Nitrogen Source and Deficit Irrigation Influence on Yield and Nitrogen Translocation of Triticale in an Arid Mediterranean Agroecosystem

V. Barati 1*, E. Bijanzadeh 1, and Z. Zinati 1

ABSTRACT

To identify the important features of triticale that contribute to improving grain and biomass Water Use Efficiency (WUEg and WUEb, respectively), grain yield, and Nitrogen (N) remobilization, a 2-year side-by-side experiment was carried out on triticale with different nitrogen sources and water regimes, in a typical Mediterranean environment of Iran. There were two levels of water regimes: Normal Irrigation (IRN) and irrigation cut off after anthesis stage (IRMD). Rain-fed treatment (IR0) was included in the second year. Four N sources including Azospirillum brasilense (Bio), Azospirillum brasilense +75 kg N ha⁻¹ as urea (Bio+N75), 150 kg N ha⁻¹ as urea (N150), and control unfertilized (N0) plots were used. This study showed that the highest grain yield (6,258 kg ha⁻¹) was achieved by chemical N fertilizer application (N150) under IRN. In contrast, the application of Bio+N75 resulted in the highest grain yield as compared with the other N sources under IRMD (4,409 kg ha⁻¹) and IR0 (2,960 kg ha⁻¹) conditions. Water stress significantly increased WUEb at all N sources. However, WUEg slightly increased by IRMD and then sharply decreased by IR0 in all N sources, except N150 plots, where WUEg drastically decreased by water stress imposed by IRMD and IR0. The Bio+N75 treatment had the highest N remobilization. Although N remobilization was not affected by IRMD in dryer year, it increased by IRMD (8.4%) in the relatively wet year. Totally, for a more sustainable farming system in arid Mediterranean conditions, integration of biofertilizer and chemical N fertilizer could be successfully used for increasing grain yield, WUE, and N remobilization of triticale, especially under deficit irrigation regimes.

Keywords: Grain yield, Nitrogen remobilization, Water Use Efficiency, X. Triticosecale Wittmack.

INTRODUCTION

Drought is a serious problem for agriculture and reduces crop productivity, particularly in arid and semi-arid areas of the world (Arseniuk, 2015). Triticale (X. Triticosecale Wittmack) usually does better than wheat under normal and a variety of drought stress conditions, although considerable variability is observed among the crop genotypes (Roohi et al., 2013; Fayaz and Arzani, 2011; Lonbanli and Arzani, 2011). For sustainable water use, considering the drought resistance of triticale crop, substantial efforts have been made to promote triticale cultivation as an alternative cereal in southern Iran, with a typical Mediterranean climate.

Generally, water deficit from double ridge to anthesis and around anthesis of cereals causes yield losses due to reductions in potential grain number per spike (Fischer, 1985; Cossani et al., 2009), while water stress and high temperatures during grain filling period, as it particularly occurs in Mediterranean regions, reduce mean kernel weight (Oweis et al., 2000).

The main concern in arid and semi-arid environments is water availability and its efficient use (Tavakkoli and Oweis, 2004). Exposing crops to a certain level of drought...
stress by withholding irrigation or reducing the amount of irrigation water either during a particular period or throughout the growing season, i.e. deficit irrigation regime, has been employed to maximize water use efficiency and achieve higher yields per unit of irrigation water in different crops (Afshar et al., 2014; Jahanzad et al., 2013). Zhang et al. (2006) reported that appropriate degree of regulated deficit irrigation could result in 33–40% higher WUE as compared to the full irrigation condition in spring wheat in an arid environment.

The N deficiency is one of the major constraints in actual farming of arid Mediterranean environment (Campbell et al., 1993; Barati and Ghadiri, 2017). Therefore, supplemental chemical N fertilizers are most widely used; however, questions have been raised about the long-term sustainability of such systems because rate of release of N in soils often does not match crop demand with fertilizer applications. Moreover, highly concentrated inorganic N inputs can have detrimental environmental impacts (Campbell et al., 1995).

In the recent years, application of natural and biological fertilizers has drawn researchers' attention due to their successful performance in crop production and their less ecological footprint compared to chemical fertilizers (Dadrasan et al., 2015). A large group of soil inhabiting microorganisms known as Plant Growth Promoting Rhizobacteria (PGPR) is able to fix atmospheric N\textsubscript{2} to a usable form for plants (Vessey, 2003). Azospirillum, a well-known free-living aerobic bacterium, which can be found in a wide range of habitats associated with various types of plants, is a member of PGPRs that can efficiently convert atmospheric N\textsubscript{2} to usable forms for plants (De Freitas, 2000; Kizilkaya, 2008). Apart from fixing atmospheric N\textsubscript{2} (Creus et al., 2010), Azospirillum can provide hormone like substances, including auxins, gibberellins and cytokinins (Creus et al., 2010; Vande Broek et al., 1999) and, consequently, stimulate both rates of root elongation and appearance of crown roots (Fallik et al., 1994). Higher root performance might increase nutrient uptake from soils, thus reducing the need for fertilizers and preventing water contamination with nitrate in agricultural areas. Furthermore, in some studies, it has been shown that the application of PGPR as seed inoculants alleviated the deleterious effects of drought stress on plant growth (Creus et al., 2004; Arshad et al., 2008).

