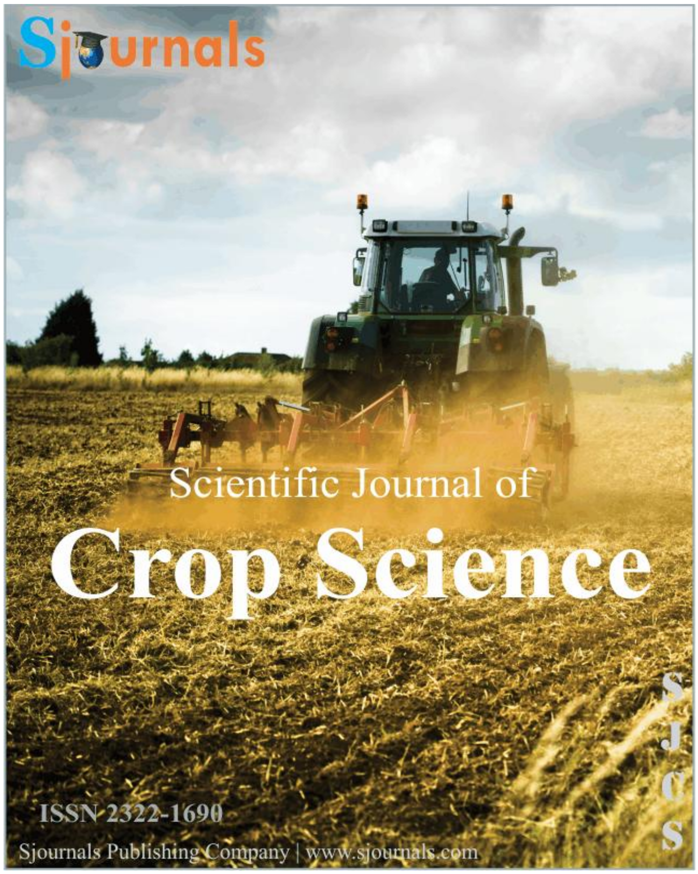
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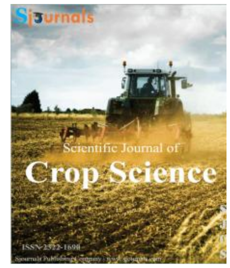

Original article

Nitrogen effects on SPAD meter and grain yield relationships in F2 durum wheat populations

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ABSTRACT

This study was conducted to evaluate the effectiveness of SPAD-502 chlorophyll meter reading as indirect selection criteria for grain yield under different nitrogen (N) conditions in F_2 durum wheat populations. In this study six durum wheat parents and their 6x6 half-sib F_2 diallel cross populations were grown under N0 (zero N), N1 (120 kg/ha) and N2 (240 kg/ha) nitrogen conditions. Chlorophyll content at heading stage was obtained by using SPAD meter (SPAD-502; Minolta, Osaka, Japan). SPAD readings and grain yield of durum wheat increased with increasing nitrogen level. Genotypic variation for grain yield and SPAD was found to be highest in N_1 and N_2 levels, respectively. General combining ability (GCA) effects were significant for plant grain yield in N1 conditions, while significant for SPAD in N1 and N2. Significant specific combining ability (SCA) effect was observed only SPAD readings in N2 condition. High GCA/SCA ratio showed that additive genes have significant role at SPAD values of N1 and N2. Grain yield correlated with SPAD of N0 (R^2 = 0.328). The results indicate that genotypic differences for SPAD reading among F_2 durum wheat progenies can be effectively detected in high nitrogen condition.

