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Original article

Response of Pearl Millet to nitrogen as affected by water deficit

O. DIOUF[a]*, Y.C. BROU[b], M. DIOUF[a], B. SARR[a], M. EYLETTERS[b], H. ROY-MACAULEY[a], J.P. DELHAYE[b]

[a] Centre d'Étude Régional pour l'Amélioration de l'Adaptation à la Sécheresse (Ceraas/Isra/Coraf), BP 3320, Thies Escale, Thies, Senegal
[b] Université Libre de Bruxelles, Av. F.-D. Roosevelt 50, CP 169, 1050 Bruxelles, Belgium

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Abstract – In the Sahelian zone, low soil N could be as limiting as drought in pearl millet production. Although growth and crop productivity depend on several biochemical reactions in which the nitrogen metabolism plays a great role, there is little information available on how N uptake and key enzymes, nitrate reductase and glutamine synthetase, are affected by nitrogen and water interaction in millet. For this purpose, the millet variety cv. Souna III was grown in the field during the dry season under three levels of nitrogen fertilization (N0 = 0.0, N1 = 17.13, and N2 = 68.50 kg N ha^{-1}) and different water regimes (well-watered and water-stressed) in a split-plot experimental design. Irrigation was stopped for water-stressed plants during tillering, and the grain formation and filling phases, thereby giving rise to two water deficit cycles. A major quantity of mobilized N (79–100%) was taken up before flowering in all N treatments. Nitrogen uptake declined significantly only during the second water deficit cycle. During the first water deficit cycle, aboveground biomass was reduced and the maintenance of the N uptake resulted in increased N and nitrate concentrations. The water deficit reduced nitrate reductase activity in all treatments and the effect was greater under high N. The increase in nitrate concentration under water deficit conditions showed that the reduction in nitrate reductase activity was probably not due to limiting nitrates. Glutamine synthetase activity was higher under the low N treatments, N1 and N0, showing the absence of a stimulating effect of glutamine synthetase activity by nitrate or ammonium. These results are discussed on the basis of their effect on grain N and grain yield.

drought adaptation / glutamine synthetase / nitrate reductase / nitrogen nutrition / *Pennisetum glaucum*

1. INTRODUCTION

The assimilation of nitrate in plants is catalyzed by the enzymes nitrate reductase (NR), nitrite reductase (NiR), glutamine synthetase (GS), glutamate synthase (GOGAT) and various aminotransferases [19, 21]. Nitrate reductase and glutamine synthetase appear as key enzymes in primary nitrogen assimilation [1, 20]. In particular, nitrate reductase is considered as the more rate-limiting enzyme [1]. On this basis, many authors have tried to correlate in cereals, notably for wheat, the dry matter production, the protein nitrogen and the grain yield with nitrate reductase activity [4, 6, 9, 23]. After contradicting reports, it now appears that nitrate reductase activity (NRA) is not directly related to yield [13] but measurement of NRA may be of some interest in studies of water stress tolerance [15, 16]. NRA is very susceptible to water stress but it recovers rapidly when water becomes available [15, 16, 29]. On the contrary, other enzymes of the reduction process (nitrite reductase and glutamine synthetase) are reduced less under water deficit conditions [31]. Nitrate reductase and glutamine synthetase activities are also reduced when nitrogen becomes limiting [2, 36].

In other respects, low soil N and drought are considered now as the main constraints to the productivity of different crops in the sahelian zone. However, growth and crop productivity also depend on various biochemical reactions playing a great role in the nitrogen metabolism. Despite this importance, there is little information available on how NRA and GSA are affected by nitrogen and water interaction in Pearl Millet. Therefore, the objective of this study was to characterize the interaction of soil moisture and soil nitrogen availability on nitrogen uptake, and nitrate reductase and glutamine synthetase activities in Pearl Millet.

2. MATERIALS AND METHODS

2.1. Description of the experimental site

Two trials were conducted in 1998 and 1999 during the dry season at the National Center for Agronomic Research (CNRA), Bambey, Senegal (14.42°N and 16.28°W) situated in the semi-arid zone. The soil of the experimental area has a sandy texture (91–94%), with low clay content (3.5–5.6%). The low organic matter content (0.27–0.34%), together with low clay content, induce a low buffering capacity with acidic pH (H_2O) of 5.7, a low water-holding capacity (75 mm m^{-1}) and a low cation exchange capacity (CEC) of 1.7–2.2 meq 100 g^{-1}. The climatic conditions and the amount of

* Corresponding author: ceraas@sentoo.sn

Table I. Water supply by irrigation and climatic conditions.

