International Journal of
Agriculture, Environment and Food Sciences
JAEFS e-ISSN : 2618-5946 DOI: 10.31015/jaefs.2019.3.1 www.jaefs.com
Research Article Int J Agric Environ Food Sci 3(3):112-120 (2019)

Improving grain yield, protein ratio and nitrogen use efficiency of durum wheat (*Triticum Durum* Desf.) hybrids using spad meter as a selection criterion

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Abstract

Chlorophyll content can serve as a guide for nitrogen management in agricultural systems. Hence, the investigating leaf chlorophyll in crops could be of benefit to boost production. The present study evaluated 15 different hybrids of durum wheat (*Triticum durum* Desf.) combinations in F_3, F_4 and F_5 generations for nitrogen use efficiency (NUE), grain yield and protein content using chlorophyll meter index (CCI) under three different nitrogen levels (0, 120 and 240 kg N/ha). The results showed that N levels significantly influenced the grain yield and quality traits of durum wheat genotypes, and accordingly, SPAD readings could be used as an indirect selection criterion in durum wheat breeding to achieve the desired production targets. Genetic correlations among grain yield, CCI, grain nitrogen yield (GNY) and protein were high in F_3 generation under high nitrogen regimes. It was also observed that all the generations of Zenit × Menceki, Mersiniye × Menceki, Zenit × Mersiniye, Mersiniye × Spagetti and Spagetti × Menceki crosses have high yield potential and yield stability. It was concluded that the evaluation of the segregation populations at different generations in the same year and selection in the later generations might make a significant contribution to reduce the costs.

Keywords: Breeding, Durum wheat, Hybrid, Nitrogen use efficiency, SPAD-Chlorophyll

Received: 03 June 2019 Accepted: 06 August 2019 Published: 05 September 2019

Introduction

Wheat production occupies an important place for ensuring human nutrition and there is great varietal potential to increase its productivity as the area under crops cannot be increased in several regions. It is wider adaptability as well as the quality of nutritive values than other cereals (Yassin et al., 2019). Hence, it has frequently emphasized the development of appropriate breeding techniques, especially the improvement of new, high yielding quality varieties which are indispensable for vertical expunction i.e., increase productivity (Pena et al., 2002; Tester and Langridge, 2010; Kizilgeci et al., 2019a). It is widely grown food cereal worldwide, due to its wider adaptability as well as the quality of nutritional values than other cereals. Furthermore, it is as a strategic crop that plays a key role in the national economy for several countries (Yildirim et al., 2018; Kizilgeci et al., 2019b). Its demand is increasing day by day to meet the food security of an increasing population (Otu Borlu et al., 2018; Khaled et al., 2018).

Nitrogen requirement of wheat is higher owing to its role in vegetative growth and generative development (Van Keulen and Seligman, 1987; Frederick and Camberato, 1995; Kizilgeci et al., 2016). It has been reported that there is a significant association between nitrogen rates and yield components as well as the yield of wheat (Colkesen et al., 1993) and it is observed that the yield was increased in wheat more than 50% due to nitrogen application (Karaca et al., 1993). This effect is determined by the stay-green period of the spike and flag leaf (Quanyi et al., 2007). Especially in arid climates, photosynthesis in the spike provides important aids to the dry matter which contributes filling of grain (Tambussi et al., 2007). Although the yield and quality traits of wheat largely depend on the optimum nitrogen level (Dogan et al., 2008; Iqbal et al., 2012; Aydogan Cifci and Dogan, 2013) and genotypic variation (Barbottin et al., 2005).

The significant in genetic improvement of nitrogen use efficiency was considered in different studies at various N levels. Ortiz-Monasterio et al. (1997) noted nitrogen use efficiency (NUE) genetic progression of 0.4-1.1 % per year depending on the N levels in spring CIMMYT wheat varieties between 1962 and 1985. Cormier et al. (2014) estimated genetic progress of 0.30-0.37% per year between 1985 and 2010 using 195 European elite winter varieties at optimal and suboptimal N levels. Due to the law of diminishing marginal utility, the nitrogen rates beyond a threshold does not provide improves in yield but increases the production costs and leads to environmental pollution. For that reason, the development of wheat varieties with high NUE considered an essential target for the researchers.

Using SPAD readings to evaluate leaf chlorophyll

Cite this article as:
Kizilgeci, F., Akinci, C., Yildirim, M. (2019). Improving grain yield, protein ratio and nitrogen use efficiency of durum wheat (*Triticum Durum* Desf.) hybrids using spad meter as a selection criterion. Int. J. Agric. Environ. Food Sci., 3(3), 112-120.
DOI: https://dx.doi.org/10.31015/jaefs.2019.3.1
Year: 2019 **Volume:** 3 **Issue:** 3 (September) **Pages:** 112-120
Available online at : http://www.jaefs.com - http://dergipark.gov.tr/jaefs

DOI: https://dx.doi.org/10.31015/jaefs.2019.3.1

content has become common and an understanding of the association between these parameters is essential (Markwell et al., 1995). These findings suggest that the association between SPAD readings and chlorophyll concentration per leaf area remains to be established (Xiong et al., 2015). The main objective of plant breeding is to reach the targeted in a short time of frame requires starting with early generations as soon as possible in the selection of promising lines. Early generation testing is based on single-crop yields in F_2, F_3 and F_4 generations. Despite the high environmental impact in single crop selection in early generations, it has been suggested that the use of F_3 generations in early selection will be successful. Significant genetic gains have been achieved so far without the aid of physiological selection tools by conducting wheat breeding programs global.

