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Full Length Research Paper

Influence of late-season nitrogen application on chlorophyll content and leaf area index in wheat

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The research was conducted in Isparta ecological conditions during the 2006 - 2007 and 2007 - 2008 crop growing seasons. The purpose of the study was to determine the effects of late-season N application (LSN) on chlorophyll content and leaf area index (LAI) of wheat. The experiment was laid out in as Randomized Complete Block split-plot design with three replications. Gün-91, Gerek-79 and Altay-2000 wintery bread wheat cultivars were used. Nitrogen was applied as conventional N (two equal amounts at seed sowing and tillering stage) and late-season N supplies (one-third of was applied during sowing, one-third at the tillering stage and the rest was applied as foliar at post-pollination stage). Chlorophyll content and LAI were significantly affected by N applications in both years. Chlorophyll content an LAI of wheat cultivars varied, depending on cultivars, N application forms and growing period. The highest chlorophyll content was obtained from Gerek-79 x conventional N application during the ear-emergence period (51.8), and in the second year it was determined in Altay-2000 x LSN application (51.1). In the first year, the highest LAI was obtained from Gün-91 x conventional N application during flowering period (9.46), and in second year it was determined in Altay-2000 x conventional N application (9.71).

Key words: Wheat, leaf area index, chlorophyll, N application.

INTRODUCTION

Nitrogen is one of the most important nutrients that are limited to crop production. Nitrogen fertilization boosts the grain yield of wheat to a certain point through its influence on yield components, phenology and leaf traits. N availability influences the efficiency of assimilated mobilization to the sink during leaf senescence, and thus affects leaf viability and activity (Ellen, 1987). Specifically, a non-limited supply of N extends the lifespan of a leaf by delaying the onset of chlorophyll loss during grain filling (Yang et al., 2000). In addition, nitrogen increases leaf area index (LAI) and amount of net assimilation, and also improve the physiological properties of plant (Pollmer et al., 1979). The photosynthesis and reception of solar radiation in plants is determined largely by vegetative surface (Monteith and Unsworth, 1990). LAI has been defined as the total green leaf area per unit ground surface area (Watson, 1947). LAI is a significant feature for the determination of plants photosynthetic activity. LAI is a key structural characteristic of plants due to the role of green leaves in controlling many biological and physical processes in plant canopies. Leaves are the most contributing organ in the formation of yield in plants (Monyo and Whittington, 1973). Approximately 70 - 90% of the final grain yield is derived from photosynthates (products of photosynthesis) produced by the plant during the grain filling. The flag leaf and head usually contribute most, but certainly not all, of the photosynthate to the grain (Anderson et al., 1985; Yildirim et al., 2009). There is a significant relationship between the green duration of leaves after ear emergence for a long period of time, especially, this feature is more important in the arid environment (Hansen et al., 2005). Chlorophyll is a green pigment found in plants, and it is necessary for photosynthesis. Chlorophyll is of great importance for plant development, nutrient storage in different organs

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Table 1. Climatic data of the experimental region related to growing seasons.

	Years	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	Mean/Total
Mean temperature(°C)	2006/2007	13.4	6.1	2.2	1.1	3.1	7.1	9.5	17.5	21.6	9.1
	2007/2008	14.4	7.4	2.7	-1.2	0.9	8.4	11.5	14.4	20.9	8.8
	Long term	12.0	7.5	3.0	2.5	5.1	9.3	10.8	15.6	20.1	9.5
Precipitation (mm)	2006/2007	140.7	91.8	0.0	3.2	7.4	25.8	22.3	24.6	4.8	351.3
	2007/2008	30.7	79.8	97.2	88.6	41.9	51.4	49.2	32.2	25.6	496.6
	Long term	28.9	76.9	98.0	46.9	28.0	42.9	56.6	50.8	24.4	453.4

Records of the Regional Meteorology Station, Isparta.

Table 2. Soil characteristics of the experimental area.