		Irrigation	PET	T max	T min	Incoming radiation
		mm	mm d^{-1}	°C		MJ m^{-2} d^{-1}
1998 experiment	W0	585	6.45	38.1	21.1	22.63
	W1	390				
1999 experiment	W0	507	6.43	37.5	20.5	22.34
	W1	370				

PET = potential evapotranspiration; W0 = well-watered regime and W1 = water-stressed regime.

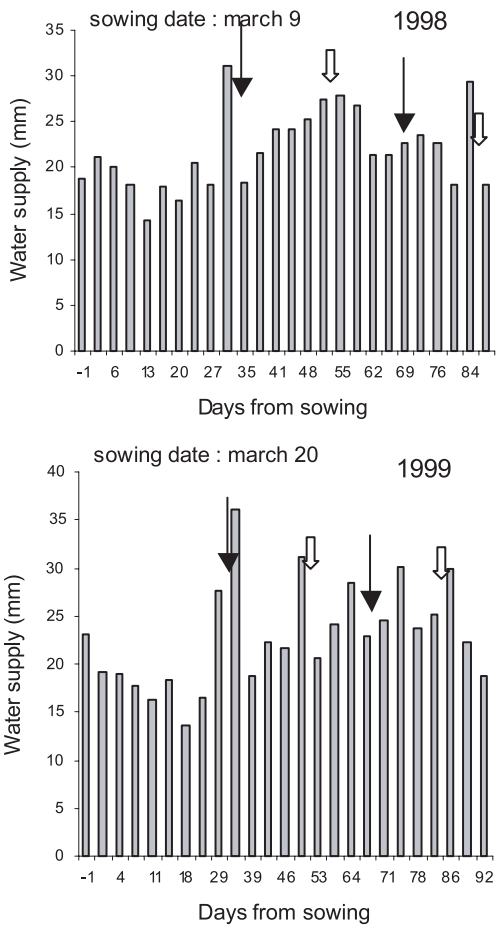

Figure 1. Pattern of water supplied by irrigation during the experiments in 1998 and 1999. The dark arrows and the white arrows indicate the date of the last irrigation of the stressed plants and the date of rewatering, respectively.

irrigation are presented in Table I and the pattern of water supply in Figure 1. The daily mean temperatures of both dry seasons were 29.6 °C in 1998 and 29 °C in 1999. These temperatures, associated with high incoming radiation, induced a high potential evapotranspiration or maximum water requirement.

The experimental design was a split-plot factorial based on three randomized complete blocks with water regimes as the main plot treatment and N rates as subplot treatment. The subplots were 10.8 m × 10.8 m separated by 2-m-wide alleys. The two water regimes were: well-irrigated (W0) control and water-stressed (W1). The water-stressed regime was applied by suspending irrigation first during the tillering and internode elongation stage (at 30 days after sowing (das) in 1998 and 29 das in 1999) for 22 d in 1998 and 21 d in 1999, and secondly during the grain formation and filling stage (at 69 das in 1998 and 67 das in 1999), for 17 d in 1998 and 14 d in 1999. Three nitrogen treatments were applied in the subplots at the rates of 0.0 (N0), 17.1 (N1) and 68.5 (N2) kg N ha^{-1}, with the maximum corresponding to the recommended dose in Senegal. The nitrogen content at sowing was 0.17‰. The nitrogen rates were incorporated into three parts as follows: one-third at seedling emergence as 15-15-15 fertilizer, one-third at 2 wk and one-third at 5 wk after planting as broadcast urea. In addition, all subplots were fertilized at seedling emergence at the same rate with 22.5 kg P ha^{-1} as ordinary superphosphate and 22.5 kg K ha^{-1} as KCl, taking into account the P and K input from the 15-15-15 fertilization. The combination of the two factors, water regime and nitrogen fertilization, led to six treatments referred to as W0N0, W0N1, W0N2, W1N0, W1N1 and W1N2.

The plant material used in this study was a 90-day variety of millet (cv. Souna III), which is cultivated throughout the Sahel region. Seeds were sown in 3- to 5-cm-deep holes made in the soil with a traditional hoe. The spacing between seed holes and between rows was 0.9 m to obtain a density of 12 345 seed holes ha^{-1}. Two weeks after planting, plants were thinned to three individuals per seed hole. Insect attack, disease development and weed proliferation were controlled using the appropriate chemicals (DECIS: deltamethrine; SPINOX TBC: thirame, benomyl and carbofuran).