1. Introduction

Nitrogen fertilization is one of the most important production factors increased substantially in wheat cultivated areas after Green Revolution. Nitrogen exists in the structure of many organic compounds of plants. Such as proteins, amino acids, nucleic acids, enzymes, ATP, ADP and chlorophyll are important organic compound, which has nitrogen containing. Chlorophyll is the green photosynthetic pigment which absorbs different wavelengths of the sun lights and transfers this energy to the reaction centre of the photosystems. SPAD chlorophyll meters estimate the relative amount of total leaf chlorophyll in intact leaves and produce values that state relative chlorophyll content (SPAD reading). The handling of SPAD meters is easy, rapid and non-destructive; therefore, the use of SPAD meters has increased remarkably in recent years. SPAD meters are used successfully with large number of crop species for estimating chlorophyll content such as rice (Lin et al., 2010), corn (Bullock et al., 1998; Ziadi et al., 2008), sorghum and pigeonpea (Yamamoto et al., 2002), soybean (Fritschi and Ray, 2007), wheat (Debaeke et al., 2006; Barutcular et al., 2015; Bahar, 2015; Kizilgeci et al., 2015), barley (Kizilgeci et al., 2016) and potato (Uddling, 2007). Follett et al. (1992) found that SPAD reading are correlated with N concentrations of leaves and grain yield. Matsunaka et al. (1997) reported that the chlorophyll content in plant leaf has been closely related to plant N content. Szabo (2014) observed that SPAD readings reached maximum level at flowering in low and normal N doses, at the high N dose the highest SPAD values were reached at Zadoks Growing Stage (ZGS) 32-33. Little effort has been devoted to investigation of the relationship between SPAD readings and genotypic performance of segregating population under different N conditions in wheat. Diallel mating designs are an important tool to provide information in early generations. The combining ability analysis provides useful information concerning the selection of parents in terms of their hybrids performance. General combining ability (GCA) provided an estimation of degree of additive gene action and specific combining ability (SCA) exhibited the performance of two particular genotypes in a specific cross, which would reflect non-additive type of gene actions. The aim of this study is to evaluate the availability of SPAD-502 chlorophyll meter reading as indirect selection criteria for grain yield under different nitrogen (N) conditions of durum wheat populations.

2. Materials and methods

Field experiment was conducted at research area of Field Crops Department, Agriculture Faculty of Dicle University, during 2010-2011 growing season. The experiment material consist of 3 landraces (Menceki, Mersiniye and Mısırı) and 3 modern cultivars (Levante, Zenit, Spagetti) and their 15 F_2 populations obtained by 6 × 6 half diallel crossing design without reciprocals. Seeds of 15 F_2's with their 6 parents (21 entries) were sown in randomized split plot design with three replicates at three nitrogen levels. Nitrogen doses were the main plots and genotypes were the subplots. Each plot consisted of two rows with 2 m long, 0.1 m intra-row and 0.2 m inter-rows. Three nitrogen doses are 0, 120 and 240 kg N/ha, and further will be called N0, N1 and N2, respectively. Each parcel took 60 kg ha^{-1} phosphorus as triple superphosphate at sowing. The half of nitrogen was applied at sowing and remaining nitrogen was applied at tillering stage as top-dressing. The meteorological data of Diyarbakır in experiment year and long-term were given in Table 1. During the experimental years, the monthly mean temperatures changed from 1.9 to 27.7 ^{0}C and the monthly mean precipitations from 4.1 to 71.4 mm. During research, total precipitation was 405.1 mm, while long-term was 484.3 mm. According to long- term, the monthly precipitation was below average in March and April of 2011. The experiment soil sampled from 0-30 cm. According to soil analysis, the soil texture was clay loam and contained 11.02 % carbonate, 0.81 % organic matter, 12.6 kg/ha P, with a pH 7.6. Herbicide, insecticide and fungicide treatments were applied to control parasites.

2.1. Measurements

Chlorophyll content was measured by using a portable chlorophyll meter (SPAD-502; Minolta, Osaka, Japan), which can indirectly measure leaf chlorophyll content. The SPAD measurements, at heading (growth stage, GS55) were made on midpoint of flag leaf of ten plants in each plot. Grain yield (g/plant) was calculated by harvesting all plants in plots.

2.2. Statistical analysis

The diallel analysis was carried out following Model I and Method 2 described by Griffing (1956) (including parents and no reciprocals). The statistical analysis of data was carried out using SAS (1998) program with genotype and treatment as fixed effects. Comparisons of means were made by using the least significant difference test (LSD) at P < 0.05. Genotypic correlations were calculated between grain yield and all SPAD readings using all genotypes.

Table 1
Monthly mean temperature (°C) and precipitation (mm) data for 2010-2011 growing seasons and long term (1960-2011) averages of Diyarbakır province.

