Original Article

Comparison of Triple Interaction of Tillage, Nitrogen Fertilizer, and Barley Varieties on Biochemical Traits and Chlorophyll Content

Amin Taherianfar[1], Esmaeil Hadidi Masouleh[2], Ali Eskandari[3*], Saeed Sayfzadeh[2], Saeed Soufizadeh[3]

[1] Ph.d. In agriculture, Department of agriculture, Islamic Azad University, Takestan Branch, Takestan, Iran. [2] Assistant Professor of Islamic Azad University, Takestan Branch, Department of Agriculture, Takestan, Iran. [3] Assistant professor of Nuclear Agriculture Research School, Nuclear Science and Technology Research Institute , Karaj, Iran.

Abstract

This study investigates the effects of different tillage methods and urea fertilizer with nitrapyrin for increasing nitrogen efficiency on quantitative and qualitative traits of barley varieties in a factorial split randomized complete block design with three replications during two crop years 2016-2017 at the Research Field of Nuclear Science and Technology Research Institute, Iran Atomic Energy Organization (Zafaranieh Farm in Karaj). Experimental treatments included tillage system at two levels (conventional tillage and minimum tillage only by disk), nitrogen fertilizer at three levels (no fertilizer or control, urea fertilizer (150 kg/ha), urea fertilizer (150 kg/ha) with nitrapyrin, and two barley varieties (Walfajer cultivar and mutant Roodasht). Tillage factor was placed in a main plot and urea fertilizer and barley varieties were factorialized in sub-plots. Results showed that tillage factor had significant effects on grain fiber and starch contents, grain protein, grain protein yield, chlorophyll content, and leaf phosphate. Grain fiber content, grain protein yield, nitrate reductase (NR), and grain nitrogen were affected significantly by triple interaction of tillage, nitrogen, and variety. Grain physiological, morphological, and chemical components decreased under conventional tillage conditions, while an increase occurred in the plant height only. Improved soil fertility with increased moisture and organic matter content at minimum tillage led to enhancements in plant physiological and morphological traits. The application of nitrification inhibitor with urea fertilizer increased NR efficiency and nitrogen uptake efficiency in the plant. However, NR, plant and grain nitrogen contents, efficiency, and harvest index were lowermost in the absence of nitrification inhibitor (nitrapyrin).

Keywords: Nitrate reductase, Biochemical traits, Chlorophyll

Introduction

Nearly 70 percent of the world population is concentrated in developing countries, whereas there is an inverse rate for food production. In advanced countries, food production and crop yield per unit area are increasing rapidly by the application of technology and new science and techniques, so that the production level often exceeds domestic consumption. Unfortunately, food shortages are expanding along with a rapid population growth in most developing countries, and major foreign exchange earnings of these countries are spent on imported food. With the current growing trend of world population, there is a pressing need to greatly increase agricultural and food production in the coming years in order to meet the food requirements of human society. According to agricultural experts, increasing food production is the only solution to the problem of hunger. In developing countries, in particular, it is necessary to invest more in food production. If food is to be supplied at the present level, these countries need to increase their agricultural productions by at least 60% in the next 30 years.

Barley, *Hordeum vulgare*, has a history equivalent to agriculture, dating back to 5,000-7,000 BC or more. Barley is the most extensively adapted plant among seed plants and is more tolerant to drought stress and soil salinity and alkalinity than other cereals. It is more adaptable to adverse climatic conditions than wheat and grows in all temperate and in many cold regions. Barley can be replaced in rainfed fields where soil moisture and rainfall are insufficient for wheat growth.

Address for correspondence: Mr. Ali Eskandari, Assistant professor of Nuclear Agriculture Research School, Nuclear Science and Technology Research Institute , Karaj, Iran.
E-mail: aieskandari@aeoi.org.ir

How to cite this article: Taherianfar, A., Hadidi Masouleh, E., Eskandari, A., Sayfzadeh, S., Soufizadeh, S. Comparison of Triple Interaction of Tillage, Nitrogen Fertilizer, and Barley Varieties on Biochemical Traits and Chlorophyll Content. Arch Pharma Pract 2020;11(S1):98-109.

Therefore, most of barley is produced in areas where adverse weather conditions are not suitable for the production of other cereals. In some developed countries, barley is used in livestock diet due to its very high contents of protein, fat, and minerals. Extensive ecological adaptability, multiple usability in human and livestock nutrition, and production of malt with optimum quality are major factors that have resulted in the continuation of barley cultivation and production in successive centuries [1].

Barley is one of the most important and oldest cereals in the country, cultivated at an area of 1.5 million hectares of irrigated (40%) and rainfed (60%) fields. This plant is highly adapted to most of rainfed fields located in cold and temperate regions. Barley is cultivated in some arid zones where rainfall is not sufficient for wheat production. Cultivation often begins with barley on newly leveled fields or on those ready for utilization after drainage. The plant is cultivated in both northern and southern hemispheres so that it extends to Norway up to 70° N in the northern hemisphere. It is highly resistant to changes in altitude and cultivated up to 4700 m in Tibet and 5000 m in India for crop production. However, as a C3 plant in temperate regions, latitudes of 30° N and S are the best range for its growth [2].

Among the three macronutrients (nitrogen, phosphorus, and potassium), nitrogen is absorbed by the plant more than the other two elements. It accounts for 2-5% of the plant dry weight and is a primary constituent of many organic compounds such as amino acids and nucleic acids. Different amounts of nitrogen and its application were studied in planting, tillering, and stem elongation or fragmentation at growth stages. It was concluded that grain yield responded positively and significantly to nitrogen consumed in planting and tillering stages; however, grain yield was less responsive to nitrogen application during stem elongation [3]. An experiment on fenugreek indicated that nitrogen fertilizer had a significant effect on leaf carbohydrate content. Comparison of mean values revealed that the highest mean amount of carbohydrate (11.36 μg/g glucose WW) was obtained from 50 kg N ha^{-1} treatment, which was higher than control treatment without nitrogen fertilizer [4]. Moreover, harvest index was affected by nitrogen fertilizer and showed an increase with rising nitrogen content. The lowest harvest index was obtained from zero nitrogen level, which was significantly different from that of 80 kg N ha^{-1}. However, spring nitrogen application reduced harvest index in rainfed conditions at cold regions of Kurdistan province. This was attributed to an increase in the ratio of straw to grain yield with increasing vegetative growth by spring nitrogen application [5]. In wheat plant, addition of an nitrification inhibitor to nitrogen fertilizer elevated total nitrogen uptake by 20% [6].

