

Variation of Nitrogen Use Efficiency, Grain Protein Concentration and Yield in Wheat Cultivars in Temperate Sub Humid

¹Ali Rahemi-Karizaki, ²Serollah Galeshi, ²Afshin Soltani and ²Behnam Kamkar

¹Student of Gorgan University of Agricultural Science and Natural Resources,
P.O. Box 386, Gorgan, Iran

²Department of Agronomy and Plant Breeding,
Gorgan University of Agricultural Science and Natural Resources, P.O. Box 386, Gorgan, Iran

Abstract: Wheat (*Triticum aestivum* L.) is the most important crops in Iran. Growing conditions, N, as one of the main inputs for cereal production systems. The increasing need to reduce pollution from N fertilizer is concomitantly strengthening the importance of improving the understanding of nitrogen use efficiency (NUE) of these crops. The objective of this study was to determine the significance and magnitude of variation in NUE, yield and grain protein concentration among diverse wheat genotypes in years of release. Field experiments were conducted during the growing seasons of 2007-2008 and 2008-2009 under well-watered conditions in Iran. Sixteen wheat cultivars were sown in a randomized complete block design with three replications. The study revealed that most breeding effects on NUE were associated with change in nitrogen utilization efficiency (NUE). Genetic improvement of grain yield was not paralleled by improvement of grain protein concentration (GPC) in a manner that with years of release grain yield improved but quality of grain not improved.

Key words:Wheat · Nitrogen use efficiency · Nitrogen utilization efficiency · Nitrogen uptake efficiency · grain protein concentration and grain yield

INTRODUCTION

It is important to define the complex trait of nitrogen-use efficiency (NUE) before considering its improvement through breeding and N management strategies. Moll *et al.* (1982) and Ortiz-Monasterio *et al.* (1997) defined NUE as grain dry matter yield per unit of N available (from the soil and/or fertilizer) and divided it into two components: (i) N-uptake efficiency (crop N uptake/N available; NUpE) and (ii) N-utilization efficiency (grain dry matter yield/crop N uptake; NutE). Nitrogen utilization efficiency comprises harvest index (HI) and biomass production efficiency (BPE). The nitrogen economy of wheat is considered in relation to both NUE and the relationships between grain protein content and composition and N supply. It is important that wheat breeders of worldwide consider selecting genotypes with high efficiency in remobilizing N from vegetative parts to the grain or genotypes with high grain protein concentration [1-6] and in developing wheat N

management strategies [7, 8] for high NUE. Recent results obtained under northern growing conditions showed that NUpE has a stronger relationship with NUE than NutE [9]. Moll *et al.* (1982) speculated that the role of NUpE is emphasized in determining NUE as soil N supply increases. Several studies showed improved NUE in modern wheat cultivars [10-12]. However, there were hardly any correlations between total N uptake and year of cultivar release [10, 11]. However, Dhugga and Waines (1989) comparing 12 spring wheat in California, found that NUpE was the most important component of NUE at all N levels. Genetic gains in NUE with breeding under low N supply have been related mainly to improvements in NUpE in spring wheat in Mexico [13] and Finland [9] and to NutE in winter wheat in France [14] and the UK [15]. In wheat, it has been found that plant breeding increased nitrogen harvest index [11, 16]. Also Genetic differences in grain protein concentration among wheat cultivars are considered as intrinsic factors to affect grain protein formation in production conditions [17]. Numerous

studies of cultivars and segregating populations have shown inverse relationships between grain yield and grain N [18, 19]. As mentioned above, an inverse genetic relationship is usually reported between grain yield and protein concentration in wheat cultivars [10, 13, 18- 20].

The objective of this study was to determine the significance and magnitude of variation in N content, NUE, NUpE, NUtE and grain protein concentration among diverse wheat genotypes in years of release. Specifically, we aimed to determine if there was a positive correlation between these factors and grain yield.

MATERIALS AND METHODS

Experimental Design: The field experiments were conducted at the Gorgan University of Agricultural Sciences Research Farm, Gorgan (36°88N, 54°27'E and 13 m asl), Iran in the growing seasons of 2007-2008 and 2008-2009 under well-watered conditions. Sixteen wheat cultivars (Table 1) were sown in a randomized complete block design with three replications.

