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Growth responses of sorghum plants to chilling temperature and duration of exposure

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Abstract

Sorghum is a species sensitive to chilling temperatures. In the cultivation areas at higher latitudes chilling sensitivity may significantly influence plants growth in early spring, resulting in significant yield reductions. The effects of chilling stress were investigated in a controlled environment experiment on sorghum plants fertilised with 0 and 44 mg per pot of N. Plants were grown at 27 °C until eighth leaf development stage, exposed to 2, 5, and 8 °C for time period varying from 1 to 8 days, and then returned to 27 °C. Dry weight of plants, leaf area and N and P concentration and content were determined before and after each period of cold treatment and after a 10-day recovery period. Plant relative growth rate (RGR), leaf relative growth rate (RLGR) and N and P uptake rates were calculated during the chilling and the recovery period. Chilling treatments greatly inhibited sorghum growth and N uptake during chilling exposure. The nature and severity of chilling damage was a function of the severity and duration of the exposure: plants suffered short chilling injury at all temperatures, when the duration of chilling was prolonged plants were able to react to chilling, but the ability of the plant to adapt decreased with the decrease of temperature. Plant shoot growth was found to be more sensitive to chilling than leaf area growth and non-fertilised plants were more tolerant to chilling than N-fertilised plants. Also the ability of the plant to recover was a function of the severity and duration of the exposure and of N availability. The recovery of growth rate decreased as temperature was lower and as exposure was longer. Non-fertilised plants were able to recover higher growth rates following chilling stress than N-fertilised plants, while for N uptake the reverse was true, with N-fertilised plants having higher N uptake rates than non-fertilised ones.

Keywords: Chilling; N level; Recovery; Relative growth rate; Relative leaf growth rate; Sorghum; Temperature

1. Introduction

Chilling stress, damage caused by low but above-freezing temperatures, has been recognized as a unique environmental impact on crop plant growth. Susceptibility to chilling injury imposes late plantings or prevents the cultivation of many crops in regions where temperatures can drop below the optimal growth temperatures for individual plant species. Early planting could contribute to a longer growing season, a more effective utilization of late spring and early summer rainfall, and to an enhanced yield potential.

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Plants exposed to chilling temperatures suffer injury, exhibiting reduction or stop of growth. This phenomenon is initially reversible, but ultimately irreversible and can result in cell death. Chilling adversely affects a wide range of processes, including crop germination and establishment, photosynthesis, flowering, plant growth and grain yield (Ting et al., 1991; Staggenborg and Vanderlip, 1996; Verheul et al., 1996). The degree of chilling injury can vary with plant species, stage of crop development and with the conditions of irradiance and mineral nutrition during chilling stress. In chilling-sensitive species it has been recognized that chilling causes greater injury under illuminated conditions, owing to photooxidative damage determined by the increase of the formation of harmful active oxygen species (Long, 1983; Wise, 1995; Aroca et al., 2001). The degree of disfunction observed after chilling of tomato plants depended on mineral nutrition, affecting the production of root-borne phytohormones (Starck et al., 1997), and plants grown with limited supply of mineral nutrients were more susceptible to chilling (Starck et al., 2000). In spinach, nitrogen deficiency greatly decreased the rate of acclimation to low temperature (Martindale and Leegood, 1997).

Chilling injury is time dependent and changes in physiological activity preceed the development of visual symptoms of injury. At chilling temperatures, the rates of respiration and ion leakage increase and photosynthetic activity and carbohydrate metabolism decrease (Karnok and Beard, 1983; White and Schmidt, 1989; Sonoike, 1999). Chilling temperatures cause starch accumulation in chloroplasts of some plant species, inhibiting the photosynthetic process because of feedback inhibition of photosynthetic enzymes (West, 1970; Rogers et al., 1977). Photoinhibition of photosynthesis is enhanced at chilling temperatures and one of the major reasons for this enhancement is the decreased utilization of excitation energy in carbon metabolism at chilling temperature (Sonoike, 1999).

Chilling damage depends not only on conditions during chilling, but also on the environment before and after chilling, such as temperature, light conditions and water status of the plants prior to chilling and the light conditions after chilling (Starck et al., 1994; Szalai et al., 1996; Smeets and Wehner, 1997).

Sorghum is one of the most economically important plants grown in Italy but, originating from the semi-arid tropics, it is susceptible to cold stress and often expresses poor early-season vigor and reduced competitive ability against weeds, owing to low temperatures after sowing. The effects of chilling stress on sorghum have been studied in relation to chilling-induced declines in photosynthetic capacity. However, there are many situations, i.e. cloudy conditions or darkness, where the photosynthetic apparatus of sorghum may not be affected by low temperatures (Taylor and Rowley, 1971). Reduced growth may result from more direct effects of temperature on growing tissues or from the reduction of photosynthate flow through the phloem. According to Wardlaw and Bagnall (1981), although low temperatures may reduce the carrying capacity of the phloem of sorghum, this is unlikely to be an important factor in regulating plant growth at low temperatures. Lang and Minchin (1986) in sequential chill-warm cycles demonstrated that the translocation process was sensitive only to the first cycle, subsequent cycles having no effect owing to sorghum adaptation mechanisms.

A better understanding of the basic mechanisms which underlie the injury and a greater comprehension of the physiological and biochemical responses of plants to chilling stress will help us design better cultural practices and develop more effective techniques for the mitigation of chilling injury.

The objective of this work was to evaluate the effects of chilling temperatures and duration of exposure on sorghum plant growth and N and P uptake at the development stage of rapid growth and nutrient uptake (eight-leaf). In addition, since it is suggested that N availability could influence chilling effects, the effect of nitrogen availability on chilling sensitivity was evaluated. In order to focus on the direct effects of low temperatures on plant growth and to minimize the secondary effects due to chilling-induced photoinhibition, experiments were carried out at low photosynthetic photon fluence rate (Long, 1983; Ting et al., 1991; Irigoyen et al., 1996).

