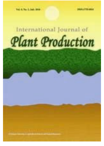

International Journal of Plant Production 10 (4), October 2016
ISSN: 1735-6814 (Print), 1735-8043 (Online)
www.ijpp.info

GUASNR

Nitrogen supply effect on emmer (*Triticum dicoccum* Schübler) ecophysiological and yield performance

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Received 23 February 2016; Accepted after revision 20 May 2016; Published online 10 August 2016

Abstract

The effects of nitrogen (N) addition on productive and physiological traits of *Triticum dicoccum* were assessed under two N levels (0, unfertilized, and 90 kg N ha^{-1}) in field conditions, in two growing seasons. In the study case, the N supply (90 kg N ha^{-1}) was applied in three-split applications (20% at pre-plant stage, 40% at tillering stage, 40% at stem elongation). In unfertilized conditions, the productive and physiological traits were consistently reduced in comparison with fertilized treatments. Indeed, control plots showed lower grain yield, biomass and protein content (about 42, 44 and 19%, respectively) and lower photosynthetic activity at light saturation level (25%) than fertilized plots. The stomatal conductance was 34.2% and 70.8% lower in unfertilized plots than in fertilized those, in the first and second year, respectively. No significant differences for the Ci/Ca ratio were evident between treatments. Emmer physiological and yield performances were affected by N supply, although the crop plasticity to N availability was emphasized.

Keywords: Crop yield; Biomass; Protein content; Photosynthetic activity; Stomatal conductance.

Introduction

Agriculture employs nearly 50% of the world labor force, defining a major impact on welfare throughout much of the world (Lowder et al., 2014). The efficiency of agriculture is defined by the ratio between the energy extracted and the energy invested (Webber, 2011). A key starting point for identifying and evaluating the efficiency of an agriculture system is to control and improve the energy input conversion that could theoretically be achieved in managed ecosystems (Zhu et al., 2008). Chemical fertilizer is one of the largest agricultural inputs to the total energy requirements of crop cultivation (Ziaei et al., 2015). Nitrogen fertilizer represents the largest component of energy consumption among agrochemicals (Hosseinpanahi and Kafi, 2012), contributing significantly to the growth and yield of crops, but posing serious challenges in relation to energy consumption in agriculture (Esengun et al., 2007). However, plant performance in relation to environmental (natural or supplied) resources provides a general picture of the strength and shape of selection in the wild (Kingsolver et al., 2001; Geber and Griffen, 2003), as well as insight into the levels of genetic variation in functional and physiological plant traits (Arntz and Delph, 2001; Geber and Griffen, 2003).

Emmer, *Triticum dicoccum* Schübler, is one of the earliest domesticated plants and has been a staple crop over centuries (Halstead, 2014; Troccoli and Codianni, 2005). Emmer is a potential crop for the sustainable development of agriculture due to interesting features for breeding, its better performance in disadvantageous pedo-climatic conditions as compared to modern cultivars, its resistance to wheat diseases, drought and lack of N in soil (Konvalina et al., 2014; Trčková et al., 2005) and for nutritional characteristics, as well as for its high seed protein content (Zaharieva et al., 2010). Interest in these ancient species has also been renewed by the increasing demand for traditional products, the demand for species suitable for growing in marginal areas and the need to preserve genetic diversity (Stagnari et al., 2008). This crop is particularly suitable for small-scale farming systems with a connection to local food processing (Konvalina et al., 2012).

In the literature, the effect of moderate nitrogen supply on emmer crop yield has generated conflicting results: some papers report that N additions (from 60 to 90 kg ha^{-1}) had a significant and positive effect on hulled and net grain yield of emmer (Marino et al., 2009; Marino et al., 2011).