The experiments were sown on December 10 and 24 of 2007 and 2008, respectively. Plots consisted of 8 rows 1.5 m long and 0.20 m apart. Seeding rate was calculated for each cultivar using germination percentage and 1000-seed weight to achieve a density of 350 plant m⁻². Prior to seeding 150 kg ha⁻¹ superphosphate was broadcasted and incorporated into the soil. Plots were top-dressed at tillering, stem elongation and heading stages with urea (each 50 kg ha⁻¹). Weeds were controlled with 2,4,D + MCPA [(4-chloro-2-methylphenoxy) acetic acid] at 1000 mL h⁻¹ in early growth stages (three leaf stage - mid tillering) [21]. Composite soil samples from 0 to 30 cm depth were taken for analyses prior to basal fertilizer application. Separate soil samples were also taken for bulk density determination. Total N was determined by the Kjeldahl method of Bremner (1960) and NH₄⁺ ±N by the method of Bremner and Keeney (1966) and other Chemical and physico-mechanical characteristics (Table 2).

Measurement: Plant samples were collected by hand at anthesis and maturity by uprooting each cultivar row. Ten plants of each cultivar in each replication were selected for further analysis. These samples were separated in two components at anthesis (leaf + culm and chaff-flowered spikes) and in three components at maturity [leaf + culm, chaff (rachis, glumes, awns) and grain]. Samples were dried at 70°C for 3 d and weighed dry matter (DM). N content of the plant parts was measured using a modified version of the Kjeldahl procedure with a Kjeltec Auto

Table 1: Characteristics of spring cultivars grown in 1969-2006 at Golestan province, Iran

Cultivar	Abbreviation	Year of release
Eniya	En	1969
Khazar1	kh	1974
Naz	Na	1979
Golestan	Go	1987
Falat	Fa	1991
Rasol	Ra	1993
Tajan	Ta	1996
Atrak	At	1996
Zagros	Za	1997
Shanhi	Sha	1997
Pastor	Pa	1997
Shirodi	Sh	1998
Kohdasht	Ko	2001
Daya	Da	2006
Moghan	Mo	2006
Arta	Ar	2006

Table 2: Soil properties in the beginning of 2-year of experiment at depth of 0-30 cm

Parameter	Year	
	2007-2008	2008-2009
Organic matter (%)	2.85	0.66
pH	7.6	8
Silt (%)	66	66
Clay (%)	34	34
Sand (%)	10	10
N (%)	0.14	0.03
EC (dSm ⁻¹)	1.7	0.85
Soil mineral N (Kg ha ⁻¹)	30.6	28.24
P (mg kg ⁻¹)	13	8
K (mg kg ⁻¹)	370	400

1030 Analyzer. At harvest, a 1.5 m² portions at the center of each wheat plot was sampled and determined biomass and harvest index. The following parameters related to yield and nitrogen use efficiency is discussed in this paper [15, 16]:

$$\text{Harvest index (HI)} = (\text{grain yield (g m}^{-2}\text{)} / \text{biological yield (g m}^{-2}\text{)}) \times 100$$

$$\text{Biomass production efficiency (BPE)} = \text{total aboveground biomass (g m}^{-2}\text{)} / \text{total aboveground N (g m}^{-2}\text{)}$$

$$\text{Proportion of the N in grain in relation to total aboveground N at maturity: nitrogen harvest index (NHI)} = \text{grain N/total N content in aboveground parts at maturity}$$

$$\text{Grain nitrogen concentration (GNC)} = ((\text{grain N (g)}) / (\text{grain dry mass (g)})) \text{ at harvest} \times 100$$

$$\text{Grain protein concentration (GPC)} = \text{GNC} \times 5.75$$

$$\text{Nitrogen use efficiency (NUE)} = \text{grain yield (gm}^{-2}\text{)} / \text{soil available nitrogen (g m}^{-2}\text{)}$$

Available nitrogen (g m^{-2}) = (N fertilizer + N soil at sowing ($\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$) + mineralized N during growing season ($\text{organic matter} \times 0.02$)) \times 0.5.

Nitrogen utilization efficiency (NUE) = grain dry mass (g m^{-2}) / aboveground N at harvest (g m^{-2})

Nitrogen uptake efficiency (NUpE) = aboveground N (g m^{-2}) at harvest / available N (g m^{-2})

Statistical Analysis: Statistical analysis was carried out using the SAS software package [24]. Differences among the treatments were evaluated with the least significant difference (LSD) test at $P=0.05$, whereas Pearson correlation coefficients were used to correlate parameters.

