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# Nitrogen remobilization in wheat as influenced by nitrogen application and post-anthesis water deficit during grain filling

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Pre-anthesis stored nitrogen in wheat (*Triticum aestivum* L.) is important because grain filling greatly depends on the remobilization of pre-anthesis nitrogen under Mediterranean growth conditions. This field study was conducted to assess the effect of post-anthesis water deficit and three N fertilizer levels on N remobilization and N losses in three wheat cultivars ('Shiraz', 'Marvdasht' and 'Chamran'). Nitrogen remobilization in plant parts decreased to 29 to 58% under water deficit compared with the well watered (WW) treatment. Grain N was 40% higher under post-anthesis water deficit than the WW treatment and with the addition of 160 kg N ha<sup>-1</sup>. The application of nitrogen fertilizer increased N remobilization to 78%. 'Shiraz' remobilized 13 and 25% more nitrogen than 'Marvdasht' and 'Chamran', respectively. Under water deficit, N remobilization efficiency increased by 13%. Leaves were more efficient than stem and spike in N remobilization efficiency either in the WW or the WD treatment. The application of fertilizer N generally lowered whole plant remobilization efficiency. The N remobilization efficiency of 'Chamran' increased when the soil moisture and/or N were limited during the grain filling period. Grain N concentration was correlated positively with N concentration or N content of vegetative parts at anthesis. In addition to nitrogen fertilizer, WD during grain filling reduced nitrogen use efficiency by 30 and 25%, respectively. In the WW treatment, 25% of the N at anthesis was lost at maturity. In contrast, under WD only 6% of the N was lost. High amount of N led to N losses at maturity. Significant negative correlations were found between grain yield and grain protein concentration in the three wheat cultivars. Results indicate that the greater the amount of N accumulated before anthesis, the higher the translocation rates of nitrogen to grain and the greater the risk of net N losses at maturity.

**Key words:** Nitrogen harvest index, nitrogen loss, grain protein, nitrogen use efficiency.

## INTRODUCTION

Nitrogen is the most expensive fertilizer used to raise crop plants (Spiertz, 2010). A reliable portion of the

(applied N is lost through leaching and denitrification Jamieson and Semenov, 2000). Increased demand for N fertilizers also raises farm input costs. Therefore, plant breeders need to develop cultivars that can uptake N more efficiently from the soil and partition most of it into the grain. Such cultivars would minimize loss of N from the soil and make more economic use of the absorbed N.

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**Abbreviations:** N, Nitrogen; NR, nitrogen remobilization; NRE, nitrogen remobilization efficiency; NHI, nitrogen harvest Index; NUE, nitrogen use efficiency; WW, well watered; WD, water deficit; CV, cultivar.

Grain N content in wheat depends on uptake of soil nitrates prior to flowering, continued uptake of soil nitrates during grain filling and remobilization of the stored vegetative N that is accumulated prior to flowering.

Anbessa et al. (2009) 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 post-anthesis N uptake (Fathi, 2005; Cox et al., 1985) and in several studies N uptake during grain filling accounted for as much as 50% of the grain N content at maturity (Rostami and Jiriaei, 1998; Kada et al., 2005). However, under conditions of high N fertility and available soil moisture, the contribution of post-anthesis N uptake may increase substantially (Perez et al., 1983). Several studies have indicated that grain N in wheat primarily originates as a result of translocation from the vegetative parts after anthesis (Hötensteiner and Feller, 2002; Haberle et al., 2008). This translocation is under genetic control (Anbessa et al., 2009), but also depends on environmental conditions (Asseng and Milroy, 2006) and can be affected by fertilizer N application (Perez et al., 1983).

Ercoli et al. (2008) found that dry matter and nitrogen increased up to maturity when fertilizer was not applied. They concluded that nitrogen in the grain was derived primarily by translocation from leaves and stems rather than by uptake from the soil during the period of grain formation. Significant differences for N remobilization after anthesis were reported in a study conducted with 95 F<sub>5</sub> breed wheat lines derived from a single cross (Cox et al., 1985). Knowles and Watkins (1993) found that most of the N that was taken up by wheat plants was translocated to the grain either directly or by mobilization from other plant parts. Since the remobilization of N from vegetative tissues is associated with the process of senescence, a large amount of vegetative N that is re-translocated to the grain could reduce the total amount of N assimilated in the plant because of reduced leaf activity (Kichey et al., 2007) which reduces the total amount of N available to the grain.