Recently, it is necessary to improve the methods and management conditions that will improve the effectiveness of existing breeding methods. The proficiency of selection could be improved if specific physiological and/or morphological properties related to yield under specific conditions can be classified and used as selection criteria to plant breeding (Acevedo, 1991; Barutcular et al., 2016; Barutcular et al., 2017). Total chlorophyll content per leaf area can be evaluated quickly, single, and non-destructively using a portable chlorophyll meter such as SPAD-502 (Minolta, Osaka, Japan). SPAD-502 indirectly measures leaf chlorophyll and nitrogen (N) content. Over the past few years, the chlorophyll meter index (CCI) has been widely used to control nitrogen nutrition in cereals. For this purpose the research using SPAD meters was focused on optimizing N application time in durum wheat, maize and rice (Shukla et al., 2004; Vetsch and Randall, 2004; Debaeke et al., 2006; Kizilgeci et al., 2016), to regulate nitrogen supplies in wheat and barley (Peltonen et al., 1995; Spaner et al., 2005), to assess nitrogen status of plant (Tosti and Guiducci, 2010; Xiong et al., 2015), and to use as a reference indicator of N deficiency (Cartelat et al., 2005).

In winter wheat, significant and positive associations were found between the chlorophyll concentration index (CCI) and the yield at the heading stage of wheat (Bavec and Bavec, 2001) and the grain filling stage (Jiang et al., 2004). Yildirim et al. (2011) and Barutcular et al. (2016) noted that CCI can be used as selection criteria to identify high production and quality durum wheat genotypes at rain-fed and irrigated environments. Genetic relationships between grain yield and CCI among F_2 durum wheat progenies was reported at low nitrogen conditions (Kizilgeci et al., 2017). Several researchers have pointed out that grain yield could be predicted by CCI in rice (Kailou et al., 2011), bread wheat (Yildirim et al., 2013) and bread wheat under stress and non-stressed conditions (Barutcular et al., 2016; Jahan et al., 2019). Liu et al. (2017) reported that it is possible to derivate the lines having higher CCI than their parents at heading and later growing stages CCI measurements. However, the present use of SPAD meter is limited in large or small-scale breeding populations and its introgression into elite backgrounds. The stabilization of SPAD meter, as an indirect method for plant selection, provides clarification and direction for breeders to identify and combine this device into new cultivars with high photosynthesis that work synergistically to enhance grain yield. The aim of this study was to assess the grain yield, protein ratio and NUE of hybrid durum wheat populations grown under different nitrogen levels and to investigate the possibility of using CCI as an indirect selection criterion for these traits.

Materials and Methods

This study was carried at Dicle University Research Station, Diyarbakir, Turkey during 2011-2012, to evaluate 15 hybrids of durum wheat combinations in F3, F4 and F5 generations for nitrogen use efficiency (NUE), grain yield and protein content using SPAD chlorophyll meter index (CCI) under three nitrogen levels. The soil of the experimental soil had pH between 7.5 and 7.6 indicating slightly alkaline in nature. The soils were classified as clay loam and salinity was low. Organic matter and phosphorus contents were very low while, potassium content was very high. The soils contain lime between 10.0-11.0% at the depth of 0-60 cm. Total precipitation was 550 mm, and 66 mm more than long-term averages. Precipitation during the critical wheat development stages (from stem elongation to heading and grain filling) was 335 mm. The temperature was 1-2°C higher than the long-term average for the March-May period in Diyarbakir.

Three durum wheat landraces viz. Misiri (1), Mersiniye (3) and Menceki (5) and three commercial durum wheat cultivars viz. Zenit (2), Spagetti (4) and Levante (6) were used as seed material. Crosses were made among the genotypes in a 6 x 6 half diallel design to obtain 15 different cross combinations. Generation advance of F_3, F_4 and F_5 populations from the cross combinations were obtained during 2008, 2009 and 2011 years. The populations were assessed under three nitrogen rates (0, 120 and 240 kg N/ha) in split plots with three replicates. The nitrogen levels were arranged as the main factor while the genotypes were placed a sub-factor. Nitrogen fertilizer was applied in splits at sowing time and tillering stage, while, phosphorus at the rate of 60 kg/ha as P2O5 was applied to all plots as a basal dose. The seeds were planted in 2-meter-long rows in each plot with 20 cm distance between the rows and 10 cm seed spacing. The experiment was conducted under rain-fed conditions without irrigation.