RESULTS AND DISCUSSION

Weather Condition: The weather was characterized by great variability. Table 3 showed monthly rainfall, minimum and maximum temperatures at the experimental site over the 2-year study. Rainfall varied considerably between years. 2008-2009 was wetter year (297.8 mm), than 2007-2009 (229.6 mm). Mean annual rainfall for the area over the last 40 years is 383.3 mm. Rainfall distribution also differed between years. The maximum monthly rainfall was recorded at February in 2008-2009 higher than the long-term average (112.1 mm versus 57.60mm) while maximum monthly rainfall was recorded at December in 2007-2008 with 71.90 mm. Differences in temperature between the two study years were relatively modest

(Table 3). The mean monthly minimum temperatures in the two growing seasons were lower than long term average. The mean monthly minimum ranged between -2.30 -1.4°C and -2.60 -11.80°C in 2007-2008 and 2008-2009, respectively versus 3.40 -19.40°C in long term. In generally mean monthly minimum temperature in long term was higher than two growing seasons. The mean monthly maximum temperature during this period was ranged between 8°C at January and 37.40°C at May in 2007-2008 and between 22.40°C at January and 38.40°C at June in 2008-2009 versus 16 -29.60°C in long term.

Nitrogen Use Efficiency: Analysis of variance for traits of NUE and NUE showed that effects year and year \times cultivar interaction were not significant even though effect of cultivar was significant (Table 4). Also effects year, cultivar and year \times cultivar interaction were not significant. Nitrogen use efficiency ranged from 10.01 to $13.54 \text{ g g}^{-1} \text{ N}$, depending on cultivar. Low yielding cultivars had the lowest NUE values. Also, high-yielding wheat cultivar had the highest NUE (Table 5). The relationships between NUE and year of cultivar release were evaluated by linear regression. As there were no significant year effects on NUE and trends in both years were similar, though slopes differed in magnitude, responses were calculated from means across 2 yr. There was clear significant linear relationship between NUE and year of release of wheat cultivars (Fig. 1). The genetic gain estimated for NUE in wheat was $0.06 \text{ g g}^{-1} \text{ Nyr}^{-1}$.

Table 3: Monthly meteorological data for 2007-2008 and 2008-2009 growing seasons and long term (40 years) averages at Gorgan, Iran.

Data	Year	DEC	JAN	FEB	MAR	APR	MAY	JUNE
Min temperature ($^\circ\text{C}$)	2007-2008	5.80	-2.30	0.40	6.10	6.60	9.80	14
	2008-2009	-0.80	-2.60	-0.60	1	-1	5.40	11.80
	Long term	6.30	3.80	3.40	6.40	9.90	14.70	19.40
Max temperature ($^\circ\text{C}$)	2007-2008	14.90	8.00	10.69	18.50	34	37.40	36.40
	2008-2009	26.80	22.40	23.60	31.40	29.40	33.20	38.40
	Long term	16	12.90	12.40	14.50	19.30	24.90	29.60
Precipitation (mm)	2007-2008	71.90	16.50	55.80	38.10	08.00	24.80	14.50
	2008-2009	51.20	15.00	112.10	13.40	63.20	29.80	13.10
	Long term	52.30	56.90	57.60	73.30	60.30	47.20	35.70

Table 4: Analysis of variance (mean square) of the effects year and genotype on traits obtained from 16 genotypes grown at Grgan; Iran, in 2007-2008 and 2008-2009

Source	df	NUE	NUE	NUpE	NHI	GPC	BPE	GY	HI
Y	1	7.10 ^{ns}	1196.39 ^{ns}	0.14 ^{ns}	198.78 ^{ns}	70.01 ^{**}	0.33 ^{ns}	424041.33 ^{**}	878.82 ^{**}
Error a	4	57.87	5.58	0.04	66.35	2.79	132.50	29035	20.68
G	15	10.28 [*]	22.44 [*]	0.01 ^{ns}	51.26 ^{ns}	4.62 [*]	153.74 [*]	159229.59 [*]	22.34 [*]
G \times Y	15	5.91 ^{ns}	30.96 ^{ns}	0.01 ^{ns}	78.86 ^{ns}	2.91 ^{ns}	138.99 ^{ns}	7089.50 ^{ns}	15.58 ^{ns}
Error b	60	5.68	14.31	0.005	95.76	2.43	165.58	5717.03	21.62
CV	-	17.44	11.41	18.09	9.79	11.85	11.69	12.96	10.46

* significant at 0.05 probability level; ** significant at 0.01 probability level, *** significant at 0.001 probability level, ns: not significant

Table 5: Means of cultivar and year on described traits in wheat.