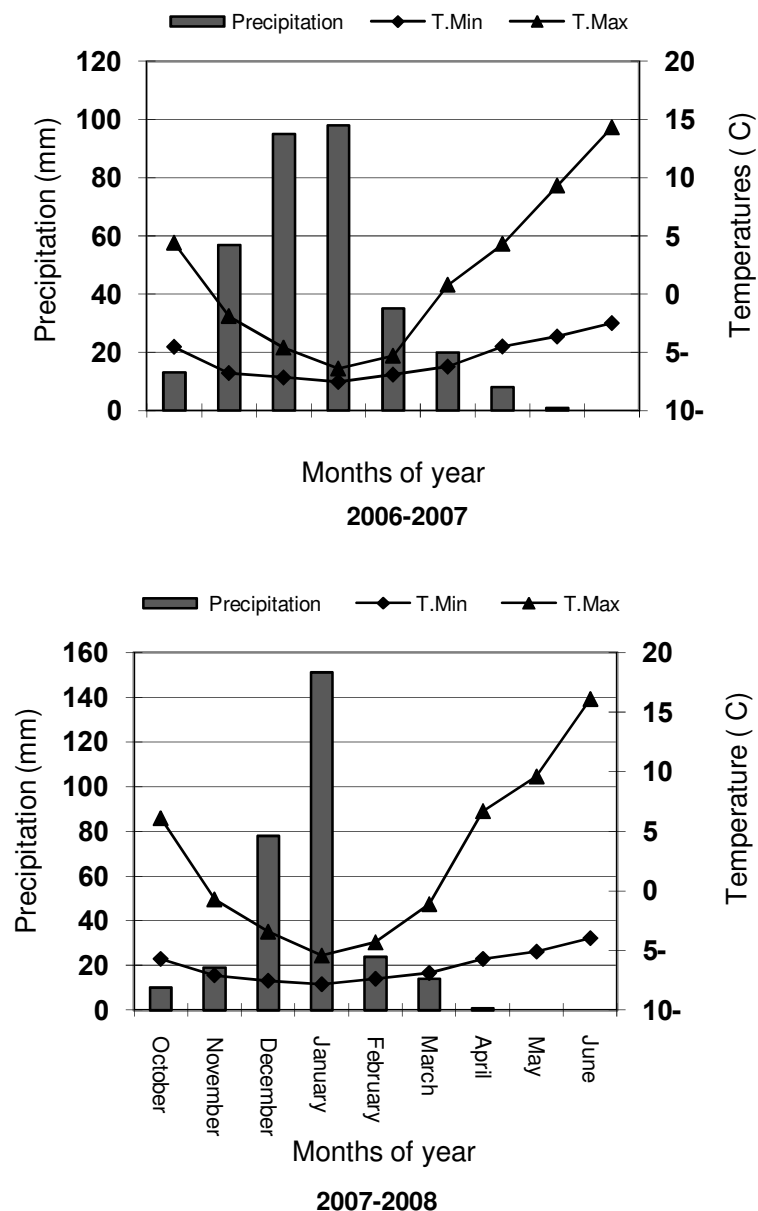
Improving grain yield and grain protein concentration simultaneously is a difficult task because of the negative relationship often found between these two characters (Charmet et al., 2005; Asseng and Milroy, 2006). Furthermore, the process of dry matter (carbohydrate) and protein accumulation may compete for assimilates and energy (Robert et al., 2001). It has been estimated that two-thirds of the grain N in wheat is derived from assimilated N before anthesis and one-third from assimilation during grain development (Barbottin et al., 2005). However, this may vary greatly depending on soil moisture and N availability during the grain filling period. Total N accumulation in cereals is maximized by the time of heading and subsequently decreases through maturity (Martre et al., 2003). One possible avenue for increasing the grain nitrogen yield in wheat is through improving the efficiency of remobilization of nitrogen from 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 (Dordas,

2009; Asseng and Milroy, 2006). Conversely, Gooding et al. (2005) and Robert et al. (2001) have reported that protein concentration in grain might be improved by selecting genotypes that translocate a higher percentage of N from the vegetative organs to the grain. Positive correlations have been observed in wheat between grain protein concentration and nitrogen harvest index (Saint Pierre et al., 2008; Paccaud et al., 1985).

Wheat grown under water deficit conditions in the Mediterranean climatic region of Iran (Figure 1) commonly fill 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. Because of the importance of the protein content and indirectly of the N fertilizer management to grain quality, this experiment was conducted to evaluate the effect of N fertilizer rate on storage, remobilization and losses of N in wheat under water deficit after post anthesis.