Investigated Traits

Chlorophyll content index was measured using the Chlorophyll meter (SPAD-502; Minolta, Osaka, Japan), which indirectly calculates the amount of total chlorophyll, called "chlorophyll concentration index" (CCI; ranging from 0 to 99.9). Measurements were made in the open air and at 12-14 hours of the day during the heading stage (ZGS 55) using flag leaf of all plants in the plot.

Plant grain yield (g/plant) was determined by dividing the value obtained after threshing all the plants in the plot by the number of plants. Protein content (%) was measured using the NIT System Infratec 1241 Grain Analyzer (Foss, Hillerod, Denmark) without grinding the grain.

Grain nitrogen yield (GNY) (mg/plant), grain yield NUE (NUEgy) and grain nitrogen yield NUE (NUEgny) were determined according to Yildirim et al. (2007).

Statistical Analysis

Data were analyzed using split-plot ANOVA, and the differences between the genotypes were analyzed by using the least significant difference test to detect the differences between the genotypes at 5% significance (SAS, 1998). Biplots analyses were realized using the software GenStat 12th (Genstat, 2009) package program.

Results

The research findings revealed that the nitrogen rates were significant for CCI, grain yield, protein content, GNY, NUEgy and NUEgny traits in all segregation populations of

F_3, F_4, and F_5 (Table 1). The differences between genotypes were significant in all traits except CCI was found to be not significant. Considering the Genotypes × N interactions were significant in F_4 for grain yield, in F_3 for protein content, in all generations for GNY, in F_5 for NUEgy and in F_3 and F_5 for NUEgny.

The range of CCI for the generations of F_3, F_4, and F_5 under different nitrogen rates were 35.6-40.4, 34.4-38.5, and 35.6-39.5 at N_0 level, 41.4-47.7, 45.3-49.3, and 41.4-47.4 at N_1 conditions, 45.1-49.3, 45.5-50.1, and 45.3-49.6 at N_2 conditions, respectively (Table 2). While, the genotypic differences for the three generations were not significant at N_0 and N_1 rates for CCI, differences among the genotypes at the N_2 rate were significant. Hybrid means of CCI produced an increase with the increase of nitrogen rate. The difference was higher between N_0 and N_1 than between N_1 and N_2 (Figure 1). The highest CCI was found in the 1×3 hybrid combination of F_4 generation at the N_2 nitrogen rate, while the lowest value was determined in 5×6 hybrid combination of F_4 at the N_0 condition. When the hybrid means of the segregation generations were examined under different nitrogen rates, the 2×6 hybrid combination achieved the highest values in the N_0 and N_1 rates for F_4 generation and in the N_1 condition for F_5 generation.

Average single plant grain yield values for the F_3, F_4, and F_5 generations under different nitrogen levels were 5.44, 5.91, and 5.26 g/plant at N_0, 7.57, 6.45, and 5.82 g/plant at N_1 and 7.15, 7.00, and 6.59 g/plant at N_2, respectively (Table 2). The results showed that genotypic differences were significant in N_1 for all three generations and in N_0 only on F_5 generation (Table 2). These differences continued following generations for the increase in grain yield due to the increase in nitrogen rates (Figure 1). The yield of the F_5 generation remained lower under all nitrogen rates compared to F_3 and F_4 generations. The maximum grain yield was produced from the 1×5 hybrid combination of F_3 and F_4 in the N_1 nitrogen dose and the lowest value was produced in the 5×6 hybrid combination of the N_1 nitrogen rate in the F_5 generation among all segregation generations. The maximum yield values were in 2×5 hybrid combinations in all rates and generations and were found to be higher than the general average except for F_4 and F_5 generations at N_2 nitrogen rate.

The protein content values for the F_3, F_4, and F_5 generations was ranged 14.7-16.2, 14.2-16.5, and 14.9-16.1% at N_0, 16.1-17.6, 16.9-17.9, and 16.2-17.7% at N_1 and 17.4-18.4 19.0, and 16.8-18.3% at N_2 (Table 3). As it is seen Figure 2, the genotypic differences were important in F_3 generation of nitrogen rates of N_0 and N_1, N_0 in F_4 generation, and N_1 and N_2 rates in F_5 generation. Protein content increased with increasing N_0 to N_2. The maximum protein content was achieved by N_2 nitrogen rate for the F_4 generation of the 3×6 hybrid combination, while the minimum value was produced by 1×2 hybrid combination at the N_0 rate of the F_4 generation. Among the hybrid combinations, the protein content of the 3×5, and 3×6 hybrid combinations were achieved the higher values than the average of the hybrid and nitrogen rates.

