Flag Leaf Role in N Accumulation and Remobilization as Affected by Nitrogen in a Bread and Durum Wheat Cultivars

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Abstract: Understanding the physiological basis of absorption and transportation of nitrogen by plants has specific importance. In this experiment, a bread and durum wheat cultivars were treated with different rates and times of nitrogen application, using split factorial on the basis of randomized complete block design with three replications at Khuzestan region during 2007-2008. Main plots were consisted of two wheat cultivars (Cahmran, *Triticum aestivum* L and Yavaros, *Triticum durum* L) and sub plots included nitrogen rates (40, 80 and 160 kg ha^{-1}) and times of nitrogen application (T1= all N fertilizer at planting, T2= 1/2 at planting + 1/2 during booting stage and T3= 1/3 at planting + 1/3 during booting stage + 1/3 at heading stage). The results showed that there were significant differences between cultivars in flag leaf nitrogen content dyring maturity stage, N remobilization and its efficiency from flag leaf to the grains and also grain protein percentage. Durum wheat was more efficient in nitrogen remobilization, therefore, had higher grain protein percentage. Increasing in rates and times of nitrogen application had significant effect on most of the traits. There were significant interactions between cultivars, rates and times of N application, indicating that durum wheat was more efficient in N remobilization from flag leaf to the grain. In general, it appeared that N remobilization efficiency was the main factor affecting the grain protein percentage under the conditions of low N absorption in this experiment.

Key words: Wheat · Nitrogen remobilization efficiency · Grain protein

INTRODUCTION

Nitrogen is the most expensive fertilizer nutrient used to raise crop plants [1, 2]. A sizable portion of applied N is lost by leaching and denitrification [3, 4]. The increase demand for N fertilizers has also raised farm input costs. Therefore, plant breeders need to develop cultivars that can absorb N more efficiently from the soil and partition most of the absorb N in to the grain. Such cultivars would minimize loss of N from the soil and make more economic use of the absorbed N.

Nitrogen transportation in plants, especially during growth and development stages, where N absorption from the soil is limited, is one of the questionable phenomena in fundamental of crop physiology. For example, several studies have indicated that grain N in wheat primarily originates as a result of translocation from vegetative parts after anthesis [5-7]. This translocation depends on environment conditions [8] under genetic control [9-11] and also can be affected by fertilizer N application [12].

Nitrogen remobilization efficiency from vegetative organs to grains was more significant in high protein cultivars [13]. It seems that, the gratest N remobilization efficiency has been the main factor in increasing grain protein in high protein cultivars [14]. Austin *et al*. [15] observed significant genotypic differences for vegetative N concentration at anthesis, yet genotypic variation for total N content at anthesis was associated primarily with variation in dry matter. Several authors have reported significant variation for postanthesis N uptake [15,16, 17] and in several studies N uptake during grain fill accounted for as much as 50% of the grain N content at maturity [18-21]. Terman [21] and Perez *et al*. [22] reported that, grain protein content maybe improved by selection of genotypes which transport more nitrogen from vegetative organs to grain. Lotfali Ayeneh and Radmehr [23] found that, there were significant differences between nitrogen application rates and genotypes in nitrogen absorption at anthesis, amount of N remobilization and N absorption at maturity stage. Patric and Smith [24] demonstrated that N

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fertilizer partitioning increase remobilization efficiency. Because the most part of nitrogen remobilization to grain raised from early nitrogen application treatment. Rostami and Jereaie [19] showed that nitrogen concentration in above ground organs of high protein cultivars was significantly more than low protein cultivars. Austin *et al*. [15] in a study with 47 genotypes of winter wheat reported that, nearly 50 percent of nitrogen in spike absorbed from the soil after anthesis stage and the rest from N remobilization. Van sanford and Mackown [10] and Tindal *et al*. [25] stated that flag leaf at maturity stage contained lower portion of nitrogen in low-N environments than enough–N environments. This probably indicates that, flag leaf nitrogen content under nitrogen stress conditions was highly transported to the grain. Waldren and Flowerdy [26] and Wada *et al*. [27] showed that, about 80 percent of nitrogen at maturity stage absorbed at anthesis stage and two third of the nitrogen was transported from leaves to the grains.

