable fertilizer (and water) absorption and appropriate yield production. A general characteristic of fast growing lines is tendency for more ammonium absorption (than nitrate). Pang et al. (2014) reported that differences in the absorption of N in wheat genotypes, (in addition to the dry matter and root structure),

associated with power and initial speed of the plant growth (Nachit 1998). So, genotypes, with high early vigor, could absorb more N in comparison with those with less early vigor. High lodging-resistant index suggests that G5 genotype can be used to increase yield production by application of N fertilizers. As a result, more N fertilizer is needed to achieve optimum yield in G5 treatment (compare with G1 and G2). But more ammonium absorption tendency may lead to toxic effects in plants (which yield reduction is one of them). DMPP application has caused high levels of ammonia in the early plant growth of G5 treatment. Preferred ammonia absorbing in this treatment depleted all ammonium ions from the soil. This leads to a small reduction of wheat yield by toxicity effect. Ofcourse, ammonium toxicity effects have not been enough to cause plant death in G5 treatment. Therefore, use of DMPP in fast growing genotypes with ammonium preferential absorption ability (like G5) should be performed with caution.

Conclusion

We observed that the nitrification inhibitor DMPP could delay nitrification process by more than 42 days. Genotypes with lower $\Delta^{13}C$ index (higher WUE) up took higher ammonium during filling stage of grain which led to higher crop yield and N fertilizer use efficiency. The results also suggested that the use of DMPP may not be beneficial in some fast growing wheat genotypes because more ammonium absorption tendency can lead to toxic effects in plants. The results of this field study are considered as the first insight to evaluate the effect of applying chemical fertilizer with DMPP using different wheat genotypes with different carbon-13 isotope discrimination. Therefore, we suggest future research activities include (i) wider range of wheat genotypes with different $\Delta^{13}C$ index in real rain-fed conditions, (ii) comparison of spring and autumn wheat genotypes together and (iii) response of different wheat genotypes to DMPP under drought or salinity stress conditions.

Notes

1. 2-Chloro 6 trichloromethyl pyridine.
2. International Maze and Wheat Improvement Center (Turkey).
3. International Center for Agricultural Research in DRY Areas (Syria).

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