

Reduction of drought stress effect in spring barley (*Hordeum vulgare* L.) by nitrogen fertilization

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ABSTRACT

An effect of nitrogen rates (0.0 g, 1.0 g, 2.0 g N per pot) on NRA (nitrate reductase activity) in leaves of spring barley (cultivar Kompakt) was investigated in a pot experiment. Plants were grown under optimum moisture regime and drought stress was induced during the growth stages of tillering, shooting and earing. Before and after respective stress period plants were grown under optimal water regime. In all the fertilized and unfertilized treatments, NRA was significantly higher under optimal water regime than in drought stress conditions. Nitrogen fertilization alleviated adverse effects of drought stress on the yields of grain; the rate of 1 g N per pot increased the grain yield of plants stressed during tillering 3.73 times compared to unfertilized and stressed treatment. When the stress was induced during shooting or earing grain yields declined by over 50% compared to optimal water regime; when compared with stressed and unfertilized treatment, the rate of 1 g N however increased yield by 29% (stress at shooting) and 55% (stress at earing). NRA values were significantly higher when plants were grown under optimum water regime than under stress conditions as well as when fertilized with nitrogen compared to unfertilized control both under optimum water regime and drought stress.

Keywords: spring barley; nitrogen fertilization; growth stage; drought stress; nitrate reductase activity (NRA)

Nitrogen is one of the most important mineral nutrients for plants and is taken up by the root system predominantly in inorganic forms (NH_4^+ and NO_3^-). Plants assimilate nitrogen as a source for growth, biomass production and development. Nitrogen is mainly absorbed as nitrate, which is the most common nitrogen source available for higher plants.

Nitrate assimilation is the primary pathway by which reduced nitrogen is accumulated in plants; that involves a consecutive action of two enzymes: nitrate reductase (NR), a cytosolic enzyme that reduces nitrate to nitrite using NADPH as electron donor, and nitrite reductase (NiR), a plastidic enzyme that reduces nitrite to ammonium (Druart et al. 2000).

Nitrate reduction and its further assimilation represent a major metabolic function (Crawford et al. 1992). Nitrate reductase activity (NRA) is hence considered to be a limiting factor for higher plant protein production, development and growth (Huppe and Turpin 1994). It is generally accepted

that drought stress has a negative effect on plant photosynthetic activity, N concentrations, free aminoacids or soluble protein contents accompanied with a decline of nitrate reductase activity in many plant species, such as winter wheat (Xu and Yu 2006), potato (Ghosh et al. 2000), maize (Foyer et al. 1998) or other plants (Xu and Zhou 2005). In all these experiments a correlation was observed between the extractable foliar NR activity and CO_2 assimilation decrease, which indicates a co-regulation of C and N metabolisms in higher plants. As energy and C skeletons required for N assimilation are provided by photosynthesis, a high rate of CO_2 assimilation favours a high rate of N assimilation (and thus high leaf NR activity) and vice versa. The NR activity decline during water stress is mainly attributed to low NO_3^- absorption and availability resulting from water uptake deprivation (Ferrario-Méry et al. 1998). The experiments with *Cassia angustifolia* Vahl. plants showed that N (in the form of urea) applied on leaves in the vegetative stage of growth at the end of the plant dehydra-

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tion period ($\Psi_w = -2$ MPa) increased the net CO_2 assimilation by 30%, compared with the plants exposed only to water dehydration. The plants treated with high N under water stress reduced leaf area but kept their abaxial stomata open; it led to maintaining the CO_2 availability for assimilation and consequently to WUE (water use efficiency) enhancement (Ratnayaka and Kincaid 2005).

In various examined crop species, NRA was often shown to decline due to water stress (Dubey and Pessarakli 1995). In field-grown wheat plants, imposition of water stress caused a gradual decline in NRA in leaves (Kathju et al. 1990); these researchers also reported that in wheat plants, the intensity of water stress increasing progressively for 3 to 9 days reduced NRA under both low and high NP fertility conditions at different plants, in a lesser extent at higher fertilizing level than in low-fertilizing treatments. In a field experiment two barley varieties and one durum wheat variety were subjected to irrigation at different rates in relatively dry Mediterranean environment with different nitrogen fertility. Although decreasing irrigation water in soil caused a drop of plant leaf water potential from -1.5 to -3.0 MPa, nitrate reductase activity of the leaves in these plants in the stage of heading was unaffected or slightly increased; on the other hand, it was the highest in the plants growing with an ample nitrogen supply irrespective of water regime (Smirnov et al. 1985).