Wheat grown under water deficit condition in the Mediterranean climatic region of Iran commonly fill their grain under increasing soil water deficits which restrict the uptake of N from the soil. Under these circumstances, most grain N in wheat may be derived from pre-anthesis stored N [7, 28].

Because of the importance of protein content and indirectly fertilizer N management to grain quality, this experiment was conducted to evaluate the effect of N fertilizer rate and time on the accumulation, remobilization and remobilization efficiency of nitrogen from flag leaf to the grain and also studying the relation between nitrogen concentration in flag leaf with grain protein.

MATERIALS AND METHODS

This study was conducted at field experiment of Islamic Azad University of Ramhormoz, Khuzestan, Iran (31°16′ N, 49°36′ E and 150.5 m above the sea level) during 2007-2008. Total precipitation was 310.8.5 mm, which is shown in Fig 1. The experiment was complete randomized block, split factorial design with three replications. Main plots consisted of two levels of cultivars ("Chamran" *Triticum aestivum L*. and "Yavarous" *Triticum durum L.)* and sub plots including nitrogen rates (40, 80 and 160 kg ha^{-1}) and times (T1= all N fertilizer at planting, T2=1/2 at planting +1/2 during booting stage and T3= 1/3 at planting + 1/3 during booting stage + 1/3 at heading stage), arranged as factorial. To determine the soil characteristics 15 samples from 30 cm depth were collected and analyzed for physical and chemical properties (Table 1). P and K fertilizers were applied according to recommendations of soil testing in forms of superphosphates and potassium sulfate, respectively. Plots were sown on 11 November 2007 with a cone seeder and were 8 m long and 1.5 m wide, with 6 rows 0.2 m apart. Plots were plowed and disked after winter wheat harvest in July. The plots were disked again before seeding in November. Apirus was applied in early April to the crop to control both broad and narrow leaved weeds. Twenty main stems that headed on same day were tagged for each treatment. As the tagged main stems of each cultivar reached anthesis, 10 plants in each plot were removed and flag leaves blade of main stems was cut. Tillers were discarded. At maturity, 10 additional tagged plants were removed and flag leaves blade of main stems was cut.
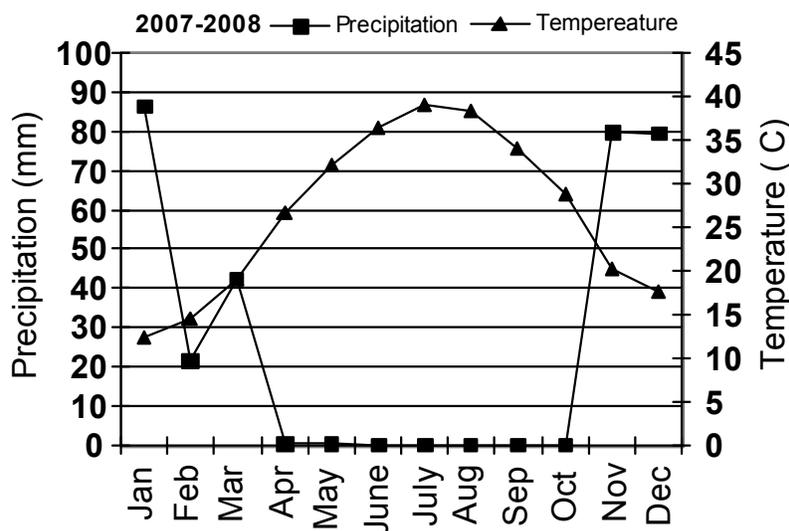