2.2. Measurements

2.2.1. Nitrate and total nitrogen contents

The standard Kjeldhal method was used to determine the nitrate and total nitrogen contents of leaves. Nitrogen content was determined at different phases of the growing cycle and nitrate content at the end of each water deficit cycle. Three plants were harvested on each plot and stems, leaves and grains were separated. After drying, the same organs of the same treatment were mixed, giving a composite sample.

2.2.2. Leaf water potential (Ψ_f)

The leaf water potential (Ψ_f) was measured on the third leaf from the top between 11:30 h and 13:30 h with a hydraulic press (Campbell J14 Instruments, [17]) which was earlier calibrated with psychrometers (C30, Wescor). Three plants were measured per plot, giving nine measurements for each treatment.

2.2.3. Enzymatic activities

Enzymatic activities were determined during periods of water deficit in the 1998 experiment only. In the field, leaf samples (about 20 g) from the third leaves of three plants of each plot were immediately fixed in liquid nitrogen and stocked at –80 °C before lyophilization.

2.2.3.1. In vitro assay of nitrate reductase activity (NRA)

Lyophilized leaf material (0.5 g) was ground with liquid nitrogen, and 4 ml of potassium phosphate extraction buffer (0.1 M, pH 7.5) containing 1 mM EDTA, 1.5% casein and 7.5 mM cystein was added as described previously [27]. After grinding, the suspension was centrifuged (35 000 *g*, 30 min, 4 °C) and the supernatant was used for assay. NRA was determined in vitro according to Conejero et al. [8]. Supernatant (0.1 ml) was added to 0.7 ml of 0.1 M potassium phosphate mixture buffer, pH 7.5, containing 150 µM NADH. With the objective of determining only actual NRA, the mixture was kept free of KNO_3. The reaction was carried out at 30 °C and after 15 min the reaction was stopped by adding 0.1 ml zinc acetate (1 M). After centrifugation for 10 min at 10 000 *g*, the nitrite formed was revealed by diazotation (1 ml sulfanilamide 1% in 1.5 N HCl + 1 ml N-naphtyl-ethylene-diamino-dichloride 0.02%) and the absorbance was measured calorimetrically at 540 nm. Each treatment was repeated three times.

2.2.3.2. Assay of glutamine synthetase (GSA)

Glutamine synthetase activity was measured by the formation of γ-glutamyl hydroxamate in the presence of hydroxylamine according to the method described by O'neal and Joy [25]. The extraction buffer contained 50 mM Tris HCl, pH 7.6, 1 mM EDTA, 1 mM DTT, 10 mM $MgCl_2$, 10 mM β-mercaptoethanol, 1 mM reduced glutathione and glycerol 10%. Glutamine synthetase activity was essayed at pH 7.6 in 2 ml 50 mM Tris HCl containing 18 mM ATP, 92 mM glutamate, 6 mM NH_2OH and 45 mM $MgSO_4$. After incubation with 250 µl of crude enzyme extract for 30 min at 30 °C, adding 1 ml of a solution containing 0.37 M $FeCl_3$, 0.67 N HCl and 0.2 M TCA stopped the reaction. The absorbance of the supernatant after centrifugation for 10 min at 10 000 *g* was measured at 540 nm. Each treatment was repeated three times. The quantity of γ-glutamyl hydroxamate was determined from a standard curve formed using authentic γ-glutamyl hydroxamate [25, 26].

2.2.3.3. Protein determination

The protein content of the samples from each plot was determined with Coomassie Blue reagent (Bio-Rad Protein Assay) and BSA as a standard [5].

2.3. Statistical analysis

The data were subjected to a factorial analysis of variance (ANOVA) with STAT-ITCF and SAS Institute software. The data were checked for normality (Lilliefors' test) and homogeneity (Bartlett's test) of variances and log or square root transformations were done when necessary. Analysis of variance was used to determine, within a season, the effects of water regimes, N fertilizer and interactions on studied parameters.

3. RESULTS

The leaf water potential (Ψ_f) of well-watered plants varied between –0.49 and –1.1 MPa in 1998 and between –0.55 and –1.04 MPa in 1999 without significant difference between N fertilization treatments (Fig. 2). In 1998, after stopping irrigation, the decrease in Ψ_f was significantly more rapid for N1 and N2 compared with N0. In fact, at 45 das for the first stress cycle, and at 77 and 80 das for the second, Ψ_f of N1 and N2 was significantly lower than that of N0 (Fig. 2a). On the contrary, in 1999, N fertilization had no significant effect on the decrease in Ψ_f during the first stress cycle (Fig. 2b). On the other hand, it induced significant differences during the second stress cycle. Thus at 72 and 77 das, the Ψ_f of N0 and N1 were greater than that of N2, while at 79 das the Ψ_f of N0 remained greater than the values of N1 and N2 (Fig. 2b).