Svobodová and Míša (2004) found that water deficit at stem elongation caused a withering out of the established tillers, drought during the formation of the florets reduced their number as well as their development into grains. In the variant comprising water stress at the beginning of stem elongation, the plants were able to compensate for stress implications by productive tillers that developed later (at stem elongation). The previous water deficit did not decrease 1000-grain weight, however protein content in grain increased due to low grain yield per pot.

In maize plants desiccation leads to a steady decrease of NRA with a concomitant decrease in leaf water potential, leaf NO_3^- flux (Wasnik et al. 1988). When rewatered, water-stressed maize plants recovered partially and showed an increase in NRA and NO_3^- flux (Shaner and Boyer 1976). To overcome, at least partially, the effect of drought stress, some measures have been suggested. By increasing soil fertility, especially with nitrogenous fertilizers the adverse effect of drought can be alleviated substantially (Lahiri 1980). In *Proposis alba*, leaf relative water content, nitrate content

and nitrate reductase activity were also decreased under salinity stress (Meloni et al. 2004). It was estimated that the stress resulting from climatic and soil conditions (abiotic factors) that are sub-optimal caused that the yield of field-grown crops in the United States is only 22% of the genetic potential yield (Boyer 1982).

Drought and limited soil nitrogen belong among the most important environmental constraints that limit both crop choice and productivity in a wide range of agricultural ecosystems. Adoption of crops that are drought-tolerant and responsive to low levels of nitrogen application is a cost-effective and environmentally sound way of farming in drought-prone soils with nitrogen deficiency.

The aim of this work was to investigate the effect of water stress applied at various growth stages of spring barley grown under different nitrogen nutrition on NRA in leaves.

MATERIAL AND METHODS

An effect of water stress and nitrogen rates on NR activity in leaves of spring barley (cultivar Kompakt) was investigated in a pot vegetation experiment at atmospheric conditions during two growing seasons (2005 and 2006). Individual pots were filled with 16 kg of sieved soil (Haplic-Luvisols) with the agrochemical characteristics stated in Table 1. Contents of P, K, Ca and Mg in the soil were determined by Mehlich II method and N_{in} as the sum of NH_4^+ -N (colorimetrically by Nessler agent) and NO_3^- -N (colorimetrically by 2,4-disulphonic acid) after previous soil extraction in 1% K_2SO_4 . Following rates of N per pot were applied: 0.0 g (treat. 1 – control), 1.0 g (treat. 2), 2.0 g (treat. 3) in the form of liquid N-fertilizer DAM-390. Originally, 32 grains were sown into surface soil layer of each pot and 7 days after emergence the seedlings were thinned to 22 most vigorous and approximately equal plants. Each treatment was repeated 4 times. The plants were grown within two blocks at the same nutrition treatments. Plants in the first block were grown under optimum soil moisture regime (60% of full soil water retention capacity – FSWRC); in the second block drought stress was applied on plants during the growth stage of tillering, shooting and earing. During respective stress period soil water content in pots was maintained on the average level of 15–20% of FSWRC. Before and after the stress period the plants were further grown under optimum water regime until the end of growing

Table 1. Agrochemical characteristics of soil used in experimental pots

Year	pH _{KCl}	N _{in}	P	K	Ca	Mg
2005	6.0	12.8	42	262	2139	492
2006	5.8	9.8	46	186	1913	404
Average	5.9	11.3	44	224	2026	448

N_{in} – inorganic nitrogen

season. Samples of leaf material were taken after the stress regime was ended and NRA was determined by Jaworski (1971) method in a modification by Barker (1974) as follows: leaf samples (200 mg) were placed into 10 ml incubation mixture containing 0.1M K-phosphate buffer (pH 7.2), 0.1M KNO₃ and 1 vol. % 1-propanol for 2 h at 25°C in dark. Colour manifestation of the reaction was achieved by adding 0.2 ml of sulphanil acid and then 0.2 ml of α-naphthylamine. Obtained colour complex was stabilized with sodium acetate. Absorption was measured at 520 nm colorimetrically. The achieved results were treated statistically by the Student *t*-test at the significance level of 0.05.