Matching N fertilization with crop water availability is essential for achieving acceptable grain yield (Latiri-Souki et al., 1998). It is largely demonstrated that the early developmental processes in cereal life cycle, such as tiller proliferation occurs during early growth sages, depend on the availability of water and N (Simane et al., 1993) (Garcia del Moral et al., 1991). Furthermore, combined water and N restrictions around anthesis stage are known to induce floret abortion resulting in a reduced kernel number per unit land area (Jeuffroy and Bouchard, 1999; Acevedo et al., 2002). Therefore, understanding of water availability\times N interaction effects, especially when slow release N sources such as bio-fertilizers are applied, is of crucial importance for stabilizing cereal production in the Mediterranean regions. Indeed, Finding the water and N fertilizing management options to fit crop requirements in areas with low water availability are necessary for sustainable use of water and N.

Mild water deficiency enhances the remobilization of pre-stored carbon and N reserves to grain and partially compensates the reduced current assimilation (Plaut et al., 2004; Barati and Ghadiri, 2017). It seems that stimulation of dry matter and N for transfer to grain by mild water stress at the later grain-filling stage, mainly related to promote whole-plant senescence (Yang et al., 2000). Heavy use of chemical N fertilizers and irrigation water leads to delayed senescence and causes crops to stay green when the grains mature, thus, it shows a low HI and NHI with much Non-Structural Carbohydrate (NSC) and N left in the straw (Bijanzadeh and Emam, 2012; Barati and Ghadiri, 2017). With respect to the negative effects of heavy use of chemical N fertilizers on pre-stored carbon and N remobilization and, consequently, on HI and NHI, studying of bio-fertilizers such as Azospirillum bacteria as a slow release N fertilizer instead of chemical N fertilizers may be an approach for increasing HI and NHI. Furthermore, as discussed above, Azospirillum bacteria may alleviate the deleterious effects of severe drought stress on plant growth and stimulate the contribution of pre anthesis reserved carbon and N to grain.
Effect of Nitrogen Source and Deficit Irrigation

Although many studies concerning water and chemical N on cereals have been performed, triticale grown under various water and N sources including bio fertilizer have not been fully investigated. The main objective of this study was to evaluate the effect of deficit irrigation and N sources (chemical and bio fertilizer) on WUE, grain yield, N remobilization and NHI (Nitrogen Harvest Index).

MATERIALS AND METHODS

Site Description

The experiments were carried out at the experimental farm of Darab Agriculture and Natural Resources College of Shiraz University located in southern Iran (29° 46′ N, 52° 43′ E, and altitude 1,603 m) during two consecutive winter triticale growing seasons in 2015–2016 and 2016–2017, referred hereafter as 2016 and 2017, respectively. Darab Area with typically arid Mediterranean climate is characterized by long-term mean annual rainfall of 257.5 mm mostly concentrated in fall and winter seasons. Furthermore, its maximum summer air temperature is 46.5°C. Climatic data were from an agro-meteorological station near the experimental site, as given in Figure 1. The soil was loam and contained 1.86% total organic matter, 0.11% total N, 20 mg kg⁻¹ available Phosphorus (P), and 340 mg kg⁻¹ available potassium (K).

Experimental Design

The experiments were laid out in a split plot pattern based on a randomized complete block design with two levels of irrigation in 2016 and three levels of irrigation in 2017 and three types of N fertilizer management with three replications. Main plots were allocated to irrigation regimes comprised of Normal Irrigation (IRₐ) and irrigation cut off after anthesis stage as a Mild Deficit Irrigation (IRₐₘₐₖ) in 2016. In 2017, rainfed treatment (IR₀) was included due to more precipitation forecasting. Sub plots were assigned to N diversity nutrition comprised of control [no N fertilizer, (N₀)], sole chemical N fertilizer [150 kg N ha⁻¹ was used as recommended N fertilizer, (N₁₅₀)], sole biological fertilizer [seed inoculated with Azospirillum brasiliense, (Bio)], and combined fertilizer [50% chemical N fertilizer (75 kg N ha⁻¹)+seed inoculated by Azospirillum brasiliense, (Bio+N₇₅)].

Agronomy

In both growing seasons, a winter triticale cultivar (Sanabad) was chosen based on the several years of experience and its well adaptation to Mediterranean climate in southern Iran. Uniform triticale seeds were hand sown at a soil depth of approximately 2 cm in rows 20 cm apart giving 250 plants m⁻² (Bijanazadeh et al., 2019) in plots of 3x5 m in both growing seasons. Adjacent plots were 1 m apart in each replication and a border of 5 m was established between the
replicates for minimizing water and N lateral movement. Dikes were established around the plots for furrow irrigation.

*Azospirillum brasilense* was provided by the Soil and Water Research Institute, Tehran, Iran. Seeds of triticale were surface sterilized in 3% NaOCl for 2 minutes and washed twice with sterile distilled water. Seeds were inoculated as previously described by Creus *et al.* (1996) and air dried in a laminar flow cabinet to 14% humidity, and stored for 2 day at 15°C in the dark until sown. Nitrogen fertilizer was applied as urea (46% N) to each plot at three splits; i.e., one-third applied at tillering stage (ZGS21) (Zadoks *et al.*, 1974), again one-third at the beginning of stem elongation stage (ZGS31) and the rest at the ear emergence stage (ZGS57) in all irrigation treatments and rainfed as well.