## MATERIALS AND METHODS

The study was conducted at Shiraz Agricultural Research Station in Iran (52°36'E, 29°33'N) for two years (2006 to 2008). The experiment was established in a randomized complete block design with a split-split plot arrangement with four replications. Main plots consisted of irrigation treatments, which were WW (well watered) and WD (post anthesis water deficit) (65% FC). Sub-plots consisted of fertilizer treatment, which were N1= 0, N2= 80 and N3= 160 kg ha<sup>-1</sup> of nitrogen (half at planting time and half at booting stage) and sub-sub plots consisted of three cultivars, that is, Shiraz, Marvdasht and Chamran. These cultivars were chosen as representative samples of winter wheat cultivars widely grown in the south of Iran that differs in anthesis dates. To determine the soil characteristics, 15 samples from 30 cm soil depth were collected and analyzed in Shiraz Soil Testing Laboratory for basic soil physical and chemical properties (Table 1). Phosphorus and potassium fertilizers were applied into the soil before sowing according to the recommendations of Soil Testing Laboratory of Shiraz Agricultural Research Station in forms of superphosphate and potassium sulfate, respectively. Plots were sown on 11 November 2007 and on 14 November 2008 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 wheat harvest in July and disked again before seeding in November. Irrigation of each main plot was measured volumetrically by field calibrated gypsum blocks. The gypsum blocks used in the experiment are made by Delmhorst Instrument Co. The blocks were cylindrical with a diameter of 25 mm and a length of 35 mm. The soil water potential at 30 cm depth was kept at -0.025 MPa (WW) and -0.1 MPa (WD) in the respective plots from anthesis to maturity. Six gypsum blocks were installed in each replication randomly. Rain fall during the post-anthesis water deficit was not occurred. Change in soil moisture was measured weekly to a depth of 30 cm. The irrigation system was operated avoiding runoff losses. Apirus herbicides were applied in early April to the crops to control both broad and narrow leaved weeds. Apirus is member of sulfonylurea (Su) herbicides which controls weeds through inhibition of the acetolactase synthase enzyme. The three most common active ingredients are chlorsulfuron, metsulfuron-methyl and triasulfuron. The four central rows (of 6 rows) of each plot were harvested at 14% grain moisture for grain yields (kg ha<sup>-1</sup>). Twenty main stems that headed on the same day were tagged for each treatment. As the tagged main stems of each cultivar reached anthesis, 10 plants



**Figure 1.** Monthly values of maximum and minimum temperatures and precipitation from planting until the end of June for two seasons.

in each plot were removed and main stems were divided into spike, flag leaf blade, lower leaves and stem. At maturity, 10 additional tagged plants were removed and the main stems were subdivided in the same way as at anthesis. Anthesis was scored when the anthers in the central florets of 50% of the spikes in each plant had dehisced and maturity when almost all the spikes in each plot showed complete loss of the green color. Anthesis date of cultivars differed by about 10 days in both years. Samples were dried to constant weight at 70°C, weighed and ground to pass a 0.4 mm mesh. 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 AACC (1971).

The various parameters related to N movement within the plant parts were as follow (Cox et al., 1986):

$NR$  ( $\text{mg plant}^{-1}$ ) = N content at anthesis – N content at maturity.

$NRE$  (%) = (N remobilization/N content at anthesis)  $\times$  100.

Nitrogen lost or gained ( $\text{kg ha}^{-1}$ ) = N content at maturity - N content at anthesis.

Nitrogen at anthesis lost or gained (%) = (N lost or gained/N content at anthesis)  $\times$  100.

(NHI) = Grain N/total N content of aboveground parts at maturity.

(NUE) ( $\text{Kg kg}^{-1}$ ) = Grain yield/nitrogen fertilizer application.

Data were analyzed by analyses of variance using the general

**Table 1.** Soil properties of the experimental plots.

Variable Year	Texture	PH	Ec dS m <sup>-1</sup>	N (%)	Organic matter (%)	P mg kg <sup>-1</sup>	K mg kg <sup>-1</sup>	Fe mg kg <sup>-1</sup>	Zn mg kg <sup>-1</sup>	Mn mg kg <sup>-1</sup>	Cu mg kg <sup>-1</sup>
2006-2007	Si-C	7.96	1.88	0.09	1.07	5.4	340	3.7	0.64	5.8	0.48
2007-2008	S-C-L	7.85	1.25	0.08	1.03	16	206	5.4	0.1	9.9	0.98

**Table 2.** Results of analysis of variance combined across years, irrigation, fertilizer and wheat cultivars to both years.

