

Effect of organic and inorganic fertilizers on grain yield and protein banding pattern of wheat

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Abstract

In order to evaluate the effects of different levels of inorganic (0, 80, 160 and 240 kg Nitrogen ha⁻¹) and organic (0, 30 and 60 Mg municipal waste compost ha⁻¹) fertilizers on wheat grain yield, gluten content, protein variability and protein banding pattern on polyacrylamide gel in different growth stages of irrigated wheat, a field experiment was conducted at research station of the School of Agriculture, Shiraz University at Bajgah in 2007. Results indicated that the highest wheat grain yield was achieved when the plants were fertilized with 160 kg N ha⁻¹ and 30 Mg compost ha⁻¹. Among yield components, spikes plant⁻¹, seeds spike⁻¹ and 1000 kernels weight were significantly increased with increasing the level of nitrogen. However, there was no significant difference between 160 and 240 kg N ha⁻¹. There was no significant effect of N fertilization on seed protein and gluten content, however the highest amount of seed protein was obtained in 60 Mg compost ha⁻¹ at all levels of nitrogen. The SDS-PAGE was performed to investigate differences between proteins banding pattern in different growth stages under different levels of N and compost. Protein banding pattern showed no polymorphism in tillering and stem elongation stages. However, in ear emergence in 160 kg N ha⁻¹ and in the all compost levels, density of a 50 kDa band was increased specifically in 60 Mg compost ha⁻¹. Furthermore, seed water soluble proteins, Albumin and Globulin, showed no polymorphism. All in all, it is possible to obtain maximum grain yield, protein and gluten, just in 160 kg ha⁻¹ nitrogen level. Thus, it shows the positive impact of compost application on reduction of chemical fertilizer use.

Keyword: Compost, Gluten, Grain yield, Nitrogen fertilization, Protein, Wheat.

Introduction

Cereals are an important dietary protein source throughout the world, because they constitute the main protein and energy supply in most countries (Bos et al. 2005). Wheat is one of the major cereal crops with a unique protein, which is consumed by humans and is grown around the world in diverse environments. Wheat seed-storage proteins according to their solubility properties are traditionally classified into four classes; albumins, globulins, prolamins and glutelins. Gluten, the most abundant wheat endosperm protein, is a large complex mainly composed of polymeric and monomeric proteins known as glutenins and gliadins, respectively (MacRitchie 1994). It has already been known that gluten proteins have a premier role in wheat flour quality. It is recognized that variation in protein content and composition significantly affect wheat quality with a subsequent influence on baking properties (Borghi et al. 1995; Daniel and Triboi 2000; Johansson and Svensson 1999; Johansson et al. 2001; Wooding et al. 2000). Wheat protein content and baking quality highly depend on genetic background and environmental factors, especially influence of drought and heat stress, during the grain filling period and nitrogen availability (soil N, rate and time of N application) (Altenbach et al. 2002; Dupont and Altenbach 2003; Luo et al. 2000; Ottman et al. 2000; Rharrabti et al. 2001; Tea et al. 2004). Nitrogen rate, type of nitrogen, and timing of its application are important factors to increase wheat yield (Garrido-Lestache et al. 2005; Grant et al. 2001; López-Bellido et al. 1998). Furthermore, N

fertilization is useful to enhance the baking quality parameters such as protein content and protein quality (Grant et al. 2001). Some studies showed that N fertilization increases the total quantity of flour proteins, resulting in an increase in both gliadins and glutenins (Dupont and Altenbach 2003; Johansson et al. 2001; Johansson et al. 2004; Martre et al. 2003; Triboi et al. 2000). The use of chemical fertilizers has been increased worldwide for cereal production (Abril et al. 2007) due to availability of inexpensive fertilizers (Graham and Vance 2000). The continued use of chemical fertilizers causes health and environmental hazards such as ground and surface water pollution by nitrate leaching (Pimentel 1996). So, reducing the amount of nitrogen fertilizers applied to the field without a nitrogen deficiency will be the main challenge in field management. One of the possible options to reduce the use of chemical fertilizer could be recycling of organic wastes. Compost as the organic waste can be a valuable and inexpensive fertilizer and source of plant nutrients. Positive effects of organic waste on soil structure, aggregate stability and water-holding capacity were reported in several studies (Jedidi et al. 2004; Odlare et al. 2008; Shen and Shen 2001; Wells et al. 2000). Furthermore, compost has a high nutritional value, with high concentrations of especially nitrogen, phosphorus and potassium, while the contamination by heavy metals and other toxic substances are very low (Asghar et al. 2006). Previous studies showed that the combination of compost with chemical fertilizer further enhanced the biomass and grain yield of crops (Sarwar et al. 2007; Sarwar et al. 2008;