**Irrigation**

In both growing seasons, the plots with IR$_N$ irrigation regimes were irrigated normally until plants reached the physiological maturity stage (ZGS95) (Zadoks *et al.*, 1974). In contrast, the plots with IR$_{MD}$ regime were irrigated normally only until plants reached full anthesis stage (ZGS69) (Zadoks *et al.*, 1974).

Before each irrigation event, the soil profile was sampled to the 90 cm depth in 30 cm increments using a post-hole auger from four spots at the center of each plot in three replicates. The volumetric water content of the soil layers was measured using the gravimetric method and the depth of irrigation was calculated using Equation (1):

$$ D = \sum_{i=1}^{n}(\theta_{eci} - \theta_i) \Delta Z_i $$  \hspace{1cm} (1)

Where, $D$ is the irrigation water Depth (mm), $i$ is equal to one layer, $n$ is the number of soil layer, $\Sigma$ is summation of amount of irrigation water depth (mm) in n number of layers, $\theta_{eci}$ is volumetric water content at field capacity (cm$^3$ cm$^-3$) in the $i$th soil layer, $\theta_i$ is volumetric water content (cm$^3$ cm$^-3$) in the $i$th soil layer and $\Delta Z_i$ is the soil layer thickness (mm) in the $i$th soil layer.

Water was applied when the mean soil moisture of the plots dropped to less than 50% of the available moisture. The amount of water applied was calculated in terms of the water needed to refill 0-90 cm depth to field capacity and was measured by time-volume technique (Barati and Ghadiri, 2017).

**Measurements**

At the physiological maturity stage, one-meter long sample were randomly harvested from the center of each plot and the number of fertile tillers was counted. The ears were threshed in a threshing machine and the number of grains per spike was determined by counting the grains using a seed counter. Mean grain weight was calculated from the weight of five sets of 1,000 grains each from the sampling area. Furthermore, plants in the area of 1 m$^2$ from center rows in each plots were hand harvested on 3 June 2016 and 10 June 2017. After harvesting, above-ground biomass and grain yield was measured. Harvest Index (HI) (%) was calculated as the ratio of the grain weight per above-ground biomass.

Oven-dried of whole plant parts samples at anthesis and grain and straw samples at maturity stage were taken from five plants randomly chosen in each plot and N concentration of each plant part was determined by the Kjeldahl method (Dordas and Sioulas, 2009). Then, the various parameters such as N uptake (kg N ha$^{-1}$), N accumulation and remobilization (kg N ha$^{-1}$), N remobilization efficiency (%) and Nitrogen Harvest Index (NHI) (%) within the triticale crop were calculated according to Equation (2) to [6] (Barati and Ghadiri, 2017):

$$ N \text{ uptake (kg N ha}^{-1} \text{)} = \text{nitrogen concentration (\%) \times dry matter (kg ha}^{-1} \text{)} $$  \hspace{1cm} (2)

Post anthesis N accumulation (kg ha$^{-1}$)

$$ = N \text{ uptake of whole plant at anthesis (kg ha}^{-1} \text{)} - \text{N uptake of whole plant at physiological maturity (kg ha}^{-1} \text{)} $$  \hspace{1cm} (3)

$$ N \text{ remobilization (kg ha}^{-1} \text{)} = \text{N uptake of whole plant at anthesis (kg ha}^{-1} \text{)} - \text{N uptake of vegetative plant parts [(leaf + culm) + chaff)] at maturity (kg ha}^{-1} \text{)} $$  \hspace{1cm} (4)

$$ N \text{ remobilization efficiency(\%)} = \frac{N \text{ remobilization (kg ha}^{-1} \text{)}}{\text{N uptake of whole plant at anthesis (kg ha}^{-1} \text{)}} \times 100 $$  \hspace{1cm} (5)

Contribution of N remobilization to grain
\[ N \text{ content (\%)} = \frac{(N \text{ remobilization (kg ha}^{-1})}{\text{grain N uptake at maturity (kg ha}^{-1})} \times 100 \] (6)

Nitrogen harvest index (NHI) (\%) = \frac{(\text{grain N uptake (kg ha}^{-1})}{\text{total aboveground N uptake at maturity (kg ha}^{-1})} \times 100 \] (7)

Grain and aboveground biomass Water Use Efficiency (WUEg and WUEb, respectively) were calculated by using the Equations (7) and (8), respectively (Barati et al., 2015). Total water use included rains in both growing seasons.

\[ \text{WUEg (kg ha}^{-1}\text{mm}^{-1}) = \frac{[\text{grain yield (kg ha}^{-1})/\text{total water use (mm)]} \times 100}{(8)} \]

\[ \text{WUEb (kg ha}^{-1}\text{mm}^{-1}) = \frac{[\text{above - ground biomass (kg ha}^{-1})/\text{total water use (mm)}]}{\times 100} \] (9)

Statistical Analysis

Separate analyses of variances were carried out due to the different irrigation regimes in 2016 and 2017 growing seasons. Statistical analyses were performed through the GLM procedure of SAS by using the correct error term to evaluate each factor and interaction. The correlation coefficients were calculated by SAS software, as well. The Least Significant Difference (LSD) at 0.05 probability level was used as mean separation test.

