Selcuk J Agr Food Sci, (2017) 31 (1), 42-47

ISSN: 2458-8377 DOI: 10.15316/SJAFS.2017.5

Selcuk Journal of Agriculture and Food Sciences

The Effects of Foliar Nitrogen Treatments at Heading Stage on Grain Protein Contents of Bread Wheat Cultivars

Erdinc SAVASLI[1], Oguz ONDER [1], Cemal CEKIC[1], Hasan Mufit KALAYCI [1], Ramis DAYIOGLU[1], Yasar KARADUMAN [1], Fatma GOKMEN [2], Nesim DURSUN [2], Sait GEZGIN [2]

[1]Transitional Zone Agricultural Research Institute, Eskisehir / Turkey

[2]Selçuk University, Faculty of Agriculture, Department of Soil Science and Plant Nutrition, 42075, Konya / Turkey

ARTICLE INFO

Article history:

Received: 23.03.2017
Accepted: 05.04.2017

Keywords:

Foliar solution
Nitrogen
SPAD
Wheat
Protein

ABSRACT

The studies were conducted at Transitional Zone Agricultural Research Institute in Eskisehir and Selçuk University in 2008-2010 growing seasons. Two bread wheat cultivars (Gerek 79 and Bezostaja 1) were used as plant material. Experimental treatments were 0, 30, 60, 90 and 120 kg N ha^{-1} applied at planting and tillering stages (early-season treatments) and 0, 20, 40 and 60 kg N ha^{-1} (urea form) applied at heading stage (late-season treatments) as foliar applications. A variation was created in terms of SPAD and TN at heading stage through different nitrogen rates in traditional nitrogen application time. Results under rain-fed conditions revealed that foliar nitrogen at heading stage could increase the protein content at least 1%, and the critical threshold NSPAD value was 0.96 for Bezostaja 1 and 0.94 for Gerek 79. On the other hand, the threshold TN value was 4.22% for Bezostaja 1 and 3.75% for Gerek 79 under the same conditions. The increase in grain protein content of Bezostaja 1 per 10 kg ha^{-1} of N was 2.0% in early-season and 3.2% in late-season treatments. The same increase rates for Gerek 79 were 1.5% in early-season and 2.7% in late-season treatments. Although both early and late-season treatments were effective on protein content and related quality traits, the application of foliar solution in heading stage was more dominant as compared to the traditional nitrogen application time.

1. Introduction

Despite the annual changes, Turkey with an annual production of 20 million tons is among the leading wheat producer countries of the world. Wheat is a significant crop in meeting nutritional needs of its own people and also a significant export commodity. International quality standards should be properly met and even surpassed to seize such an export potential. Grain protein content is the most important indicator of grain quality. Thus, protein composition is a highly significant factor for the end-use quality of wheat grains. While the qualitative composition is depending on genetics of the genotype, quantitative value is largely influenced by the environmental conditions. With regard to grain protein compositions, significant genotype x environment x treatment interactions were reported in previous studies (Zhu & Khan 2001).

Late-season foliar solution treatments may improve protein content of grains. Diluted urea ammonium nitrate is commonly used to prevent blight or salt effects of high concentration urea treatments. Spiertz (1983) indicated that 65-80% of grain nitrogen came from post-flowering translocation of the nitrogen taken up before flowering and also reported decreased post-flowering root activity and consequent decreased nitrogen uptake especially under dry conditions (Ellen & Spiertz 1980). While the nitrogen-use efficiency for the nitrogen provided at sowing was between 30-55%, the ratio for the nitrogen provided at flowering increased to