Table 1. Interaction effects of nitrogen and compost on wheat grain yield (kg ha^{-1})

Treatment Nitrogen (kg ha^{-1})	Compost (Mg ha^{-1})			Mean
	0	30	60	
0	1274.19 f	3600.90 de	3312.65 de	2720.20 c
80	2893.58 e	4206.67 cde	5274.42 bc	4124.80 b
160	3439.00 de	7546.19 a	6548.55 ab	5844.59 a
240	4507.00 cd	6089.94 b	5307.09 bc	5301.00 a
Mean	3021.70 b	5110.60 a	5360.90 a	

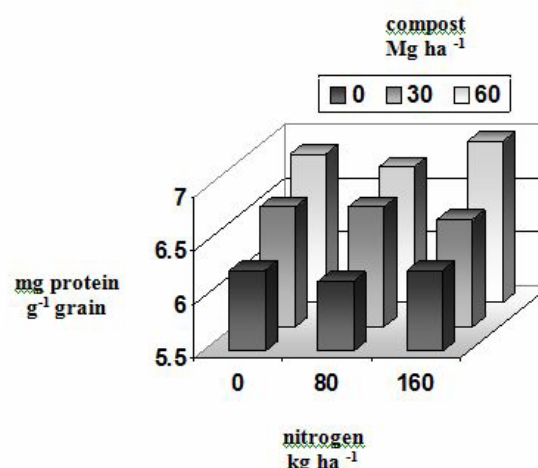
Means in the same column and the last row followed by the same letters are not significantly different ($P < 0.05$), according to Duncan's test.

Cheuk et al. 2003). Furthermore, positive changes have been reported in the quality of wheat flour, because of increasing the amount of gluten after compost treatment (Gopinath et al. 2008). It is important to conduct research to determine the plant response to N fertilization and to develop rational practices for more N use efficiency in this plant, in order to improve the nitrogen application management. The objective of this study is to investigate the influence of inorganic (nitrogen) and organic (compost) fertilizers on yield, yield components, gluten content, protein concentration and its banding pattern in various growth stages of bread wheat. The main aim of this study is to improve nitrogen management practices to maximize grain yield production with no reduction in protein content and its quality and alleviate the environmental hazards by extra nitrogen leaching.

Material and Method

Field Experiments

The field experiment was conducted on silty loam soil at the Experimental Farm of School of Agriculture, Shiraz University, Shiraz, Iran, is located at bajgah (1810 m above the sea level with longitude $52^{\circ} 35'$ and latitude $39^{\circ} 40'$) in the autumn of 2007. The experiment was laid out in a randomized complete-block design with four replications. The treatments included nitrogen at four levels (0, 80, 160 and 240 kg N ha^{-1}) and municipal waste compost at two levels (0, 30 and 60 Mg ha^{-1}) pre planting and soil incorporated. Nitrogen was supplied from urea and was added to plots in two periods (50% at planting time and 50% at stem elongation stage) and compost was supplied from compost factory in Esfahan, Iran. After land preparation plowing, disking and ridging the plots (6 m long and 2 m wide) were done and winter wheat (CV. Shiraz) was planted at seeding rate of 180 kg ha^{-1} in the rows. Spikes per plant were determined from 1 m^2 area of each plot by clipping the plants at the soil surface. After over drying and weighing, grain was threshed from the straw, cleaned, and weighed. Kernel weight was determined by counting weighing four 250 kernel samples taken from the harvested grain of each plot. Kernels per ear were calculated by dividing the number of kernels per harvested area by the number of ears per harvested area. After harvesting the grain yield and yield component (spikes plant^{-1} , seeds spike^{-1} and 1000 kernels weight) were determined for all plots. As our results showed no significant differences between 160 and 240 kg N ha^{-1} on yield components and grain yield, we considered just three levels of all N treatments (0, 80 and 160 kg N ha^{-1}) to investigate protein content and its pattern. Samples from all replications in different growth stages were used to determine the gluten and protein content and electrophoresis analyses.