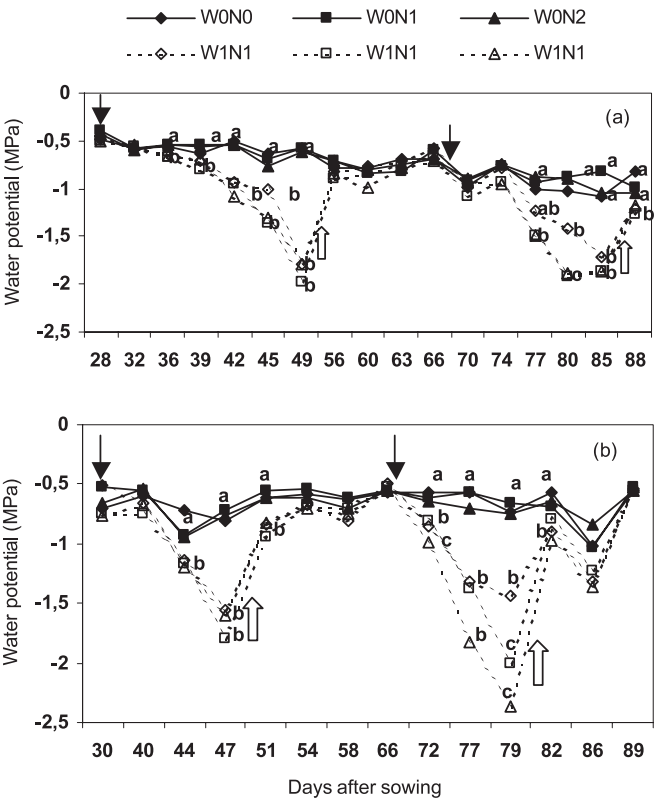

Figure 2. Changes in leaf water potential *(l)* in 1998 (a) and 1999 (b) dry seasons. N0 = 0.0, N1 = 17.1 and N2 = 68.5 kg N ha^{-1}; W0 = well-watered regime and W1 = water-stressed regime. The dark arrows and the white arrows indicate the date of the last irrigation of the stressed plants and the date of rewatering, respectively. For a given date of measurement, means followed by different letters are significantly different at $P < 0.05$ according to the Student Newman Keul's range test.

In 1998, the analysis of variance within growth stages indicates a significant effect of N fertilization on N uptake from 49 days after sowing (das) to the end of the growth cycle (Fig. 3). During this period, N uptake increased with the increase in the N rate. The effect of the first water deficit cycle on N uptake was not significant ($P < 0.05$); however, the N uptake by the W1N2 treatment during the period of rewatering (Fig. 3) was greater than by W0N2 (positive delay-effect). The second water deficit induced a reduction in N uptake that became statistically significant at the end of the growth cycle.

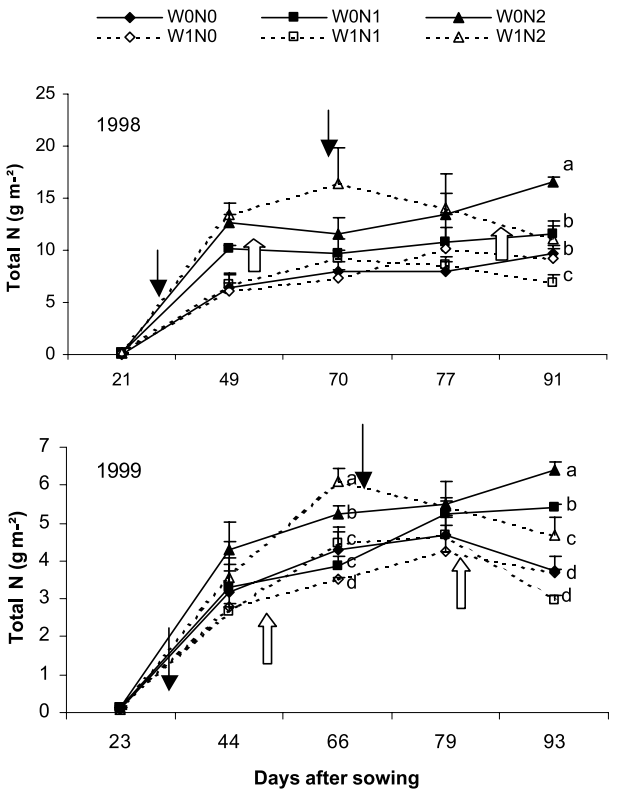

Figure 3. N accumulated by aboveground dry matter for all treatments. N0 = 0.0, N1 = 17.1 and N2 = 68.5 kg N ha^{-1}; W0 = well-watered regime and W1 = water-stressed regime. For a given date of measurement, means followed by different letters are significantly different at $P < 0.05$ according to the Student Newman Keul's range test. The dark arrows and the white arrows indicate the date of the last irrigation of the stressed plants and the date of rewatering, respectively.