	NUE	NUpE	NUtE	NHI	GPC	GY	HI	BPE
Year								
1	13.39	0.36	29.61	70.36	14.03	516.88	34.09	85.76
2	13.93	0.42	36.67	73.23	12.32	649.81	40.14	85.88
Pr > F	0.73	0.12	0.001	0.001	0.008	0.02	0.004	0.97
Cultivar								
En	10.01	0.37	32.28	75.92	13.67	465.56	34.99	84.54
kh	12.10	0.47	29.38	73.43	14.93	561.39	36.33	78.11
Na	11.21	0.44	34.70	75.40	12.70	528.47	37.02	88.52
Go	11.64	0.49	32.27	72.15	13.09	543.40	36.07	80.41
Fa	12.72	0.44	33.24	72.17	12.74	606.04	38.34	87.58
Ra	11.26	0.44	33.43	77.33	13.35	529.70	38.23	84.18
Ta	12.22	0.44	32.93	73.14	12.53	579.52	39.08	86.92
At	12.34	0.39	34.18	70.98	12.09	584.93	37.83	93.60
Sha	13.02	0.43	34.34	73.87	13.67	599.44	38.58	75.01
Pa	12.62	0.43	35.11	82.53	12.41	669.31	38.78	88.31
Za	11.90	0.39	30.29	73.25	14.59	598.33	33.33	87.45
Sh	10.76	0.40	30.08	71.12	14.41	587.43	34.20	82.21
Ko	12.71	0.43	33.75	73.61	12.70	602.15	38.85	89.17
Da	12.88	0.40	32.79	75.32	13.30	661.88	34.71	86.36
Mo	12.03	0.44	35.19	75.76	12.47	577.78	39.27	86.70
Ar	13.54	0.38	36.21	75.52	12.08	638.20	38.27	94.07
LSD	2.75	0.09	4.36	5.80	1.76	87.32	4.45	11.88

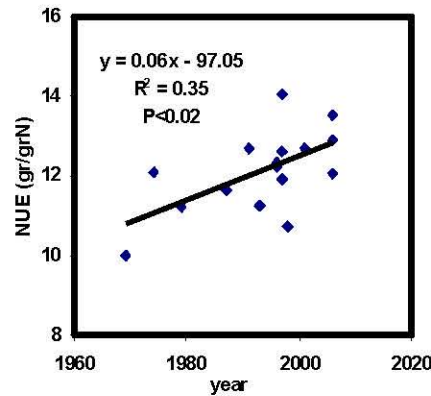


Fig. 1: Changes in nitrogen use efficiency with year of release for cultivars of wheat (The values are the means from 2 yr)

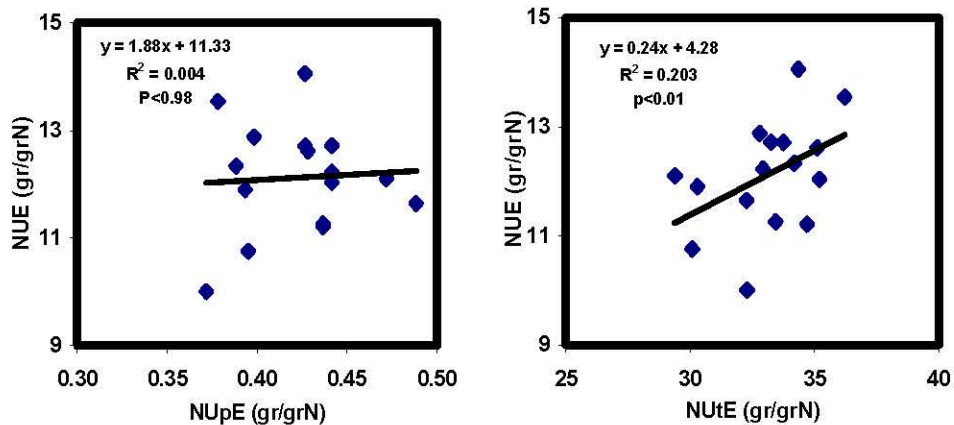


Fig. 2: Relationships between nitrogen use efficiency (NUE), nitrogen uptake efficiency (NUpE) and nitrogen utilization efficiency (NUtE) for wheat (The values are the means from 2 yr)

Table 6: Correlation matrix among traits obtained from 16 wheat cultivar grown at Gorgan, Iran, in 2007-2008 and 2008-2009.