GNY values of F_3, F_4 and F_5 generations were varied from 107.4 to174.5, 112.7 to 180.8, and 95.5 to 175.3 mg/plant at N_0, from 149.3 to 258.9, 134.3 to 250.9, and 92.4 to 197.2 mg/plant at N_1, from 173.2 to 249.2, 152.0 to 246.2, and 154.9 to 236.3 mg/plant at N_2 (Table 4). It was observed GNY was too low at N_0 (Figure 2). The optimum value for GNY was produced by 1×4 hybrid combination of F_5 generation at N_2 level, while the minimum value was produced by 3× 6 hybrid combination of F_5 generation at N_0. The GNY values of the 1× 5, and 2×5 hybrid combinations were achieved higher value than the other hybrid averages.

The highest NUEgy value was found in the 1×2 hybrid combination at N_0 nitrogen rate in the F_5 generation, while the lowest was obtained from the 3×5 hybrid combination in the F_5 generation at N_2 nitrogen dose (Table 4). NUEgy and NUEgny significantly decreased at N_1 and N_2 respect to N_0 (Figure 3) The NUEgy and NUEgny values of 1×5, 2×3, and 2×5 hybrid combinations were found to be higher than the average of hybrids and nitrogen levels.

The results pertaining to GNY, protein and CCI had a significant positive relationship with grain yield estimated at F_3, F_4, F_5 segregations and N rates (Figures 4 and 5), while NUEgy and NUEgny did not show significant relationships with grain yield and CCI. In contrast, protein and CCI did not show a correlation with grain yield on hybrid means based on evaluation under investigation (Figure 6). The CCI had a stronger positive relationship with the grain yield, GNY and protein content at F_3 generation and N_2 rate, while they did not correlate to NUE traits.

Table 1. Mean squares for variables recorded on 15 hybrid populations evaluated at three nitrogen rates at F_3, F_4 and F_5generation

	Mean Squares																		
		CCI			Grain Yield			Protein content			GNY			NUEgy			NUEgn		
Source of Variation	d.f.	F3	F4	F5	F3	F4	F5	F3	F4	F5	F3	F4	F5	F3	F4	F5	F3	F4	F5
Nitrogen (N)	2	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***
Replication	6	***	**	***	**	***	***	**	**	***	***	***	***	***	***	***	***	***	***
Genotype (G	14	ns	ns	ns	**	*	**	*	**	***	***	*	***	**	*	***	**	*	***
G×N	28	ns	ns	ns	ns	*	ns	**	ns	ns	*	*	**	ns	ns	***	**	ns	***
Error	84	5.31	5.08	5.22	1.49	1.29	1.53	3.33	0.38	0.22	653.95	1030.63	645.11	70.80	102.67	51.96	0.04	0.07	0.04
C.V.%		5.26	5.09	5.27	18.44	17.58	20.97	0.32	3.64	2.78	14.00	18.24	16.15	18.96	22.48	17.85	18.19	23.35	18.25

*,**, ***, indicates data significant at P ≤ 0.05, P ≤ 0.01, P ≤ 0.001, respectively. ns: non-significant

DOI: https://dx.doi.org/10.31015/jaefs.2019.3.1

Table 2. Average values of CCI and grain yields of three segregation level at different nitrogen rates.

Hybrid	CCI									Grain yields (g/plant)								
	N0			N1			N2			N0			N1			N2		
	F3	F4	F5	F3	F4	F5	F3	F4	F5	F3	F4	F5	F3	F4	F5	F3	F4	F5
(1x2)	37.8	37.2	38.2	43.3	45.3	44.4	46.8	50.0	45.7	5.592	5.150	7.214	7.105	6.634	7.468	6.932	6.645	6.658
(1x3)	37.5	37.5	37.9	46.3	47.6	46.6	48.6	50.1	49.2	5.348	5.900	6.145	8.078	5.891	6.217	6.440	8.213	5.695
(1x4)	36.7	37.8	37.7	47.7	46.3	43.5	45.1	49.4	48.8	4.623	5.720	5.793	7.027	6.747	6.749	6.015	8.005	6.513
(1x5)	39.4	38.1	36.8	46.0	46.7	42.4	46.2	46.9	46.5	5.201	6.654	4.692	9.211	9.286	6.550	8.176	6.678	6.526
(1x6)	35.6	37.1	35.6	44.5	46.5	44.5	48.6	48.8	48.4	4.263	4.735	4.923	7.983	7.272	5.756	7.175	5.227	6.451
(2x3)	39.3	38.0	39.3	44.8	46.9	43.6	49.2	48.6	46.6	5.704	6.390	6.890	7.800	6.087	5.813	6.577	7.036	6.803
(2x4)	40.5	38.2	39.3	45.8	46.4	44.4	48.3	49.7	45.3	4.900	6.526	4.902	6.219	5.427	5.605	6.834	7.164	7.347
(2x5)	36.0	36.1	38.4	46.2	48.1	46.5	49.0	48.9	45.7	6.306	6.548	5.980	8.568	8.095	7.064	7.234	6.626	6.330
(2x6)	37.6	38.5	38.1	46.1	49.3	47.4	47.4	47.3	49.1	4.494	5.841	5.248	6.846	6.352	6.227	7.116	7.138	6.648
(3x4)	40.4	38.2	38.9	46.4	49.1	45.8	46.7	46.9	46.9	5.662	6.913	5.337	8.544	6.370	4.411	8.575	6.875	6.351
(3x5)	38.1	38.5	39.0	45.2	46.6	44.8	49.2	46.8	46.9	6.026	7.164	4.800	8.361	5.812	5.388	7.371	8.401	5.508
(3x6)	37.5	36.1	38.4	45.9	46.5	45.4	46.4	45.9	49.6	6.160	5.129	4.048	5.390	5.655	5.263	7.691	7.214	6.670
(4x5)	38.7	36.9	37.3	44.0	47.6	44.8	49.3	47.2	46.8	6.956	5.673	4.307	6.872	6.290	6.595	6.707	5.552	8.236
(4x6)	39.5	36.8	39.5	41.4	48.9	41.4	48.6	49.7	47.9	4.577	4.957	4.711	6.737	4.807	4.747	6.119	7.034	6.346
(5x6)	37.4	34.4	36.0	45.8	46.6	45.8	48.6	48.6	47.8	5.821	5.375	3.884	8.748	5.980	3.401	8.210	7.192	5.843
Hybrid mean	38.1	37.3	38.0	45.3	47.2	44.8	47.9	48.3	47.4	5.442	5.912	5.258	7.566	6.447	5.817	7.145	7.000	6.528
$LSD_{0.05}$ (G)	ns	ns	ns	ns	ns	ns	1.871	2.007	1.775	ns	ns	1.269	1.608	1.925	1.644	ns	ns	ns
				F_3	F_4	F_5							F_3	F_4	F_5			
$LSD_{0.05}$ (N)				0.97	0.94	0.96							0.400	0.475	0.395			
Dose mean	37.8			45.8			47.9			5.537			6.610			6.891		