However, this reduction occurred later for the W1N0 treatment comparatively with W1N1 and W1N2. At the end of the crop cycle, the effect of nitrogen rates on total N uptake was significantly affected by water regime.

In 1999, the first water deficit cycle reduced N uptake but this effect was not significant (Fig. 3). During the period of rewatering, at 66 das, the effect of nitrogen rates on total N uptake was significantly affected by water regime. As previously, the first water deficit induced a greater N uptake for W1N2 during the period of rewatering (positive delay-effect). During the second water deficit, N uptake declined significantly. As in 1998, the effect of nitrogen rates on total N uptake was significantly affected by water regime, and the decline in N uptake began earlier with W1N2. These effects of N fertilizer rates with respect to accumulated N were also shown by the variations in the rate of N uptake (Tabs. II and III) as a function of the water regime.

The first water deficit cycle induced an increase in N concentration in organs of stressed plants, especially in the W1N2 treatment (Tab. IV). The increase in total N concentration under water deficit was associated with an increase in nitrate concentration.

Table II. Rate of N uptake (mg m^{-2} d^{-1}) between the different dates of measurement in 1998 (calculated from means).

	Well watered conditions			Water stressed conditions		
das	N0	N1	N2	N0	N1	N2
21-49	228	361.2	444.1	211.9	233.0	478.3
49-70	75.3	–28.5	–45.9	65.5	124.9	141.5
70-77	19.4	158.4	267.1	382.7	–102.1	–347.3
77-91	126.2	64.1	215.8	–130.5	–251.5	–428.8

Table III. Rate of N uptake (mg m^{-2} d^{-1}) between the different dates of measurement in 1999 (calculated from means).

	Well watered conditions			Water stressed conditions		
Das	N0	N1	N2	N0	N1	N2
23-44	147.3	152.7	199.8	128.4	122.0	165.8
44-66	50.6	25.2	42.0	31.9	80.8	115.4
66-79	27.7	103.8	21.5	58.5	16.1	–49.2
79-93	–67.8	13.6	64.3	–41.4	–120.7	–57.1

The effect of nitrogen rates on biomass N accumulation and grain N yield was significantly affected by the water regime (Tab. V). Thus, under well-watered conditions, the amount of N exported by both biomass and grain increased with N fertilization. However, under water deficit conditions, although N2 presented more biomass N, N0 allocated a greater amount of N to the grains.

Under well-watered conditions and at the first stages of the growth cycle, NRA was generally higher in the N2 treatment than the N0 and N1 treatments (Tab. VI). This was also the case at the end of the growth cycle (85 and 88 das) when senescence increased and NRA decreased accordingly. This decrease was more significant for N0 and N1 than for N2, probably due to the higher N uptake in N2.

Under water deficit conditions, the NRA of all N treatments decreased with the decrease in leaf water potential (Tab. VI). During the first water deficit, the reduction in NRA was most significant in N2 and even decreased down to zero at the end of the water deficit period (49 das). During the second water deficit cycle, the nitrate concentration being least significant in N2 (Tab. IV), the NRA of stressed plants was reduced in a similar manner in all N treatments. Measurements made 5 days after rewatering showed that NRA was completely recovered and, surprisingly, plants which had been submitted to water shortage previously, presented a higher NRA than those which had not.

In both watering regimes, glutamine synthetase activity (GSA) was higher under low N treatments (Tab. VII). Towards the end of the first water deficit cycle, the GSA of water-stressed plants had recovered after its reduction at 45 das. This behavior was also observed during the second water deficit cycle with even higher GSA in stressed plants (W1N1 and W1N2) compared with watered plants.

Table IV. Total N and nitrate concentrations of leaves at the end of each water deficit cycle.