**Fig 1.** Effect of nitrogen and compost fertilizers on seed water soluble protein

Protein Extraction

In different growth stages, the leaves were harvested, frozen in liquid nitrogen and then stored at -80°C . The frozen leaves were ground to a fine powder in liquid nitrogen and total protein extracted with ice-cold 0.1 M Tris - HCl buffer (pH 7.5) containing 5% (w/v) PVP (4:1 buffer volume/fresh weight). The homogenate was centrifuged at 13000 g for 15 min, at 4°C , and the supernatant was used for determining protein concentration and SDS-PAGE analysis. Furthermore, the seeds were ground to a fine powder and 100 mg of powder was used for protein extraction. 1 ml extraction buffer was added to each micro tube containing 100 mg powder and centrifuged at 13000 g for 10 min at 4°C .

Protein Measurement

The concentration of protein was measured by the Bradford method (1976). BSA was used as a standard.

SDS-PAGE

Prior to electrophoresis, samples were mixed with SDS sample buffer (62.5 mM Tris-HCl, pH 6.8, 2% [w/v] SDS, 10% [w/v] glycerol, 5% [v/v] β -mercaptoethanol, 0.001% [w/v] bromophenol blue) and boiled for 5 min. Protein samples were separated on 5- 20% SDS- PAGE gradient gels based on method of Laemmli (1970). Staining and destaining of the gels were carried out by the standard Coomassie brilliant blue G-250 (CCB) staining method.

Table 2. Effect of nitrogen on wheat yield and yield components

Nitrogen (kg ha ⁻¹)	Grain yield (kg ha ⁻¹)	Spike number/ m ²	Seed number/ spikes	1000 grain Weight (g)
control	2720.20 c	509.50 c	13.73 b	38.49 b
80	4124.90 b	600.50 b	17.20 ab	39.86 a
160	5844.59 a	762.70 a	18.96 a	39.60 a
240	5301.00 a	748.30 a	18.21 a	40.05 a

Means in the same column followed by the same letters are not significantly different ($P < 0.05$), according to Duncan's test.

Table 3. Effect of compost on wheat yield and yield components

Compost (Mg ha ⁻¹)	Grain yield (kg ha ⁻¹)	Spike number/ m ²	Seed number/ spikes	1000 grain Weight (g)
0	3025.80 b	479.20 b	15.21 b	39.42 b
30	5110.60 a	735.60 a	17.90 ab	38.69 c
60	5360.90 a	841.30 a	19.96 a	40.23 a

Means in the same column followed by the same letters are not significantly different ($P < 0.05$), according to Duncan's test.

Gluten Measurement

Flour was hand washed according to the standard method (AACC 1983) with 30 min of resting time. Isolated drying gluten contained was evaluated.

Statistical Analysis

All data were analyzed by analysis of variance (ANOVA) procedures using MSTATC software package. Treatment means were separated by Duncan's multiple range tests at (Duncan 0.05).

Results

Yield and Yield Components

Results showed that, with increasing levels of nitrogen from 80 to 160 and 240 kg ha⁻¹, wheat grain yield significantly increased (52%, 115% and 95% over control in 80, 160 and 240 kg N ha⁻¹ treatments, respectively), compared to control (Table 1). Considerable point is insignificant difference between grain yield in 160 and 240 kg N ha⁻¹ treatments. Results revealed a significant increase in grain yield when compost was applied. However, there was no significant difference between 30 and 60 Mg compost ha⁻¹ application (Table 1). The use of chemical fertilizer (N) in combination with compost increased the grain yield significantly (Table 1). The maximum yield increases were observed in 160 kg N ha⁻¹ in combination with 30 or 60 Mg compost ha⁻¹ (Table 1). Result of nitrogen and compost interaction effects indicated that maximum wheat grain yield was obtained from 30 Mg ha⁻¹ compost application and 160 kg N ha⁻¹ (7546.19 kg ha⁻¹) and increasing nitrogen levels from 160 to 240 kg N ha⁻¹ had no significant effect on wheat yield. Results clearly indicate that nitrogenous fertilizer had a significant effect on yield components (spikes plant⁻¹, seeds spike⁻¹ and 1000 kernels weight) and grain yield (Table 2). From 0 to 80 and 160 kg N ha⁻¹ all of these parameters significantly enhanced with increasing nitrogen levels, but there was no significant increase from 160 to 240 kg N ha⁻¹ (Table 2). Data in table 3 shows that 30 and 60 Mg compost ha⁻¹ application lead to a same significant increase in spikes plant⁻¹ and seeds spike⁻¹, compared to no compost application. As well as that, the maximum amount of 1000- grain weight was obtained in 60 Mg compost ha⁻¹ application.

