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THE RESPONSE OF CHLOROPHYLL AND PROTEIN CONCENTRATION IN WINTER WHEAT TO DIFFERENT LEVELS OF IRRIGATION AND NITROGEN APPLICATION

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ABSTRACT

Crop production in arid and semi-arid regions is restricted by soil deficiencies in moisture and plant nutrients. Therefore, in order to investigation response of chlorophyll and protein concentration in winter wheat to different levels of irrigation and nitrogen application a field experiment in split plot based on Randomized Complete Block Design with three replications was conducted at 2008-2010 in the research field of Karaj, Iran. Treatment included: two wheat cultivars (Gascogne and MV-17), three levels of nitrogen (0, 75 and 150 kg N ha^{-1}), and three levels of withholding irrigation (I_1: regular irrigation in total growth stages, I_2: withholding irrigation at anthesis stage, and I_3: withholding irrigation at grain filling period stage). The results were shown that chlorophyll concentration increased with nitrogen application and decreased when that water stress was occurred. Minimum chlorophyll concentration was observed in 0 kg N ha^{-1} and irrigation omitted at grain filling period. Water deficit reduced chlorophyll concentration from 24.9 in check treatment to 22.7 in I_2 and 21.3 in I_3. There were no significantly different between cultivars in terms of chlorophyll concentration. Assessing protein content of wheat grain showed that, nitrogen rates had relatively major effects on grain protein.

Increases in grain wheat protein content were recorded by 11.7% and 18.4% in N_{75} and N_{150} kg N ha^{-1}treatments. Also, withholding irrigation at anthesis and grain filling stages caused that protein content increased by 5.6% and 8.8% respectively.

Keywords: Bread, Drought, Grain Filling Period, Quality Traits, *Triticum aestivum*.

INTRODUCTION

Photosynthetic pigments are colored complex organic molecules which absorb radiant energy in the visible range of visible spectrum (400-700 nm) and convert into chemical form of energy. Significant correlations between Chl and other physiological traits such as SLA, leaf N content [1], apparent photosynthesis rate [2] have been reported for many crop species [3 and 4]. Commonly, photosynthetic pigments content could be estimated with spectrophotometry and fluorometry. In spectrophotometry, Chl from area known leaf pieces were extracted with various chemical solutions such as acetone [5], NN-dimethyformamide and dimethylsulphoxide [6], and estimated by subjected to spectrophotometer at specified wave length of light which absorbed by particular pigment. Recently, chlorophyll meter; SPAD (Soil Plant Analysis Development) has been widely used in estimation of Chl content. Numerous studies reported that SPAD reading had high correlation with Chl content and developed regression equations to estimate Chl content by measuring SPAD for several crops [7]. Thus, in this experiment was used from the SPAD 502 for the measurement of the chlorophyll content in wheat leaves that were used as a diagnostic tool for the evaluation of plant nutrients, such as nitrogen and other nutrients related to chloroplast pigment contents. Also, Nitrogen frequently limits grain yields and grain protein percentage, and additional N inputs are required to optimize productivity and profitability. Adequate N fertilization is necessary to produce high yields of wheat and to increase the quality such as grain protein concentration. In this investigation, the irrigation and nitrogen fertilization management was evaluated on SPAD value and protein concentration in two wheat cultivars.

MATERIALS AND METHODS

Wheat cultivars (*Triticum aestivum L.* cv. Gascogne and MV-17*)*, supplied by the Karaj agricultural administration, Iran), varieties widely cultivated in Karaj Province, Iran were selected as the experimental material. This study was conducted at 2008-2010 in the research field of Karaj agricultural administration province, Iran ($35^0 48^{'}$ N, 51.00^0 E; 1360 m elevation). Before conduct of experiment, composite soil samples from experimental sites were collected from 0-30 cm depth. Soil samples were analyzed for physico-chemical properties. The results of soil analysis were shown in Table (1). There were 18 treatments in three replications. Treatment included: cultivars (Gascogne and MV-17), three levels of nitrogen (0, 75 and 150 kg N ha^{-1}), and three levels of withholding irrigation (I_1: regular irrigation in total growth stages, I_2: withholding irrigation at anthesis stage, and I_3: withholding irrigation at seed filling period stage). The field experiment was laid out in split plot based on Randomized Complete Block Design with three replications. Cultivars placed in Main plot, nitrogen levels in sub plot and irrigation regimes in sub subplot. Leaf chlorophyll content was estimated non-destructively by measuring leaf greenness using a portal SPAD (Soil Plant Analysis Development)-502 chlorophyll meter (Monilta Camera Co. Ltd., Japan). SPAD readings were collected from first fully expanded flag leaf from the top of the plant. Five plants were selected for recording the observations. In each plant five readings were recorded from leaf and they were averaged across each plot and expressed as SPAD reading per plant. Moreover, at harvesting time, protein content in grains was analyzed by Near Infrared Transmittance Based Protein Analyzer (Model: Intratec 1241 grain analyzer). The data collected from the experiment was subjected to statistical analysis. The level of significance used in 'F' and't' test was *P=0.05*. Critical difference values were calculated using Duncan's Multiple Range Test (DMRT) wherever the 'F' test was significant.