In Particular, the increasing N addition (30, 60 and 90 kg ha^{-1} of Nitrogen) had a significant and positive effect on hulled and net grain yield of emmer, through a higher number of spikes m^{-2}, spikelets per spike and plant height (only compared with N0), number of kernels m^{-2}, biomass, lodging and protein content (Marino et al., 2009). Furthermore, Marino et al. (2011) have found that the optimal N rate (from 60 to 90 kg ha^{-1}) relays on the commercial target in the typical climatic conditions of central Italy.

Although others studies report that N addition did not improve grain yield (Codianni et al., 1993), de Giorgio et al. (1995) found that unfertilized crop produced as much as that supplied with the minimum amount and others even report a yield reduction in southern and central Italy at high N rates (Castagna et al., 1996).

Conversely, the positive effect of N supply on protein content were previously documented by many authors (Castagna et al., 1992; Castagna et al., 1996; Mariani et al., 1992; de Giorgio et al., 1995; Marino et al., 2009, 2011).

Nitrogen supply at anthesis has been found to increase grain yield in winter wheat, although dry matter allocation to grain was unrelated to current photosynthetic rate (Madani et al., 2010). In the first half of the grain filling period, the crop has been found sensitive to source reduction leading to an increase in net photosynthetic rate of 10%, with little effect of sink reduction (Yin et al., 1998). No data are available in the literature on the effect of nitrogen fertilization on emmer physiological parameters. We hypothesized that low N availability should not impair crop performance of emmer grown in poor environmental conditions and, thus, the gain in dry matter and its allocation to grain filling processes. This would help to define the suitability of emmer cultivation to low-input agricultural systems, for sustainable crop production in marginal lands. The plant performance was assessed through the study of gas exchange and plant productivity of an emmer crop cultivated in a marginal area and with or without N supply during the crop cycle.

Materials and Methods

Experimental conditions

Field experiments were carried out in Central Italy in 2 years (2012-2013). Emmer crop (*Triticum dicoccum* Schübler, Molise population) was used in both years. According to the USDA classification, the soil at the experimental field sites was clayey (sand 35%, silt 23 %, clay 42%), with a uniform soil profile. Soil sampling and analysis were performed at the beginning of the first cropping season. The mean content of N was 0.7 g kg^{-1} (Kjeldahl), available P was 19 mg kg^{-1} (Olsen); available K was 168 mg kg^{-1} (ammonium acetate), organic matter was 0.9 % (Blake–Walkley) and pH was 6.7.

The experiment was set up as a completely randomized block design, with unfertilized control (NF, 0 kg N ha^{-1}) and fertilized treatment (F, 90 kg N ha^{-1}, NH_4NO_3). Nitrogen was applied at seeding (20%), tillering stage (40%) and at stem elongation (40%). Sowing was carried out in the third decade of November, in both years.

The size of each plot was 150 m^2 replicated three times; each plot received 200 seeds m^{-2}. Grain was harvested in the second decade of July, in the first year and in the first decade of July, in the second year.

Daily maximum and minimum temperatures and rainfall were recorded through a standard agro-meteorological station (Skye instruments Ltd, Llandrindod Wells, UK) placed in the experimental fields.

Crop traits

Structural traits were recorded on three replicate plants per each sampling subplot. Plants of each sampling subplot (1 m^2) were harvested for the measurement of yield-related traits. The plant dry mass (root, stem and spikes) was determined after oven drying plant material for 24 hours at 75 °C. The phenological stage was recorded following Zadoks et al. (1974). The number of spikelets of 100 spikes was counted at heading. Total number of culms and spikes was determined at physiological maturity. Threshed and hulled grain yield, the hulled index (%), the thousand kernel weight, the number of kernels per spike and of spikes per square meter and the kernels per square meter, as yield components were determined at harvest.

The protein content of kernels was determined by the Dumas method using the automatic system LECO FP-428 (LECO Co, St. Joseph, USA) (see D’Egidio et al., 1990). Ash content was determined in duplicate on 5 g of sample by dry combustion for 16 h at 580 °C (AACC approved method 08-01-01). Following Keller et al. (1999), lodging was recorded using a scoring scale from 0 (all plants in the plot completely upright) to 9 (all plants in the plot completely lodged), depending on the deviation of the plants from the vertical and on the percentage of lodged plants per plot.