* Corresponding author email:erdinc.savasli@tarim.gov.tr

55-80% levels (Wuest & Cassman 1992). Westcott *et al (*1997) carried out a study with spring wheat in Montana under irrigated conditions and indicated that additional fertilization increased protein contents when the normalized SPAD values decreased to 0.93-0.95 levels and such an increase was almost 1% at an NSPAD value of 0.89. Researchers also reported that high correlations between flag leaf total nitrogen (TN) values and supplemented nitrogen indicates grain protein response to supplemented nitrogen when the flag leaf TN contents were below 4.2-4.3%. However, they were not able to express the level of response with the tests employed. Lorbeer *et al* (2000) carried out a study with two different winter wheat cultivars at two different locations (dry and wet) of Montana and reported that every 0.1% increase in flag leaf total nitrogen content yielded 0.26% increase in grain protein content of one cultivar and 0.55% increase in protein content of the other cultivar. The researchers also indicated the threshold flag leaf total nitrogen content as 4.2%.
In this study aimed to determining the critical thresholds by using NSPAD and flag leaf nitrogen value to increase the bread wheat grain protein content.

2. Materials and Methods

The present study was conducted at 2008-2010 growing seasons at 4 different locations over the experimental fields of Eskişehir Agricultural Research Institute and farmer's fields. The factors in this study were 0, 30, 60 and 90 kg N ha^{-1} doses applied at traditional nitrogen application periods (half with sowing and the other half at tillering) to create a variation; and 0, 20, 40 and 60 kg N ha^{-1} foliar solutions applied at heading stage. Experiments were conducted in split plots in randomized complete block experimental design with 4 replications. Early-season treatments to create a variation were placed in main plots and foliar solution treatments (major factors) were placed in sub-plots. Seeding density of the experimental treatments was 450 kernel m^{-2}. In foliar solution treatments, 4% urea solution was used at heading stage, and solutions were applied in two equal portions (each portion was applied in one-week interval) to prevent leaf blights. Common Minolta SPAD-502 chlorophyll meter (Markwell *et al* 1995) was used to measure leaf chlorophyll concentration in SPAD units, and assessments were made over SPAD units.

SPAD readings at heading stage were performed on flag leaves over main shoot of randomly selected 10 plants. Readings were performed at 3 different sections. The SPAD readings taken from experimental plots were then divided by SPAD readings taken from the plots with the highest fertilization dose of the same genotype. This process is called normalization of SPAD values and expresses the relative SPAD values as follows:

NSPAD (Normalized SPAD) = SPAD (plot) / SPAD (maximum) *(1)*

Total nitrogen measurements were performed over 30 flag leaves taken from main shoots. Measurements were made in accordance with Dumas method (AACC 2000). Monthly precipitations for the experimental years of the research institute are provided in Table 1.

Table 1
Monthly average precipitation of the experimental site at the Institute during 2007-2010 growing seasons.

Years	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Annual Total (mm)
Long-Term	14.7	25.2	30.6	45.6	38.4	32.6	33.3	35.0	42.1	29.3	13.8	6.5	347.1
2007-08	0.0	19.2	92.4	49.9	15.7	1.0	42.4	38.5	11.7	9.3	0.0	5.5	286.6
2008-09	30.7	6.4	49.6	34.5	66.3	82.0	40.9	28.0	15.4	10.2	19.4	2.0	385.4
2009-10	7.1	9.0	29.5	65.1	36.0	42.8	32.6	23.9	20.7	79.0	7.4	0.9	354.0

Soil samples were subjected to NO_3 and NH_4+NO_3 nitrogen analyses with two different methods recommended by Bremner (1965). Samples were not dried and analyses were performed at field soil moisture contents. Soil chemical characteristics of the experimental fields are provided in Table 2.

Nitrate nitrogen levels of the experimental soils were low. These low levels were taken into consideration while selecting the experimental fields. Experimental soils were fine textured (clay, clay-loam) with low organic matter contents. Soil lime levels were ranged from medium to high. Soils had slight alkaline reaction and were slightly saline or nonsaline.