ns: non-significant

Table 3. Average values of protein content and grain nitrogen yield (GNY) of three segregation level at different nitrogen levels.

Hybrid	Protein content (%)									GNY (mg/plant)								
	N0			N1			N2			N0			N1			N2		
	F3	F4	F5	F3	F4	F5	F3	F4	F5	F3	F4	F5	F3	F4	F5	F3	F4	F5
(1x2)	16.1	14.2	14.9	16.7	17.0	16.5	17.9	18.0	16.8	144.7	116.9	173.4	189.1	180.9	197.2	199.4	191.9	177.3
(1x3)	17.1	15.2	16.0	16.8	17.7	17.0	18.4	18.5	17.7	145.3	143.5	157.1	217.1	167.0	167.9	190.1	242.5	161.6
(1x4)	14.7	16.4	15.9	16.7	17.5	17.1	18.0	18.3	18.3	108.7	150.5	147.4	186.8	187.2	184.6	173.2	234.7	189.7
(1x5)	16.1	14.8	16.0	17.5	16.9	17.0	17.4	18.4	18.0	133.9	157.6	120.6	258.9	250.9	178.8	227.4	196.2	188.2
(1x6)	15.9	14.8	15.0	16.1	17.2	17.1	17.6	18.1	18.1	108.2	112.7	117.9	205.7	199.8	157.7	201.2	152.0	186.9
(2x3)	15.2	15.4	15.9	16.9	17.4	16.9	17.6	17.9	17.6	138.7	156.3	175.3	209.7	168.7	156.2	184.8	200.8	191.5
(2x4)	15.6	15.1	15.0	17.0	17.5	16.2	18.2	17.9	17.1	122.6	156.4	118.1	168.6	151.8	144.0	199.3	205.2	201.6
(2x5)	15.6	16.1	15.6	17.6	17.4	16.3	18.2	18.6	17.5	156.7	167.9	149.4	241.4	225.6	183.8	210.4	196.9	177.5
(2x6)	14.7	15.1	15.4	17.4	17.4	17.7	18.2	17.9	18.3	107.4	140.6	128.8	189.8	177.1	175.3	207.4	205.0	194.6
(3x4)	15.7	15.9	15.1	17.5	17.0	16.6	18.2	18.0	17.7	142.5	175.1	129.4	238.6	173.2	116.1	249.2	197.7	179.7
(3x5)	16.2	15.8	15.1	17.3	17.5	16.9	18.0	18.3	17.6	157.6	180.8	116.6	231.2	162.2	145.4	212.3	246.2	154.9
(3x6)	14.9	16.0	15.7	17.3	17.9	17.0	18.3	19.0	17.8	137.6	129.0	95.5	149.3	160.9	140.8	225.6	219.3	190
(4x5)	15.8	15.9	16.1	17.1	17.8	17.4	18.0	18.0	17.9	174.5	143.9	110.4	188.1	179.0	182.6	193.6	160.2	236.3
(4x6)	15.2	14.6	15.2	17.0	17.6	17.1	17.9	17.5	17.3	112.4	117.4	114.6	183.3	134.3	130.7	176.4	196.6	175
(5x6)	15.7	16.5	15.9	16.9	17.3	17.0	18.2	17.6	17.6	145.1	141.0	98.3	236.6	164.9	92.4	238.5	203.1	164
Hybrid mean	15.6	15.5	15.5	17.1	17.4	16.9	18.0	18.1	17.7	135.7	146.0	130.2	206.3	178.9	156.9	205.9	203.2	184.6
$LSD_{0.05}$ (G)	1.223	1.174	ns	0.778	ns	0.755	ns	ns	0.580	37.94	ns	33.23	42.08	54.68	44.77	47.73	ns	ns
				F3	F4	F5							F3	F4	F5			
$LSD_{0.05}$ (N)				0.235	0.259	0.194							10.7	13.5	10.7			
Dose mean	15.5			17.1			17.9			137.3			180.7			197.9		