RESULTS AND DISCUSSIONS

Grain Yield, Yield Components, and Harvest Index

Averaged across the Normal and Deficit Irrigation (IR35 and IR50, respectively) treatments, grain yield of triticale crop was greater in 2017 (4,548 kg ha\(^{-1}\)) than 2016 (3,671 kg ha\(^{-1}\)), which may be attributed to the higher rainfall in the vegetative stage of the second year (572 mm) as compared with the first year (86.4 mm) (Figure 1). Average grain yield in both years was in the range of triticale grain yield reported by the other authors for different level of irrigation treatments (Irandoust, 2015).

In this study, deficit irrigation significantly decreased the fertile tiller and grain number per spike in both years (Table 1). Similar results were found by Acevedo et al. (1991). Regardless of the source, N fertilizer improved the fertile tiller and grain number per spike of the triticale crop at all irrigation treatments (Table 1). It has been largely proven that the early development stages, such as tiller proliferation, depend on the availability of N (Jeuffroy and Bouchard, 1999; Garcia del Moral et al., 1991). Also, in line with our results, Okon (1984) showed that the inoculation of cereal plants with Azospirillum spp. increased the number of tillers. In our study, among the biological, chemical, and integrated fertilizers, grain number per spike of triticale best responded to Bio+N\(_{75}\) at all irrigation conditions. Similar results were reported by Millet and Feldman (1984) and Ozturk et al. (2003).

Averaged over all irrigation conditions, the correlation coefficient between grain yield and grain weight was significant and higher than that between grain yield and the other yield components for all N treatments (r= 0.96, r= 0.80 and r= 0.87 for N\(_{150}\), Bio and Bio+N\(_{75}\), respectively). Across the 2-year period, higher decrement in grain weight was observed in N\(_{150}\) than the other N treatments with increasing water stress (significant N fertilizer x irrigation interaction) (Table 2). Drought stress and high temperatures during grain filling period, as it often occurs in Mediterranean conditions (such as our study environment), reduce mean grain weight (Oweis et al., 2000; and Acevedo et al., 2002). Gibson and Paulsen (1999) reported that the late water stress shortens the grain-filling period because it leads to premature desiccation of the endosperm and limits the embryo size. Therefore, reduction in yield is mainly due to a reduction in the weight of the produced grains.

The higher reduction of grain weight in N\(_{150}\) as compared with the Bio and Bio+N\(_{75}\) as a consequence of water deficit in grain filling period in the first year, and especially the second year (Table 2), may be attributed to "haying-off" effect. The conditions that most commonly lead to "haying-off" are rapid vegetative growth in response to adequate soil water and N, followed by terminal water deficit that expose cereal crops to severe water stress during grain filling period (Van Herwaarden et al., 1998). Also, Frederick and Camberato (1995) concluded that the soil water must be available for applied N treatments to have a positive effect on the effective filling.
Table 1. Above Ground biomass (AG-biomass), Harvest Index (HI), grain yield and yield parameters as influenced by irrigation regime and N fertilizer source in 2016 and 2017.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>AG-biomass</th>
<th>HI (%)</th>
<th>Grain yield</th>
<th>Fertile tiller</th>
<th>Grains</th>
<th>Grain weight</th>
</tr>
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<tbody>
<tr>
<td>Irrigation regime</td>
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<tr>
<td>IR&lt;sub&gt;90&lt;/sub&gt;</td>
<td>11033</td>
<td>12430</td>
<td>40.3</td>
<td>42.1</td>
<td>4536</td>
<td>5296</td>
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<tr>
<td>IR&lt;sub&gt;95&lt;/sub&gt;</td>
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<tr>
<td>N Fertilizer source</td>
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<td>12845</td>
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<td>3955</td>
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<sup>a</sup> IR<sub>90</sub>: Normal irrigation, IR<sub>95</sub>: Mild Deficit irrigation and IR<sub>20</sub>: Rain fed. <sup>b</sup> N<sub>0</sub>: No N fertilizer (control), N<sub>20</sub>: Sole chemical N fertilizer (150 kg N ha<sup>-1</sup>), Bio: Sole biological fertilizer (seed inoculated with Azospirillum brasilense). Bio+N<sub>20</sub>: Combined fertilizer (75 kg N ha<sup>-1</sup> seed inoculated with Azospirillum brasilense). *** Significant at P<sub>0.001</sub>, ** Significant at P<sub>0.01</sub>, * Significant at P<sub>0.05</sub> and NS: Not Significant.

Table 2. Grain N uptake, total N uptake, N accumulation after anthesis, Nitrogen Harvest Index (NH<sub>H</sub>), grain weigh, Harvest Index (HI) and grain yield as influenced by irrigation regime-N fertilizer source interaction in 2016 and 2017.