Source	Anthesis	Maturity	NR † of spike mg plant <sup>-1</sup>	NR of flag leaf mg plant <sup>-1</sup>	NR of stem mg plant <sup>-1</sup>	NR of lower leaves mg plant <sup>-1</sup>	Total NR mg plant <sup>-1</sup>	NRE ‡ of spike (%)	NRE of flag leaf (%)	NRE of stem (%)	NRE of lower leaves (%)	Total NRE (%)	NHI §	NUE ¶ Kg kg <sup>-1</sup>	Grain protein percent (%)
	Total N content mg plant <sup>-1</sup>	Total N content mg plant <sup>-1</sup>													
Year (Y)	**	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	*	*	*	*
Irrigation (I)	NS	*	NS	NS	*	NS	NS	NS	NS	*	NS	*	*	*	**
Y×I	NS	*	NS	NS	*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Nitrogen (N)	**	*	*	*	*	NS	*	NS	*	*	NS	*	*	*	**
Y×N	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
I×N	*	NS	NS	NS	*	NS	*	NS	NS	NS	NS	*	*	*	*
Y×I×N	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	*
Cultivar (C)	*	*	*	*	*	NS	*	NS	*	*	NS	*	*	*	*
Y×C	*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
I×C	*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	*	*
N×C	*	*	NS	NS	*	*	*	NS	NS	NS	NS	*	*	NS	*
Y×I×C	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	*
Y×N×C	*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	*	*
I×N×C	*	NS	NS	NS	*	NS	*	NS	NS	NS	NS	*	NS	NS	*
Y×I×N×C	*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	*
CV, %	18.5	19.2	16.2	21.3	16	19.6	18.9	14.6	19.2	12.1	15.6	16.5	9.8	10.1	6.6

†: Nitrogen remobilization; ‡: nitrogen remobilization efficiency; §: nitrogen harvest index; ¶: nitrogen use efficiency. \*, \*\* Significant at 0.05 and 0.01 probability levels, respectively. NS= non-significant at P > 0.05.

linear model (GLM) procedure provided by the statistical analysis system (SAS 1982). Combined analysis of variance between years was computed with replication considered random, whereas irrigation, N fertilizer and cultivars were considered fixed. When significant differences were found ( $P=0.05$ ), the Duncan's multiple range test (DMRT) were tested.

## RESULTS AND DISCUSSION

### Nitrogen content and remobilization

The three cultivars differed in their ability to remobilize N from the vegetative parts in both

years (Tables 2 and 3). 'Shiraz' remobilized 13 and 25% more nitrogen than 'Marvdasht' and 'Chamran', respectively (Table 3). Nitrogen remobilization in all plant parts decreased to 29 to 58% under water deficit compared with the WW treatment (Table 3). Remobilization within the

**Table 3.** Total N content at anthesis and maturity, N remobilization and its efficiency of spike, flag leaf, stem, lower leaves, total N remobilization and its efficiency, nitrogen harvest index (NHI), nitrogen use efficiency (NUE) and grain protein percent of three wheat cultivars under three N and two water regimes in 2006 to 2008.

Treatment	Anthesis	Maturity	NR † of spike mg plant <sup>-1</sup>	NR of flag leaf mg plant <sup>-1</sup>	NR of stem mg plant <sup>-1</sup>	NR of lower leaves mg plant <sup>-1</sup>	Total NR mg plant <sup>-1</sup>	NRE ‡ of spike (%)
	Total N content mg plant <sup>-1</sup>	Total N content mg plant <sup>-1</sup>						
<b>Irrigation *</b>								
WW	35	25	1.5	2.7	4.7	2.1	10	75
WD	34	28	1.1	1.7	3.5	0.9	7	77
LSD (0.05)	ns	2.1	ns	ns	0.8	ns	1.0	Ns
<b>Nitrogen(N) Kg ha<sup>-1</sup></b>								
0	18	10	1.9	1.6	3.1	1.4	8	72
80	36	16	3.5	6.2	7.1	4.2	20	70
160	39	14	4.6	7.7	8.4	5.3	25	69
LSD (0.05)	2.9	0.9	0.4	0.6	0.5	ns	1.7	Ns
<b>Cultivar(C)</b>								
Shiraz	37	19	2.5	5.2	6.1	3.2	18	74
Marvdasht	32	17	1.7	4.4	5.3	2.4	15	73
Chamran	21	9	0.9	3.6	4.5	1.6	12	68
LSD (0.05)	3.1	1.0	0.2	0.4	0.5	ns	1.4	Ns
Treatments	NRE of flag leaf (%)	NRE of stem (%)	NRE of lower leaves (%)	Total NRE (%)	NHI §	NUE ¶ Kg kg <sup>-1</sup>	Grain protein (%)	
<b>Irrigation(I)</b>								
WW	75	65	72	70	0.79	55	10.1	
WD	77	78	74	79	0.64	42	11.4	
LSD (0.05)	ns	8.2	ns	8.6	0.03	6	0.9	
<b>Nitrogen(N) Kg ha<sup>-1</sup></b>								
0	79	72	74	76	0.78	58	9.6	
80	70	67	72	71	0.74	50	10.3	
160	67	61	71	66	0.66	41	11	
LSD (0.05)	9.1	7.8	ns	8	0.04	6.1	0.8	
<b>Cultivar(C)</b>								
Shiraz	81	67	65	67	0.80	40	11.3	
Marvdasht	79	65	70	70	0.77	51	11.2	
Chamran	70	73	77	76	0.68	55	10.1	
LSD (0.05)	10	8	7.9	7.9	0.04	6	0.9	