Leaf gas exchange

Leaf gas exchange was measured at booting stage on three replicate plants per each sampling subplot, using a LI-COR photosynthesis system (LI-6400; Li-COR Inc., Lincoln, NE, USA). Photosynthetic response curves to light were measured on fully expanded leaves of the two treatments using a portable photosynthesis system (LI-6400, Li-Cor, Lincoln, NE). Photosynthetic light response curves were obtained by measurements at PPFD (photosynthetic flux density) values of 1200, 800, 600, 400, 300, 200, 150, 120, 80, 40 and 0 μmol m^{-2} s^{-1} (LED source, red blue 6400-02B). Irradiance was changed at intervals of 120-200 s. The CO_2 entering the cuvette was adjusted to maintain a chamber CO_2 concentration of 350 μmol mol^{-1}.

Photosynthetic CO_2 response curves were obtained by changing the CO_2 concentration entering the cuvette with an external CO_2 cartridge mounted on the LI-6400 console and automatically controlled by a CO_2 injector. The CO_2 assimilation rate was measured by setting the reference CO_2 concentration at 0, 50, 100, 150, 200, 250, 300 and 350 µmol mol^{-1}. Gas exchange was determined at each step after exposure of the leaf to the new CO_2 concentration for 60-120 s; PPFD was maintained at 1500 µmol m^{-2} s^{-1}.

Statistical analysis

The analysis of variance (ANOVA) was applied in order to evaluate the effects of N supply. Statistical analysis was conducted with OriginPro version 8.5.1 (OriginLab, Northampton, MA).

Results

Meteorological conditions

Decadal minimum and maximum air temperature and rainfall during the study period were computed for each year. From sowing to harvesting, the total amount of rainfall was 720 mm and 742 mm in 2011-12 and 2012-13, respectively. In both years, rainfall was uniformly distributed from November to March, with a reduction in rainfall from April to July. Minimum temperatures were recorded in February, in the first decade in 2012 (-1.8 °C) and in the third decade in 2013 (-1.2 °C). Maximum temperatures did not exceed 32 °C in July in both years, mean values during the crop cycle was of 9.6 and 9.2 °C for 2012 and 2013, respectively.

Yield, yield components and protein content

The dry biomass at harvest was slightly different between the two years with 10% lower biomass in the first than in the second year; whereas, biomass was 44% higher in fertilized than in unfertilized plots (Figure 1).

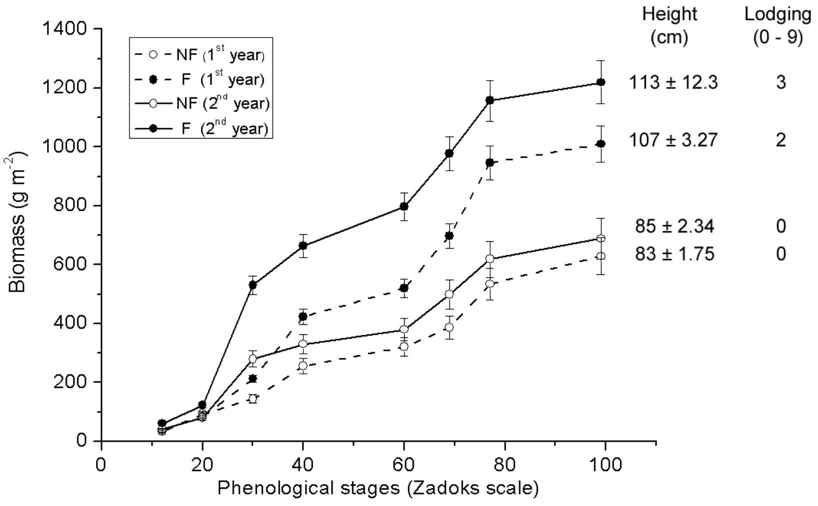

Figure 1. Dry-mass accumulation during the *Triticum dicoccum* crop cycle. Each point represents a single destructive measurement. On the right side the plant height (cm) and lodging value (0-9) related to the fertilized treatment (F) and the unfertilized treatment (NF), in both years. Mean values (± standard deviation) were reported.