Table 2

Soil characteristics of experimental sites (0- to 30-cm soil layer)

Charactertics	Unit	2008 Loc1	2008 Loc2	2009 Loc3	2009 Loc4
Texture		C	C	CL	SCL
pH (1:2.5 Soil:Water)		7.52	7.36	7.35	7.67
EC (Salt) (1:5, Soil:Water)	µS/cm	140	130	203	180
$CaCO_3$ (Lime)	(%)	9.6	13.5	19.1	23.3
Organic Matter	(%)	1.1	1.31	2.06	1.79
Phosphorus (P)	mg kg^{-1}	32.1	8.39	18.1	20.5
Potassium (K)	mg kg^{-1}	667	365	405	250
Phenoldisulphonic Acid Met. (NO_3)	mg kg^{-1}	3.3	2.29	3.83	6.6
KCl (NH_4^+ , NO_3)	mg kg^{-1}	15.9	20.6	21	13.7

Data analysis: JMP statistical software (JMP, SAS Institute, Cary, NC) was used for data analyses. Significance of the effects of treatments was tested through variance analysis and Student's t-test was used to compare the means. Regression and correlation analyses were performed following the variance analysis as it was in every study with numerical independent variables. Stepwise multiple regression analysis was performed to compare relative impacts of early and late-season treatments on grain protein contents.

3. Results and Discussion

Yield averages of foliar solution treatments for Bezostaja 1 and Gerek 79 cultivars are presented in Table 3.

Early (soil) x Late (foliar) season nitrogen treatments interaction was significant in both cultivars. As the general average, yield response to early-season nitrogen treatments ceased at 30 kg N ha^{-1} dose in both cultivars. On the other hand, the optimum dose in foliar solution treatments was observed as 60 kg N ha^{-1}. A quadratic increase was observed in grain yields at heading nitrogen treatments. Such an impact of late-season nitrogen treatments were thought to be related to ecological conditions of the last two years. The precipittions especially during the grain-fill stage of the last two years were higher than the seasonal averages of the region. Higher precipitations might have prolonged grain-fill stage and the nitrogen used might affect the grain yields. Yields of both cultivars were generally close to each other but heading of Beztaya1 was 6 to 8 days later than Gerek 79. Slightly higher yields of Bezostaja 1 might be resulted from this late heading and better use of precipitations received during grain-fill stage.

Table 3

The main effects of early and late-season foliar N treatments on grain yield of two winter wheat cultivars (2008-2010) (average of 4 experiments)

Nitrogen Early-Season kg N ha^{-1}	**BEZOSTAJA 1** Grain Yield (kg ha^{-1}) Nitrogen Late-Season (kg N ha^{-1}) 0	20	40	60	Aver.
0	364	380	398	354	374 B
30	380	426	423	419	412 A
60	416	400	442	415	418 A
90	384	403	426	400	404 A
Aver.	387c	403b	423a	398bc	403
CV (%) = 8.7	$LSD_{(0.05)}$ Early=25*		$LSD_{(0.05)}$ Late=13.5**	$LSD_{(0.05)}$ ExL=27.0*	
Nitrogen Early-Seson kg N ha^{-1}	**GEREK 79** Grain Yield (Kg ha^{-1}) Nitrogen Late-Season (kg N ha^{-1}) 0	20	40	60	Aver.
0	352	340	358	309	340 B
30	336	373	371	375	366 A
60	377	367	389	357	373 A
90	319	327	397	376	356AB
Aver.	347b	353b	379 a	355b	359
CV (%) = 12.2	$LSD_{(0.05)}$ Early= 18.6*		LSD $_{(0.05)}$ Late=14.9*	LSD $_{(0.05)}$ ExL= 29.9*	

Means with the same the letter are statistically non-significant, ** Significant at p<0.01 level, * Significant at p<0.05 level by Fisher's LSD, NS: Non-significant

Grain protein contents were determined through multiplying grain nitrogen contents by a factor of 5.83 and results for the cultivars Bezostaja 1 and Gerek 79 are given in Table 4.