Protein and Gluten Content

Figure 1 shows no significant effects of nitrogen fertilizer on seed protein content. However, compost had a significant effect on seed protein and the maximum amount of seed protein was observed in 60 Mg compost ha⁻¹ treatment. Results indicated that there were no significant differences in seed gluten content between different nitrogen and compost treatments (data not shown).

SDS-PAGE Protein Analysis

Figure 2 demonstrates the SDS PAGE protein profile of wheat leaves in different growth stages and wheat seed under different fertilization treatments. The results clearly indicated that there is no polymorphism between different treatments in tillering and stem elongation stages and seed. However, in ear emergence stage, intensity of a 50 kDa molecular weight band was increased in 160 kg N ha⁻¹ and all compost levels especially in 60 Mg compost ha⁻¹ (Fig. 2).

Discussion

The rapid increase in the world population demands parallel increases in food production, particularly of wheat. In order to preserve the environment and the present natural resources, further increases in global wheat production must be along with a proper management of fertilization. Integrated use of organic wastes and chemical fertilizers is beneficial in improving crop yield, soil pH, organic carbon and available N, P and K in soil (Rautaray et al. 2003). The results of present experiments indicated that wheat grain yield and yield components increased significantly with the application of N fertilizer and compost (Table 1, 2 and 3). However, there were no significant enhancements in these parameters under last two levels of N and compost applications (160 and 240 kg N ha⁻¹) or (30 and 60 Mg ha⁻¹ compost). Additionally, results showed that the use of chemical fertilizer, N, in combination with organic materials, compost, further enhanced the grains yield. Similar results were obtained by other scientists (Cheuk et al. 2003; Parmer and Sharma 2002; Sarwar et al. 2007; Sarwar et al. 2008). Besides the positive effect of organic fertilizer on soil structure that lead to better root development that result in more nutrient uptake, compost not only slowly releases nutrients but also prevents the losses of chemical fertilizers through denitrification, volatilization and