Climatical data / Years	Temperature (°C)		Precipitation (mm)	
	2010-2011	Long-Term (1960-2011)	2010-2011	Long-Term (1960-2011)
September	25	24.8	9.2	4.1
October	16.4	17.2	11.8	34.7
November	6.4	9.2	73	51.8
December	2.3	4	40.2	71.4
January	2.4	1.8	78.3	68
February	1.9	3.5	74.4	68.8
March	5.1	8.5	44	67.3
April	15.2	13.8	26.2	68.7
May	19.6	19.3	41	41.3
June	27.7	26.3	7	8.2
Total/average	12.2	12.8	405.1	484.3

3. Results and discussion

According to variance analysis made to split plot design, there were significant differences between nitrogen doses for SPAD and grain yield. Genotypic and GCA effects were significant only grain yield. Genotype x doses interaction was found significant for SPAD (data not shown). Split plot design variance analysis was insufficient to explore the genotypic variance over each nitrogen doses. Therefore, each nitrogen doses was analysed separately to show suitable nitrogen dose in which breeders can use unique environment for SPAD and grain yield based selection.

Genotypes grown at N2 conditions had significant differences (P<0.01) for SPAD reading, while genotypic differences were not appeared at N0 and N1 conditions (Table 2). Similarly, general combining ability (GCA) and specific combining ability (SCA) effects of genotypes was significant at N2 conditions. The reliability of the results was highest at N2 conditions. Genotypic differences for grain yield disappeared at N0 and N2 conditions, but it was significant at N1 conditions. Significant GCA effects appeared only N1 conditions. The GCA/SCA ratio was found higher than 1 at N0 and N1 levels for grain yield, while was higher than 1 at N1 and N2 conditions for SPAD reading, indicating additive gene effects in the inheritance of grain yield and SPAD reading. Non-additive (dominance) gene effect mediated the heritance of SPAD reading at N0 and grain yield at N2. Suggesting that the data of SPAD measurements were mediated mainly dominance gene action. According to the result of 288 spring wheat advanced lines tested 12 environments; SPAD had high heritability in wheat (Lopes et al., 2012). Significant genotypes x N interaction were observed for SPAD readings, not for grain yield (data not shown). Therefore, genotypic response of each genotype to SPAD reading will be different at changing N level. However, favourable genotypes at the grain yield basis selection can be reliably determined at changing N conditions, due to similar response of genotypes to different N conditions.

The increase at N rates positively affected mean SPAD readings. The highest SPAD reading was obtained from Mersiniye x Spagetti at N2 application (Table 3). Significant differences determined among hybrid populations and parents at N2 condition. SPAD readings of some parents decreased at N2 conditions, due to significant genotype x N dose interaction, while only one hybrid significantly decreased at N2 conditions. Genotypic differences of hybrid were more remarkable at N2 and some hybrids with low SPAD reading at N0 and N1 conditions had highest values at N2 conditions. Generally, the response of parent and hybrid populations changed independently at each N level. Spaner et al. (2005) reported increases at ZGS 59, SPAD readings by top-dressing N increase in winter wheat. They obtained 38.45 and 48.0 SPAD values which are similar to our data, from 0, 30 and 60 kg/ha N top-dress application, respectively. ZGS 65 SPAD reading also reported as 42.5, 49.4 and 51.6 by 0, 30 and 60 kg ha^{-1}, respectively (Szabo, 2014).

Table 2
Analysis of variance of SPAD reading and grain yield in parents and F2 populations at three N levels.

Source of variation	df	Mean squares: SPAD reading N0	N1	N2	Grain yield N0	N1	N2
Replication	2	165.8***	18.8*	13.3*	12.1***	6.9*	0.3
Genotypes	20	6.6	6.9	11.1**	0.8	3.5*	18.3
GCA	5	3.3	14.2*	16.5**	11.5	6.5**	1.5
SCA	15	7.7	4.5	9.3*	0.6	2.5	1.9
Error	40	6.3	6.1	4.3	0.6	1.7	1.8
GCA/SCA		0.42	3.15	1.77	19.16	2.6	0.79
CV (%)		6.7	5.5	4.4	17.6	17.0	16.2

*, **, *** Significant at 0.05, 0.01 and 0.001 probability levels, respectively.

Table 3
Means for SPAD reading and grain yield in parents and F_2 populations at three N levels.