Grain protein contents of both genotypes and the early and late-season nitrogen treatments were different. All doses of early and late-season nitrogen treatments increased the grain protein contents of both cultivars. The genotype had the least effect on differences in grain protein contents based on the assessment genotype, environmental factors and nitrogen treatments effects. Bezostaja 1 had a grain protein content of 12.0% and Gerek 79 had a grain protein content of 11.5%. Several studies indicated higher impacts of genotype on grain protein content than environmental conditions (Triboi & Triboi-Blondel 2002; Foulkes *et al* 2009). However, quite low level of difference between the average values (0.5%) does not imply that genotype was totally ineffective. Bezostaja 1 had higher protein contents than Gerek 79 at all doses of early and late-season nitrogen treatments. The difference in grain protein contents created by nitrogen treatments was higher than the difference created by genotype (Table 4). As the average of 4 treatments, while both cultivars had 10.5% grain protein content in control treatments without any nitrogen applications, grain protein content of Bezostaja 1 increased by 2.8% and reached to 13.3% and protein content of Gerek 79 increased by 2% and reached to 12.5% in treatments with the maximum grain protein content. Both maximum values were obtained from 90 kg N ha^{-1} early + 60 kg N ha^{-1} late-season nitrogen treatment combinations. With regard to contributions of early and late-season applications to grain protein contents, the contribution of each unit of nitrogen was higher in late-season applications and this comparison was presented graphically in Figure 1.

Table 4
The main effects of early and late-season foliar N treatments on grain protein contents of two winter wheat cultivars (average of 4 experiments)

Nitrogen Early-Season kg N ha^{-1}	BEZOSTAJA 1 Grain Protein (%)				
	Nitrogen (Late-Season) (kg N ha^{-1})				
	0	20	40	60	Aver.
0	10.5	10.8	11.7	12.2	11.3 C
30	10.9	11.6	12.1	12.8	11.9 B
60	11.1	11.8	12.4	12.8	12.0 B
90	12.1	13.0	13.1	13.3	12.9 A
Aver.	11.2d	11.8c	12.3b	12.8a	12.0
CV (%) = 5.8	LSD (0.05) Early=0.55**		LSD (0.05) Late=0.25**		LSD (0.05) ExL=0.51*

Nitrogen Early-Season kg N ha^{-1}	GEREK 79 Grain Protein(%)				
	Nitrogen (Late-Season) (kg N ha^{-1})				
	0	20	40	60	Aver.
0	10.5	10.6	11.2	11.8	11.0 B
30	10.2	11.0	11.2	12.1	11.2 B
60	10.9	11.8	11.7	12.4	11.7 A
90	11.7	11.7	12.2	12.5	12.0 A
Aver.	10.8d	11.3 c	11.6b	12.2a	11.5
CV (%) = 4.9	LSD (0.05) Early=0.33*		LSD (0.05) Late=0.22*		LSD (0.05) ExL= 0.44*

Means with the same the letters are statistically non-significant, ** Significant at p<0.01 level, * Significant at p<0.05 level by Fisher's LSD, NS: Non-significant

BEZOSTAJA 1:
Grain Protein (%) = 10.3 + 0.20 x *E.S. (kg N ha^{-1})* + 0.32 x *L.S. (kg N ha^{-1})* – 0.012 x (E.S. x L.S.)
(R^2 =0.95**, n = 16). *(2)*
GEREK 79:
Grain Protein (%) = 10.1 + 0.15 x *E.S. (kg N ha^{-1})* + 0.27 x *L.S. (kg N ha^{-1})* – 0.010 x *(E.S. x L.S.)*
(R^2 = 0.92**, *n* = 16). *(3)*
(Where, E.S. = Early-Season Nitrogen;
L.S. = Late-season Nitrogen)

The increase created in grain protein content of Bezostaja 1 by each unit of kg ha^{-1} N was 2,0% in early-season and 3,2% in late-season applications (Equation 2). These increase rates in Gerek 79 were 1,5% in early-season and 2,7% in late-season applications. Several other studies have also indicated higher impacts of late-season applications on grain protein contents (Wuest & Cassman 1992). "Equation 2 and 3" also indicate a negative interaction between early and late-season applications in both cultivars. Contributions of late-season nitrogen treatments to grain protein content decreased with increased early-season treatments The late-season treatments may not provide any contributions when the early-season treatments exceeded a certain level. Although current findings present the existence and level of such relations, the equations still represent the current experimental conditions. Thus, there is a need for wider practical indicators. The objective of the present study was also to determine such a threshold value. Similar responds of both parameters to nitrogenous fertilization present the relationship between them. There were highly significant correlations between SPAD and TN values of the present study (Figure 2).