Combined application of a nitrification inhibitor with ammonium nitrate sulfate fertilizer resulted in significant increases in wheat biological yield and grain yield compared to inhibitor-free application of this fertilizer. This was attributed to nitrate supply needed by the plant during early planting, gradual oxidation of ammonium to nitrate during planting, and supplying part of the plant required nitrogen in the form of ammonium as a result of nitrate inhibitor application [7].

The use of different tillage systems was found to result in changes in the soil structure by crushing aggregates, structural or size changes of the pores, and the arrangement of soil particles, all of which change other soil physical properties [8]. In a study on wheat, the highest (43.50 g) and the lowest (41.77 g) 1000-grain weights were obtained in low and conventional tillage systems, respectively [9].

The pressing need of food supply for growing population of the country requires increasing agricultural productions as much as possible. Therefore, application of a conservation tillage system, selection of different barley varieties, and the use of nitrification inhibitors (with a 50-year history worldwide) have not been used in the agricultural production of Iran. This research, therefore, investigated the effect of using nitrapyrin (a nitrification inhibitor) on nitrogen use efficiency (NUE), yield and yield components, protein content, and some morphological and physiological traits of barley.

Materials and Methods

Barley seeds of Walfajr and Roodasht varieties irradiated with gamma rays were used in this study. Roodasht variety is obtained from two barley varieties, Walfajer and Zarjoo, and accounts for the highest area of barley cultivation in cold and temperate regions of the country. The advantages of this variety include average growth period, resistant to logging and grain loss, resistant to salinity, resistant to spike axis fragility, moderately cold-resistant in non-stress conditions, resistant to barley leaf diseases in saline conditions, and semi-sensitive to barley leaf diseases (e.g. brown spots and powdery mildew disease) in non-saline conditions. This cultivar has the spring-fall growth type, with six-row spikes, and salinity tolerance is one of its prominent traits.

Half of the field was plowed in October 2017 and 2018, and then plowed twice by perpendicular discs to crush lumps and unify the field soil, followed by leveling operations. The other half of the filed was only plowed once by perpendicular discs followed by leveling operations. The field was then furrowed at a distance of 15 cm by a furrower. Total area of the experimental field was 540 m^2 (18 × 30 m).

Soil samples were taken in a zigzag and randomized from at a depth of 0-40 cm from different parts of the Zafaranieh research field affiliated to Karaj Nuclear Agricultural Research Center. The samples were then combined and sent to the soil laboratory. The recorded results are presented in Table 1.

Table 1: Soil profile of Zafaranieh research field, Karaj, Iran

CCE	OC	TDS	EC	pH	SP	PWP	FC	Soil texture (%)			Soil depth
(%)		mg/L	Ms			(%)		Clay	Silt	Sand	cm
16·0	0·42	375	0·6	7·8	26·8	9·5	16·8	21·2	20·1	58·7	0 -20
16·6	0·25	417	0·7	7·7	26·0	9·2	16·5	18·8	20·0	61·2	20 -40

CCE	OC	TDS	EC	pH	SP	PWP	FC	Soil texture (%) ·			Soil depth
(%)		mg/L	Ms			(%)		Clay	Silt	Sand	cm
16·0	0·42	375	0·6	7·8	26·8	9·5	16·8	21·2	20·1	58·7	0 -20
16·6	0·25	417	0·7	7·7	26·0	9·2	16·5	18·8	20·0	61·2	20 -40

Characteristics and type of statistical design used in field study

The present experiment was implemented as a split plot factorial design based on a randomized complete block design with three replications. The main factor in this study was tillage system (T) in main plots and secondary factors of nitrogen fertilizer (N) and barley varieties (V) were placed as factorial in sub-plots. T, N, and V were evaluated at two, three, and two levels, respectively.

Main factor

T at two levels
t_0: Conventional tillage (with disk)
t_1: Minimum tillage (by disk)
Secondary factor
N at three levels
n_0: No use of urea fertilizer (zero)
n_1: 150 kg/ha of urea fertilizer
n_2: 150 kg/ha of urea fertilizer with an inhibitor (nitroperne)
V at two levels
v_o: Walfair Cv.
v_1: Roodasht C.

The traditional irrigation method was used in this study. The field was prepared to apply the main factor after determining the field area to calculate a half for the tillage with disk operation and the other half for disk only operation before cultivation.

Data from the two crop years were collected, tested for experimental error uniformity, and analyzed by combined analysis of variance using the SAS statistical software. Mean values were compared by Duncan's test at 5% level using the same software. Charts were drawn by the Excel software.

Measurement of physiological traits

Chlorophylls b, a, and a + b
Chl. a = $(12.25 \times A_{663}) - (2.79 \times A_{647})$
Chl. b = $(21.5 \times A_{647}) - (5.1 \times A_{663})$
Chl. a +b= $(7.15 \times A_{663}) - (18.71 \times A_{647})$

Measurement of biochemical traits

1) Grain fiber
2) Grain starch
3) Grain protein content
4) Calculation of grain protein yield per hectare

Grain protein yield per hectare was calculated by the following formula.

$$\text{Grain yield} \times \text{protein (\%)}$$

5) Nitrate reductase (NR)
6) Grain N content
7) Plant N content

Results and Discussion

Chlorophyll a (chl. a)

During minimum tillage, the leaves were fresher, retained their green color, and increase chlorophyll a. On the other hand, photosynthetic rate and chlorophyll synthesis decreased in conventional tillage.

Nitrogen deficiency in plants causes premature aging of plants, breakage of chloroplasts, hydrolysis of thylakoid proteins, and decrease of chlorophyll a content. Chlorophyll degradation can also be considered as a preliminary step in protein degradation. Therefore, application of N fertilizer increases nutrients and photosynthetic ability of the plant. It also increases leaf number and area and number of stomata, thereby increasing the amount of photosynthetic enzymes and chlorophyll a in the plant, which is in agreement with those of, for example, Mohammadzadeh et al. (2012) and El-Tayeb (2006) [10, 11].

Chlorophyll content decreased under N deficiency due to chloroplast degradation under these conditions and declined pigment production. Freshness and bold color of the plant leaves with an increase in N fertilizer result from the fact that more supply of suitable nutrients enabled the plant to increase pigment production and further facilitate the transfer of photosynthates in the plant. Obviously, increased chlorophyll synthesis and improved photosynthesis will provide favorable conditions for achieving better performance.

Chlorophyll b

In N deficient conditions, chlorophyll b decreases due to the degradation of chloroplasts in these situations resulting in decreased pigment synthesis. N deficiency at the post-flowering period also causes rapid aging in many plant species, the most noticeable sign of which is leaf yellowing and reduced chlorophyll, which corresponds to those of, for example, Mohammadzadeh et al. (2012) and El-Tayeb (2006) [10, 11].