From tillering onwards, the dry biomass differed between treatments; the N supply significantly increased the plant biomass in fertilized plots (+69%) in comparison with unfertilized plots, in both years. Biomass accumulation in unfertilized plots had a similar pattern in both years, throughout the whole crop cycle. The dry mass accumulation during the crop cycle did not change between treatments, although higher values (+22%) were recorded in the second than in the first year. Leaf area index showed significant differences between treatments, with higher values (about 50%) in fertilized than in unfertilized plots.

Nitrogen supply increased plant height and lodging; unfertilized plants were 25% shorter than fertilized plants; a lodging mean value of 3 for fertilized plots was recorded vs. a value of 0 for unfertilized plots.

Hulled grain yield (Table 1) ranged between 1.51 and 3.46 t ha^{-1} and the N supply resulted in a significantly higher yield (+83%) in fertilized than in unfertilized plots. Differences between years were minor. The hulled index highlighted significant differences between treatments, following the same trend of grain yield, with a better performance of fertilized plots (75% of naked grain yield) in comparison with unfertilized plots (66% of naked grain yield).

The spike number per square meter was not significantly different between years. It was lower (by 41%) in unfertilized than in fertilized plots; on the contrary, the thousand kernel weight was higher in the unfertilized treatment (2% in the first year and 8% in the second year). The thousand kernel weight was higher in the second year than in the first year (41 g vs 54 g), although minor differences for the number of spikelets per spike were recorded between years and between treatments.

The grain protein content was significantly different between years and between treatments. Higher protein contents were recorded in the first than in the second year (about 4.5%); whereas, a decrease in protein contents in the unfertilized treatment in comparison with the fertilized treatment was observed (15% in the second year and 21% in the first year).

Physiological traits

Nitrogen supply positively influenced photosynthetic rate ($P=0.003$) and stomatal conductance ($P=0.002$), measured at saturating light intensity (1500 µmol m^{-2} s^{-1}) and ambient CO_2 concentration; whereas, no significant differences in Ci/Ca were found between treatments (Table 2). The fertilized plants showed a distinct response of photosynthesis to Ci in comparison with the unfertilized plants (Figure 2). Differences between treatments were noticeable in both years, though more evidently in the second one, when the effect of fertilization was effective even at Ci lower than 200 µmol mol^{-1}, where photosynthesis responds linearly to CO_2. The linear response of photosynthesis to light (at PPFD lower than 200 µmol m^{-2} s^{-1}) was not affected by the fertilization (Figure 2). However, photosynthesis of fertilized plants was higher than that of unfertilized plants at high irradiance, particularly in the second year.

Table 1. Yield and yield components in *Triticum dicoccum,* grown with N fertilization (F) and without N supply (NF) in the two-year experiment. Mean values (± standard errors) were reported. Significance of treatment effect (ANOVA) for each variable is indicated by F-value and P-level.