Fig. 1: Monthly values of temperature and precipitation in the field experiment

Table 1: Soil physical and chemical characteristics of the experimental site

Variable	
Texture	Silty-Clay
PH	8.96
EC dS m $^{-1}$	3.88
Organic matter (%)	0.55
N(%)	0.042
P mg Kg $^{-1}$	5.4
K mg Kg $^{-1}$	340
Fe	2.6
Zn	0.56
Mn	5.2
Cu	0.66

Anthesis was scored when anthers in the central florets of 50% of the spikes in plant had dehisced and maturity when almost all the spikes in plot showed complete loss of green color. Samples were dried to constant weight at 70°C, weighed and ground to pass a 0.4-mm screen. Grain samples were ground using the same screen. Total nitrogen concentration was determined by standard macro-Kjeldhal procedure. Grain protein concentration was calculated by multiplying grain N concentration by 5.7 [29].

The various parameters referring to N movement within the flag leaf of wheat plant that are discussed in this paper were evaluated as fallows: [30].
Flag leaf nitrogen content at anthesis stage (mg plant $^{-1}$).
Flag leaf nitrogen content at maturity stage (mg plant $^{-1}$).
Nitrogen remobilization (mg plant $^{-1}$) = N content at anthesis – N content at maturity.
Nitrogen remobilization efficiency (%) = (N remobilization/N content at anthesis) × 100.
Data were analyzed by analysis of variance [31].When significant differences were found (*P=0.05*) among means, Duncan's multiple range test (DMRT) were applied.

RESULTS AND DISCUSSION

The results showed that, there were significant differences between two wheat cultivars in nitrogen remobilization, its efficiency and grain protein content (Table 2). Mean comparisons (Table 3) indicated that, there were no significant differences between cultivars in nitrogen content of flag leaf at anthesis stage. This probably presents both cultivars absorbed the same amount of nitrogen from the soil. But, at maturity stage, durum wheat reserved less nitrogen content in flag leaf than bread cultivar, indicating more N remobilization

in durum wheat than bread wheat. Consequently, grain protein percentage in durum wheat increased. Branlard *et al.* [32] and Cox *et al.* [14] found that the differences among wheat cultivars in the amount of residual N in the straw resulted mainly from the differences in straw DM, since the differences in straw N concentrations were small. Rostami and Jereaie [19] reported that N remobilization was the reason of increasing grain protein percent. It seems that a genetic difference between two cultivars also is another factor in N remobilization from vegetative organs to the grains. Significant differences for N remobilization after anthesis were reported in a study conducted on 95 F_5 breed wheat lined derived from a single cross [33]. Grain protein in low protein cultivars depended on translocation of N already present in lower leaves at anthesis. In contrast, high protein cultivars required continued assimilation of N by flag leaves after anthesis [34,13]. One possible avenue for increasing the grain nitrogen yield in wheat through improving the efficiency of remobilization of nitrogen in the plant to the grain. Other studies have shown that the relationship between grain protein concentration and N translocation or N-translocation efficiency is not consistent [35, 36]. Conversely, Robert *et al.* [37], McNeal *et al.* [38] and Bhatia and Rabson [39] have reported that protein concentration in grain might be improved by selecting genotypes that translocate a higher percentage of N from vegetative organs to the grain. Positive correlations have been observed in wheat between grain protein concentration and nitrogen harvest index [40-43]. The descending ranking of sources of advantage high protein wheat cultivars would seem to be: viability and continued assimilation of N by flag leaves after anthesis; efficiency of translocation of vegetative N present at anthesis; and amount of vegetative N present at anthesis [44, 9, 43, 21, 25].