ns: non-significant

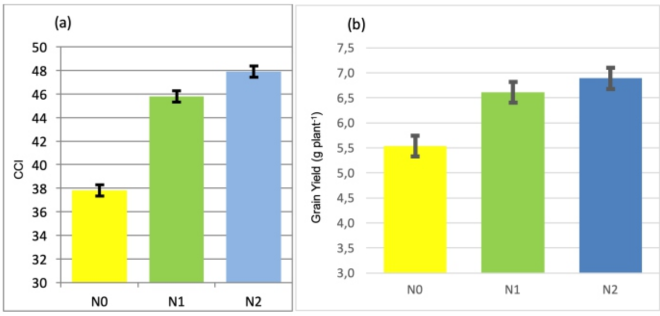

Figure 1. (a) Chlorophyll content (CCI) and (b) grain yield of all hybrids over all segregations. Values of LSD for CCI: 0.96 and plant grain yield: 0.42

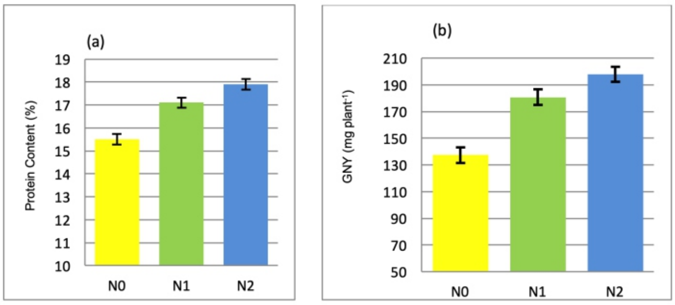

Figure 2. (a) Protein content and (b) GNY of all hybrids over all segregations. Values of LSD for Protein content: 0.23 and GNY: 11.6

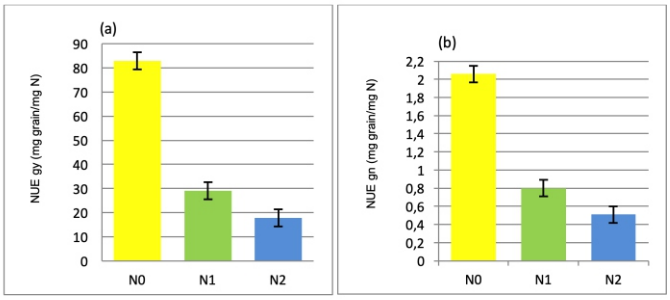

Figure 3. (a) NUEgy and (b) NUEgn of all hybrids over all segregations. Values of LSD for NUEgy: 3.56 and NUEgn: 0.095

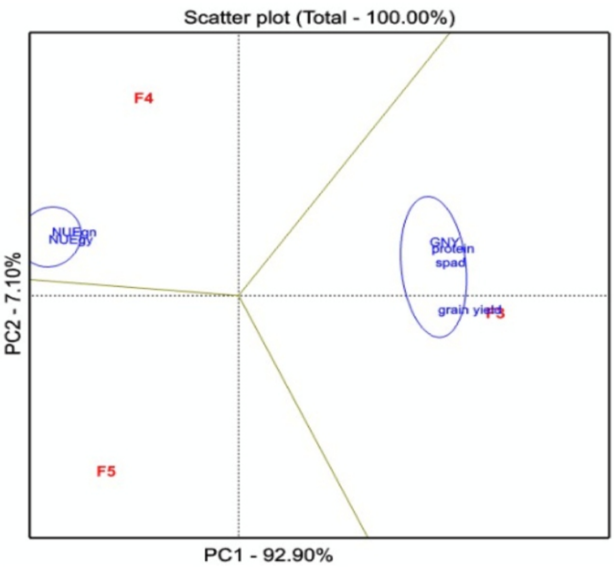

Figure 4. Biplot of traits by segregation population interaction evaluated over all hybrids