<table>
<thead>
<tr>
<th>Source of variation&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Grain N uptake &lt;sup&gt;b&lt;/sup&gt;</th>
<th>Total N uptake &lt;sup&gt;b&lt;/sup&gt;</th>
<th>N accumulation after anthesis &lt;sup&gt;b&lt;/sup&gt;</th>
<th>NH&lt;sub&gt;H&lt;/sub&gt;</th>
<th>Grain weight &lt;sup&gt;c&lt;/sup&gt;</th>
<th>HI</th>
<th>Grain yield &lt;sup&gt;d&lt;/sup&gt;</th>
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<td>0.98</td>
<td>3.0</td>
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<sup>a</sup> Symbols for irrigation and N treatments are the same as under Table 1 and the main text.
Effect of Nitrogen Source and Deficit Irrigation

period and, consequently, on individual kernel weight. In contrast, the high performance of kernel weight of Bio or Bio+N treatments as compared to N150 under water stress conditions may be attributed to positive effects of Azospirillum on water absorption of roots in grain filling period. Indeed, Azospirillum can provide hormone like substances, including auxins, gibberellins, and cytokinins (Creus et al., 2010; Vande Broek et al., 1999) and consequently stimulate both rates of root elongation and appearance of crown roots (Fallik et al., 1994). Wu et al. (2013) demonstrated that the adequate P uptake in biological fertilizer can enhance crop drought tolerance through different mechanisms, including enhancing root growth and development, which extends the volume of soil that can be explored for water. Furthermore, increasing root hydraulic conductance in biofertilizer treatments was reported by Singh and Sale (2000).

In both growing seasons, under IRN, the highest grain yield (5,913 and 6,602 kg ha⁻¹ in 1st and 2nd years, respectively) was achieved by chemical N fertilizer application (N150), followed by combined N fertilizer (Bio+N75) (Table 2). When the mild water stress occurred by Deficit Irrigation (IRMD), grain yield decreased with increasing water shortage. However, the decrease was more in N150 (44.7% averaged over two years) as compared with Bio and Bio + N75 (27.6% and 28.3% averaged over two years, respectively). Similar trend was shown when the severe deficit irrigation (IR0) was imposed in the second year (63.7, 36.9, and 39.7% reduction for N150, Bio and Bio+N75, respectively). As a consequence, the application of Bio+N75 and Bio resulted in higher grain yield (2960 and 2558 kg ha⁻¹, respectively) as compared with N150 (1,463 kg ha⁻¹) under IR0 condition (Table 2).

More grain yield reduction by high amount of the chemical N fertilizer as compared with the other N treatments when triticale crop suffered water stress (IRMD and IR0) was in line the finding of Garabet et al. (1998), Pandey et al. (2001a) and Ercoli et al. (2008). Also, Frederick and Camberato (1995) concluded that under non-water-limiting conditions, the quantity of chemical N fertilization is often the yield-determining factor, but under drought stress conditions, high chemical N increases severity of water stress and, consequently, reduces growth and yield of wheat crops. In contrast, as some authors (Dadrasan et al., 2015; Creus et al., 2004) reported, using sole biological and integrated chemical and biological N fertilizer could lead to reducing deleterious effects of severe water stress on crop growth by some mechanisms as discussed above and consequently increases grain yield. Therefore, optimal N fertilizer source depends on irrigation regimes and precise understanding of crop responses to water and N interaction.

The above ground biomass of triticale crop significantly varied in relation to N application (the average increments by N150, Bio and Bio+N75 as compared with the control unfertilized crops were 119.6, 78.7 and 114.1%, respectively) and the amount of water availability (the average decrements in IRMD and IR0 as compared with IRN condition were 13.8 and 24.3%, respectively) (Table 1), as reported in previous studies on other crops (Van Herwaarden et al., 1998; Barati and Ghadiri, 2017; Dadrasan et al., 2015). When crops face water restriction, they tend to minimize water loss through closing their stomata, which in turn limits CO₂ availability for photosynthesis and dry matter production (Sun et al., 2013). With respect to N sources, the highest above ground biomass was achieved in N150 (13,281 and 12,845 kg ha⁻¹, in 1st and 2nd years, respectively) while it did not have significant difference with Bio+N75.

Harvest index increased with each N source application in IRN condition in both years. Also, The Bio+N75 treatment had the highest HI (42.6 and 43.9% in the 1st and 2nd years, respectively) under IRN, while it was not significantly differed from N150 (Table 2). Significant N fertilizer×irrigation interaction for HI (Table 2) in both years showed that the water stress (IRMD and IR0) decreased HI in all N treatments, however, it had the highest negative effect under N150 (32.5 and 60.6% for IRMD and IR0, respectively) as compared with the other N fertilizing systems. Therefore, the HI had the lowest value in N150 treatment under IRMD (24.3 and 33% in the 1st and 2nd years, respectively) and rainfed (13%) conditions than the other N sources.

With respect to the highest correlation coefficient between grain yield and mean kernel weight as compared to the other grain yield components under IRMD and IR0 (r= 0.657*** and
increase, respectively), the decrease in HI was mainly due to the lower individual grain weight than the other yield components under water stress. Our results further confirmed the finding of Gonzalez et al. (2007). Water stress from anthesis to maturity, which occurs under deficit irrigation or rainfall conditions of Mediterranean regions (such as our study environment), closes stomata and, consequently, limits photosynthesis (Bijanzadeh and Naderi, 2014), hastens leaf senescence, reduces the duration and rate of grain filling, decreases the time for localization of carbohydrate reserves to the grain (Oweis et al., 2000) and, consequently, reduces mean kernel weight (Acevedo et al., 2002) and HI.