RESULTS AND DISCUSSION

In this experiment was used from the SPAD 502 for the measurement of the chlorophyll content in wheat leaves that were used as a diagnostic tool for the evaluation of plant nutrients, such as nitrogen and other nutrients related to chloroplast pigment contents. Many researches were conducted on the usefulness of SPAD 502 as a nondestructive analysis method for determination of chloroplast pigments [8 to 13]. In this experiment, nitrogen application increased the evaluated traits the highest value of which obtained by application 75 and 150 kg N ha^{-1} in three years (16.3, 25.0 and 27.5 in control N_{75} and N_{150} respectively) (Table2). But, water deficit reduced chlorophyll concentration from 24.9 in check treatment to 22.7 in I_2 and 21.3 in I_3. In the other side, there were no significantly different between cultivars in terms of chlorophyll concentration. This is important to note that, there is positive correlation between chlorophyll concentration and protein content. As expected, chlorophyll concentration (SPAD value) increased with nitrogen application and decreased when that water stress was occurred. Maximum SPAD value was obtained in 150 kg N ha^{-1} (27.5) and regular irrigation (24.9) treatments (Table2). In contrast, minimum chlorophyll concentration (based on SPAD value) was observed in 0 kg N ha^{-1} (16.3) and irrigation omitted at seed filling period (21.3). As can be seen, responses of cultivars chlorophyll concentration to different levels of nitrogen and irrigation regimes were similar (Table3). Indeed, the lowest SPAD value in both cultivars was observed in N_0 and withholding irrigation at seed filling period treatments (Table4). Minerals are required in the biosynthetic pathway and essential for the synthesis of chlorophyll [14 to 16]. Assessing protein Content of wheat grain showed that, nitrogen rates had relatively major effects on grain protein (Table 2). Increases in grain wheat protein content was recorded by 11.7% and 18.4% in N_{75} and N_{150} kg N ha^{-1} treatments compared to check treatment. The effects of different irrigation regimes on protein content were less than the nitrogen fertilization. Withholding irrigation at anthesis and seed filling stages caused that protein content increased by 5.6% and 8.8% respectively compared to regular irrigation (Table2). Withholding irrigation and high levels of N result in an improved grain quality [17]. Limited irrigation can also induce better utilization of soil moisture [18 and 19] and improve grain quality such as protein content [17]. A trend of increasing protein content with N rates was observed for both cultivars with the lowest protein content occurring at 0 kg N ha^{-1}. Increasing the rate of N fertilization from 0 to 75 and 150 kg N ha^{-1} increased the protein content by 10.9% and 18.3% (in MV-17 cultivar) and by 11.4% and 16.9% (in Gascogne cultivar), respectively compared to N_0 (check treatment) (Table3). Also, water stress caused that protein content increased when that withholding irrigation at anthesis and grain filling times occurred by 5.8% and 10.6% (in MV-17 cultivar) and by 4.5% and 7.6% (in Gascogne cultivar), respectively. Higher protein and good ratios of gliadin and glutenin are fundamental traits to making high quality breads [20].

CONCLUSION

The grain protein concentration is an important component of the quality of the produced grain, and the trait most commonly used to measure it, since it is considered to be of great importance for both human nutrition and bread quality. Adequate N fertilization is necessary to produce high yields of wheat and to increase the quality (grain protein concentration). Limited irrigation in present study improved mean grain protein percentage. Overall a significant increase (5.40 to 8.85%) in grain protein contents was recorded by nitrogen application and (11.2 to 17.9%) for limited irrigation treatments as compared to N_0 (control) and regular irrigation. These results indicated that the genotypes not only differed in yield, but also in grain protein response to nitrogen and water soil availability. Also, Nitrogen deficiency is closely related to restricted chlorophyll synthesis, and it typically results in a reduction in plant growth.