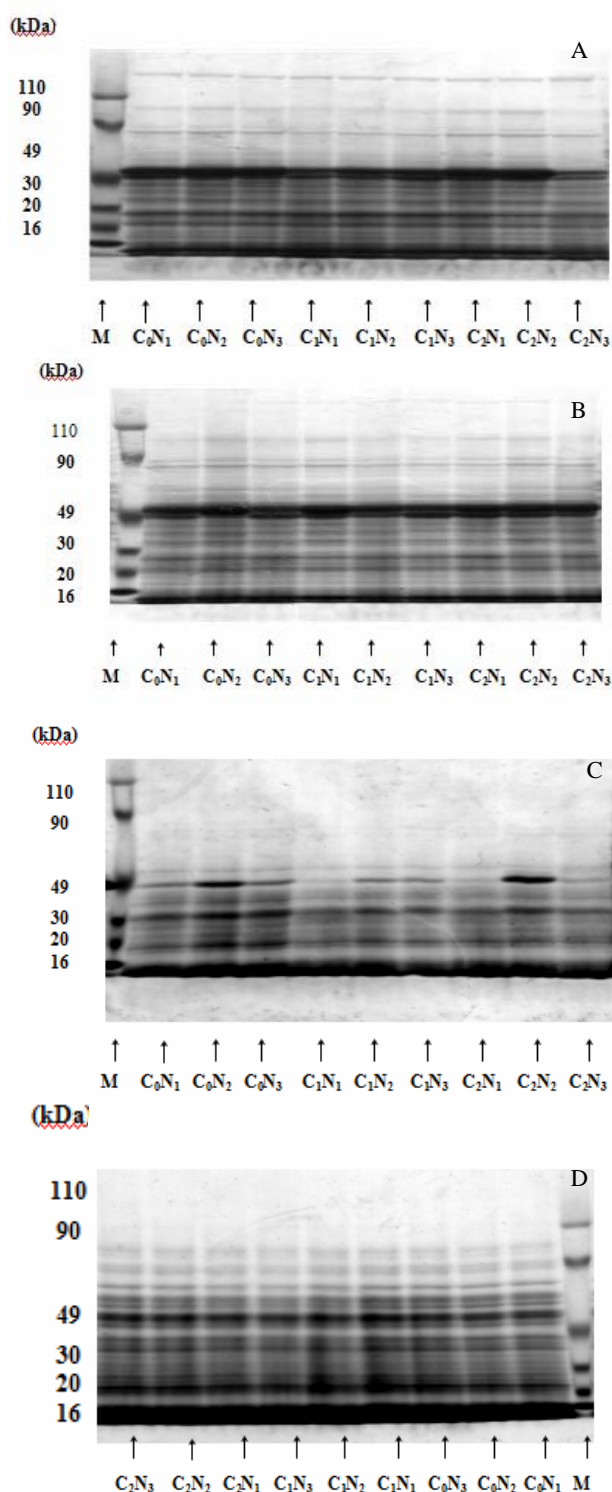


Fig 2. Water soluble protein banding patterns of wheat in (A) tillering stage, (B) stem elongation stage, (C) ear emergence stage and (D) seed; M: Protein marker; C: Compost; C₁: 0 mg ha⁻¹, C₂: 30 mg ha⁻¹, C₃: 60 mg ha⁻¹; N: Nitrogen; N₁: 0 kg ha⁻¹, N₂: 80 kg ha⁻¹, N₃: 160 kg ha⁻¹, N₄: 240 kg ha⁻¹

leaching by binding to nutrients and releasing with the passage of time (Arshad et al. 2004). Thus, it is very likely that when we apply enriched compost along with chemical fertilizers, compost prevents nutrient losses. Consequently, integrated use of chemical fertilizers and recycled organic

waste may improve the efficiency of chemical fertilizers and thus reduce their use in order to improve crop productivity as well as sustain soil health and fertility. Increasing yield potential without negative effect on the quality of the grain is difficult, mainly because increases in grain yield are generally accompanied by a decrease in the seed protein content, which is strongly associated with bread-making quality. Therefore, the protein and gluten content were determined under different fertilization treatments. The results indicated that seed water-soluble protein (Fig. 1) and gluten contents were unaffected by N strategy. These results are in accordance with the conclusions of other researchers who found that the quantity of albumins-globulins is scarcely influenced by N nutrition (Dupont and Altenbach 2003; Johansson et al. 2001; Pedersen and Jorgensen 2007; Wieser and Seilmeier 1998). As noted previously, protein composition of the wheat grain is influenced by genotype, as well as by cultivation system and environmental conditions. In other words, although increased nitrogen supply correlated significantly to an increase in all protein components, its effect on grain protein also depends on the cultivar sown, due to different uses of available soil N, especially during stem elongation. One important point in our finding was significant increase in content of grain protein when we used organic material in combination with N fertilization (Fig. 1). It seems that organic matter can improve the physical properties of the soil and would have caused increased root development that acted positively in more uptakes of water and nutrients (Brady and Weil 2005). In a word, the effect of combination of compost and chemical fertilizers on seed protein was positive, because of more nutrients availability. In order to find out the effect of organic and inorganic fertilizer on protein pattern of leaf and seed, we carried out SDS electrophoresis in various growth stages and seed. As regards Figure 2, we observed no polymorphism in leaf protein pattern in all growth stages exception in ear emergence stage that density of a 50 kDa molecular weight band was increased in some treatments. Furthermore, seed water soluble proteins such as Albumin and Globulin showed no polymorphism, which confirmed insensitivity of these proteins to nitrogenous fertilization. To sum up, results described here confirm application of 160 kg N ha⁻¹ in composition with compost as an organic fertilizer result in the maximum amount of wheat yield component which lead to the highest grain yield. Moreover, we found negligible influence of N supply on grain protein and gluten content, while compost application lead to grain protein content enhancement due to its effect on soil structure and consequently increase in plant nutrients uptake with no negative effect on seed protein pattern. In conclusion, it's strongly suggested to use combination of organic and inorganic fertilizer to achieve highest yield without negative effect on seed quality. It is clear from the results of the present study that 30% of the required nitrogen fertilizer could be replaced by compost, because compost improved the use efficiency of recommended nitrogen fertilizer and reduced its cost. In this way, by carefully managing N fertilization, less N may be needed while grain wheat yields and protein may be maintained or increased. In addition, less use of N fertilization will lead to environmental conservation.

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