Traits	NUE	NUpE	NUtE	NHI	GPC	GY	HI	BPE
NUE	1							
NUpE	0.07 ^{ns}	1						
NUtE	0.44*	-0.12 ^{ns}	1					
NHI	0.04 ^{ns}	-0.03 ^{ns}	0.46 ^{ns}	1				
GPC	-0.47*	0.1 ^{ns}	-0.50*	-0.03 ^{ns}	1			
GY	0.88**	-0.14 ^{ns}	0.23 ^{ns}	-0.08 ^{ns}	-0.27 ^{ns}	1		
HI	0.50*	0.33 ^{ns}	0.73***	0.26 ^{ns}	-0.63***	0.18 ^{ns}	1	
BPE	0.32 ^{ns}	-0.48 ^{ns}	0.42 ^{ns}	-0.42 ^{ns}	-0.64***	0.28 ^{ns}	0.20 ^{ns}	1

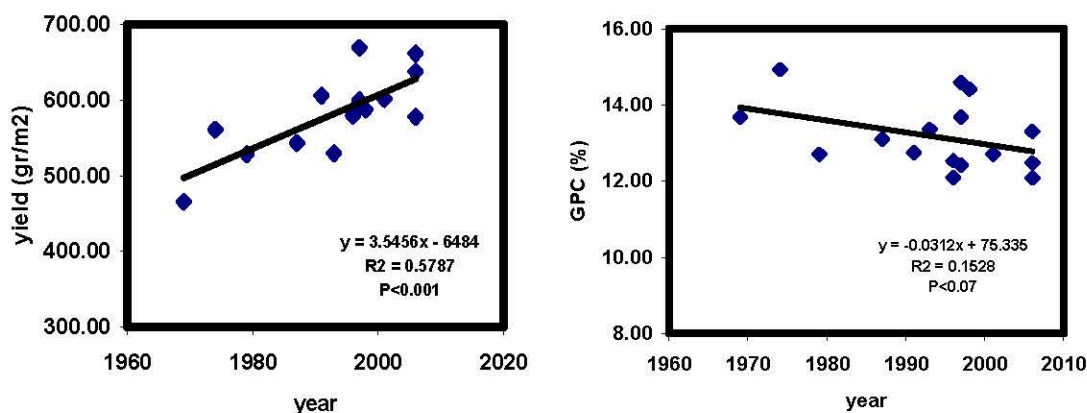


Fig. 3: Relationships between grain yield (GY) and grain protein concentration (GPC) with years of release for wheat cultivars (The values are the means from 2 yr)

The relationships between NUE measurements with NUtE and NUpE were evaluated by linear regression. There was a strongly significant relationship between NUE with NUtE for all cultivars wheat but relationship between NUE with NUpE not significant (Fig. 2).

Correlations between NUpE, NUtE, NUE and other traits measured from mature plants were calculated using average values across 2 yr. The NUpE had not significant correlation with other traits. The NUtE had positively significant correlation with harvest index (HI). Also it had negatively significant correlation with grain protein concentration (GPC) (Table 6).

Abbreviations and units: nitrogen use efficiency (NUE; $gg^{-1}N$), nitrogen utilization efficiency (NUtE; $gg^{-1}N$), nitrogen uptake efficiency (NUpE; $gg^{-1}N$), nitrogen harvest index (NHI; %), production efficiency (BPE; $gg^{-1}N$), grain protein concentration (GPC; %), harvest index (HI; %), grain yield (GY; gm^{-2}), Year (Y), replication (R) and genotype (G).