Structure	Clay (%)	Silt (%)	Sand (%)	pH 1:1	EC 10^6 (dS/m)	$CaCO_3$ (Lime)	Organic Matter (g kg^{-1})	P (mg kg^{-1})	Nitrogen (%)
Loamy	23.1	33.9	43.0	8,1	400	255	13.4	199	0.14

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and nutrient cycling. Chlorophyll content of varieties with higher leaf area index is also high, and therefore, increases the rate of photosynthesis. Pulkrabek (1998) reported that chlorophyll content had changed throughout the growing season of plants. Chlorophyll content of plants begins to decline at the start of aging in plant leaf (Matile et al., 1988). Changes in accumulation of chlorophyll in plants depend on growth conditions, and chlorophyll content reduces in negative conditions (Masuda et al., 2002).

Photosynthesis capacity is affected from other nutrient elements, especially nitrogen. Nitrogen is one of the basic components of chlorophyll inside. In addition, Nitrogen is also effective on the enzyme of carbon metabolism and photosynthetic electron carriers. Net photosynthesis rate of C-3 and C-4 plants varies depending on the amount of nitrogen (Ozen and Onay, 2007). Nitrogen accumulated in the leaves delays aging of the leaf. In wheat, leaves remain green for long, especially in the greens for a long time in period of ear emergence increases photosynthetic activity. The aim of the study was to determine the effects of conventional late-season nitrogen application on the chlorophyll content and LAI of wheat.

MATERIALS AND METHODS

The experiment was conducted in Isparta ecological conditions in Turkey during the 2006 - 2007 and 2007 - 2008 growing seasons.

Climatic data for crop growing seasons are shown in Table 1. Isparta has a territorial climate (cold winters and dry hot summers) with an annual total rainfall of 500 mm. The long-term average temperature from October to June was 9.5°C. Precipitation was 453.4 mm for the same period. Average temperatures and total precipitation were 9.8 and 8.8°C and 351.3 and 496.6 mm from sowing in October to anthesis in June for 2006 - 07 and 2007 - 2008 years, respectively (Table 1).

Soil was sampled in a depth of 60 cm before the beginning of the experiment for physicochemical analysis. The experimental soil was low in nitrogen (0.14% N) and phosphorus (199 mg kg^{-1} P_2O_5), and had alkaline (pH: 8.1) and loamy contents (Table 2). The data given in Table 2 indicates that there were other soil fractions in the experimental area.

Experiment was laid out in Randomized Complete Block split plot design with 3 replications. Wheat cultivars were main plots, the nitrogen application form were subplots split within main blocks. The winter bread wheat cultivars intensely sown in the area (Gün-91, Gerek-79 and Altay-2000) were used.

Seeds were sown with 15 × 5 cm row spaces using a parcel sowing machine. The net plot size was 9 m^{-2}. Sowing was made in October in both years. All cultural practices were kept regular and uniform for all treatments in both years. The experiments were not irrigated in both growing seasons.

Gün-91

The variety is white spiked, awned, winter, and resistant to lodging, drought, diseases and pests, with 90 - 100 cm in height.

Gerek-79

The variety is a winter cultivar, awned, resistant to drought, cold, disease, pests, and the variety posses' wide adaptation ability.

Altay-2000

The variety is a brown seeded, awned, wintery cultivar, with 1000 grain weight equaling to 33 - 35 g., and resistant to lodging, drought, cold, stress condition, disease, pests, and the variety is 100 - 110 cm in height.

Soil N was analyzed before sowing. Nitrogen and ammonium nitrate (NH_4NO_3; 33.5%N) at a rate of 80 kg ha^{-1}, was applied as conventional (two equal amounts at seed sowing and tillering stage) and late-season foliar N application (one-third of was applied during

Table 3. Chlorophyll content (SPAD) of wheat cultivars of N application forms.