		Biomass yield		Hulled grain yield		Hulled index		Spike number		Thousand kernel weight		Spikelets per spike		Grain protein content	
		($t\ ha^{-1}$)		($t\ ha^{-1}$)		(%)		($n.\ m^{-2}$)		(g)		(n.)		(%)	
1st year	NF	6.2	± 0.24	1.51	± 0.6	66.9	± 4.07	224	± 63	41.8	± 1.03	15.5	± 3.31	15.3	± 0.82
	F	10.98	± 0.34	3.46	± 0.16	74.2	± 0.61	461	± 17.6	40.7	0.27	18.5	± 0.18	19.4	± 0.7
2nd year	NF	6.87	± 0.45	2.37	± 0.22	65.6	± 1.98	250	± 20.1	56.6	± 0.42	16.7	± 0.74	11.6	± 0.2
	F	12.24	± 0.36	3.25	± 0.25	74.8	± 0.93	348	± 22.8	51.8	± 1.05	18	± 0.46	13.7	± 0.51
		p-level	F-value	p-level	F-value	p-level	F-value	p-level	F-value	p-level	F-value	p-level	F-value	p-level	F-value
	treatment	0.000	301.4	0.001	24.2	0.003	18.7	0.000	32.2	0.002	21.9	2.33	0.166	0.000	40.2
	year	0.011	10.8	0.299	1.23	0.873	0.027	0.181	2.14	0.000	420.4	0.069	0.800	0.000	92.0
	interaction	0.352	0.975	0.101	3.42	0.629	0.252	0.046	5.55	0.020	8.39	0.355	0.568	0.092	3.671

Table 2. Instantaneous gas exchange values under saturating light conditions in *Triticum dicoccum,* grown with N fertilization (F) and without N supply (NF) in the two-year experiment. Mean values (± standard errors) were reported. Significance of treatment effect (ANOVA) for each variable is indicated by F-value and P-level.

		Photosynthetic rate		Stomatal conductance		Ci/Ca	
		μmol m^{-2} s^{-1}		mol m^{-2} s^{-1}		μmol $μmol^{-1}$	
1st year	NF	11.64	± 0.72	0.50	± 0.04	0.84	± 0.01
	F	14.99	± 0.49	0.76	± 0.08	0.85	± 0.01
2nd year	NF	12.83	± 0.04	0.19	± 0.01	0.66	± 0.06
	F	17.83	± 0.57	0.65	± 0.02	0.78	± 0.02
		p-level	F-value	p-level	F-value	p-level	F-value
	treatment	0.02	7.57	0.01	9.07	0.00	22.93
	year	0.00	32.07	0.00	24.51	0.05	5.21
	interaction	0.00	19.82	0.00	16.79	0.00	14.07

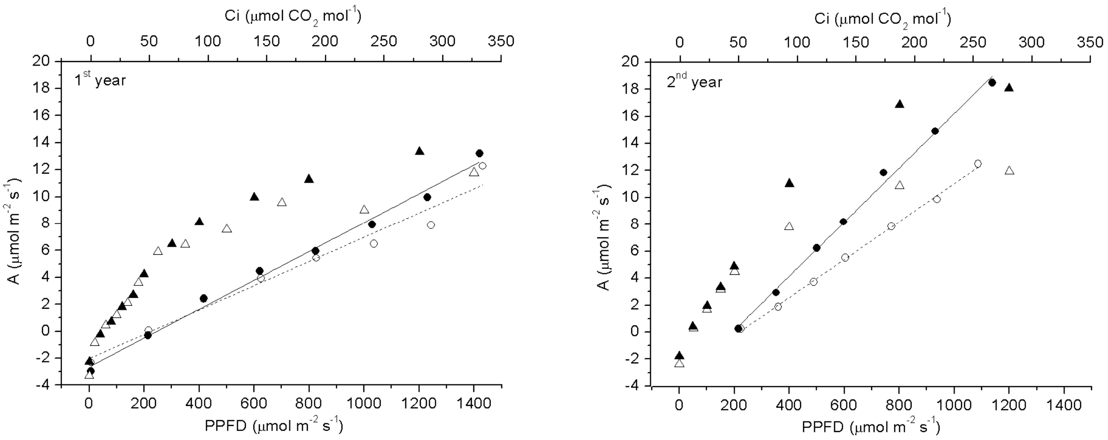

Figure 2. Representative light response curve of photosynthesis (A/PPFD) (A, y-axis; PPFD, lower x-axis) and response of photosynthesis to intercellular CO_2 concentration (A/Ci) (Ci upper x-axis) for each treatment, in the two years. Black and white symbols refer to the fertilized treatment (F) and the unfertilized treatment (NF), respectively. Triangles are for A/PPFD and circles for A/Ci.