The relatively higher decrease in grain yield and HI observed in N$_{(150)}$ in comparison with Bio and Bio+ N$_{(75)}$ in IR$_{(75)}$ and rainfed conditions could be attributed to the "haying off" effect, which can occur when chemical N is applied excessively high in water stress conditions. This encourages the crop to produce excessive biomass and use extra water, reducing water availability during the grain filling process (Van Herwaarden et al., 1998). In this case, the number of tillers and ears per square meter increases, however, the additional ears, derived from higher shoot categories, yield less than the main stem, causing a reduction in the grain yield to total above-ground biomass ratio or HI (Sieling et al., 1998). In contrast, the higher plant’s ability to tolerate drought in the Bio or Bio+ N$_{(75)}$ treatment may be attributed to producing a deeper and expanded root system, facilitating water uptake from the soil (Dadrasan et al., 2015), especially under drought environments. Therefore, "haying off" effect declined and HI was not limited by terminal water stress.

Water Use Efficiency

The WUE$_{e}$ and WUE$_{b}$ varied between 2.19 and 8.19 kg$_{\text{grain}}$ ha$^{-1}$ mm$^{-1}$, and between 7.47 and 24.98 kg$_{\text{biomass}}$ ha$^{-1}$ mm$^{-1}$, respectively, over the two growing seasons (Table 3 and Figure 2). The WUE$_{b}$ significantly increased with IR$_{(75)}$ (24.5% for average of two growing seasons), and with increasing water stress by the rain fed condition (IR$_{0}$) in the second year, it slightly increased (6.5%) as compared with IR$_{(75)}$ (Table 3). Other studies have shown that water stress can either decrease WUE (Johnson et al., 1984) or increase WUE (Singh and Kumar, 1981). Barati et al. (2015) demonstrated that moderate water stress (25% water reduction from normal irrigation) in the reproductive period of barley could improve WUE$_{e}$ and WUE$_{b}$, however, the values decreased with more diminishing water supply (50% water reduction from normal irrigation or rain fed). Ritchie (1983) reported that the effects of water deficit on transpiration loss are greater than the effects on the photosynthesis, such that WUE may improve under dry conditions compared to wet conditions, although total crop production is severely restricted. Additionally in another study on wheat, Zhang et al. (1998) reported that although the grain yield was restricted by 15% in the severe drought stress conditions as compared to the well watered conditions, WUE for total water consumption was improved by 24%.

Irrespective of the irrigation treatment and growing seasons, WUE$_{b}$ positively responded to N fertilization (Table 3). Among the biological, chemical, and integrated fertilizers, the highest WUE$_{b}$ was achieved by the integrated fertilizers (Bio+ N$_{(75)}$) and N$_{(150)}$ (Table 3). With respect to WUE$_{e}$ there was a significant N fertilizing system×irrigation interaction in either season (Figure 2). The IR$_{(75)}$ slightly increased WUE$_{e}$ in the integrated fertilizer, sole biofertilizer, and the control, however, it significantly decreased WUE$_{e}$ (21.1 and 16.2% in first and second years, respectively) in N$_{(150)}$. Further water stress (IR$_{0}$) in the second year, drastically decreased WUE$_{e}$ in all N treatments, however, this decrement was the greatest in N$_{(150)}$ (56.9%) (Figure 2).

These results further confirmed findings of Van Herwaarden et al. (1998) who showed WUE$_{e}$ of rain-fed wheat crop decreased with applied 150 kg N ha$^{-1}$ as chemical fertilizer at a location exposed to water deficit at the time of anthesis because the pre anthesis evapotranspiration was stimulated in the crops that received high N. The other authors (Pandey et al., 2001a; Barati et al., 2015) also reported that the WUE$_{e}$ of high N crops was lowered when exposed to water stress. In our study, it seems that the biological and the integrated fertilizers provided N demand during the tricotile life cycle. Therefore, the growth of vegetative parts was induced slowly and, consequently, rate of soil water extraction was
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<table>
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<tr>
<th>Source of variation</th>
<th>WUE&lt;sub&gt;b&lt;/sub&gt;</th>
<th>Grain N uptake</th>
<th>Straw N uptake</th>
<th>N uptake of whole plant at anthesis</th>
<th>Total N uptake</th>
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</table>

* Symbols for irrigation and N treatments are the same as under Table 1 and the main text. ** Significant at P≤ 0.001, ** Significant at P≤ 0.01, * Significant at P≤ 0.05 and NS: Not Significant.
lower compared with the sole chemical N fertilizer ($N_{150}$). In this situation, the grain filling period did not encounter severe water stress in IRMD conditions and $WUE_g$ slightly increased in the biological and the integrated fertilizer treatments (Figure 2). With increasing water stress in rain-fed (IR0) condition, crops experienced severe water stress, especially in high N treatment ($N_{150}$). Therefore, the photosynthesis rate and dry matter deposition in grain was severely restricted (Ercoli et al., 2008; Bijanzadeh and Naderi, 2014; Barati and Ghadiri, 2017) and consequently grain yield and $WUE_g$ were diminished in all N treatments, especially in sole chemical N ($N_{150}$).