Growth stages		Tillering		Stem elongation		Ear emergence		Flowering		Milk development	
Cultivars / N application forms		**Ist Year**	**2nd Year**	**Ist Year**	**2nd Year**	**Ist Year**	**2nd Year**	**Ist Year**	**2nd Year**	**Ist Year**	**2nd Year**
Gün-91	CN	44.4^{klm}	39.4^{o}	48.3^{def}	46.1^{f-m}	50.7^{abc}	47.0^{c-j}	48.1^{d-g}	46.5^{e-k}	44.8^{j-m}	45.0^{h-n}
	LSN	41.8^{no}	39.8^{o}	44.1^{lm}	45.9^{g-m}	48.2^{def}	49.6^{a-e}	46.1^{i-k}	47.0^{d-j}	45.1^{i-m}	46.2^{f-m}
Cultivar 1 mean		43.1ef	39.6e	46.2cde	46.0a-d	49.5ab	48.3a-c	47.1b-d	46.8a-d	44.9d-f	45.6a-d
Gerek-79	CN	44.3^{mn}	43.2^{mn}	45.3^{h-l}	45.0^{h-n}	51.8^{a}	50.1^{abc}	49.7^{j-m}	48.0^{b-h}	46.3^{g-j}	46.6^{e-k}
	LSN	40.0^{p}	42.5^{n}	43.8^{lm}	44.1^{j-n}	50.7^{abc}	49.2^{a-f}	49.4^{cd}	48.0^{b-h}	46.9^{e-i}	47.6^{b-i}
Cultivar 2 mean		42.2 f	42.8 de	44.5d-f	44.5b-e	51.3 a	49.6 ab	49.5ab	48.0a-d	46.6b-d	47.1a-d
Altay-2000	CN	45.1^{i-m}	43.5^{k-n}	44.9^{j-m}	44.9^{h-n}	51.4^{ab}	49.9a-d	48.1^{d-g}	48.5^{a-g}	46.6^{f-j}	48.3^{a-g}
	LSN	40.3^{op}	43.3^{lmn}	47.1^{e-h}	44.7^{i-n}	49.4^{cd}	51.1^{a}	48.5^{de}	50.2^{ab}	46.9^{e-i}	46.4^{f-l}
Cultivar 3 mean		42.7p	43.4cde	46.0c-e	44.8b-e	50.4a	50.5a	48.3a-c	49.3ab	46.7b-d	47.3a-d

CV $\%_{\text{first year}}$ = 2.09; CV$\%_{\text{second year}}$ = 3.47. CN: Conventional N; LSN: Late-season N.

Table 4. Effect on chlorophyll content (SPAD) in different growth stages of N application forms.

Growth stages	Tillering		Stem elongation		Ear emergence		Flowering		Milk development	
/N application Forms	**Ist Year**	**2nd Year**	**Ist Year**	**2nd Year**	**Ist Year**	**2nd Year**	**Ist Year**	**2nd Year**	**Ist Year**	**2nd Year**
CN*	44.6^{d}	42.0^{e}	46.2^{cd}	45.3^{cd}	51.2^{a}	49.0^{ab}	48.7^{b}	47.7^{a-d}	45.9^{d}	44.6^{de}
LSN	40.7^{e}	41.9^{e}	45.0^{d}	44.9^{de}	49.5^{b}	49.9^{a}	48.0^{bc}	48.4^{abc}	46.3^{cd}	46.7^{bcd}
Mean	42.6d	41.9c	45.6c	45.1b	50.4a	49.4a	48.3b	48.0ab	46.1c	45.6b

CN: Conventional N; LSN: Late-season N.

sowing, one-third at the tillering stage and the rest was applied to foliar at post-pollination growth stage) (Zadoks et al., 1974). Liquid N foliar-applied was by diluted and supplied via spraying. All the phosphorous fertilizer (60 kg P_2O_5 ha^{-1}) was given as triple super phosphorus with sowing.

Chlorophyll content was identified by using Minolta SPAD-502 chlorophyll meter in the flag leaf of randomly selected 10 plants in tillering, stem elongation, ear emergence, flowering and milk development growing stages (Zadoks et al., 1974; Hussain et al., 2000; Singh et al., 2002) in each wheat variety and N application.

Plants were harvested in a length of 40 × 15 cm at each growing stages (tillering, stem elongation, ear emergence, flowering and milk development) for LAI in both years, and brought to the laboratory. Leaf areas of all samples were measured using portable AM 300 (leaf area measuring device).

All the data were analyzed with the analysis of variance (ANOVA) using SAS Statistical Package Program. Means were compared using the DUNCAN test.