Nitrogen application rates had significant effect on all traits (Table 2). By increasing nitrogen levels all traits increased, except N remobilization efficiency (Table 4). Increasing N remobilization due to N fertilization was also reported by Pritchard and Bhandari [2], Spiertz and Ellen [45] and Cox *et al.* [14]. N content of flag leaf enhanced at anthesis and maturity stages by increasing of nitrogen. N remobilization from flag leaf to the grain increased by increasing N application rate, resulted in increasing grain protein percent. However, N remobilization efficiency was decreased by increasing nitrogen application rate. Grain N content in wheat depends on uptake of soil nitrate prior to flowering, continued uptake of nitrate during grain fill and remobilization of stored vegetative N accumulated

Table 2: Analysis of variance of cultivars, rate and time of nitrogen application and interaction between them on nitrogen content of flag leaf at anthesis and maturity stage, N remobilization, N remobilization efficiency and grain protein percent

s.o.v	d.f	N content of flag leaf at anthesis (mg g^{-1})	N content of flag leaf at maturity (mg g^{-1})	N remobilization (mg g^{-1})	N remobilization efficiency (%)	Grain protein (%)
Replication	2	0.457 ns	0.14 ns	0.003 ns	0.574 ns	0.211 ns
Varieties	1	4.932**	14.97**	131.7**	1633.5**	32.3**
Error (a)	2	8.65	0.035	0.033	0.722	0.335
N Rates	2	69.73**	14.23**	20.4*	43.6**	22.7**
N Times	2	69.23**	11.35**	3.99**	3.019*	8.13**
Varieties x N Rates	2	33.22**	0.16**	3.20**	12.06*	0.65**
Varieties x N Times	2	15.01**	1.57**	3.90**	100.05**	5.72**
N Rates x N Times	4	11.70**	0.85**	10.81**	59.96**	1.39**
Varieties x N Rates x N Times	4	16.32**	4.43**	13.78**	74.94**	6.38**
Error (c)	32	2.644	0.005	0.002	0.419	0.122

Ns (Non significant), * (Significant at 5%) and **, 1% (Significant at 1%) levels.

Table 3: Mean nitrogen content of flag leaf at anthesis and maturity stage, N remobilization, N remobilization efficiency and grain protein percent in bread and durum wheat cultivar

Cultivars	N content of flag leaf at anthesis (mg g^{-1})	N content of flag leaf at maturity (mg g^{-1})	N remobilization (mg g^{-1})	Remobilization N Efficiency (%)	Grain protein (%)
Chamran	16.44a	6.51a	9.67b	58.7b	12. 29b
Yavarous	16.97a	5.46b	11.59a	69.7a	13.70a

Means with similar letters in each column are not significantly different at 5% level of probability. (Duncan)

Table 4: Effect of nitrogen application rates on mean nitrogen content of flag leaf at anthesis and maturity stage, N remobilization, N remobilization efficiency and grain protein percent in bread and durum wheat cultivar

N application Rates (kg ha^{-1})	N content of flag leaf at anthesis (mg g^{-1})	N content of flag leaf at maturity (mg g^{-1})	N remobilization (mg g^{-1})	Remobilization N Efficiency (%)	Grain protein (%)
40	15.12b	5.15c	10.45 b	66a	11.93c
80	16.22b	5.88b	10.29c	63.39b	12.95b
160	18.85a	6.92a	12.21a	63.22b	13.48a

Means with similar letters in each column are not significantly different at 5% level of probability. (Duncan)

Table 5: Effect of nitrogen application times on mean nitrogen content of flag leaf at anthesis and maturity stage, N remobilization, N remobilization efficiency and grain protein percent in bread and durum wheat cultivar

N application Time *	N content of flag leaf at anthesis (mg g^{-1})	N content of flag leaf at maturity (mg g^{-1})	N remobilization (mg g^{-1})	Remobilization N efficiency (%)	Grain protein (%)
T1	14.48b	5.19c	9.56c	64.06ab	11.97c
T2	17.38a	5.98b	11.22b	64.67a	12.87b
T3	18.22a	6.78a	12.16a	63.89b	13.63a

Means with similar letters in each column are not significantly different at 5% level of probability. (Duncan)

* T1= All N fertilizer at planting, T2= 1/2 at planting + 1/2 during booting stage and T3= 1/3 at planting + 1/3 during booting stage + 1/3 at heading stage

prior to flowering. However, under condition of high N fertility and available soil moisture, the contribution of post anthesis N uptake may increase substantially [5, 46]. Palta and Filery [4] reported that N remobilization efficiency decreased, when the amount of N increased. N absorption after flowering in Mediterranean climate conditions, similarly climate of Iran, is slow because of drought conditions, which is generally happens after anthesis stage. Other studies found that N absorption reduced 25% during hot and drought summer [47-49]. But when the summer was moderate this amount was about 50% [20, 28, 50]. It seems that environmental differences

lead to different patterns to N remobilization from flag leaves to the grains. In general, when plants exposed to low nitrogen in the soil the mechanism of N remobilization was more efficient.