**Nitrogen Uptake and Remobilization**

The grain N content responses to the different treatments and experimental years ranged from 20.3 to 86.7 kg ha$^{-1}$ (Table 2). Generally, N uptakes were higher in 2017 than 2016; this may be attributed to higher rainfall at triticale vegetative growth stages in the second year. Indeed, crop response to N fertilization is heavily reliant to water availability and rainfall distribution (Pala et al., 1996; Tilling et al., 2007) and it is diminished during growing seasons with low rainfall (Rasmussen and Rohde, 1991). In our study, disregarding the growing season, N source, and irrigation regime, grain yield was positively related to total N uptake (Figure 3). The positive relationship that was observed between grain yield and total N uptake coincides with the limits reported for other cereals (barley, durum and bread wheat) in Mediterranean areas (Cossani et al., 2012).

In both years, grain, straw, and total N uptake decreased with successive water stress treatments that imposed by deficit irrigation, and increased with increasing N fertilizer application (Tables 2 and 3). In agreement with this result, Pandey et al. (2001b) and Garabet et al. (1998) reported that the N uptake by wheat crops was reduced under dry environment, even when mineral N was present in the soil. In our study, the integrated N fertilizer had the highest total N uptake, followed by the sole chemical N fertilizer in 2016 averaged over all irrigation treatments (Table 3). Concerning the grain N uptake (in both years) and total N uptake (in 2017), there were significant N fertilizer × irrigation interactions for these traits (Table 2). The highest total N uptake and grain N uptake was achieved by Bio+$N_{75}$ application, followed by $N_{150}$ under...
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Regardless of the N source, N remobilization during the grain filling was affected positively by the N fertilizer (in both years) and Mild Deficit Irrigation (IRMD) (in 2017) and negatively by severe water stress (IR0) (in 2017) (Table 4). The Bio+N$_5$ had the highest N remobilization (40.0 kg ha$^{-1}$ and 51.1 kg ha$^{-1}$, in the 1$^{st}$ and 2$^{nd}$ years, respectively) and significantly differed from Bio (in 2016 and 2017) and N$_{150}$ (in 2017). As a consequence, the Bio+N$_5$ remobilized about 113.9 and 110.3% more N than the unfertilized crop in the first and second years, respectively. Also, the highest level of water stress in 2016 (IRMD) and 2017 (IR0) decreased remobilized N by 8.8% and 10.3% as compared to IR3, respectively. Ercoli et al. (2008) and Giuliani et al. (2011) also reported increasing N remobilization due to N fertilization in durum wheat. Several researchers (Seligman and Sinclair, 1995; Xu et al., 2006; Ercoli et al., 2008) demonstrated the reduction of N remobilization amount as affected by severe water stress and its dependence on climatic conditions (Giuliani et al., 2011). Furthermore, Yang and Zhang (2006) concluded that when severe water stress was improved, the pattern of dry matter and nitrogen deposition in grain was substantially modified, since the severe water stress may seriously disrupt phloem function.

The water stress treatments from IR$_0$ to IRMD slightly decreased N translocation efficiency (1.1%) in dry year (2016), however, IRMD and IR$_0$ increased N remobilization efficiency by 8.4% and 1.5%, respectively, in wet year (2017) (Table 4). With respect to the increase in N remobilization efficiency and N remobilization (as mentioned in the above paragraph) by mild deficit irrigation, several studies have shown the modest water stress (such as the mild deficit irrigation in wet year of our study) is considered likely to enhance N remobilization from leaf to grain (Palta et al., 1994; Sinclair et al., 2000; Barati and Ghadiri, 2017). Furthermore, Xu et al. (2006) showed that the water deficit increased the N translocation ratio in various vegetative organs to grains, confirming the enhancement of N transfer from the vegetative organs to grains in our study. Generally, N application decreased the N remobilization efficiency in both growing seasons as compared to unfertilized plots. The highest decrement of N remobilization efficiency belonged to N$_{150}$ (35.7 and 29.2% in the first and second year, respectively) and the lowest was achieved by sole biofertilizer in 2016 (21.9%) and integrated N fertilizer in 2017 (17.1%) as compared with the unfertilized control (Table 4).

The contribution of N remobilized to N content of grain ranged from 50.1 to 92.7% across the different irrigation and N fertilizing systems (Table 4), indicating the importance of pre-anthesis storage of N for attaining high grain N. The contribution of N remobilized to N content of grain decreased by the N fertilizer application and increased with improving water stress by the consecutive deficit irrigation treatments. Although it decreased by N application, there was no significant difference among the N sources (Table 4). This result further confirmed the finding of Barati and Ghadiri (2017) on barley. The results from Xu et al. (2006) showed that the water deficit remarkably increased the contributions of N in various organs to grain N. They suggested that the water deficit would weaken the availability of N fertilizer [bearing in mind that the N uptake was decreased since the water deficit occurred in our study (Tables 2 and 3)], however, enhance the remobilization of pre-stored N to grain and, consequently, increase contribution of N remobilized to N content of grain.