RESULTS

The results regarding the influence of late-season nitrogen applications on chlorophyll content and leaf are index of wheat are shown in Tables 3, 4, 5 and 6.

Chlorophyll content of wheat cultivars was significant at 0.05 level in the both years. In the first year, the highest chlorophyll content was obtained from Gerek-79 cultivar (51.3), as in Altay-2000 in the second year (50.5) during ear emergence stage. In the first year, the lowest chlorophyll content was obtained from Gerek-79 cultivar (42.2), in the second year it was determined in Gün-91 cultivar (39.6) during the tillering stage (Table 3).

Nitrogen application form had significant effect ($p \leq 0.05$) on chlorophyll content in both years. The highest chlorophyll content was obtained from conventional N application (51.2) in the first year, in the second year, it was determined in late-season N application (49.9) at ear emergence stage (Table 4).

The highest chlorophyll content, depending on growth stages of wheat, was obtained from the ear emergence stage (50.4 and 49.4, respectively) in both years (Table 4).

Chlorophyll content of wheat was affected by cultivar x N application and form x growth stage interaction significantly ($p \leq 0.01$). In the first year, the highest chlorophyll content was obtained from Gerek-79 x conventional N x

Table 5. Effects to leaf are index of wheat cultivars of N application forms.

Growth stages		Tillering		Stem elongation		Ear emergence		Flowering		Milk development	
Cultivars / N application forms		**Ist Year**	**2nd Year**	**Ist Year**	**2nd Year**	**Ist Year**	**2nd Year**	**Ist Year**	**2nd Year**	**Ist Year**	**2nd Year**
Gün-91	CN	2.94^{f}	2.95^{e}	5.32^{cde}	5.69^{d}	8.76^{ab}	9.07^{bc}	9.46^{a}	9.24^{abc}	5.40^{cd}	5.72^{d}
	LSN	2.56^{f}	2.61^{e}	4.38^{e}	5.54^{d}	8.74^{ab}	8.73^{c}	9.18^{ab}	9.10^{bc}	5.26^{cde}	5.48^{d}
Cultivar 1 mean		2.75c	2.78c	4.85b	5.61b	8.75a	8.90a	9.32a	9.17a	5.33b	5.60b
Gerek-79	CN	2.42^{f}	2.67^{e}	4.86^{cde}	5.95^{d}	8.61^{ab}	9.01^{bc}	8.68^{ab}	9.64^{ab}	5.07^{cde}	5.31^{d}
	LSN	2.27^{f}	2.42^{e}	4.69^{de}	5.56^{d}	8.89^{ab}	9.31^{abc}	8.33^{b}	9.83^{a}	4.98^{cde}	5.34^{d}
Cultivar 2 mean		2.34c	2.54c	4.77b	5.75b	8.75a	9.16a	8.50a	9.73a	5.02b	5.32b
Altay-2000	CN	2.78^{f}	2.92^{e}	4.95^{cde}	5.75^{d}	8.89^{ab}	9.88^{a}	8.87^{ab}	9.71^{ab}	5.75^{c}	5.51^{d}
	LSN	2.71^{f}	2.85^{e}	4.80^{cde}	5.47^{d}	8.60^{ab}	9.02^{bc}	8.43^{b}	9.09^{bc}	4.69^{de}	5.48^{d}
Cultivar 3 mean		2.74c	2.88c	4.87b	5.61b	8.74a	9.45a	8.65a	9.40a	5.22b	5.49b

CV $\%_{first\ year}$: 9.31; CV$\%_{second\ year}$: 6.64. CN: Conventional N; LSN: Late-season N.

Table 6. Effect on leaf is index in different growth stages of N application forms.