\*:WW= well watered treatment; WD= post anthesis water deficit; †: nitrogen remobilization; ‡: nitrogen remobilization efficiency; §: nitrogen harvest index; ¶: nitrogen use efficiency.

main stem under post-anthesis water deficit itself provided most of the pre-anthesis N that was accumulated in the grain (Table 3). Plants received 160 kg N ha<sup>-1</sup> remobilized 78% more nitrogen than the plants received 0-N, resulting in grain protein (Table 3). Remobilized N from the flag leaf could have accounted for 19, 15 and 12% of the N accumulated by the developing grain of 'Shiraz', 'Marvdasht' and 'Chamran',

respectively (Table 3). At zero nitrogen level, nitrogen levels of total leaves for the three cultivars were generally higher than that of the Culm (Table 3). Fan et al. (2005) reported that decrease in grain N of the high-protein cultivars when flag leaves were removed at anthesis indicated the importance of flag leaves in the mobilization and translocation of the vegetative N to the developing grain. Remobilization of nitrogen from culms to the grain

**Table 4.** Total N content at anthesis, N remobilization of stem, total N remobilization and its efficiency, nitrogen harvest index (NHI), nitrogen use efficiency (NUE) and grain protein percent as affected by irrigation and N fertilizer treatments in 2006 to 2008.

Treatment	Irrigation* Nitrogen Kg ha <sup>-1</sup>	Anthesis	NR† of stem mg plant <sup>-1</sup>	Total NR mg plant <sup>-1</sup>	Total NRE (%)	NHI §	NUE ¶ Kg kg <sup>-1</sup>	Grain protein (%)
		Total N content mg plant <sup>-1</sup>						
WW	0	27	3.9	9	73	0.79	57	9.8
	80	36	5.9	15	71	0.77	53	10.2
	160	37	6.5	18	68	0.73	48	10.5
WD	0	26	3.3	8	78	0.71	50	10.5
	80	35	5.3	14	75	0.69	46	10.8
	160	36	5.9	16	72	0.65	42	11.2
LSD(0.05) ¥		3	0.6	1.6	8	0.03	5.9	1.0

\*: WW= well watered treatment; WD= post anthesis water deficit; †: nitrogen remobilization; §: nitrogen harvest index; ¶: nitrogen use efficiency; ¥: between nitrogen for the same I treatment.

was essentially the same in 'Shiraz' and 'Marvdasht'. The N content at anthesis in spike, flag leaf, stem and lower leaves was higher in the N fertilized plants (Table 3). Nitrogen in 'Shiraz' and 'Marvdasht' was substantially higher in the flag leaf and the lower leaves than 'Chamran' at 80 and 160 kg N ha<sup>-1</sup> levels. 'Chamran' retained less N in its vegetative parts at maturity than 'Shiraz', although, only the leaf plus Culm component differed significantly (Table 3). Nitrogen remobilization of spike for the cultivars was generally much lower than that for total culm nitrogen at the same nitrogen level (Table 3). In post-anthesis of WD along with 160 kg N ha<sup>-1</sup>, the grain N arising from pre-anthesis storage was 40% higher than the WW treatment (Table 4), whereas post-anthesis uptake of N from the soil was higher in the WW treatment. Increased N remobilization due to N fertilization was also reported by and Cox et al. (1986). Increased nitrogen remobilization caused by nitrogen application was greatest for 'Shiraz' and least for 'Chamran' (Table 5). 'Shiraz' showed 10% increase in N remobilized in response to the highest rate of N fertilization (Table 5). The three cultivars under the WD treatment had an increase in nitrogen remobilization to the grains under the 160 kg N ha<sup>-1</sup> (Table 6). No significant difference between cv. Shiraz and cv. Marvdasht for N concentration in vegetative components at maturity was found, although, cv. Chamran was lower in the low N treatments (Table 5). Cox et al. (1986) found that 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. The positive relationship between rate of fertilizer N and the amount of residual straw N at maturity is consistent with the results of others (Gooding et al., 2007; Ehdai and Waines, 2001).