	SPAD reading (Unit)			Grain yield (g $plant^{-1}$)		
Parents	**N0**	**N1**	**N2**	**N0**	**N1**	**N2**
Mısırı	36.7	46.3	45.7	3.797	6.242	7.398
Zenith	39.2	43.8	43.0	3.937	6.958	7.906
Mersiniye	36.7	42.2	44.9	4.923	9.126	8.630
Spagetti	37.0	45.2	47.7	4.916	5.983	8.359
Menceki	39.7	46.2	44.0	5.244	7.799	9.583
Levante	36.9	46.5	47.5	4.618	7.547	9.801
Parent Mean	37.7	45.0	45.5	4.570	7.280	8.610
F_2 Populations						
(Mısırı x Zenith)	36.1	45.9	45.6	4.622	6.638	7.903
(Mısırı x Mersiniye)	37.8	45.8	46.5	3.985	5.842	8.803
(Mısırı x Spagetti)	39.8	46.2	49.4	4.050	6.744	7.554
(Mısırı x Menceki)	34.1	46.2	50.2	4.261	5.998	7.699
(Mısırı x Levante)	39.1	46.6	47.3	4.726	8.604	7.567
(Zenith x Mersiniye)	37.4	45.7	45.8	4.320	8.059	7.387
(Zenith x Spagetti)	38.8	43.7	46.1	4.990	8.425	8.756
(Zenith x Menceki)	37.6	41.7	46.1	5.048	7.496	8.335
(Zenith x Levante)	38.2	44.0	47.5	4.354	7.189	7.760
(Mersiniye x Spagetti)	36.3	43.5	48.3	5.785	9.545	8.701
(Mersiniye x Menceki)	38.8	42.9	47.3	3.998	8.352	6.871
(Mersiniye x Levante)	35.5	43.8	48.3	4.666	8.406	8.378
(Spagetti x Menceki)	36.9	43.2	49.0	5.207	7.409	8.261
(Spagetti x Levante)	38.5	43.9	48.3	4.413	8.733	7.124
(Menceki x Levante)	38.9	45.1	43.5	4.131	8.319	9.126
Hybrid mean	37.6	44.5	47.3	4.570	7.710	8.020
Mean	37.7	44.7	46.8	4.570	7.590	8.190
$LSD_{0.05}$ Genotypes	ns	ns	3.4	ns	2.13	ns
$LSD_{0.05}$ Doses		0.99			0.43	

N0, N1 and N2 are 0, 12 and 24 g m^{-2} nitrogen, respectively.

Grain yield was raised by adding of N fertilizer, but mean differences between N1 and N2 applications were non-significant for hybrids (Table 3). Genotype x dose interaction was non-significant and general response of parent and hybrids was similar to N changes with some exceptions like landrace Mersiniye and hybrids of Mısırı x Levante, Zenith x Mersiniye, Mersiniye x Spagetti, Mersiniye x Menceki and Spagetti x Lavante. Significant GCA effects for SPAD reading were found at N1 and N2 level and the N levels significantly altered the sign of GCA effects of some genotypes (Table 4). GCA effects of genotypes changed at positive and negative direction depending on N level. Mersiniye had negative contribution to SPAD reading at all N level, while Levante had positively contribution. Mısırı and Spagetti had significantly positive GCA effects for N1 and N2 levels, respectively. Depending on N levels, significant positive and negative SCA effects were identified for SPAD reading in some cross combinations. Significant differences for GCA effects were obtained for grain yield and the sign of effects were positive or negative, depending on the N levels. The sign of GCA effects of each genotypes for grain yield was generally same for all N level. It shows that the genotypes including positive GCA can be selected or used in early breeding stages ignoring nitrogen level in soil. Mersiniye had significantly positive contribution to increase grain yield at N1 level. The differences for SCA effects were significant for some cross combinations. The hybrid of Mersiniye x Spagetti had significant SCA effect for N1 and N2 levels. Four hybrids have positive SCA effects for all N levels and the sign of SCA effects changed depending on N level.