Growth stages / N application forms	Tillering		Stem elongation		Ear emergence		Flowering		Milk development	
	Ist Year	**2nd Year**	**Ist Year**	**2nd Year**	**Ist Year**	**2nd Year**	**Ist Year**	**2nd Year**	**Ist Year**	**2nd Year**
CN	2.71^{c}	2.85^{c}	5.04^{b}	5.80^{b}	8.75^{a}	9.32^{a}	9.00^{a}	9.53^{a}	5.41^{b}	5.51^{b}
LSN	2.51^{c}	2.63^{c}	4.62^{b}	5.22^{b}	8.74^{a}	9.02^{a}	8.64^{a}	9.34^{a}	4.94^{b}	5.43^{b}
Mean	2.61c	2.74c	4.83b	5.51b	8.74a	9.17a	8.82a	9.43a	5.17b	5.47b

CN: Conventional N; LSN: Late-season N.

ear emergence interaction (51.8), and in the second year it was determined in Altay-2000 x late-season N x ear emergence interaction (51.1) (Table 4).

LAI of bread wheat was significant at 5% level in the both years. In the first year, the highest LAI was obtained from Gün-91 (9.32) during flowering stage, and in second year it was determined in Gerek-79 (9.73). The lowest LAI were obtained from Gerek-79 cultivar (2.34 and 2.54, respectively) during tillering stage in both years (Table 5).

Nitrogen application form had significant effect ($p \leq 0.05$) on LAI in both years. The highest LAI were obtained from conventional N application (9.00 and 9.53, respectively) during flowering stage in both years (Table 5).

Growth stages of wheat had significant effect ($p \leq 0.01$) on LAI in both years. The highest LAI were determined from the flowering stage (8.82 and 9.43, respectively) in both years (Table 6).

The LAI of wheat was significantly ($p \leq 0.01$) affected by cultivar x N application and form x growth stage interaction. In the first year, the highest LAI was obtained from Gün-91 x conventional N x flowering stage interaction (9.46), and in second year it was determined in Gerek-79 x late-season N x flowering stage interaction (9.83) (Table 5).

DISCUSSION

In the study, chlorophyll content and LAI of wheat cultivars was affected by N application forms. Differences in chlorophyll content and LAI of wheat cultivars could be resulted due to genetic structures, morphological and tillering characteristics (Cardenas and Gausman, 1973). The findings agreed with those of Singh et al. (2002) who stated that chlorophyll content and LAI varied depending on morphological and tillering characteristics of cultivars.

Conventional N application had higher chlorophyll content and LAI than the late-season N application. It is thought that applied N in early period to plant will stimulates vegetative growth than late-season N application. Therefore, the increased in chlorophyll content and LAI of wheat, depends on the increased vegetative parts. Thomas and Gausman (1977) determined that leaf pigment concentration and nutrition elements in different parts of plant changed by different N applications.

The highest chlorophyll content was obtained from conventional N x ear-emergence stage interaction in all the cultivars. The highest LAI was obtained from conventional N x flowering stage interaction in all the cultivars. Both chlorophyll content and LAI increased until the period ear emergence and flowering, and afterwards it began to decrease. Our findings agree with those of Pulkrabek (1998) who observed that chlorophyll content and LAI varied depending on the years, N application forms and growth period.

Net photosynthesis is affected by the quantity applied N, nutrition conditions of soil, and decrease in the quantity of nitrogen is affecting the quality and quantity of photosynthetic enzymes (Ozen and Onay, 2007). Similarity, Bavec et al. (2007) reported that there were significant and positive correlation between nitrogen and chlorophyll content. Matile et al. (1988) reported that chlorophyll content of plants begins to fall by the start of an aging plant leaf. Similarity, Thimann (1985) reported that loss of chlorophyll in plants begins with hydrolysis of RNA as well as the aging of plant leaves. Bavec et al. (2007) observed that LAI varied depending on N fertilization, growth period and genetic structures of cultivar. In another study, Bariga (1980) and Sharma et al. (2003) reported that size and number of plant leaves depends on their genotype.

Conclusion

The results obtained from present study indicated that the effects of N application form were found to be significant on both chlorophyll content and LAI. Chlorophyll content and LAI of wheat cultivars varied according to years, N application forms and growth period. Based on the results of the research, conventional N application had higher chlorophyll content and LAI as compare to the late-season N application. In the study, chlorophyll content increased until the period ear emergence, and afterwards it began to decrease. LAI increased until to the period of flowering, and afterwards it began to decrease.

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