Grain Yield and Protein Concentration: ANOVA showed significant effects of year and cultivar on GY and GPC ($P < 0.001$) but non-significant effects of year \times cultivar (Table 4). These differences for among cultivars ranged from 12.08 to 14.93 percentage and GY ranged from 465.56

to 669.31 gm^{-2} values (Table 5). The relationships between GY with years of cultivar release were evaluated by linear regression. There was clear significant linear relationship between GY and GPC with year of release of wheat cultivars (Fig. 3). These relationships between GY and GPC with years release showed that during years release GPC reduced and GY increased. The genetic gain estimated for GY and GPC in wheat was 3.55 $gm^{-2}yr^{-1}$ and -0.03 percentages per year. Grain yield was significantly positive correlated with NUE. Coefficient correlation GPC with NUE was significant (Table 6).

Abbreviations and units: nitrogen use efficiency (NUE; $gg^{-1}N$), nitrogen utilization efficiency (NUtE; $gg^{-1}N$), nitrogen uptake efficiency (NUpE; $gg^{-1}N$), nitrogen harvest index (NHI; %), production efficiency (BPE; $gg^{-1}N$), grain nitrogen concentration (GNC; %), grain protein concentration (GPC; %), harvest index (HI; %), grain yield (GY; gm^{-2}), Year (Y), replication (R) and genotype (G).

DISCUSSION

According to our hypothesis wheat cultivars in Gorgan, Iran would differ in their NUE and yield due to differences in release year. However, our results indicated

that NUE values for modern wheat cultivar than old wheat cultivar was improved (Fig. 1). Nitrogen use efficiency ranged from 10.01 to 13.54 g g⁻¹ N, depending on cultivar. Low yielding cultivars had the lowest NUE values. Also the high-yielding wheat cultivar had the highest NUE (Table 5). The NUE values for wheat (average of all wheat cultivars, 12.06 g g⁻¹N) in our study were lower than mean values (26-44 g g⁻¹N) reported under different growing conditions [13,16]. However, the soil mineral N estimation used in this study did not include the mineralization of N during the growing season exactly and therefore calculated NUE values are not unambiguously comparable with values obtained with other methods. The genetic gains in NUE for wheat attributable to plant breeding reflect genetic gains in grain yield [13, 15, 16, 25]. Total mineral N levels in the experiments turned out to be high. This could have given better advantages for the modern cultivars with the higher response to high N levels and therefore promote the higher NUE of modern wheat cultivars compared with old cultivars. Nevertheless, similar to our results, Ortiz-Monasterio *et al.* (1997) demonstrated that under moderate fertilization rate, the NUE values of the modern wheat cultivars were higher than on old cultivars. However, the postulate of Ortiz *et al.* (2002) of improvement in two-row barley grain yields in the Nordic areas in is not supported by our results. It appears that NUE values for old cultivars at the beginning of the 20th century have influenced the extent of NUE improvement during the subsequent breeding process under temperate sub-humid growing conditions (Fig. 1).

Although wheat showed improved NUtE during the 2 yr. genotypic differences in NUE seem to have been mostly associated to NUtE for wheat (Fig. 2). Our result was differed with studies in the UK [15], Mexico [13] and Finland [9] found that increases in NUE were explained approximately equally by NUpE and NUtE. Also in contrast various studies worldwide have identified genetic associations between grain yield and NUE components under contrasting conditions of high and low N input supply. In general, these studies indicated that NUpE accounts for more of the genetic variation in NUE at low N than at high N supply, e.g. amongst 10 spring wheat cultivars in Mexico [13], oat cultivars in Finland [9] and 20 winter wheat cultivars in France [27]. However, Dhugga and Waines (1989) comparing 12 spring cultivars wheat in California, found that NUpE was the most important component of NUE at all N levels.

However, Foulkes *et al.* (2009) defined NUtE as a combination of HI and BPE and showed that new wheat

cultivars had improved HI rather than BPE. Also in our study NUtE and NUE had positively significant correlation with HI (Table 6) whereas NUtE had positive correlation with BPE but it no significant. Earlier studies on spring wheat and barley grown in the Nordic region showed that improvement in NUtE was achieved through reduced plant height and lodging and improving yields via improved HI [13, 26]. Also Muurinen *et al.* (2007) indicated that NUtE and NUE were positively correlated with HI for wheat and oat. Therefore, the NUE would be improved through NUtE. However, they showed there was no evidence for improvement of NUtE for barley. Also our result same, studies several studies worldwide concluded that wheat breeding did not result in consistent improvements in NUpE but in improved NUtE associated with higher harvest index, e.g. in Mexico [29], Argentina [10], France [14] and in various countries [30].