N content of flag leaf at anthesis and maturity stages was increased by N split application (Table 2). Also N remobilization was increased by N split application, resulted in higher grain protein percentage. However, N remobilization efficiency decreased by N split application (Table 5). It has estimated that two-thirds of the grain N in wheat is derived from N assimilated before anthesis and one-third from assimilation during grain development [51, 52]. However, this may vary greatly depending on soil moisture and N availability during the grain-filling period. Vansanford and Mackown [10] reported that N remobilization efficiency in flag leaf was greater under nitrogen deficiency condition. Radmehr *et al*. [53] also, reported that splitting of N fertilizer raised N remobilization efficiency. Knowles and Watkins [54] found that most of the N taken up by the wheat plants was translocated to the grain either directly or by mobilization from other plant parts. When N was probably abundantly available, no significant difference in remobilization efficiency was observed. One explanation for differences in remobilization efficiency is that during grain filling period, the plant retains an amount of N at anthesis that is essential for survival and various biological functions, while the remainder is available for remobilization. It appears that N retained depends on cultivars and prevailing growth conditions, although genetic variability in nitrogen remobilization has been reported [55-57].

The interaction between cultivars and nitrogen application rates, cultivars and N application times and rates and times of N application were significant in all traits (Table 2). It seems that, durum wheat was more efficient in low N application rates, probably due to genetic characteristic of durum wheat. N remobilization in durum wheat was higher than bread wheat by increasing of N application rate. Lotfaly ayeneh and Radmehr [23] and Le Gouis *et al*. [58] reported that there are significant differences between durum wheat genotypes and N application rates in N remobilization efficiency. The interaction between cultivars and N application time showed that by splitting N application durum wheat had more N remobilization efficiency than bread wheat. Under N deficiency conditions in the soil, the mechanism of N remobilization was more efficient in durum wheat. The results also showed that, N remobilization was increased by splitting N application

in two wheat cultivars. As, durum wheat had better response by splitting N application. The highest N remobilization efficiency was obtained with durum wheat at the rate of 80 kg N application at the first time of N application level.

CONCLUSION

In summary, N content was always greater at anthesis than maturity for the Mediterranean climate conditions of this study. Wheat grown under hot and dry weather condition (Fig 1) in the Mediterranean climatic region of Iran commonly fills their grain under increasing soil water deficits which restrict the uptake of N from the soil. Under these circumstances, most grain N in wheat may be derived from pre-anthesis stored N.

Remobilization efficiency of plant nitrogen to grain differed between cultivars over the range of nitrogen levels used in this study. As, durum wheat was more efficient than beard wheat in nitrogen remobilization efficiency. There was a general trend for a decreased efficiency in flag leaf remobilization with increasing of soil nitrogen in beard and durum wheat cultivars. The minimum level of nitrogen obtained in flag leaf tissue of the durum wheat under the lowest nitrogen level at maturity in the present study enables speculation to be made on the potential for increasing nitrogen transport out of these tissues by breeding for increased remobilization efficiency.

In comparing the nitrogen remobilization efficiency values for this tissue obtained at low soil nitrogen level with the values at the higher nitrogen levels, a large potential appears for increasing remobilization efficiency. Harper at al., [59] reported that decrease in grain N of the high-protein cultivars when flag leaves were removed at anthesis indicated that the importance of flag leaves in mobilizing and translocation vegetative N to the developing grain.

ACKNOWLEDGMENTS

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