Discussion

Meteorological conditions during the crop cycle did not reveal any particular environmental constraints condition (for either water availability or air temperature) in both years. In the pedo-climatic conditions of the experimental area, N fertilization had a positive effect on emmer productivity. The effect of N supply in satisfying the crop N demand depends on crop species, type of fertilizer, timing of application, seasonal trends, etc. (Borghi, 2000; Blankenau et al., 2002). Ancient cereals, as emmer, were found to be less dependent on N supply (e.g., Castagna et al., 1992; Mariani et al., 1992) and suitable for growing in less favorable conditions (montane areas, dry regions) or in low-input and organic farming systems (Moudrý et al., 2011). However, in this experiment, N supply was split into three applications (showing, tillering and stem elongation), resulting in increased biomass, yield, protein content and gas exchange.

The total amount of biomass at harvest was higher in fertilized than in unfertilized plots, as shown by Castagna et al. (1996) who found higher biomass production in emmer with N applications (100 kg ha^{-1}). Marino et al. (2009) observed similar response in emmer crops grown in field conditions similar to the present experiment. In this study, the effect of high N availability due to fertilization on dry mass accumulation was evident from the tillering stage onwards. To the best of our knowledge, we have observed for the first time a positive effect of N fertilization on emmer at tillering. Nitrogen supply at tillering stage sets the efficiency of fertilization in the most important phenological stage for crop production (i.e., the number of plants per square meter), as known for other cereals (Cabrera-Bosquet et al., 2009; Madani et al., 2010). In this experiment, the unfertilized treatment showed the same pattern of dry mass accumulation in both years, during the crop cycle, with a reduction of about 40% in productive traits, such as biomass, spikes for square meter and LAI (data not shown), suggesting a contextual reduction in growth of plants and number of tillers.

Although long and weak culms of emmer are susceptible to lodging as reported by many authors(e.g. Konvalina et al., 2010, Volpe et al., 2005), in the present experiment the N supply did not induce appreciable lodging, probably because the maximum plant height reached under moderate fertilization and on marginal land (113 cm) was lower than reported for emmer crops grown in more different pedo-climatic conditions (e.g., Stagnari et al., 2008; Castagna et al., 1996; Pagnotta et al., 2009, Konvalina et al., 2010). Furthermore, varieties with short and strong down internodes and high number of nodes on the stems and high width of the stalks are more resistant to lodging (Konvalina et al., 2009).

The range of yield values were in agreement with those reported in the literature (from 1.8 kg t ha^{-1} to 3.4 t ha^{-1} reported by Pagnotta et al., 2009; Codianni et al., 1996; Stagnari et al., 2008; Castagna et al., 2009, de Giorgio et al., 1995; Konvalina et al., 2012; Laghetti et al., 1999), moreover hulled grain yield was improved by a moderate N supply. On the contrary, a reduced grain yield was obtained with no N supply (42% lower in unfertilized than in fertilized conditions), in agreement with previous studies by Marino et al. (2009, 2011) on emmer crops cultivated in similar environmental conditions. A few studies have reported that grain yield did not increase with N supply. Thus Codianni et al. (1993) found no significant differences in grain yield between N treatments, although higher plant biomass and protein content were observed. Castagna et al. (1996) reported a yield reduction in southern and central Italy at high N rates. De Giorgio et al. (1995) reported that increasing N application did not improve grain yield and indeed the unfertilized crop produced as much as that supplied with the minimum amount. It must be noted, however, that such studies have generally presented pooled data, bringing together the joint response to N supply of different accessions (e.g., *Triticum monococcum*, *Triticum dicoccum*, *Triticum spelta* and free-threshing wheat), different experimental locations (of variable yield potential), different environmental conditions (often lowland areas subjected to drought stress and high temperature), and/or different seeding rates (e.g., 200 or 400 seeds per square meter) and often reporting only the main effects. In the present study, the yield components that had major effects on grain yield in fertilized plants were the number of spikes per square meter (405 spikes m^{-2} in F vs 240 spikes m^{-2} in NF) and the hulled index (75% vs 66%). Moreover, the spikes of fertilized plants were much more filled than those of unfertilized plants. Laghetti et al. (1999) and Castagna et al. (1996) reported husk values between 25 and 32% of hulled yield, as a mean of applied N treatments.