As mentioned an inverse genetic relationship is between grain yield and protein concentration in wheat cultivars in a manner that high-yielding cultivars (new cultivar) had lower protein concentration than low-yielding cultivars. Also with years of release grain yield improved but quality of grain not improved which may be attributed in part to the higher glucose costs for synthesis of protein than of carbohydrates [15]. This negative relationship is also in part due to the balance between the processes of carbon capture and N remobilization from the vegetative tissues to the grain. On the other hand, differences in NHI were mostly due to the effect of cultivars. Modern cultivars showed higher NHI than the oldest cultivar, which indicates that along years there was an improvement in the N partitioning to the grains. Numerous studies of cultivars and segregating populations have shown inverse relationships between rain yield and grain N concentration [10, 13, 15, 16, 18- 20]

The cultivars currently utilized have been selected because of their greater ability to respond to applied N. However, because use of high rates of N fertilizer associates with a range of environmental problems, the need to reduce N fertilizer rates has further emphasized the need for genotypes with improved NUE. As HI and BPE determine NUtE, to increase NUtE in the future would need more efficient improvement of BPE, while simultaneously maintaining a high HI. However, BPE was negatively correlated with NUE in this study. Therefore, increased N uptake would be the avenue to address NUE in wheat.

REFERENCES

1. Guarda, G., S. Padovan and G. Delogu, 2004. Grain yield, nitrogen-use efficiency and baking quality of old and modern Italian bread-wheat cultivars grown at different nitrogen levels. *Eur. J. Agron.*, 21: 181-192.
2. Hirel, B., J. Le Gouis, B. Ney and A. Gallais, 2007. The challenge of improving nitrogen use efficiency in crop plants: towards a more central role for genetic variability and quantitative genetics within integrated approaches. *J. Exp. Bot.*, 58: 2369-2387.
3. LaPerche, A., M. Brancourt-Hulmel, E. Heumez, O. Gardet and J. Le Gouis, 2006. Estimation of genetic parameters of a DH wheat population grown at different N stress levels characterized by probe genotypes. *Theor. Appl. Genet.*, 112: 797-807.
4. Li, Z., B. Li and Y. Tong, 2008. The contribution of distant hybridization with decaploid *Agropyron elongatum* to wheat improvement in China. *J. Genet. Genomics*, 35: 451-456.
5. Ortiz-Monasterio, J.I., G.G.B. Manske and M. Van Ginkel, 2001. Nitrogen and phosphorus use efficiency. In: Reynolds, M.P. Ortiz-Monasterio, J.I. McNab, A. (Eds.), *Application of Physiology in Wheat Breeding*. CIMMYT, Mexico, pp: 200-207.
6. Stanley Omar, P.B., Samonte, Lloyd T. Wilson, James C. Medley, Shannon R.M. Pinson, Anna M. McClung and Joveno S. Lales, 2006. Nitrogen Utilization Efficiency: Relationships with Grain Yield, Grain Protein and Yield-Related Traits in Rice. *Agron. J.*, 98: 168-176.
7. Raun, W.R., J.B. Solie, G.V. Johnson, M.L. Stone, R.W. Mullen, K.W. Freeman, W.E. Thomason and E.V. Lukina, 2002. Improving nitrogen-use efficiency in cereal grain production with optical sensing and variable rate application. *Agron. J.*, 94: 351-815.
8. Shanahan, J.F., N.R. Kitchen, W.R. Raun and J.S. Schepers, 2008. Responsive in-season nitrogen management for cereals. *Comput. Electron. Agric.*, 61: 51-62.
9. Muurinen, S., G.A. Slafer and P. Peltonen-Sainio, 2006. Breeding effects on nitrogen use efficiency of spring cereals under northern conditions. *Crop. Sci.*, 46: 561-568.
10. Calderini, S. D.F., Torres-Leon and G.A. Slafer, 1995. Consequences of wheat breeding on nitrogen and phosphorus yield grain nitrogen and phosphorus concentration and associated traits. *Ann. Bot.*, 76: 315-322.
11. Slafer, G.A., F.H. andrade and S.E. Feingold, 1990. Genetic improvement of bread wheat (*Triticum aestivum* L.) in Argentina: relationships between nitrogen and dry matter. *Euphytica*, 50: 63-71.
12. Reynolds, M.P. and N.E. Borlaug, 2006. Impacts of breeding on international collaborative wheat improvement. *J. Agric. Sci.*, 144: 95-110.
13. Ortiz-Monasterio, J.I., K.D. Sayre, S. Rajaram and M. McMahon, 1997. Genetic progress in wheat yield and nitrogen use efficiency under four nitrogen rates. *Crop. Sci.*, 37: 898-904.
14. Brancourt-Hulmel, M., G. Doussinault, C. Lecomte, P. Be' rard, Le B. Buanec and M. Trottet, 2003. Genetic improvement of agronomic traits of winter wheat cultivars released in France from 1946 to 1992. *Crop. Sci.*, 43: 37-45.
15. Foulkes, M.J., M.P. Reynolds and R. Sylvester-Bradley, 2009. Genetic improvement of grain crops: yield potential. In: V.O. Sadras and D.F. Calderini, (Eds.), *Crop Physiology Applications for Genetic Improvement and Agronomy*. Academic Press, Amsterdam, pp: 355-386.
16. Muurinen, S., J. Kleemola and P. Peltonen-Sainio, 2007. Accumulation and Translocation of Nitrogen in Spring Cereal Cultivars Differing in Nitrogen Use Efficiency. *Agron. J.*, 99: 441-449.
17. Jamieson, P.D. Zyskowski, R.F. Semenov, M.A. 2004. Modelling genetic variability in wheat quality. In: VIII European Society for Agronomy, *Book of Proceedings*. pp. 275-276.
18. Kibite, S. and L.E. Evans, 1984. Causes of negative correlations between grain yield and grain protein concentration in common wheat. *Euphytica*, 33: 801-810.
19. Triboi E., P. Martre, C. Girousse, C. Ravel and A.M. Tribot"-Blondel, 2006. Unravelling environmental and genetic relationships between grain yield and nitrogen concentration for wheat. *Eur. J. Agron.*, 25, 108-118.
20. Kramer, T., 1979. Environmental and genetic variation for protein content in winter wheat (*Triticum aestivum* L.) *Euphytica* 28, 209-218.
21. Zadoks, J.C., T.T. Chang and C.F. Konzak, 1974. A decimal code for the growth stages of cereals. *Weed. Res.*, 14: 415-421.
22. Bremner, J.M., 1960. Determination of nitrogen in soil by the Kjeldahl method. *J. Agric. Sci.*, 55: 11-33.