### Nitrogen remobilization efficiency

Under water deficit, N remobilization efficiency increased by 13% (Table 3). Leaves, especially the flag leaves, were more efficient than stem and spike in N remobilization either in the WW or the WD conditions. The application of fertilizer N generally lowered remobilization efficiency of the whole plant where N was available during grain filling (Table 3). Krček et al. (2008) reported in wheat that N remobilization efficiency to the grain decreased by increasing availability of soil N. With the WD treatment, N remobilization efficiency increased with 80 kg ha<sup>-1</sup> N rate, but further increase of N decreased N remobilization efficiency (Table 4). The N remobilization efficiency of 'Chamran' increased when the soil moisture and/or N were limited during the grain filling period. When N and soil moisture were abundant, no significant differences in remobilization efficiency were observed between 'Shiraz' and 'Marvdasht'. Differences among cultivars in mean remobilization efficiency were observed, ranging from 76% for 'Chamran' to 67% for 'Shiraz'. 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 retention depends on cultivars and the prevailing growth conditions, although, genetic variability in nitrogen remobilization has been reported (Kirda et al., 2001).

### Grain N yield and grain N concentration

Grain N yield and total N content at maturity increased with the addition of N fertilizer by 15 and 40%,

**Table 5.** Total N content at anthesis and maturity, N remobilization of stem and lower leaves, Total N remobilization and its efficiency, nitrogen harvest index (NHI) and grain protein percent of three wheat cultivars as affected by N fertilizer.

Treatment		Anthesis	Maturity	NR† of stem mg plant <sup>-1</sup>	NR of lower leaves mg plant <sup>-1</sup>	Total NR mg plant <sup>-1</sup>	Total NRE ‡ (%)	NHI §	Grain protein (%)		
		Total N content mg plant <sup>-1</sup>	Total N content mg plant <sup>-1</sup>								
Nitrogen Kg ha <sup>-1</sup>	Cultivar										
		0	Shiraz	28	15	4.6	2.3	13	72	0.79	10.5
			Marvdasht	25	14	4.2	1.9	12	73	0.78	10.4
	Chamran	20	10	3.8	1.5	10	76	0.73	9.9		
80	Shiraz	37	18	6.6	3.7	19	69	0.77	10.8		
	Marvdasht	34	17	6.2	3.3	18	71	0.76	10.8		
	Chamran	29	13	5.8	2.9	16	74	0.71	10.2		
160	Shiraz	38	17	7.3	4.3	22	67	0.73	11.2		
	Marvdasht	36	16	6.9	3.9	20	68	0.72	11.1		
	Chamran	30	12	6.5	3.5	19	71	0.67	10.6		
LSD (0.05) ¥		2.9	1.4	0.9	0.4	1.1	9	0.04	0.9		

†: Nitrogen remobilization; ‡: nitrogen remobilization efficiency; §: nitrogen harvest index; ¥: between nitrogen for the same I treatment.

**Table 6.** Total N content at anthesis, N remobilization in stem, total N remobilization and its efficiency and grain protein percent of three wheat cultivars as affected by N fertilizer and irrigation treatments.

Treatment			Anthesis	NR† of stem mg plant <sup>-1</sup>	Total NR mg plant <sup>-1</sup>	Total NRE ‡ (%)	Grain protein (%)		
		Total N content mg plant <sup>-1</sup>							
Irrigation*	Nitrogen Kg ha <sup>-1</sup>	Cultivar							
			0	Shiraz	32	4.6	12	71	10.3
				Marvdasht	28	4.4	11	72	10.3
		Chamran	25	4.1	10	74	9.9		
WW	80	Shiraz	38	6	16	69	10.5		
		Marvdasht	34	5.7	15	70	10.5		
		Chamran	31	5.4	14	72	10.2		
	160	Shiraz	39	6.4	18	68	10.8		
		Marvdasht	35	6.1	17	69	10.9		
		Chamran	32	5.9	16	71	10.5		
WD	0	Shiraz	32	4.2	11	74	10.8		
		Marvdasht	28	4	10	75	10.7		
		Chamran	25	3.7	9	77	10.4		
	80	Shiraz	38	5.6	15	72	11		
		Marvdasht	34	5.3	14	73	11		
		Chamran	31	5	13	75	10.6		
	160	Shiraz	39	6	17	71	11.2		
		Marvdasht	35	5.7	16	72	11.2		
		Chamran	32	5.5	15	74	10.8		
LSD(0.05) ¥			3.1	0.8	1.3	8.4	1.1		