According to the present results, it can be concluded that increasing N fertilizer led to increased chlorophyll b in Rhoodasht V compared to the control in both conventional and minimum T conditions, resulting from better nutrient supply and more transfer of photosynthates in the plant. Therefore, application of N fertilizer combined with nitrapyrin in both conventional and minimum T conditions increased soil nitrate uptake to the plant root and elevated plant nitrogen content, thus affecting stomatal conductance and transpiration, stimulating further photosynthesis, and synthesizing more chlorophyll b.

Total chlorophyll

N deficiency in the plant causes reduced leaf area, early leaf aging, reduced leaf number, declined chlorophyll content, and most importantly, decreased photosynthetic rate during the growth stage, which is in agreement with those of Shokhmgar et al. (2013), El-Tayeb (2006), and others [4, 11]. According to the results, it can be concluded that increasing N fertilizer resulted in increased total chlorophyll in Rhoodasht V in both conventional and minimum tillage conditions compared to the control. This resulted from the use of nitrapyrin inhibitor that increases the plant chlorophyll content by providing nutrients, improving soil pH, and increasing photosynthesis capacity. Therefore, it stores materials, prevents nutrient leaching and loss, and raises chlorophyll content in these conditions. N is an essential element for chlorophyll formation and plays an important role as an energy carrier during photosynthesis.

Figure 1: Comparison of mean chlorophyll a contents in two tillage methods

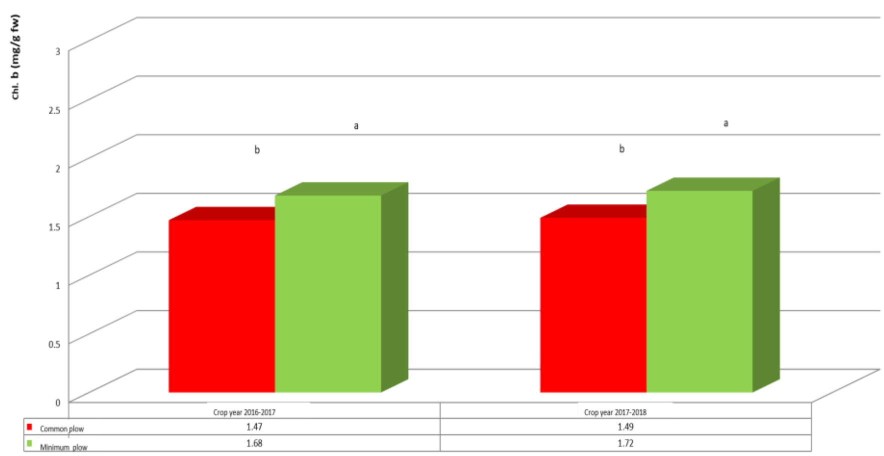

Figure 2: Comparison of mean chlorophyll b contents in two tillage methods

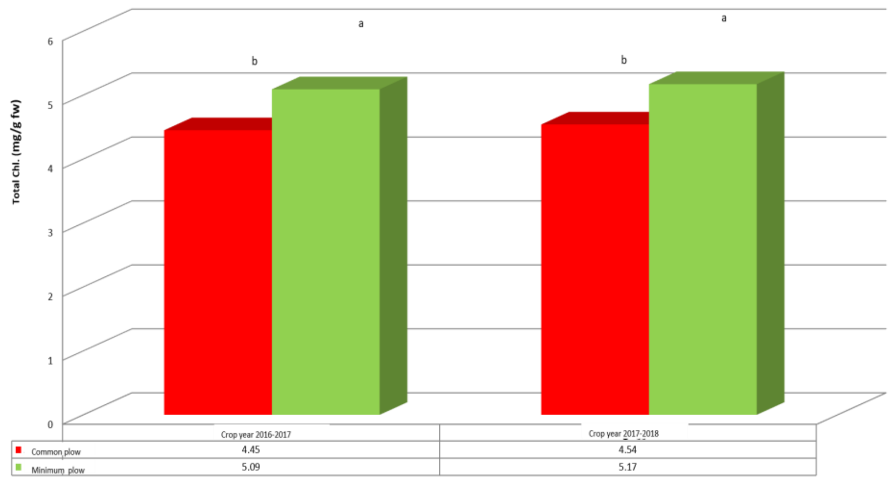

Figure 3: Comparison of mean total chlorophyll in two tillage methods

Table 2: Two-year composite analysis of chlorophyll a, chlorophyll b, and total chlorophyll

Source of changes	df	Mean of squares		
		Chl. a	Chl. b	Total Chl.
Year (Y)	1	0.049 $^{n.s}$	0.017 $^{n.s}$	0.125 $^{n.s}$
Repeat (year)	4	0.028	0.010	0.026
Tillage (T)	1	3.108**	0.864**	7.251**
Y × T	1	0.004 $^{n.s}$	0.002 $^{n.s}$	0.0004 $^{n.s}$
Main error	4	0.012	0.004	0.019
N fertilizer (N)	2	4.114**	1.254**	9.903**
Variety (V)	1	0.172*	0.227**	0.795**
Y × N	2	0.002 $^{n.s}$	0.001 $^{n.s}$	0.006 $^{n.s}$
Y × V	1	0.012 $^{n.s}$	0.000001 $^{n.s}$	0.012 $^{n.s}$
T × N	2	0.143**	0.030 *	0.296**
T × V	1	0.008 $^{n.s}$	0.015 $^{n.s}$	0.047 $^{n.s}$
V × N	2	0.025 $^{n.s}$	0.009 $^{n.s}$	0.062 $^{n.s}$
Y × N × T	2	0.0007 $^{n.s}$	0.0006 $^{n.s}$	0.00004 $^{n.s}$
Y × V × T	1	0.0008 $^{n.s}$	0.00003 $^{n.s}$	0.001 $^{n.s}$
Y × N × V	2	0.012 $^{n.s}$	0.00001 $^{n.s}$	0.012 $^{n.s}$
V × N × T	2	0.035 $^{n.s}$	0.003 $^{n.s}$	0.018 $^{n.s}$
Y × N × T × V	2	0.005 $^{n.s}$	0.0003 $^{n.s}$	0.007 $^{n.s}$
Sub error	40	0.026	0.009	0.026
Total	71	-	-	-
CV (%)		5.05	5.94	3.39

** and **: significant at the 5% and 1% probability levels, respectively.
n.s: non-significant difference

Table 3: Comparison of Triple Interaction of Tillage, Nitrogen and Barley Cultivars on Chlorophyll a, Chlorophyll b and Total Chlorophyll