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Table 1: Soil characteristics of experimental field

Soil properties	2008	2009	2010
Sand (%)	15	16	13
Clay (%)	40	43	41
Silt (%)	45	41	46
Soil texture	Silty clay	Silty clay	Silty clay
Organic matter (%)	2.0	2.2	2.1
pH	7.1	7.0	7.1
Electrical conductivity (dsm^{-1})	0.63	0.67	0.52
N (%)	0.13	0.13	0.11
P (ppm)	8.2	8.6	8.9
K (ppm)	359	382	354
Zn ($mg.kg^{-1}$)	0.55	0.63	0.51
Fe ($mg.kg^{-1}$)	2.8	2.5	3.2
Mn ($mg.kg^{-1}$)	2.5	2.5	2.7

Table 2: The effect of year and other treatment on evaluated traits (average of 2008-2010)

Treatment components (Variety Nitrogen and Irrigation)		Treatment	TRAITS: CHL: Chlorophyll concentration (SPAD value)	TRAITS: Protein (%)
Year		Y	23.0	12.9
V_1:Gascogne		V_1	22.5	13.0
V_2:MV-17		V_2	23.5	12.9
N		N_0	16.3	11.9
		N_{75}	25.0	13.3
		N_{150}	27.5	14.1
I		I_1	24.9	12.5
		I_2	22.7	13.2
		I_3	21.3	13.6
N_0	I_1	N_0I_1	18.1	11.3
	I_2	N_0I_2	16	11.9
	I_3	N_oI_3	14.9	12.6
N_{75}	I_1	$N_{75}I_1$	26.8	12.5
	I_2	$N_{75}I_2$	24.7	13.4
	I_3	$N_{75}I_3$	23.8	13.9
N_{150}	I_1	$N_{150}I_1$	29.7	13.7
	I_2	$N_{150}I_2$	27.6	14.2
	I_3	$N_{150}I_3$	25.4	14.4
Average			23.0	13.1
Cv.: (Coefficient of variation) (%)			7.77	1.01

Table 3: Evaluated traits affected by cultivars, nitrogen and cultivars, irrigation interaction (average of 2008-2010)

Variety	Levels of Nitrogen and Irrigation	Treatment	CHL: Chlorophyll concentration (SPAD value)	Protein (%)
V_1: GASCOGNE	N_0	V_1N_0	16.2	11.8
	N_{75}	V_1N_{75}	24.5	13.2
	N_{150}	V_1N_{150}	26.85	13.85
V_2: MV-17	N_0	V_2N_0	16.5	12.05
	N_{75}	V_2N_{75}	25.65	13.4
	N_{150}	V_2N_{150}	28.25	14.35
V_1: GASCOGNE	I_1	V_1I_1	24.4	12.4
	I_2	V_1I_2	22.15	13.05
	I_3	V_1I_3	21	13.4
V_2: MV-17	I_1	V_2I_1	25.35	12.55
	I_2	V_2I_2	23.35	13.3
	I_3	V_2I_3	21.65	13.95
	Average		22.99	13.11
Cv.: (Coefficient of variation) (%)			7.77	1.01

Table 4: Evaluated traits affected by cultivars, nitrogen and irrigation interaction (average three years 2008-2010)

VARIETY	NITROGEN FERTILIZER	IRRIGATION	TREATMENT	CHL: CHLOROPHYLL CONCENTRATION (SPAD value)	PROTEIN (%)
V_1: GASCOGNE	N_0	I_1	$V_1N_0I_1$	18.5	11.2
		I_2	$V_1N_0I_2$	15.7	11.9
		I_3	$V_1N_0I_3$	14	12.4
	N_{75}	I_1	$V_1 N_{75} I_1$	26.2	12.6
		I_2	$V_1 N_{75} I_2$	23.9	13.3
		I_3	$V_1 N_{75}I_3$	23.5	13.7
	N_{150}	I_1	$V_1 N_{150} I_1$	28.5	13.5
		I_2	$V_1 N_{150} I_2$	26.9	14
		I_3	$V_1 N_{150} I_3$	25.2	14
V_2: MV-17	N_0	I_1	$V_2 N_0 I_1$	17.8	11.5
		I_2	$V_2 N_0 I_2$	16.3	12
		I_3	$V_2 N_0 I_3$	15.4	12.8
	N_{75}	I_1	$V_2 N_{75} I_1$	27.5	12.4
		I_2	$V_2 N_{75} I_2$	25.5	13.5
		I_3	$V_2 N_{75}I_3$	24	14.2
	N_{150}	I_1	$V_2 N_{150} I_1$	30.9	13.8
		I_2	$V_2 N_{150} I_2$	28.3	14.5
		I3	V2 N150 I3	25.5	14.8
		Average		23	13.1
Cv.: (Coefficient of variation) (%)				7.77	1.01