Because of genotypic differences in chlorophyll contents of cultivars and effects of factors other than nitrogen fertilization, SPAD values were usually converted into NSPAD values, and critical threshold values were determined over these values. Therefore, it is determined that which NSPAD or TN values were critical and at which NSPAD or TN levels result in certain increase in grain protein contents through foliar solution treatments. NSPAD values calculated from SPAD values measured over flag leaves at heading period and TN values measured over the flag leaves sampled at the same time. Increases in protein contents provided by foliar solution treatments were provided in Table 5.

Table 5
The main effects of early and late-season foliar N treatments on increases in grain protein contents and flag leaf total nitrogen contents of two winter wheat cultivars (2008-10) (Average of 4 experiments)

Nitrogen Early-Season kg N ha^{-1}	**BEZOSTAJA 1**		
	NSPAD	Flag Leaf TN (%)	Increase In Grain Protein %
0	0.86	3.52	1.68
30	0.89	3.74	1.91
60	0.91	3.87	1.63
90	0.95	4.09	1.18

Nitrogen Early-Season kg N ha^{-1}	**GEREK 79**		
	NSPAD	Flag Leaf TN (%)	Increase In Grain Protein (%)
0	0.86	3.26	1.35
30	0.87	3.51	1.92
60	0.91	3.63	1.57
90	0.94	3.70	0.84

TN (total Nitrogen)

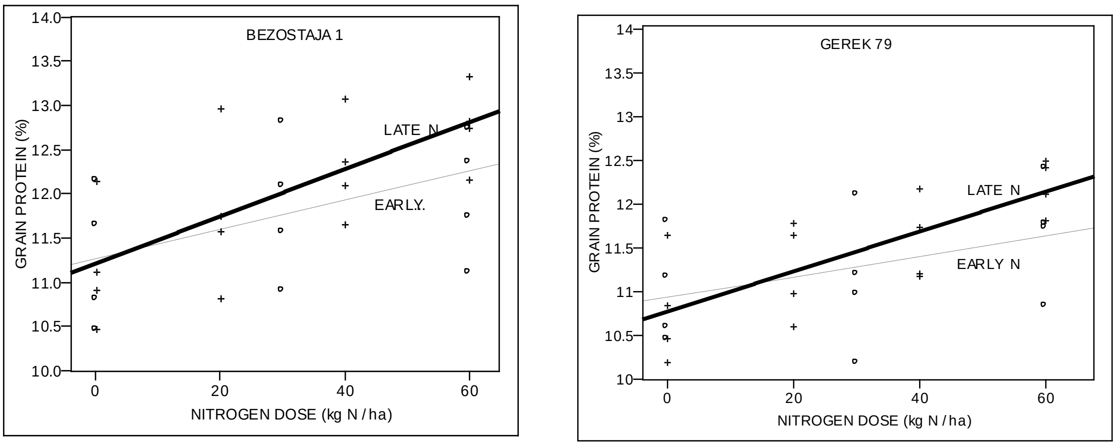

Figure 1
The main effects of early and late-season N treatments on grain protein content of two winter wheat cultivars