Total Chl. (mg/g fw) 2017-2018	Total Chl. (mg/g fw) 2016-2017	Chl. b (mg/g fw) 2017-2018	Chl. b (mg/g fw) 2016-2017	Chl. a (mg/g fw) 2017-2018	Chl. a (mg/g fw) 2016-2017	V	N fertilizer	Tillage

3.79 h	3.73 g	1.18 g	1.17 g	2.61 f	2.56 f	V0	N0	T0
4.61 e	4.56 d	1.52 ef	1.51 ef	3.09 cd	3.05 cd	V0	N1	
4.79 e	4.69 cd	1.57 de	1.53 de	3.21 c	3.15 c	V0	N2	
4.09 g	4 fg	1.36 f	1.35 f	2.73 ef	2.65 ef	V1	N0	
4.74 e	4.67 cd	1.62 cde	1.60 cde	3.12 cd	3.07 cd	V1	N1	
5.20 d	5.04 bc	1.71 bcd	1.69 bcd	3.48 b	3.34 bc	V1	N2	
4.20 g	4.11 ef	1.39 f	1.36 f	2.81 e	2.75 def	V0	N0	T1
5.35 cd	5.29 b	1.78 bc	1.75 bc	3.57 b	3.54 ab	V0	N1	
5.66 b	5.67 a	1.88 ab	1.82 ab	3.78 a	3.85 a	V0	N2	
4.45 f	4.39 de	1.46 ef	1.43 ef	2.98 d	2.95 cde	V1	N0	
5.41 c	5.37 ab	1.81 b	1.80 ab	3.59 b	3.57 ab	V1	N1	
5.94 a	5.69 a	2.03 a	1.94 a	3.91 a	3.75 a	V1	N2	

Numbers with at least one common letter in each column have no significant difference based on Duncan's test at 5% level.

Grain fiber content

ANOVA results showed that simple tillage effect on grain fiber was significant at 1% Duncan level. Also, minimum tillage treatment increased grain fiber by 11.21% and 8.62% in 2017 and 2018, respectively. Interaction of three factors (T × N × V) on grain fiber was also significant at 1% level. Comparison of means revealed the highest grain fiber content (2.85% and 2.92% in 2017 and 2018, respectively) was observed for Roodasht V in treatments with minimum tillage and urea fertilizer plus nitrapyrin. In minimum tillage conditions, therefore, grain fiber in T1N2V1 increased 41.8% and 39.71% in 2017 and 2018, respectively, compared to control (T1N0V0). Thus, N fertilizer can increase grain fiber content by providing nutrients for plant and transferring material to grains in minimum tillage condition.

Grain starch content

ANOVA showed significant simple effect of tillage on grain starch at 1% Duncan level. According to Duncan's test at 5% level, the highest mean grain starch content (68.11%) belonged to minimum tillage in 2017 and no significant differences were observed between the two tillage treatments in 2018, hence, the two levels were assigned to a single statistical group.

The interaction of three factors (T × N × V) was not significant on the grain starch. Comparison of means also indicated that the highest mean grain starch content (67.56% and 68% in 2017 and 2018, respectively) was observed for Roodasht V in treatments with conventional tillage and urea fertilizer plus nitrapyrin.

Grain protein content

As shown by ANOVA results, simple effect of tillage on seed protein was significant at 1% Duncan level. Since soil absorbs more moisture and organic matter and provides to the plant in minimum tillage condition, grain protein content will increase due to increased transfer of photosynthates to the grain.

The simple effect of N fertilizer on grain protein was significant at 1% level. The grain filling period increases as a result of N fertilizer application, and grain protein content also rises because of increased production and transfer of photosynthates to grains. Barley can absorb nitrogen and synthesize protein until its late growth period. For this reason, the NR enzyme, responsible for reduction of nitrate absorbed by the plant and incorporation into protein production during wheat aging, is abundantly present in the plant, which is in line with the results of Moraghebi et al. (2011) [12].

With an increase in the amount of consumed nitrogen, grain N content and consequently protein content increased in late stages of the plant growth using the residual nitrogen in the soil and plant tissues. This is because NR enzyme (responsible for reduction of nitrate absorbed by the plant and its incorporation into protein production) is abundantly present at the time of plant aging.

In both conventional and minimum tillage conditions, the use of urea fertilizer plus nitrapyrin increased nutrient availability and elevated protein synthesis in Roodasht V by increasing cell polysomes. Also, grain soluble protein content decreased without the use of N fertilizer due to the lack of nutrients in the plant, which reduced the protein content of grains. Accordingly, the use of urea fertilizer plus nitrapyrin improves the photosynthesis and total dry weight of the plant, thereby increasing the grain protein content with rising plant dry weight.

Grain protein yield per hectare

Results of ANOVA showed that simple effect of tillage on grain protein yield was significant at 1% Duncan level. According to mean comparisons with Duncan's test at 5% level, minimum tillage treatment led to increased grain protein yield by 31.52% and 33.72% in 2017 and 2018, respectively. The simple effect of N fertilizer on grain protein yield was significant at 1% level. In fact, N fertilizer deficiency at vegetative growth stage decreases plant yield due to decreased leaf area and low dry matter content produced at this stage. Low leaf area index at the early flowering and grain filling stages decreases the rate of current photosynthesis, which forms a major part of grain yield, and ultimately reduces grain yield. Consequently, a reduction in grain protein yield should be expected with the declined grain yield under these conditions compared to those of N fertilizer application. These results show that grain protein yield increased significantly with the remarkable rises of grain yield and protein content by the application of urea fertilizer plus nitrapyrin. Increased grain protein yield to some extent was reported by some researchers [12].

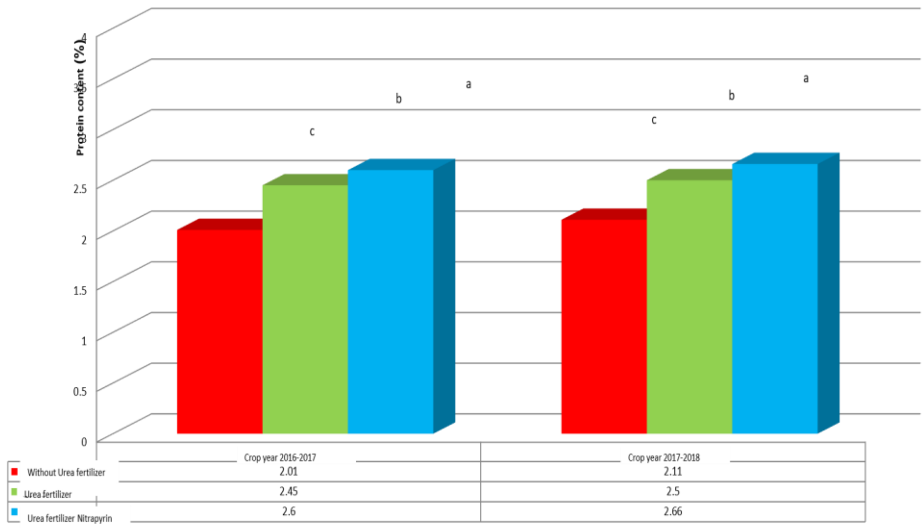

Figure 4: Comparison of average grain fiber contents at different levels of N fertilizer