\*: WW= well watered treatment and WD= post anthesis water deficit; †: nitrogen remobilization; ‡: nitrogen remobilization efficiency; ¥: between nitrogen for the same I treatment.

**Table 7.** Simple correlation coefficients between various N related parameters for the three wheat cultivars in 2006 to 2008.

Parameter	Cultivar			
	Shiraz	Marvdasht	Camran	Combined
	Correlation coefficient			
Grain N concentration vs.				
N concentration at anthesis	0.76**	0.83**	0.96**	0.80**
N content at anthesis	0.90**	0.87**	0.75**	0.79**
N- remobilization efficiency	0.48*	0.40*	0.86*	0.53*
Grain N yield vs.				
N content at anthesis	0.89**	0.89**	0.75**	0.77**
N remobilized	0.88**	0.85**	0.39*	0.65**
N remobilization efficiency	0.29*	0.38*	0.55*	0.36*
NHH † vs. N content at anthesis	0.57*	-0.40*	-0.42*	-0.50*

\*, \*\* Significant at 0.05 and 0.01 probability levels, respectively; †: nitrogen harvest index.

respectively (Table 3). Simple correlation coefficients between N related parameters are shown in Table 7. Grain N concentration was correlated positively with N concentration or N content of the vegetative parts at anthesis in the three wheat cultivars. Correlation coefficients of grain N concentration versus N-remobilization efficiency were lower for 'Shiraz' and 'Marvdasht' than 'Chamran'. Grain N yield was correlated with N content at anthesis and remobilized N in 'Shiraz' and 'Marvdasht', while no correlation was observed in 'Chamran'. Correlations of grain N yield with N remobilization efficiencies were lower and variable. Low correlations of N assimilation prior to anthesis and grain protein concentration or grain protein yield were reported by Charmet et al. (2005). There were significant differences in 2006 to 2007 and in 2007 to 2008 in grain protein percent. Highest grain protein was obtained in the WW treatment with 160 kg ha<sup>-1</sup> in 2006 to 2007.

### Nitrogen harvest index

Nitrogen treatment and cultivar differences for NHI were observed (Table 3). The NHI was significantly affected by N application in 'Shiraz' and 'Marvdasht' (Table 5). The reduction in NHI with the use of higher rates of N fertilizer is consistent with observations in wheat (Sepaskhah and Hosseini, 2008). Hötensteiner and Feller (2002) concluded from the data of other studies that this index was consistent for rice, maize and wheat over a wide range on N regimes. The correlation coefficient of NHI versus N content at anthesis was significant only for 'Shiraz' and 'Marvdasht' (Table 7). In both years, 'Shiraz' and 'Marvdasht' showed the highest NHI. These are high protein cultivars and therefore, had greater N-remobilization.

### Nitrogen use efficiency

Analysis of NUE is presented in Table 2. Stress during grain filling reduced NUE by 30% due to reducing nitrogen uptake and N remobilization (Table 3). NUE decreased with the addition of N fertilizer by 25% in 160 kg N ha<sup>-1</sup> treatment. 'Shiraz', which had the lowest N remobilization efficiency, had also the lowest NUE (15 to 25%) (Table 3).

### Nitrogen losses between anthesis and maturity

A major finding of this study was that in the post-anthesis, water deficits affected the apparent volatilization and leaching losses of N by wheat. With the WW treatment, 25% of the N in the plant at anthesis was lost at maturity. In contrast, with WD treatment only 6% of the N was lost. The loss of N from foliage during the post anthesis period has been documented previously (Haberle et al., 2006; Egle et al., 2008). Losses by volatilization and leaching of mobile nitrogenous compounds appear to be related to the duration of green leaf area. Palta et al. (1994) concluded that more rapid stomatal closure and senescence with post-anthesis water deficits resulted in smaller losses of N in this period. The reduced uptake, the reduced losses and the increased demand for pre-anthesis stored N in the post-anthesis period at WD compared with the WW treatments resulted in the total grain N not being affected. Nitrogen losses from the wheat plants increased as the amount of N was increased. N loss from anthesis to maturity ranging from 20 to 80 kg ha<sup>-1</sup> where no N and 160 kg of N ha<sup>-1</sup> were applied respectively. According to Martre et al. (2003), N losses over 40 kg ha<sup>-1</sup> are sufficient to be of practical significance. Nitrogen content change showed a loss of



**Table 8.** Correlation coefficients for grain protein concentration with N remobilization, N remobilization efficiency and grain N yield in 2006 to 2008.