	1998 dry season				1999 dry season			
	1st water stress		2nd water stress		1st water stress		2nd water stress	
	N (%)	NO_3 (%)	N (%)	NO_3 (%)	N (%)	NO_3 (%)	N (%)	NO_3 (%)
W0N0	1.83	0.10	0.91	0.06	2.38	0.22	0.79	0.014
W0N1	2.26	0.14	1.38	0.07	2.42	0.11	0.89	0.095
W0N2	2.43	0.18	1.46	0.07	3.02	0.15	0.90	0.018
W1N0	2.10	0.17	1.74	0.07	2.33	0.11	0.88	0.031
W1N1	2.63	0.25	1.95	0.10	2.58	0.30	0.99	0.053
W1N2	3.15	0.57	2.54	0.21	3.18	0.14	1.4	0.053

N0 = 0.0, N1 = 17.1 and N2 = 68.5 kg N ha^{-1}; W0 = well-watered regime and W1 = water-stressed regime.

Table V. Biomass N and grain N at harvest in 1998 and 1999.

	1998		1999	
	Biomass (g N m^{-2})	Grain (g N m^{-2})	Biomass (g N m^{-2})	Grain (g N m^{-2})
		Water regime		
W0	12.60a s.e. 3.68	5.35a s.e. 1.52	5.18a s.e. 1.37	2.11a s.e. 0.72
W1	9.01b s.e. 2.69	1.7b s.e. 0.90	3.78b s.e. 0.99	0.70b s.e. 0.27
		Nitrogen fertilization		
N0	9.42b s.e 1.2	3.16a s.e. 0.95	3.71b s.e. 0.35	1.08b s.e. 0.32
N1	9.22b s.e. 2.64	3.36a s.e. 2.26	4.24b s.e. 1.22	1.53a s.e. 1.02
N2	13.77a s.e. 2.97	4.06a s.e. 2.58	5.53a s.e. 0.97	1.61a s.e. 0.89
		Interaction		
W0N0	9.67b s.e. 0.34	3.91b s.e. 0.40	3.72d s.e. 0.06	1.33b s.e. 0.02
W0N1	11.62b s.e. 0.90	5.57a s.e. 0.30	5.42b s.e. 0.08	2.52a s.e. 0.19
W0N2	16.51a s.e. 0.41	6.57a s.e. 0.51	6.46a s.e. 0.13	2.47a s.e. 0.19
W1N0	9.17b s.e. 1.12	2.41c s.e. 0.42	3.69d s.e. 0.35	0.82cb s.e. 0.19
W1N1	6.82c s.e. 0.63	1.15c s.e. 0.38	2.98d s.e. 0.08	0.53c s.e. 0.09
W1N2	11.02b s.e. 1.05	1.54c s.e. 0.28	4.66c s.e. 0.41	0.74cb s.e. 0.07

For a given parameter and effect, along the columns, means followed by the same letter are not significantly different at $P < 0.05$ according to Student Newman Keul's range test; N0 = 0.0, N1 = 17.1, and N2 = 68.5 kg N ha^{-1}; W0 = well-watered regime and W0 = water-stressed regime; s.e. = standard error of means.

4. DISCUSSION

The N uptake pattern showed that the majority of mobilized N (79 to 100%) was taken up before flowering in all treatments. These results are similar to those obtained in wheat by Clarke et al. [7]. The decline in N mobilized at the end of the growth cycle could be explained by the decrease in the rate of N uptake, and the loss of dead leaves.

Contrary to low N, the increasing effect of high N on leaf area index (LAI) and biomass (data not shown) could be related to greater transpiration and therefore to lower water potentials. The improvement in N uptake during rewatering for stressed plants compared with well-watered plants was consistent with the fact that water deficit promoted the downward growth of roots [37] and also had effects on soil mineralizing and, therefore, would allow a better uptake of N. The maintenance of better water status in low N treatments was associated with the late decline in N uptake.

Aboveground biomass being reduced by the water deficit, the increase in N concentration within organs of stressed plants could be due to the maintenance of the N uptake observed during the first water deficit cycle, or to the lesser dilution of N (already absorbed) in the tissues due to lesser growth. This increase in N concentration was more significant during

Table VI. Nitrate reductase activity (NRA) and corresponding leaf water potential (Ψ_f) during periods of water deficit in 1998.