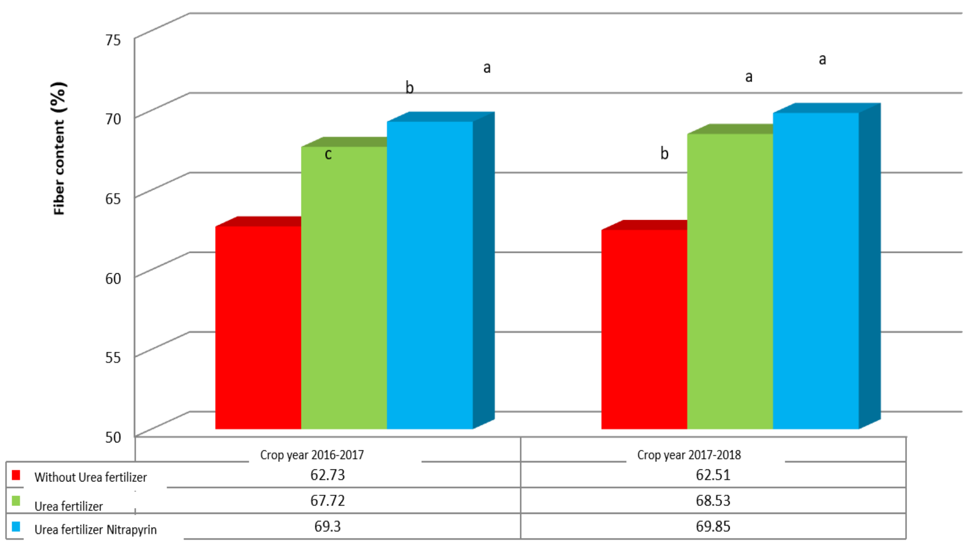

Figure 5: Comparison of average grain starch yield at different levels of N fertilizer

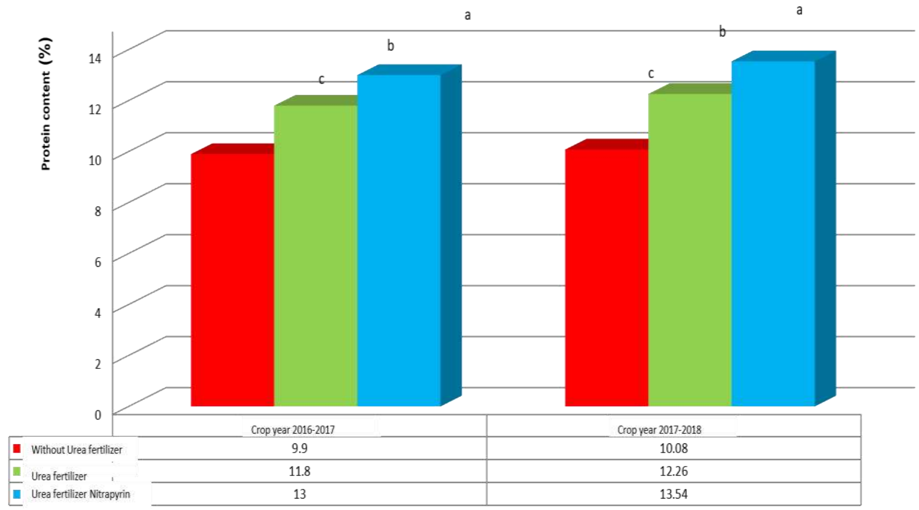

Figure 6: Comparison of average grain protein contents at different levels of N fertilizer

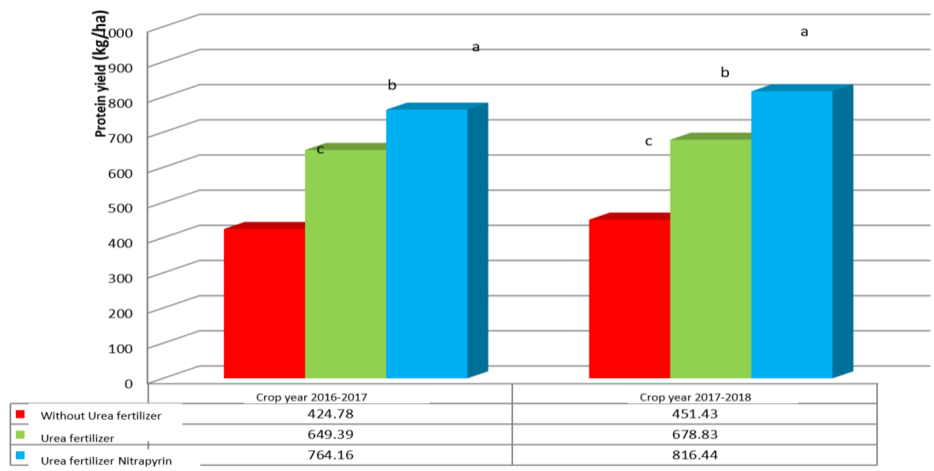

Figure 7: Comparison of mean grain protein yield at different levels of N fertilizer

Table 4: Two-year composite analysis of grain fiber, starch, and protein contents and protein yield per hectare

Source of changes	df	Mean of squares			
		Grain fiber (%)	Grain starch (%)	Grain protein (%)	Grain protein yield/ha
Year (Y)	1	0.076 n.s	2.606 n.s	2.860 **	24331.812**
Repeat (year)	4	0.012	4.019	0.436	1360.713
Tillage (T)	1	0.968**	174.533**	44.730**	568492.514**
Y × T	1	0.010 n.s	0.061 n.s	0.175 n.s	2109.143 n.s
Main error	4	0.018	4.384	0.111	630.892
N fertilizer (N)	2	2.092**	323.315**	65.886**	767846.306**
Variety (V)	1	0.255**	58.500**	4.575**	49789.583**
Y × N	2	0.003 n.s	1.705 n.s	0.217 n.s	1379.963 n.s
Y × V	1	0.0008 n.s	0.911 n.s	0.025 n.s	291.974 n.s
T × N	2	0.109**	4.753 n.s	3.092**	32043.470**
T × V	1	0.022 n.s	15.773 n.s	0.042 n.s	1073.621 n.s
V × N	2	0.007 n.s	0.094 n.s	0.221 n.s	2164.508 n.s
Y × N × T	2	0.001 n.s	0.480 n.s	0.222 n.s	1161.863 n.s
Y × V × T	1	0.002 n.s	0.823 n.s	0.120 n.s	16.695 n.s
Y × N × V	2	0.007 n.s	0.092 n.s	0.096 n.s	680.066 n.s
V × N × T	2	0.053**	3.155 n.s	0.120 n.s	9164.513**
Y × N × T × V	2	0.002 n.s	0.364 n.s	0.005 n.s	113.554 n.s
Sub error	40	0.007	3.990	0.283	1060.424