23. Bremner, J.M. and D.R. Keeney, 1966. Determination and isotoperatio analysis of different forms of nitrogen in soils: 3. Exchangeable ammonium, nitrate and nitrite by extraction distillation methods. *Soil Sci. Soc. Am. Proc.* 30: 577-582.
24. SAS Institute Inc, 1989. SAS user' guide: Statics, Version 6, 4th editions, SAS Inst. Inc. Cary, N.C.
25. Slafer, G.A. and P. Peltonen-Sainio, 2000. Yield trends of temperate cereals in high latitude countries from 1940 to 1998. *Agric. Food. Sci. Finl.*, 10: 121-131.
26. Ortiz, R., M. Nurminiemi, S. Madsen, O.A. Rognil and A. Bjo' mstad, 2002. Genetic gains in Nordic spring barley breeding over sixty years. *Euphytica*, 126: 283-289.
27. Le Gouis, J., D. Be' ghin, E. Heumez and P. Pluchard, 2000. Genetic differences for nitrogen uptake and nitrogen utilization efficiency in winter wheat. *Eur. J. Agron.*, 12: 163-173.
28. Dhugga, K.S. and J.G. Waines, 1989. Analysis of nitrogen accumulation and use in bread and durum wheat. *Crop. Sci.*, 29: 1232-1239.
29. Fischer, R.A. and P.C. Wall, 1976. Wheat breeding in Mexico and yield increases. *J. Aust. Inst. Agric. Sci.*, 42: 139-148.
30. Paccaud, F.X., A. Fossanti and H.S. Cao, 1985. Breeding for quality and yield in winter wheat: consequences for nitrogen uptake and nitrogen partitioning efficiency. *Z. Pflanzenzucht*, 94: 89-100.