	W0N0	W1N0	W0N1	W1N1	W0N2	W1N2
Days of stress			First water deficit			
6 (36 das)	2.41b s.e 0	1.31d (54) s.e. 0.03	2.29b s.e. 0.14	1.71c (75) s.e. 0.08	2.78a s.e. 0.10	2.07b (74) s.e. 0.05
Ψ_f	–0.55	–0.64	–0.54	–0.67	–0.54	–0.65
15 (45 das)	1.13b s.e. 0.02	0.58d (51) s.e. 0.09	1.81a s.e. 0.11	0.98cb (54) s.e. 0.02	2.04a s.e. 0.06	0.82c (40) s.e. 0.06
Ψ_f	–0.64	–1.00	–0.68	–1.37	–0.75	–1.30
19 (49 das)	1.73b s.e. 0.01	0.83e (48) s.e. 0.05	1.56c s.e. 0.05	1.09d (70) s.e. 0.04	1.89a s.e. 0.02	0.0f (0) s.e. 0
Ψ_f	–0.57	–1.79	–0.57	–1.98	–0.61	–1.80
			Second water deficit			
5 (74 das)	1.69a s.e. 0.06	0.77c (46) s.e. 0.04	1.80a s.e. 0.04	1.03b (57) s.e. 0.04	1.76a s.e. 0.06	0.61c (35) s.e. 0.03
Ψ_f	–0.74	–0.78	–0.77	–0.92	–0.75	–0.96
8 (77 das)	2.15a s.e. 0.05	0.72c (33) s.e. 0.04	2.17a s.e. 0.07	0.87c (40) s.e. 0.06	2.06a s.e. 0.02	0.83c (40) s.e. 0.03
Ψ_f	–1.00	–1.23	–0.93	–1.49	–0.88	–1.49
15 (85 das)	0.45c s.e. 0	0.21c (47) s.e. 0.03	0.80b s.e. 0.14	0.35c (44) s.e. 0	1.18a s.e. 0.28	0.42c (36) s.e. 0.01
Ψ_f	–1.08	–1.72	–0.82	–1.86	–1.05	–1.87
			Rewatering			
88 das	0.46c s.e. 0.03	2.45a s.e. 0.06	0.39c s.e. 0.02	2.55a s.e. 0.12	1.39b s.e. 0.21	2.62a s.e. 0.02
Ψ_f	–0.81	–1.21	–1.00	–1.28	–1.04	–1.18

NRA, (µmol NO_2 g^{-1} h^{-1}); Ψ_f = water potential (negative values) in MPa; NRA of stressed plants are expressed as percentage of activity of well-watered plants (values between brackets); Das = day after sowing. For a given variable and effect, along the rows, means followed by the same letter are not significantly different at $P < 0.05$ according to Student Newman Keul's range test. N0 = 0.0, N1 = 17.1, and N2 = 68.5 kg N ha^{-1}; W0 = well-watered regime and W1 = water-stressed regime; s.e. = standard error of means.

Table VII. Glutamine synthetase activity (GSA) and corresponding leaf water potential (Ψ_f) during periods of water deficit in 1998.

	W0N0	W1N0	W0N1	W1N1	W0N2	W1N2
Days of stress			First water deficit			
6 (36 das)	2.62a s.e. 0.002	2.42b (92) s.e. 0.08	2.72a s.e. 0.06	1.87c (69) s.e. 0.02	1.71d s.e. 0.04	1.4e (82) s.e. 0.02
Ψ_f	–0.55	–0.64	–0.54	–0.67	–0.54	–0.65
15 (45 das)	1.12a s.e. 0.01	1.05b (94) s.e. 0.01	0.79d s.e. 0.01	0.71e (90) s.e. 0.01	0.85c s.e. 0.005	0.68e (80) s.e. 0.002
Ψ_f	–0.64	–1.00	–0.68	–1.37	–0.75	–1.30
19 (49 das)	1.59a s.e. 0.05	1.61a (100) s.e. 0.01	1.34b s.e. 0.02	1.29b (96) s.e. 0.004	1.06c s.e. 0.02	1.04c (98) 0.01
Ψ_f	–0.57	–1.79	–0.57	–1.98	–0.61	–1.80
			Second water deficit			
5 (74 das)	1.18a s.e. 0.04	0.60c (36) s.e. 0.02	0.91b s.e. 0.02	0.43d (47) s.e. 0.01	0.67c s.e. 0.05	0.69c (100) s.e. 0.03
Ψ_f	–0.74	–0.78	–0.77	–0.92	–0.75	–0.96
8 (77 das)	2.44a s.e. 0.05	1.75c (71) s.e. 0.01	1.92b s.e. 0.03	1.57d (82) s.e. 0.004	1.59d s.e. 0.04	1.43e (90) s.e. 0.03
Ψ_f	–1.00	–1.23	–0.93	–1.49	–0.88	–1.49
16 (85 das)	1.70a s.e. 0.03	1.29c (76) s.e. 0.01	1.18d s.e. 0.01	1.36b (115) s.e. 0.03	0.68f s.e. 0.01	1.00e (147) s.e. 0.006
Ψ_f	–1.08	–1.72	–0.82	–1.86	–1.05	–1.87
			Rewatering			
88 das	1.16b s.e. 0.02	0.96c (83) s.e. 0.009	1.68a s.e. 0.06	0.96c (57) s.e. 0.007	0.58e s.e. 0.006	0.81d (140) s.e. 0.02
Ψ_f	–0.81	–1.21	–1.00	–1.28	–1.04	–1.18