The relationships between yield and SPAD readings under different N conditions have been the subject of several studies (Vidal et al., 1999; Lopez-Bellido et al., 2004; Arregui et al., 2006). The correlation between SPAD reading and grain yield was significant (r^2=0.328, P<0.01) at N0 conditions, while there was no significant relationships at N1 and N2 conditions (r^2= -0.116 and -0.171 for N1 and N2 levels, respectively) (Table 5). Significant relationships between grain yield and SPAD reading were found by Spaner et al. (2005) at wheat, except N0 application. Interestingly, there were no significant relationships among SPAD reading. This shows that SPAD values of each genotypes change depend on N level. Genotype x N interaction (data not shown) in genotype rankings is main cause of non-significant correlations for N1 and N2. Similar results predicted by Hamblin et al. (2014). SPAD rarely had significant contributions to yield in heat stressed environment (Lopes et al., 2012).

Table 4
Estimates of GCA and SCA effects for SPAD and grain yield at three N levels.

Parents	SPAD reading			Grain yield		
	N0	N1	N2	N0	N1	N2
Mısırı	-0.37	1.31*	0.39	-0.35*	-0.85**	-0.37
Zenith	0.40	-0.52	-1.29**	-0.10	-0.18	-0.17
Mersiniye	-0.53	-0.82	-0.16	0.08	0.66**	0.01
Spagetti	0.11	-0.24	1.15**	0.29	-0.04	-0.02
Menceki	0.31	-0.16	-0.42	0.14	0.00	0.27
Levante	0.08	0.44	0.33	-0.06	0.40	0.28
F_2 Populations			**SCA**			
(Mısırı x Zenith)	-1.52	0.45	-0.27	0.49	0.08	0.26
(Mısırı x Mersiniye)	1.11	0.62	-0.47	-0.32	-1.56*	0.98
(Mısırı x Spagetti)	2.47	0.41	1.06	-0.46	0.05	-0.24
(Mısırı x Menceki)	-3.46**	0.36	3.49**	-0.11	-0.74	-0.38
(Mısırı x Levante)	1.58	-0.83	-1.98	0.47	1.82*	-0.57
(Zenith x Mersiniye)	-0.09	2.39	0.51	-0.23	-0.02	-0.64
(Zenith x Spagetti)	0.67	-0.23	-0.53	0.23	1.05	0.76
(Zenith x Menceki)	-0.70	-2.31	1.01	0.43	0.08	0.05
(Zenith x Levante)	0.88	-0.46	0.50	-0.50	-0.91	-0.48
(Mersiniye x Spagetti)	-0.93	-0.09	0.58	0.85*	1.33*	0.53
(Mersiniye x Menceki)	1.40	-0.78	1.08	-0.79	0.09	-1.60*
(Mersiniye x Levante)	-1.60	-1.33	-0.19	0.28	-0.04	0.32
(Spagetti x Menceki)	-1.14	-1.12	1.51	0.21	-0.15	-0.17
(Spagetti x Levante)	-0.21	0.04	-1.28	-0.61	-0.75	-1.10
(Menceki x Levante)	2.41	1.99	-5.13***	-0.13	0.52	1.25

*, **, *** Significant at 0.05, 0.01 and 0.001 probability levels, respectively.

Significant relationships were existed between N0 and N1 grain yields (r^2=0.38, P<0.01), while N2 grain yield did not show correlation with grain yield of N0 and N1 conditions, in spite of non-significant genotype x N interaction.

Table 5
Correlation coefficient among SPAD readings and grain yield at different nitrogen conditions.

Traits		SPAD			Grainyield		
	Nitrogen doses	**N0**	**N1**	**N2**	**N0**	**N1**	**N2**
SPAD	**N0**	1					
	N1	0.063	1				
	N2	-0.191	0.183	1			
Grainyield	**N0**	**0.328****	-0.651	0.113	1		
	N1	0.181	-0.116	-0.025	**0.387****	1	
	N2	0.126	-0.103	-0.171	0.163	-0.006	1

** Significant at 0.01 probability level.

4. Conclusion

SPAD measured at N0 conditions showed lower genotypic variation and better correlations with grain yield, contrary SPAD had higher genotypic variation at high N condition, but lower genetic correlations. Due to significant relationship between SPAD readings and grain yield under low N conditions, it will be hard to estimate grain yield by using SPAD readings in trials which applied conventional N rate. Therefore, nitrogen balance, which changes between N0 and N1, must be carefully adjusted to get accurate results at studies in which SPAD meter used as yield predictor.

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