Results of this study showed that the percentage of husk was influenced by field location and N amount at ear filling. No significant differences were recorded in the number of spikelets per spike. The thousand kernel weight showed slightly lower values (3%) in unfertilized plots (46 g) than in fertilized plots (49.2 g); whereas, the number of kernels per square meter in fertilized plots was double that in unfertilized plots.

Furthermore, a positive effect of N supply on the grain protein content was observed, as previously documented by Castagna et al. (1992, 1996), Mariani et al. (1992), de Giorgio et al. (1995) and Marino et al. (2009, 2011).

In this study, the improvement in productive traits in fertilized conditions was consistent with changes in gas exchange. The N supply induced an increase in photosynthetic rate and stomatal conductance in this Italian emmer population. However, the functionality of the photosynthetic processes, defined by the response of photosynthesis to increasing irradiance and C_i, was not impaired without N supply. Nevo et al. (1991) found that the highest photosynthetic efficiency was displayed by emmer populations of the xeric marginal region of Israel, but most variation for photosynthetic capacity occurred between accessions within ecogeographical regions and populations. Populations and genotypes of emmer, having intrinsically higher photosynthetic capacity could, therefore, provide different responses to N supply. About 20% of total leaf N constitutes the RuBP carboxylase protein and, thus, a differential N allocation could alter kinetic properties of the enzyme and consequently affect the CO_2 concentration at the site of carboxylation. The N supply can play a relevant role in determining high photosynthetic capacity, as an increased allocation of N would probably induce an up regulation of Rubisco in leaves (Gutiérrez et al., 2013). In a Mediterranean environment, N uptake has been found to decrease in enriched-CO_2 and/or water-limiting conditions due to stomatal closure, reducing transpiration (Long et al., 2004). Again, higher N availability in the soil may be immediately used by the developing grains, instead of being first incorporated into the leaf photosynthetic proteins (Madani et al., 2010). The N supply improved gas exchange and grain yield, suggesting a direct effect of N in the grain filling process of this emmer crop; high rates of N remobilization from leaves to grains could be associated with high grain protein concentration in emmer (Uauy et al., 2006).

Conclusion

Triticum dicoccum has great adaptability to severe environmental conditions not impairing of gas exchange and crop yield in marginal areas with low N availability. Emmer could be a potential crop for the development of low-input agriculture in internal poor lands in Italy. However, there is a lack of knowledge on the complex link among physiology, biomass and production of emmer under optimal and deficient N supply. The absence of N fertilization reduced yield performance, although physiological traits were only partially affected, emphasizing the plasticity of emmer and its adaptive potential to low-input farming systems. The main factors that led to differences in grain yield between N treatments were the number of spikes per square meter (N at tillering) and the hulled index (filling of the ear). Therefore, the spikes of fertilized plants were much more filled than those of unfertilized plants. Interesting results were obtained in unfertilized conditions, showing similar patterns in dry mass accumulation and photosynthetic capacity in both years of experiment. As reported for other cereals, N supply improved yield and physiological traits of emmer.

No fertilization emphasized the crop's plasticity and its adaptability to different environmental conditions, suggesting the value of using emmer in sustainable cultivation systems.

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