GSA (µmol GH mg^{-1} min^{-1}); Ψ_f = water potential (negative values) in MPa; GSA of stressed plants are expressed as percentage of activity of well-watered plants (values between brackets); Das = day after sowing. For a given variable and effect, along the rows, means followed by the same letter are not significantly different at $P < 0.05$ according to Student Newman Keul's range test. N0 = 0.0, N1 = 17.1, and N2 = 68.5 kg N ha^{-1}; W0 = well watered regime and W1 = water-stressed regime; s.e. = standard error of means.

the first water deficit when the major quantity of mobilized N was absorbed. Total N uptake was more significant in 1998 (minimum temperature 20.6 °C) than 1999 (minimum temperature 17 °C), probably because of low temperatures occurring during the first growth stages in 1999, resulting in low rates of N uptake (Tabs. II and III). Therefore, the increase in N and nitrate concentration were higher in 1998. Such a nitrate accumulation under water deficit conditions has previously been reported [31, 33] and occurred under conditions of high N availability when N absorbed was high. The accumulation of nitrate suggests the existence of decreasing nitrate reductase activity, the decrease not being due to limited nitrate availability. Similar responses have been observed in maize [22, 31], barley [14] and wheat [11, 16]. The greatest decrease (down to zero) in NRA observed under the W1N2 treatment at the end of the water deficit (49 das) occurred when the water potential values of stressed plants had reached low values similar for all N treatments. The higher reduction of NRA in W1N2 is associated with a higher nitrate concentration [31] which could have induced a retro-inhibition action or an unbalance of ionic strength. These results were similar to those obtained by Golberg et al. [11] studying the response of wheat to drought and nitrogen availability.

The complete recovery in NRA has also been reported in wheat by Jonas et al. [16] and Golberg et al. [11]. The decrease during the stress period could be due to an inactivation or degradation of the enzyme or an inhibition of its synthesis [22, 31]. The fast recovery of NRA suggested that the enzyme was probably preserved by inactivation (no degradation or inhibition of synthesis capacity) and therefore, de novo synthesis should not be an adaptive response [22]. Furthermore, the increase in NRA after rewatering could also be due to a modification of the cellular compartment that gives nitrate more access to induction sites [11]. In this case, the nitrate accumulation observed in stressed plants may be related to the role of nitrate as osmoticum [33] although osmotic adjustment is low in millet [10].

The higher GSA under low N treatments, N1 and N0, showed the absence of the stimulating effect of GSA by nitrate or ammonium as reported by other authors [12, 24]. However, an increase in GSA was noted after addition of nitrate but not of ammonium to the roots of pea [36]. In pine, the stimulating effect of nitrate was observed only in the roots and not in other organs [28]. In this last species, nitrate fertilizer also induced a small increase in GSA. The stimulation of GSA by nitrate and ammonium appears, therefore, difficult to generalize across species. Also, its variability between plants is high. An advantage in water-stressed plants of higher GSA under high N conditions than under low N conditions could be to avoid the accumulation of ammonium [34, 35] and the resulting ammonium-toxicity syndrome [18].

The second water deficit cycle, corresponding to a period of low N uptake, reduced NRA in a similar way for all N treatments. Despite the higher biomass N observed in high N treatments, grain N was higher in low N treatments (Tab. V). This response suggested a higher remobilization of nitrogen compounds from stems and leaves to grains under low N. In fact, GS plays a major role in the reassimilation of endogenously-generated ammonium during the N remobilization process [3, 28, 30]. In addition, these N remobilization processes and the transport of soluble N compounds such as glutamine require an adequate water status [32]. The higher GSA and adequate water status observed under the W1N0 treatment favor the hypothesis that this remobilization could contribute to the higher grain N observed (Tab. V) in low N treatments. This high grain N was associated with higher grain yield (data not shown).

These results showed a significant interaction effect between water and nitrogen on water potential, biomass N, grain N, NRA and GSA, and suggest that a low fertility level reduces the risk of crop failure in drought-prone areas. The improvement of cropping systems aimed at reducing variability in crop productivity demands the implementation of N fertilization practice as a function of climatic risks.

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