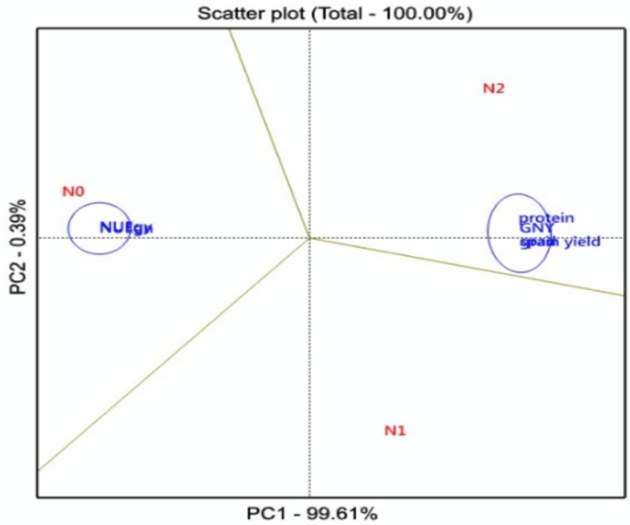

Figure 5. Biplot of traits by N dose interaction evaluated over all hybrids

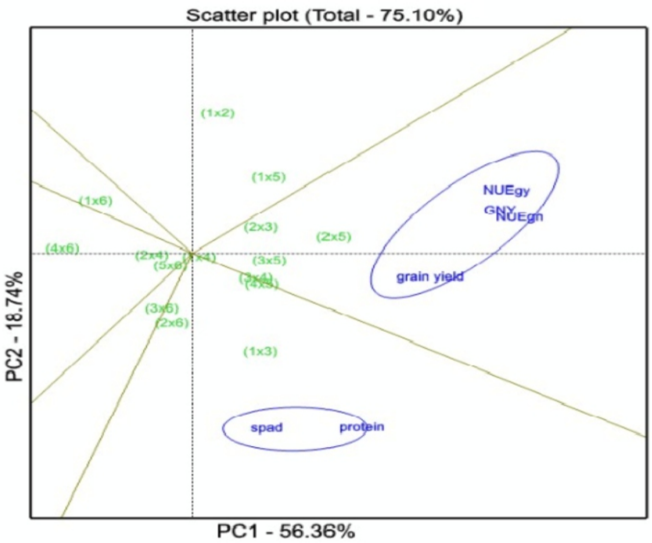

Figure 6. Biplot of traits by genotype interaction over all segregation level and N rates

Discussion

In this study, in spite of 15 different hybrids of durum wheat combinations at F_3, F_4 and F_5 generations were evaluated in terms of grain yield, protein content and NUE at three different nitrogen rates. The use of the chlorophyll meter (Minolta 502-SPAD) as a selection criterion was investigated for the traits under study. Meanwhile, the increasing amounts of nitrogen (N) fertilizers have been used for expanding to increase of CCI in hybrids. According to Bavec and Bavec (2001), Lopez-Bellido et al. (2004), Ziadi et al. (2008), Lin et al. (2010) there was a positive relationship between CCI and nitrogen concentration in the leaf. According to findings, genotypic differences for flag leaf chlorophyll content (CCI) in all generations (F_3, F_4, F_5) appeared only in high nitrogen conditions. Therefore, as in harmony with an earlier study (Kizilgeci et al., 2017), selection can be suggested at high levels of nitrogen to identify differences among the lines in breeding programs.

The results of the study indicated there was a significant relationship among the grain yield of hybrids and nitrogen rates application, which was in agreement with the results of A Ortiz-Monasterio et al. (1997), Le Gouis et al. (2000) and Van Ginkel et al. (2001), Bonfil et al. (2004), Guarda et al. (2004), on the other hand, some researchers reported that, fertilization over a certain nitrogen rate leading to reduction in grain yield, with consider the effect of genotype and environmental conditions (Ellmer et al., 2001;Cossey et al., 2002).

Several studies detected significant G×N interactions for agronomic performances (Barraclough et al., 2010; Cormier et al., 2014), meaning that the genetic values of varieties differ among the N levels. Significance of G×N interactions directly affects the correlations of genetic values between N levels, and hence, the best varieties at high N may not be the best at low N. In our study G×N interaction of the traits changed depending on F stage and traits (Table 1). Non-significant G×N interactions for CCI indicates that it is a stable trait as an indirect selection criterion, when it would be significantly associated with desired traits, like yield, protein content etc. (Figures 4-5). Given that we observed relatively high heritability prediction (71.5 h^2) of CCI (Tahmasebi et al., 2014) also supported our suggestion.

The significant differences among the genotypes for grain yield in the segregation populations were observed under varying levels of nitrogen. Although, yield increment was recorded with increasing nitrogen rate compared to moderate nitrogen, the difference between genotypes was rather narrow. Therefore, the chances of success for the selection at the optimum level of nitrogen fertilization for plant breeding could be increased. These results indicate that selection can be made independently on segregation stages.

As pointed out, in wheat, different studies indicated that indirect selection at high N can be an effective strategy to breed for low N conditions (Brancourt-Hulmel et al., 2005; Laperche et al., 2006). According to this scenario, if the genetic correlation is high then the selection is made on the basis of yield or other agronomic characteristics in high N rates. It was observed that the genotypic correlation of all other properties was low except CCI under high N rates. So, the selection should be made under normal nitrogen conditions, except for CCI, to increase the efficiency of selection according to the current study.