RESULTS AND DISCUSSION

Measurements of NRA (nitrate reductase activity) in leaves of fertilized and unfertilized plants of spring barley showed that NRA values were significantly higher under optimal water regime than in respective treatments of plants exposed to drought stress (Figures 1–3). Decreasing NRA of leaves under drought stress was observed at the

end of each investigated growth stage during which the barley plants were exposed to the stress. These results are in accordance with the findings of Kathju et al. (1990) who reported reduced N uptake from the soil and decreased activities of N assimilatory enzymes including nitrate reductase in plants growing in water stressed environment.

When the barley plants were exposed to drought stress during tillering phase (Figure 1) the highest value of NRA (15.9 nmol NO₂⁻/g fw/min) (fw = fresh weight) was obtained at treatment 3 (2 g N per pot). It was due to the stress-eliminating effect of applied nitrogen. Half rate of N (1 g per pot) acted in a very similar way (14.9 nmol NO₂⁻/g fw/min) and the difference was not significant. Within the same growth stage, but under optimum water regime, fertilization with N significantly increased NRA amounting to 34.3 nmol NO₂⁻/g fw/min in treatment 2; double rate of N was less effective compared to treatment 2 (25.0 nmol NO₂⁻/g fw/min) although NRA was still higher than in fertilized treatments under stress conditions.

In growth stage of shooting, NRA under optimal moisture conditions generally increased compared to growth stage of tillering (Figure 2).

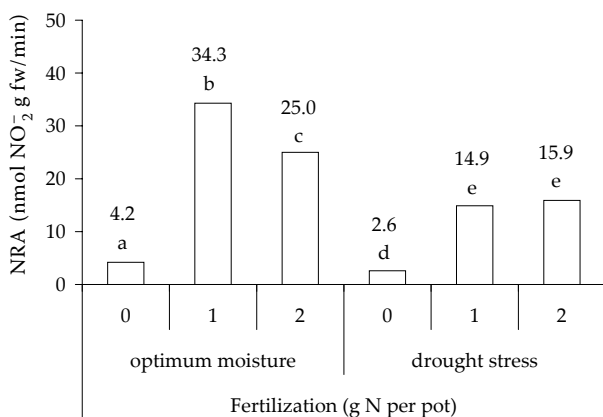


Figure 1. Activity of nitrate reductase in barley leaves in the growth stage of tillering – average of 2 years (the same letters in value columns indicate non-significant differences)

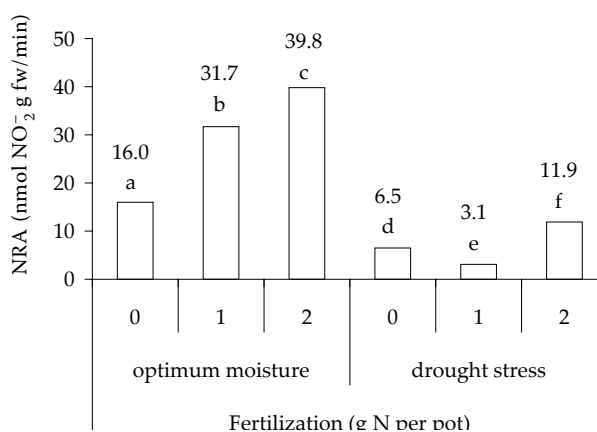


Figure 2. Activity of nitrate reductase in barley leaves in the growth stage of shooting – average of 2 years (the same letters in value columns indicate non-significant differences)

Contrary to the earlier growth stage the higher rate of N increased NRA more than the lower one. In this growth stage, but under drought stress, the plants showed significantly reduced level of NRA in leaves when fertilized by 1 g N per pot (3.1 nmol NO₂⁻/g fw/min) compared to the stressed control (6.5 nmol NO₂⁻/g fw/min). Contrariwise, double rate of N (2 g per pot) was capable to increase NRA significantly (by 83%) compared to stressed control; it reached the value of 11.9 nmol NO₂⁻/g fw/min. Figure 2 shows that only in this growth stage the highest NRAs were achieved under optimum water regime in comparison with the growth stage of tillering and earing.

Similar results were achieved by Kathju et al. (2001) in the experiment with different genotypes of pearl millet (*Pennisetum glaucum*) subjected to drought and higher N fertilization. These plants displayed significantly higher photosynthetic rate and NRA as compared to unfertilized control plants in all genotypes. Fertility-induced improvement of metabolic efficiency coupled with higher photosynthesis and NRA for efficient N utilization seems to be the control mechanism for enhanced growth and yield of pearl millet under limited water conditions.