Total	71	-	-	-	-
CV (%)		3.68	2.99	4.52	5.15

** and **: significant at the 5% and 1% probability levels, respectively.

Table 5: Comparison of triple interaction between tillage (T), N fertilizer, and barley varieties (V) on grain fiber, starch, and protein contents and protein yield per hectare

Grain protein yield (kg/ha) 2017-2018	Grain protein yield (kg/ha) 2016-2017	Grain protein (٪) 2017-2018	Grain protein (٪) 2016-2017	Grain starch (٪) 2017-2018	Grain starch (٪) 2016-2017	Grain fiber (٪) 2017-2018	Grain fiber (٪) 2016-2017	V	N fertilizer	Tillage
366.02 i	334.71 j	9.46 g	9.26 i	60.86 f	60.70 i	2.05 f	1.84 h	V0	N0	T0
555.75 ef	549.79 g	11 ef	10.76 fg	66.26 cde	65.66 efg	2.28 e	2.19 fg	V0	N1	
684.22 d	628.69 ef	12.30 d	11.70 de	67.70 bcd	67.53 cde	2.53 cd	2.50 cde	V0	N2	
437.36 h	405.20 i	10.03 fg	9.70 hi	62.33 ef	62.40 hi	2.13 ef	2.07 g	V1	N0	
603.29 e	591.90 fg	11.30 e	11.20 ef	67.13 bcd	66.50 def	2.42 d	2.35 ef	V1	N1	
684.98 d	665.68 de	12.66 cd	12.33 cd	68 bcd	67.56 cde	2.50 d	2.42 de	V1	N2	
479.27 gh	467.08 h	10.06 fg	10.23 gh	61.93 ef	63.26 gh	2.09 f	2.02 g	V0	N0	T1
769.53 c	714.24 cd	13.16 cd	12.53 cd	68.96 bcd	68.30 bcd	2.58 bcd	2.55 bcd	V0	N1	
896.76 b	812.27 b	14.16 ab	13.50 b	70 abc	69.33 bc	2.67 bc	2.65 bc	V0	N2	
523.09 fg	492.12 h	10.76 ef	10.40 fgh	64.93 def	64.56 fgh	2.17 ef	2.12 g	V1	N0	
786.74 c	741.63 c	13.60 bc	12.70 bc	71.76 ab	70.43 b	2.73 b	2.71 ab	V1	N1	
999.81 a	949.98 a	15.05 a	14.46 a	73.73 a	72.80 a	2.92 a	2.85 a	V1	N2	

Values with at least one similar letter in each column have no significant difference based on Duncan's test at 5% level.

Nitrate reductase (NR)

Interaction of three factors (T × N × V) was significant on NR at 1% level. Comparison of means showed that the highest average NR (12.19 and 12.48 mmol/g WW in 2017 and 2018, respectively) belonged to Roodasht V in treatments with conventional tillage and urea fertilizer plus nitrapyrine. Therefore, average NR increased in T0N2V1 treatment by 71.93% and 58.17% in 2017 and 2018, respectively, compared to control treatment (T0N0V0) under conventional tillage conditions.

The highest average NR (17.27 and 18.13 mmol/g WW in 2017 and 2018, respectively) was recorded for Roodasht V in treatments with minimum tillage and urea fertilizer plus nitrapyrine. Therefore, average NR increased in T0N2V1 treatment by 63.69% and 68.02% in 2017 and 2018, respectively, compared to control treatment (T0N0V0) under minimum tillage conditions.

Grain N content

Preferred ammonium uptake versus nitrate, increased ammonium retention in soils treated with nitrate inhibitors, synergism between ammonium and nitrate, and increased N uptake rate were some of the reasons that increased grain N uptake in the present study, which is in agreement with those of Kiani (2012) and Fangueiro et al. (2009) [13, 14].

Interaction of three factors (T × N × V) was significant on grain N content at 1% level. Comparison of means indicated that the highest average grain N levels (119.56 and 121.73 mg/kg in 2017 and 2018, respectively) belonged to Roodasht V in treatments with conventional tillage and urea fertilizer plus nitrapyrin. Therefore, average grain N contents increased in T0N2V1 treatment by 43.01% and 43.16% in 2017 and 2018, respectively, compared to control treatment (T0N0V0) under conventional tillage conditions.

According to the results, both conventional and minimum tillage with the application of nitrification inhibitor increased grain N levels in Rhoodasht V compared to the control. This results from reduced N loss through denitrification and consequently improved N supply to grains, an increase in the grain nitrogen availability over a longer period due to relatively higher amounts of soil ammonium, and root ammonium nutrition.

Plant N content

High levels of organic matter and nutrients in the soils of minimum tillage treatment led to a significant response to the plant N content. Barley N contents increased in N_2 and N_3 fertilizer treatments by 14.95% and 18.59% in 2017, and by 14.08% and 18.77% in 2018, respectively, compared to N1. This can be attributed to a decline in nitrification rate and prolongation of N usability in the soil as a result of using the nitrification inhibitor, which corresponds to that of Kiani (2012) [13].

Interaction of three factors (T × N × V) was not significant on the plant N content. Comparison of means revealed that the highest average plant N levels belonged to Roodasht V (361.46 mg/kg) in 2017 and Walfajr (373.26 mg/kg) in 2018 within treatments with conventional tillage and urea fertilizer plus nitrapyrin. Therefore, it can be concluded that average plant N content increased in Roodasht V compared to control treatment under both conventional and minimum tillage conditions. This resulted from the use of nitrapyrin, which reduced the energy for absorption and synthesis by the use of ammonium compared to nitrate. Additionally, nitrapyrin acidifies the root environment and facilitates nitrate uptake through simultaneous transfer of proton/nitrate, eventually improving plant yield by increasing nitrogen uptake in the plant.