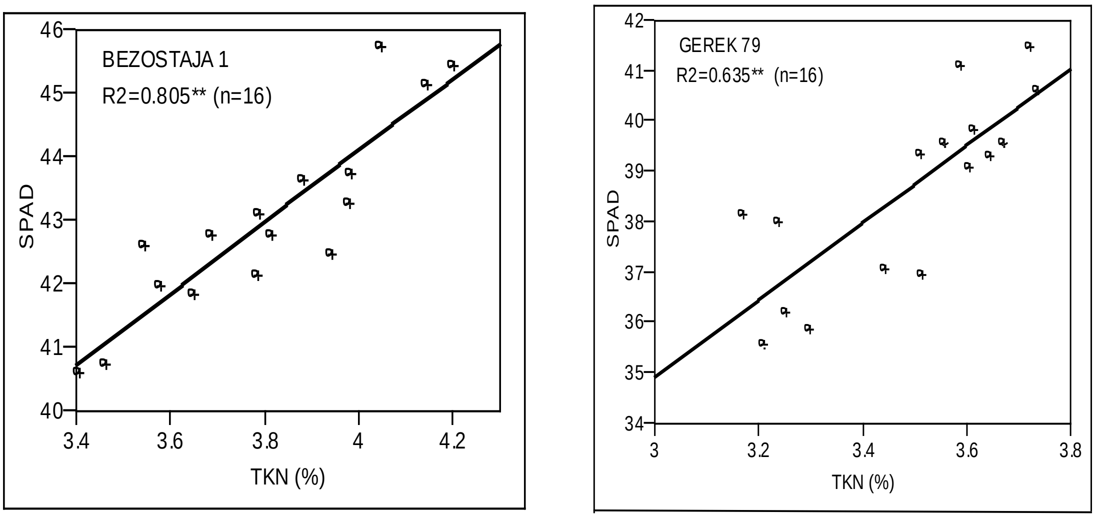

Figure 2
Relationships between flag leaf SPAD and total N content of two bread wheat cultivars at heading stage

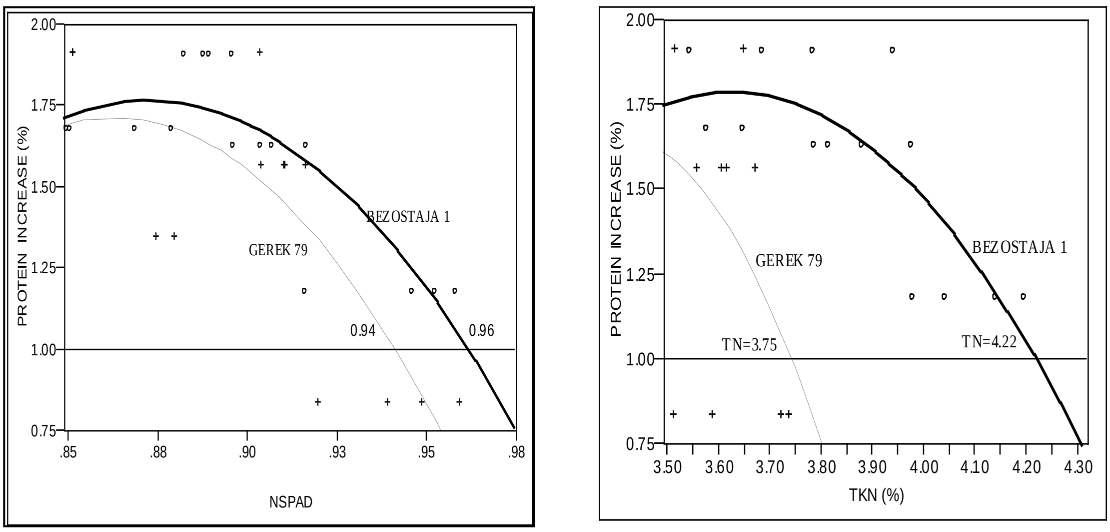

Figure 3
Critical threshold values for NSPAD and TN of two bread wheat cultivars treated with foliar solution under dry conditions

In a study carried in Montana, USA, the critical threshold NSPAD values were determined to be between 0.93 and 0.95 and the authors indicated that 1% increase in grain protein contents could only be reached when the NSPAD value decreased to a value of 0.89 (Westcott *et al* 1997). In the present study, such 1% increase was achieved at critical threshold NSPAD values of between 0.94 and0.96 and about 1.7% increase in grain protein contents was achieved for both cultivars when the NSPAD values decreased to 0.89 (Figure 3) Similar critical threshold NSPAD values were observed in both cultivars with slight differences. However, critical threshold TN values of cultivars were quite different from each other. While the critical threshold TN value was 4.22% in Bezostaja 1 and it was 3.75% for Gerek 79. The reason for such different values was the direct use of TN values while normalized SPAD values (NSPAD) are used through dividing SPAD values of each cultivar by maximums of them. Another reason was the higher nitrogen (and chlorophyll) contents of Bezostaja 1 leaves than Gerek 79 leaves. Although critical threshold NSPAD values can be used for several cultivars because of normalization process, critical threshold TN values should be determined for each cultivar separately because of genotypic differences between the cultivars, The TN values should also be subjected to normalization process like NSPAD values to obtain the percentage of maximum. In two previous studies carried out in Montana, critical threshold TN value was between 4.2 and 4.3% (Westcott *et al* 1997) in one of them and was found to be as 4.2% in the other (Lorbeer *et al* 2000). These values were similar to values observed in this study for Bezostaja 1. The NSPAD method was more appropriate since it was more practical than leaf analyses and exhibited already high correlations with TN values.