As far as the latest growth stage is concerned, a positive effect of N fertilization on NRA was found also during growth stage of earing, particularly when the plants were fertilized by 2 g N per pot. In this case NRA represented the value of 21.0 nmol NO₂⁻/g fw/min which was even slightly over the value achieved in optimally irrigated treatment under 1 g N application (19.7 nmol NO₂⁻/g fw/min). On the other side, the rate of 1 g N per pot showed to be ineffective (4.0 nmol NO₂⁻/g fw/min) and decreased NRA even in comparison with plants grown under unfertilized conditions in stress environment (7.2 nmol NO₂⁻/g fw/min) (Figure 3). Ferrario-Méry et al. (1998) reported more negative drought-related response of plants in NRA during reproductive rather than vegetative growth stages. This trend was confirmed by our results (see above).

Lahiri (1980) demonstrated that N application to the soil reduced the adverse effect of drought on dry matter and grain yield of pearl millet. When fertilized with N, sorghum (*Sorghum halepense* L.) recovered faster after relief from water stress. In the growth stage of flowering Brestič and Olšovská (2001) observed a decline of spring barley (cultivar Kompakt) grain yield in dependence on the duration of applied drought stress even by 32% at 11 days of plant stress in comparison to the control

treatment. Under 3-day application of drought stress the grain yield increased by 3% compared to unstressed control.

At the site where the wheat encountered little water deficit, dry grain yield increased from 607 g/m² for a low-N control crop to 798 g/m² for a high-N crop. At the site with severe terminal drought, dry grain yield decreased by 24% from 374 g/m² for the control to 284 g/m² for the highest N crop. At the third site, yields increased with small applications of N, whereas greater applications resulted in a negative yield response (Van Herwaarden et al. 1998).

Evaluating individual years it can be said that NRA under optimum water regime showed the same response to nitrogen fertilization in all investigated growth stages in both evaluated years. It means that N-rates of nitrogen proportionally increased values of NRA except for the rate of 2 g N in growth stage of tillering that acted depressively on NRA. Average of 2 years expressed in terms of NRA reflects adequately the situation in individual years (Figures 1–3).

Under drought stress conditions the rate of 2 g N per pot showed a slightly depressive effect on NRA (statistically insignificant) in comparison to 1 g N per pot in the growth stage of tillering in 2005. Nevertheless, next year the rate of 2 g per pot slightly stimulated NRA compared to 1 g N in the same growth stage (but also insignificantly). These trends are quite well reflected in the 2-year average showing nearly the same (and insignificant) values of NRA amounting to 14.9 nmol NO₂⁻/g fw/min (1 g N per pot) and 15.9 nmol NO₂⁻/g fw/min (2 g N per pot) (Figure 1).

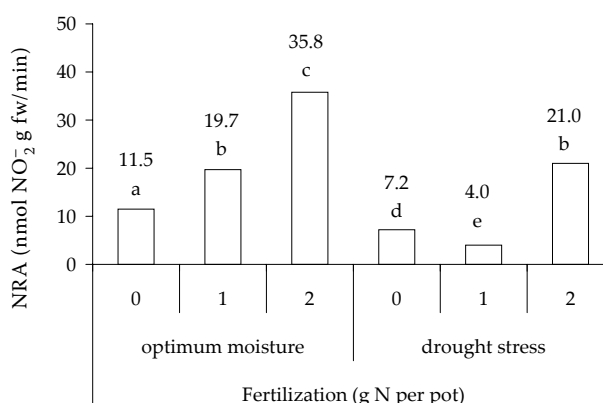


Figure 3. Activity of nitrate reductase in barley leaves in the growth stage of earing – average of 2 years (the same letters in value columns indicate non-significant differences)

Table 2. Spring barley grain yields (g per pot) and their relative expression to unfertilized treatment (%) – average of 2 years

Rate of N per pot	Optimum soil moisture		Stress induced during					
			tillering		shooting		earring	
	(g)	(%)	(g)	(%)	(g)	(%)	(g)	(%)
0 g	7.44 ^a	100	7.14 ^a	100	7.90 ^a	100	7.00 ^a	100
1 g	24.44 ^b	328	26.63 ^b	373	10.16 ^b	129	10.86 ^b	155
2 g	15.76 ^c	212	19.92 ^c	279	9.51 ^b	120	2.60 ^c	37

Stress was induced only during the respective growth stage. Before and after the stress period plants were grown under optimum moisture conditions. The same letters at the average values indicate non-significant differences

As far as the growth stage of shooting is concerned the comparison between years shows that there were achieved very similar results of NRA under drought stress and average values of 2 years reflects the situation very well (Figure 2).