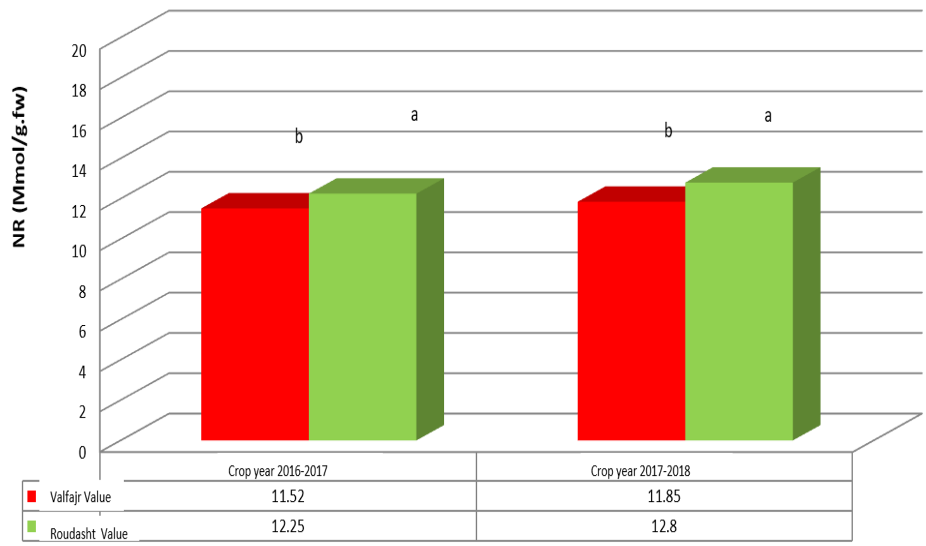

Figure 8: Comparison of mean nitrate reductase (NR) in two barley cultivars

Figure 9: Comparison of mean grain nitrogen in two barley cultivars

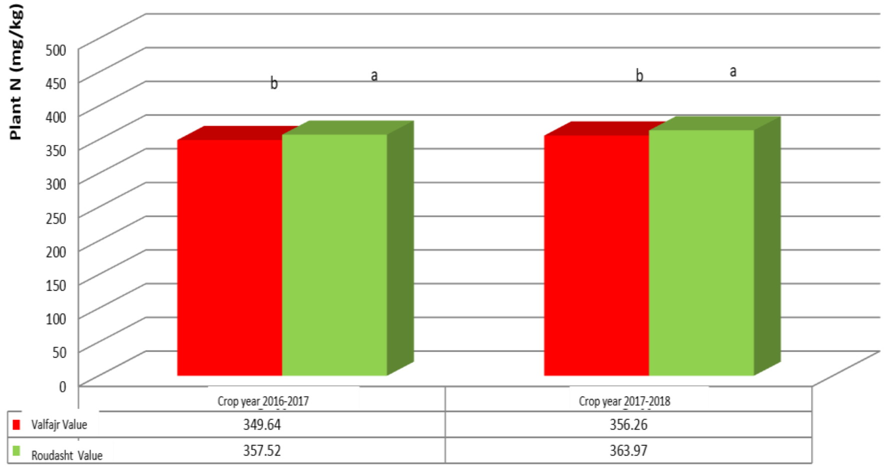

Figure 10: Comparison of average plant nitrogen in two barley cultivars

Table 6: Two-year composite analysis of nitrate reductase (NR), grain nitrogen, and plant nitrogen

Source of changes	df	Mean of squares		
		NR	Grain N	Plant N
Year (Y)	1	3.822**	379.960*	767.666*
Repeat (year)	4	0.391	18.307	8.983
Tillage (T)	1	157.679**	11120.375**	10860.923**
Y × T	1	0.001$^{n.s}$	97.068$^{n.s}$	14.133$^{n.s}$
Main error	4	0.173	18.629	55.057
N fertilizer (N)	2	153.113**	12086.587**	23832.915**
Variety (V)	1	12.013**	928.805**	1094.340**
Y × N	2	0.296$^{n.s}$	27.107$^{n.s}$	20.167$^{n.s}$
Y × V	1	0.292$^{n.s}$	1.027$^{n.s}$	0.133$^{n.s}$
T × N	2	11.065**	1209.113**	209.893$^{n.s}$
T × V	1	0.005$^{n.s}$	0.375$^{n.s}$	21.233$^{n.s}$
V × N	2	2.517**	18.465$^{n.s}$	134.433$^{n.s}$
Y × N × T	2	0.621$^{n.s}$	7.722$^{n.s}$	49.540$^{n.s}$
Y × V × T	1	0.075$^{n.s}$	8.000$^{n.s}$	7.933$^{n.s}$
Y × N × V	2	0.315$^{n.s}$	2.78$^{n.s}$	15.853$^{n.s}$
V × N × T	2	3.636**	206.929**	92.223$^{n.s}$
Y × N × T × V	2	0.160$^{n.s}$	1.402$^{n.s}$	5.015$^{n.s}$
Sub error	40	0.253	19.736	68.736
Total	71	-	-	-
CV (%)		4.16	3.72	2.32

** and **: significant at 5% and 1% probability levels, respectively.
n.s.: non-significant difference

Table 7: Comparison of triple interaction of tillage (T), nitrogen (N) fertilizer, and barley varieties (V) on nitrate reductase (NR), grain nitrogen, and plant nitrogen

Plant N (mg/kg) 2017-2018	Plant N (mg/kg) 2016-2017	Grain N (mg/kg) 2017-2018	Grain N (mg/kg) 2016-2017	NR (Mmol/g.fw) 2017-2018	NR (Mmol/g.fw) 2016-2017	V	N fertilizer	Tillage

309.53 h	304.23 f	85.03 i	83.60 f	7.89 i	7.09 i	V0	N0	T0
349.83 e	343.40 d	109 ef	103.56 e	11.28 ef	11.16 ef	V0	N1	
373.26 cd	360.50 c	120.66 d	118 d	12.09 de	11.80 de	V0	N2	
319.20 gh	316.23 ef	96.13 h	95.13 e	9.62 h	8.95 h	V1	N0	
366.66 d	359.33 c	115.23 de	114.30 d	11.75 de	11.32 def	V1	N1	
371.16 d	361.46 c	121.73 d	119.56 d	12.48 d	12.19 d	V1	N2	
332.03 fg	321.13 e	98.03 gh	95.16 e	10.79 fg	10.55 fg	V0	N0	T1
377.33 cd	377.70 b	143.20 c	133.53 c	13.59 c	14.02 c	V0	N1	
395.56 ab	390.90 ab	152.56 b	145.63 b	16.13 b	15 b	V0	N2	
337.50 ef	330.50 de	103.56 fg	99.60 e	10.15 gh	10.07 g	V1	N0	
387.26 bc	381.90 ab	145.23 c	135.16 c	14.03 c	13.22 c	V1	N1	
402.03 a	395.73 a	168.26 a	160.26 a	18.13 a	17.27 a	V1	N2	

Values with at least one similar letter in each column have no significant difference based on Duncan's test at 5% level.