Although protein was positively correlated with nitrogen application, the protein is a nitrogenous compound, it can be said that the increase of the protein content in the plant organs with the increase in nitrogen rates, it is an expected result as reported in previous studies (Delogu et al., 1998; Ottman et al., 2000; Sade and Soylu, 2001; Woolfolk et al., 2002).

Grain protein change was effective due to environmental factors such as temperature, light intensity and soil moisture (Gauer et al., 1992; Sajo et al., 1992; DuPont and Altenbach, 2003), and the agronomic management factors like the soil tillage (De Vita et al., 2007); Zebarth and Sheard (1992), nitrogen application rates, application time, and application form as well as genotypes. Nitrogen rates and segregation level were not predictive of genotypic differences in protein proportion. Since genotype x nitrogen rate interactions for the protein content was generally unimportant, successive selection in breeding can be done at different nitrogen rates.

The genotypic differences in varied segregation populations for GNY, NUEgy and NUEgny were observed to be low in high nitrogen rates (Table 2). With low and moderate nitrogen rates, the response of the genotypes changed depending on the segregation level, and genotypic differences were higher in moderate nitrogen rate than in low nitrogen rate. The findings provided the success of the selection process for NUE. Protein content and GNY indicated that genetic responses varied according to nitrogen rates and level of segregation and that both properties had to be assessed separately in breeding.

In general, the chlorophyll content was observed high in the hybrid combination of Misiri genotype used as a parent. Spagetti and Menceki varieties used in the hybrids achieved the maximum yield potential. It was observed that protein ratio was high for each segregation (F_{3}-$_{5}$) and different nitrogen rates in the hybrids in which the Zenit variety was used as a parent. It appears that hybrids with higher nitrogen rates were different from hybrids that stand out in terms of protein content. As a parent variety Spaghetti produced an enhancing effect on GNY, NUEgy and NUEngy in all its hybrid combinations. On the other hand, Mersiniye × Spagetti hybrid achieved high combination ability especially in early segregation levels. It could be acknowledged that the use of landraces in breeding programs would allow a positive contribution to the success of durum wheat breeding and enhance the narrow range variation. The landraces that used in the current study had considerable enhancing effects on the grain yield and GNY, NUEgy and NUEgny in the hybrid combinations.

There is a growing interest for using chlorophyll meter in durum wheat breeding might enable to breeders for evaluating the current variability more effectively, both by reducing labour and by allowing the more single plant to be screened and development of kind of tools is important to continue progress through plant breeding. It is possible to reach the favourite yield and quality results by using SPAD meter as an indirect selection criterion in durum wheat breeding, these results were supported by the findings of Yildirim et al. (2009) which indicated that CCI could be used as a selection criterion to classify the high yielding durum wheat breeding lines at early segregation of progenies.

The results showed that the genotypes that were examined under different nitrogen rates in wheat breeding would be crucial to reveal the genotypic effect or inactive genes of a genotype. In the present study, there were no genotypic variations in grain yield in several combinations at low and high nitrogen rates. A similar situation had been observed at low and medium nitrogen rates for CCI, at high nitrogen rates for protein content GNY, NUEgy and NUEgny traits. The optimum of environmental factors such as heat and drought stress, management techniques and soil factors, as well as the nitrogen content of the soil, in order to reveal genotypic effects at the highest level, would be leading to the existing genetic variability to be used more effectively in durum wheat breeding.

One of the most significant practical outputs of this research, a low budget and a limited number of hybrid populations, this method is based on the fact that 3-4 generations from F_3 to F_6 growing in the same year and at different nitrogen rates as a result of the production of their seeds without being subjected to selection in different segregation levels, as the hybrid populations in this investigation. According to this method, the selection at the last segregation combination with the highest value for investigated traits for all generations and more than one nitrogen rate will allow both high genetic progress and high stability to be achieved. All these facilities will be provided with low input, time and labour.

Conclusion

Testing the genotypes at different nitrogen rates in wheat breeding is crucial to reveal the genotypic effect or inactive genes of a genotype. In this study there were no genotypic differences in grain yield in many combinations at low and high nitrogen rates. Similar findings were also observed at low and medium nitrogen rates for CCI, at high nitrogen rates for protein content GNY, NUEgy and NUEngy traits.

This it is suggested that in addition to environmental factors such as heat and drought stress, management techniques and soil factors, the nitrogen content of the soil, has the potential to achieve the varietal potential of wheat genotypes. This method is based on the fact that 3-4 generations from F_3 to F_6 growing in the same year and at different nitrogen rates as a result of the production of their seeds without being subjected to selection in different segregation levels, as the hybrid populations in this study. According to this method, the selection at the last segregation combination with the highest value for investigated traits for all generations and more than one nitrogen rate will allow both high genetic progress and high stability to be achieved. All these benefits will be attained with low input, time and labour.

Acknowledgement

This work was supported by Dicle University research supporting foundation (DUBAP-11-ZF-13).

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