The greatest differences in NRA in leaves of spring barley between two experimental years were found in the growth stage of earing under drought stress conditions. While in 2005 the NRA values were quite high amounting to 7 nmol NO₂⁻/g fw/min (1 g N) and 39 nmol NO₂⁻/g fw/min (2 g N), in 2006 they were much lower, i.e. 1 nmol NO₂⁻/g fw/min (1 g N) and 3 nmol NO₂⁻/g fw/min (2 g N). In this case the average of 2 years (Figure 3) does not illustrate the situation realistically showing NRA at 2 g N per pot to be 21 nmol NO₂⁻/g fw/min.

The differences could have been caused by different course of temperature during the growth stage of earing in both years.

Our results achieved in the pot trial show that fertilization of spring barley with nitrogen increased grain yield in comparison to unfertilized control when barley was grown under optimum soil moisture as well as in drought stress condi-

tions. Comparing a fertilizing effect of nitrogen, it is obvious, that lower rate of N (1 g per pot) increased yield of grain more (in larger extent) than double rate (2 g per pot) both under optimum moisture and drought stress treatments. The N effect on elimination of drought stress consequences depended on the time of stress induction; it was the most significant when the barley plants were exposed to stress during the growth stage of tillering. In this case the gained grain yields were even higher than those achieved when barley was grown under optimum soil moisture regime in the course of the whole growing period (Table 2). When drought stress was induced later in the growing season the elimination effect of N fertilization on stress impact was observed as well, but in a much lesser extent than at tillering. Moreover, the stress induced during shooting or earing resulted in a decrease in the yields of grain, compared to optimally irrigated treatments (Table 2).

Adverse effect of drought stress on grain yield was the lowest in unfertilized treatment, the difference between the yield of moistened and stressed treatments was relatively small, but the grain yields

Table 3. Spring barley grain yields (g per pot) and their relative expression to optimally moistened treatment (%) – average of 2 years

Water regime	N fertilization per pot					
	0 g		1 g		2 g	
	(g)	(%)	(g)	(%)	(g)	(%)
Optimum	7.44 ^{a, b}	100	24.44 ^a	100	15.76 ^a	100
Stress during tillering	7.14 ^a	96	26.63 ^a	109	19.92 ^b	126
Stress during shooting	7.90 ^b	106	10.16 ^b	42	9.51 ^c	60
Stress during earing	7.00 ^a	94	10.86 ^b	44	2.60 ^d	16

Stress was induced only during the respective growth stage. Before and after the stress period plants were grown under optimum moisture conditions. The same letters at the average values indicate non-significant differences

were low (Table 3). The rate of 1 g N per pot increased the yield of grain in the treatment where the plants were stressed during tillering by 9% compared to the plants moistened optimally during the whole vegetation (Table 3), and even several times (3.73 times) compared to unfertilized and stressed treatment (Table 2).

However, when the drought stress was applied during shooting or earing the grain yields sharply declined by over 50% in comparison with optimal water regime (Table 3). When compared with stressed and unfertilized treatment the rate of 1 g N per pot increased yield by 29% (stress applied at shooting) and 55% (stress applied at earing) (Table 2).

Under the conditions of drought stress the grain yield of spring barley was the most affected by NRA at the growth stage of shooting when correlation coefficient $r = +0.85$ ($n = 24$) was determined. At the growth stage of tillering a correlation coefficient between NRA and grain yield was lower ($r = +0.60$, $n = 24$); a negative relation was observed when the stress was induced in the second half of growing season (growth stage of earing), i.e. $r = -0.56$.

When water regime was optimal during the whole growing season the correlation coefficients between NRA and grain yield were as follows: $r = +0.64$ ($n = 24$) in tillering, $r = +0.74$ ($n = 24$) in shooting; these values are very near and differ from those of stress conditions. A weak correlation was found between NRA and grain yield in the growth stage of earing ($r = +0.31$, $n = 24$), although in this case, in contrast to stress conditions, the correlation was positive.

The above-mentioned facts show that the spring barley plants were the least sensitive to water deficit during growth stage of tillering fertilized with the rate of 1 g N per pot. In later growth stages the sensitiveness of barley plants to water deficit considerably increased. Stress-eliminating effect of applied nitrogen acted in opposite direction; the earlier were barley plants stressed by drought, the better eliminating effect of N fertilization on negative impact of this stress on yields was observed.

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