In 2017, N accumulation after anthesis in triticale during grain filling was affected positively by each form of N fertilizer and negatively by water stress (Table 4). In line with our results, Ercoli et al. (2008) found similar results about effects of water stress and chemical N fertilizer on the N accumulation. The highest N accumulation was achieved in Bio+N$_5$ (28.3 kg ha$^{-1}$), followed by Bio (21.3 kg ha$^{-1}$) (Table 4). In 2016, similar to the second year, drought stress after anthesis reduced N accumulation, however, the reduction trend by water stress was not consistent in different forms of N fertilizer (nitrogen×irrigation interaction, $P \leq 0.0734$) (Table 2). The highest reduction was achieved by N$_{150}$ (42.7%) as compared with the other N sources (N$_0$ (10.8%), Bio (22.8%) and Bio+N$_5$.
Table 4. Nitrogen remobilization, N remobilization efficiency, Contribution of N remobilized to N content of grain and N accumulation after anthesis as influenced by irrigation regime and N fertilizer source in 2016 and 2017.

<table>
<thead>
<tr>
<th>Source of variation*</th>
<th>N remobilization kg ha(^{-1})</th>
<th>N remobilization efficiency %</th>
<th>Contribution of N remobilized to N content of grain %</th>
<th>N accumulation after anthesis kg ha(^{-1})</th>
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<td>9.3</td>
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</table>

* Symbols for irrigation and N treatments are the same as under Table 1 and the main text. *** Significant at P≤ 0.001, ** Significant at P≤ 0.01, * Significant at P≤ 0.05 and NS: Not Significant.

(25.1%) when IR\(_{50}\) treatment was imposed. Low N accumulation in N\(_{50}\) under post-anthesis drought stress could be attributed to drastically increasing shoot/root ratio (data not shown) before anthesis stage as compared with the other N sources, therefore, higher transpiration surface coupled to a lower root surface area decreased N and water absorption ability of root. In contrast, biofertilizers enhances plants ability to tolerate water stress through helping plants to produce expanded and deeper root system, which facilitates N and water uptake from the soil profile (Sharma, 2002).

**Nitrogen Harvest Index**

The NHI has significance for maximizing the grain protein content for a given amount of plant N (Dawson et al., 2008) and it is a measure of N translocation efficiency. In our study, The NHI increased linearly and positively with increases in the N remobilization efficiency in all irrigation regimes (Figure 4). However, the magnitude of response to the N remobilization efficiency differed for each irrigation regime. On the other hand, increasing N remobilization efficiency at IR\(_{50}\), IR\(_{50}\)MD, and IR\(_0\) treatments increased NHI by 0.15, 0.39, and 0.78%, respectively (Figure 4). Furthermore, the N remobilization efficiency explained approximately 30, 50, and 60% of the variability observed in NHI, respectively (R\(^2\)= 0.26, R\(^2\)= 0.49 and 0.62).

The NHI had constant values or decreasing trend when N sources were applied (Table 2). In line with our result, several authors (Delogu et al., 1998; Muurinen et al., 2007; Giuliani et al., 2011) observed that NHI significantly declined as N fertilizer increased, mainly because of a more proportional increase in straw N uptake than grain N uptake. In our study, the nitrogen×irrigation interaction for NHI (Table 2) showed that the
Deficit Irrigation (IRMD and IR0) significantly decreased NHI, however, the decrease was higher in N150 plants (12.5% for IRMD averaged over two years and 36.3% for IR0) than N0, Bio, and Bio+N8. Therefore, the lowest NHI was achieved by N150 under IRMD (49.9 and 53.6% in 1st and 2nd years, respectively) and IR0 (37.8%). The decrease in NHI as affected by water stress could be attributed to the negative effects of drought stress on N remobilization from straw to grain at grain filling period, especially in the crops that received high N (Table 3). In line with our results, Ercoli et al. (2008) and Giuliani et al. (2011) demonstrated that the severe post-anthesis water stress (such as IR0 in our study) greatly reduced the N accumulation, remobilization, and NHI of durum wheat genotypes.

CONCLUSIONS

Cutting off irrigation after anthesis (IRMD) decreased grain yield by 33%. However, IRMD increased WUEgrain (in sole bio-fertilizer and integrated fertilizer), WUEbiomass and N remobilization efficiency as compared with the normal irrigation. Therefore, applying IRMD in relatively wet years (such as the second year of our study with 572 mm precipitation) may be acceptable. This approach will provide water to a larger number of the farmers in arid Mediterranean areas. Regardless of the irrigation regime, integrated fertilizer (50% of chemical N fertilizer+seed inoculation with Azospirillum brasilense) outperformed sole biofertilizer and sole chemical N fertilizer with respect to grain yield, WUEg, WUEs, N uptake, N remobilization efficiency, and N harvest index. The current study can provide useful information for farmers in order to decrease irrigation water and N chemical fertilizer consumption up to 50% in water-limited regions, which will improve the sustainability of the agro-ecosystems in Mediterranean arid area ultimately.

REFERENCES


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