CONCLUSIONS

The present results clearly put forth the positive contributions of nitrogen treatments at heading stage under rain fed conditions to grain protein content and relevant bread-making quality attributes. To put this approach into practice, critical threshold values for both chlorophyll meter readings and total nitrogen contents were also presented. The foliar urea treatments at heading stage increased the grain protein content of Bezostaja 1 from 11.2 to 12.8% with 1.6% increase and increased grain protein content of Gerek 79 from 10.8 to 12.2% with 1.4% increase. A 1% increase was achieved at NSPAD values of 0.94-0.96 and the increase in grain protein contents of both cultivars was about 1.7% when the NSPAD value decreased to 0.89. The critical threshold TN value was 4.22% for Bezostaja 1 and 3.75% for Gerek 79.

4. Acknowledgements

The present paper was prepared by using the results of experiments carried out within the scope of 106G111-numbered TÜBİTAK-KAMAG funded project. Authors wish to thank project owner General Directorate of Agricultural Researches and Policies of the Ministry of Food, Agriculture and Livestock and TÜBİTAK for financial supports provided for the project.

5. References

AACC (2000). Approved methods of the American Associationnof Cereal Chemists, 10th ed. Method 38-12, Method 38-12A, Method 54-50, Method 54-30A. St. Paul, MN, USA

Bremner J M (1965). Nitrogen Ed.: C.A. Black. In: Method of Soil Analysis. Part: II. Chemical and Microbiological Properties. Agronomy Series. No:9. Agron. Inc. Madison. Wisconsin. USA.

Ellen J & Spiertz J H J (1980). Effects of rate and timing of nitrogen dressings on grain yield formation of winter wheat (T. aestivum L.). Fert. Res. 1:177–190.

JMP. 13.0.0. Scintilla - Copyright (C) 1998-2014 by Neil Hodgson;neilh@scintilla.org

Foulkes M J, Hawkesford M J, Barraclough P B, Holdsworth M J, Kerr S, Kightley S & Shewry P R (2009). Identifying traits to improve the nitrogen economy of wheat: Recent advances and future prospects. Field Crops Res. 114: 329-342.

Lorbeer S, Acobsen J, Bruckner P, Wichman D & Berg J (2000). Capturing the genetic protein potential in winter wheat. Fertilizer Facts. Number 23. Montana State Univ.

Markwell J, Osterman J C & Mitchell L J (1995). Calibration of the Minolta SPAD-502 leaf chlorophyll meter. Photosyn. Res. 46, 467–472.

Spiertz J H J (1983). Agronomical and physiological aspects of the role of nitrogen in yield formation of cereals. Plant Soil 75:379–391.

Triboi E & Triboi-Blondel A M (2002). Productivity and grain or seed composition: a new approach to an old problem. Eur. J. Agron;16:163–186.

Westcott M, Eckhoff J, Engel R, Jacobsen J, Jackson G & Stongaard B (1997). Flag leaf diagnosis of grain protein response to late-season N application in irrigated spring wheat. Fertilizer Facts. Number 12. Montana State University.

Wuest S B & Cassman K G (1992). Fertilizer-nitrogen use efficiency of irrigated wheat: I. Uptake efficiency of preplant versus late-season application. Ag.J.84:682–688.

Zhu J & Khan K (2001). Effects of genotype and environment on glutenin polymers and bread making quality. *Cereal Chemistry* 78: 125-130.