Parameter	Nitrogen Kg ha <sup>-1</sup>			Combined
	0	80	160	
	Correlation coefficient			
Grain protein concentration vs.				
N remobilization	0.09 <sup>ns</sup>	0.19 <sup>ns</sup>	0.32 <sup>*</sup>	0.20 <sup>ns</sup>
N remobilization efficiency	0.90 <sup>**</sup>	0.83 <sup>**</sup>	0.86 <sup>**</sup>	0.50 <sup>**</sup>
NHI †	0.48 <sup>*</sup>	0.40 <sup>*</sup>	0.36 <sup>*</sup>	0.41 <sup>*</sup>
Grain yield	-0.29 <sup>*</sup>	-0.37 <sup>*</sup>	-0.40 <sup>*</sup>	-0.35 <sup>*</sup>

<sup>\*</sup>, <sup>\*\*</sup> Significant at 0.05 and 0.01 probability levels, respectively; NS= non-significant at P > 0.05; †: nitrogen harvest index.

42 kg of N ha<sup>-1</sup> from the leaves and stems while the head gained 18 kg ha<sup>-1</sup> representing a net loss of 24 kg ha<sup>-1</sup> or 18%. Of the total loss of 42 kg, 73% was associated with stems and 27% with leaves. It therefore apparent that N loss was primarily associated with stems. It is probable that the N decline in plant tops reflects a lack of carbohydrates, a situation which delays conversion of NH<sub>3</sub> to the amines or a limited capacity of inflorescences to store N (Da Silva and Stutte, 1981). Another factor that may contribute to the loss of high amounts of N from vegetative parts is the combination of high temperature and low air humidity which prevails under Mediterranean climate from the grain filling period to physiological maturity (Figure 1). Master (2002) observed that the evolution of NH<sub>3</sub> from plants was greater under condition of high evapotranspiration rates and Spiertz et al. (2006) reported that the decline of N concentrations was accelerated by high air temperatures.

### Relation between grain protein and grain yield

An inverse relationship between grain yield and grain protein concentration is discussed widely in the literature and is frequently confirmed in studies in grain yield and protein (Cox et al., 1985; Charmet et al., 2005; Asseng and Milroy, 2006). This negative correlation probably arises from higher energy costs associated with protein synthesis when compared with carbohydrate synthesis (Robert et al., 2001). This relationship does not always hold, depending on soil fertility, water availability and other environmental factors (Triboi et al., 2006). In this study, significant negative correlations existed between grain yield and grain protein concentration in three wheat cultivars (Table 8). However, these relationships were only low to moderate ( $r = -0.29, -0.37$  and  $-0.40$ ) for 0, 80 and 160 kg N ha<sup>-1</sup>, respectively. Grain protein concentration in these cultivars showed no correlation with the N remobilization, little with NHI, high with remobilization efficiency and negative with the grain yield (Table 8). These results are in agreement with studies by Asseng and Milroy (2006), Kade et al. (2005) and Cox et

al. (1986), but differ from the results of Paccaud et al. (1985).

### Conclusion

In summary, N content of wheat is always higher at anthesis than maturity for the Mediterranean climate conditions. Hot and dry weather during the post anthesis period (Figure 1) was unfavorable to high nitrogen uptake from the soil and thus, nitrogen was determined to a great extent by remobilization to the grain. The post anthesis water deficit clearly affected the utilization and remobilization of pre-anthesis stored N. Despite the reduction in post-anthesis uptake of N, grain N accumulation was not affected by WD, because N remobilization efficiency to the grain was increased. The major effect on N remobilization arises from the high crop content on N and not on the greater water deficits at high levels of applied N. The minimum levels of nitrogen obtained in flag leaf, lower leaf, spike and stem tissues of the 'Chamran' under low nitrogen level at maturity enables speculation to be made on the potential for increasing nitrogen transport by breeding for increased remobilization efficiency. In comparing the nitrogen percentage obtained at low soil nitrogen level with the percentage of high nitrogen levels, a large potential appears for increasing remobilization efficiency.

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