Discussion and Conclusion

Barley is one of the most adaptable cereals produced in favorable weather conditions in fertile soils with high water retention and a pH of 7-8. The plant is more resistant to drought than wheat hence it can produce the highest yield in a climate where water restricts cereal production. The water use efficiency (WUE) in barley is higher than that of wheat under drought stress. In addition, WUE is higher in varieties with rootlet than rootlet-free ones. Barley is sensitive to flood conditions. The grain filling pattern of barley is similar to that of wheat. Current photosynthesis supplies a large portion of the carbohydrate needed for grain filling. Spike photosynthesis provides 30-43% of final grain weight [15].

This study was conducted to investigate the effect of two tillage levels and urea fertilizer with nitrapyrin (a nitrification inhibitor) for increasing NUE on quantitative and qualitative traits of barley varieties.

In both conventional and minimum tillage, the use of urea fertilizer plus nitrapyrin conditions increased nutrient availability and elevate protein synthesis in Roodasht V by increasing cell polysomes. Also, the grain soluble protein content decreased in the absence of N fertilizer due to the lack of nutrients in the plant, leading to declined protein content of the grains. As a result, the use of urea fertilizer with nitrapyrin improved both the photosynthesis and total dry weight of the plant, along with elevated grain protein content with rising the plant dry weight.

Since grain protein yield is a function of grain protein content and grain yield, increases in these two traits can improve grain protein yield. According to the results, it can be concluded that the conventional and minimum tillage conditions with the use of urea fertilizer plus nitrapyrin led to increased grain protein yield compared to the control. This resulted from the use of more accessibility of N fertilizer in the plant, resulting in the transfer of water and photosynthates from the source to the destination (grains) for grain filling, which creates larger grains and increases grain yield and protein content, thereby raising grain protein yield.

Due to the fact that nitrogen fertilizer deficiency reduces the number of cells and photosynthates in the plant, NUE in the application of nitrapyrin inhibitor was higher than that under the nitrapyrin-free condition. The concomitant use of nitrapyrin with urea fertilizer could reduce the amount of N fertilizer consumption and consequently increased further transfer to the plant, which is economically of high importance and reduces production costs. Nitrapyrin inhibitor, therefore, can be identified as an effective agent in reducing N fertilizer loss and thus increasing both N transfer from soil to the plant and its yield.

The NR enzyme rises with increases of nutrients and nitrogen content in the plant, which provide greater amount of nitrogen to the grain and plant, thus producing more chlorophyll and increasing grain yield.

A positive significant correlation was also observed between NR and grain yield at 5% level. NR increased with rising nitrogen content and led to improved grain and plant nitrogen levels, thereby raising harvest index, N efficiency, grain yield, and chlorophyll production.

References

1. Akhavan Samimi, K. Effects of different levels of foliar application with ascorbic acid (vitamin C) and application of superabsorbent polymer on the eco-physiological traits of barley in lead-contaminated soils. MSc thesis in Agroecology, Faculty of Agriculture, Varamin University, 2014.
2. Fateh, H. Effects of nitrogen and zinc fertilizers on physiological traits of two autumn barley varieties under drought stress conditions. MSc thesis, Faculty of Agriculture, University of Kurdistan, 2010.
3. Hosseini, R, Galeshi, S, Soltani, A, Kalate, M. Effect of nitrogen fertilizer on grain yield and yield components of old and new wheat varieties. Electronic Journal of Crop Production, 2011; 4, 1. 187-199.
4. Shokhmgar, M, Baradaran, R, Mousavi, Gh, Pouyan, M, Arzamjo, A. Effects of irrigation period and nitrogen fertilizer on grain yield

changes and physiological traits of fenugreek. Iranian Journal of Medicinal and Aromatic Plants Research, 2013; 29, 3, 527-538.

5. Sadeghi, H., Kazemaini, A. Effects of crop residues and nitrogen fertilizer management on grain yield and yield components of two barley varieties under rainfed conditions. Iranian Journal of Crop Sciences, 2011; 3, 13.
6. Mousavi Shalmani, M, A, Lakzian, R, Khorasani Khavazi, K. The influence of nitrate inhibitory 3, 4- dimethyl pyrazol phosphate on nitrogen-15 dynamics and yields of different wheat genotypes with different carbon-13 discrimination index. Iranian Journal of Dryland Agriculture,2015; 4, 2.
7. Ehsanpour, F, Kiani, Sh, Hosseinpour, A. Effect of 3,4-dimethyl pyrazol phosphate and phosphorus inhibitors on phosphorus use efficiency and yield in wheat. Journal of Water and Soil (Agricultural Science and Technology), 2012; 26, 6, 1473-1482.
8. Gaghnia Gh, Koochaki, A. Sustainable Soil Management (translation), Mashhad University Jihad Press, 204 p., Mashhad, Iran, 1996.
9. Amini, A, Rajaie, M, Farsinejad, K. Effects of different tillage methods and plant residue management on yield and yield components of wheat. Journal of Plant Ecophysiology, 2014; 6, 16.
10. Mohammadzadeh, A, Hosseini, NM, Moghaddam, H., Akbari, M. Effects of drought stress and nitrogen fertilizer levels on physiological traits of two red bean genotypes. Iranian Journal of Crop Sciences, 2012; 14, 3.
11. El-Tayeb, M. A. Differential responses of pigments, lipid peroxidation, organic solutes, catalase and peroxidase activity in the leaves of two Viciafaba L. cultivars to drought.Int. J. Agric. Biol. 2006; 8: 116-122
12. Moraghebi, F, Akbari M and A, Hooshmandfar. Effect of nitrogen use levels and time on grain protein content and nitrogen efficiency of wheat (Pishtaz Cv.) in Saveh. Research Journal of Plant and Biome, 2011; 7, 1-29.
13. Kiani, Sh. Effect of 3 and 4-dimethylpyrazol phosphate nitrification inhibitor on nitrogen uptake, yield components, and wheat yield under greenhouse conditions. Journal of Water and Soil, 2011; 26, 5: 1226-1235.
14. Fangueiro D., Fernandes A., Coutinho J., Moreira N., Trindade H. Influence of two nitrification inhibitors (DCD and DMPP) on annual ryegrass yield and soil mineral N dynamics after incorporation with cattle slurry. Communications in Soil Science and Plant Analysis, 2009; 40:3387-3398.
15. Khodabandeh, N. Cereal Farming. University of Tehran Publications, 2000; 537 p.