2. Materials and methods

The research was conducted in 1999 in a climatic chamber located at the Department of Agronomia

e Gestione dell'Agroecosistema, Pisa, Italy, where sorghum plants were grown in pots containing 1 kg soil. Soil chemical–physical properties were: 76.9% sand; 12.9% silt; 10.2% clay; pH 7.9; 1.4% organic matter (Lotti method); 2.2‰ total nitrogen (Kjeldahl method); 18 mg kg^{-1} NO_3-N; 16 ppm assim. P_2O_5 (Olsen method); 18 ppm assim. K_2O (Dirks–Sheffer method). The Venturoli Aralba hybrid sorghum (class 400/500) was used. Three seeds per pot were planted, and seedlings were thinned at stage 1 (scale of Vanderlip and Reeves, 1972) to one per pot. Plants were first grown until the third-leaf stage. Growing conditions were 14-h day/10-h night photoperiod regime at 27 °C, which was the optimal temperature for plant growth estimated on the same genotype in a previous controlled environment research (Ercoli et al., 1996). Lighting was provided by fluorescent lamps (Osram Fluora 77) characterized by high emission in the blue and red bands. Photosynthetic photon flux density at the top of the plant canopy was 250 μmol m^{-2} s^{-1} measured using a hand-held spectroradiometer model LI-1800 (LI-COR Inc., Lincoln, NE, USA). Relative humidity was kept at 65 ± 5%. Pots were watered regularly to avoid water limitation.

Treatments consisted in three air temperatures (2, 5, and 8 °C), three lengths of duration of exposure to chilling (1, 4, and 8 days), and two N fertilization rates (0 and 44 mg per pot of N, corresponding to 0 and 200 kg N ha^{-1}). The experiment was a split-plot design, with chilling temperatures serving as main plots, chilling durations as sub-plots and N levels as sub-subplots with three replications. Fertilisers were applied before seeding and were uniformly distributed throughout the volume of soil. Nitrogen was applied as urea. Phosphorus fertiliser (triple mineral perphosphate) and potassium, as K_2SO_4, were applied at rates of 50 mg per pot of P (100 kg ha^{-1} of P_2O_5) and 94 mg per pot of K (100 kg ha^{-1} of K_2O).

After the treatment period, plants were back transferred for recovery to 27 °C for 10 days. Plants grown at 27 °C for 11, 14 and 18 days were used as recovery controls. Determinations were carried out before and immediately after each period of cold treatment and after the recovery period. The aerial part dry weight and the photosynthetically active leaf area of plants were measured as well as the visual symptoms of chilling injury. Plant parts were dried for dry weight determination at 75 °C to constant weight. Green leaf area was determined by means of a Leica Quantimet 500 image analyser. Plant samples were analyzed for P and N content, with total P determined colorimetrically in triacid extract by the ammonium–molybdophosphoric blue color method, and total N determined by the microKjeldahl method. The P and N contents of the aerial plant part were calculated by multiplying the nutrient concentrations by dry weights.

In order to take into account the original difference in size of plants due to N levels, following Hunt (1981), plant growth and leaf area expansion rate were determined as relative growth rate (RGR) and relative leaf growth rate (RLGR) for the chilling and recovery periods:

$$\mathrm{RGR} = \frac{\ln W_2 - \ln W_1}{T_2 - T_1}$$

$$\mathrm{RLGR} = \frac{\ln A_2 - \ln A_1}{T_2 - T_1}$$

where W_1 and A_1 are the above-ground dry weight and green leaf area at time T_1 and W_2 and A_2 at time T_2. Weight of necrotic leaf area was considered in RGR calculation whereas necrotic leaf area was not considered in RLGR calculation.

To allow comparison across treatments at the end of the experiment, the observed plant dry weight and leaf area were standardized by calculating values relative to the control and expressed as percent.

Data recorded before chilling treatments, immediately after chilling treatments and after the 10-day recovery period were analyzed separately by analysis of variance in order to test the effects of N level for data measured before chilling treatments, and of chilling temperature, chilling duration, N level and their interactions for data measured immediately after chilling treatments and after the 10-day recovery period. Plant dry weight and leaf area values expressed as percent of controls were arc-sine transformed before analysis (Steel and Torrie, 1980). Significantly different means were separated at the 0.05 probability level by the least significant difference test (Snedecor and Cochran, 1980).

3. Results

3.1. Effect of nitrogen on the growth of sorghum before chilling treatments

In stage 3, the accumulated dry weight and leaf area of sorghum plants were greatly affected by nitrogen availability (Table 1). Leaf area of N-fertilised plants, compared to not fertilised plants, increased by 47%, dry weight by 49% and N concentration in the plant by 23%, while P concentration was unaffected (Table 2). As a consequence, the increase of N uptake in N fertilised plants compared to non-fertilised ones was higher (83%) than P uptake (42%).

3.2. Effects of chilling injury during and after exposure

All plants exposed to the chilling treatments survived and no visual symptoms of injury (necrotic areas, chlorosis on leaves and stems, etc.) were evidenced on any plant throughout or at the end of chilling exposure, thus sorghum plants could be held for 8 days at the lower temperature without apparent damage.

Table 2
Effect of nitrogen fertilization on sorghum plants at stage 3

Character	Nitrogen level	
	N_0	N_{44}
Dry weight (mg per plant)	1089.2 a[a]	1618.5 b
N concentration ($g\,kg^{-1}$)	23.2 a	28.6 b
N content (mg per plant)	25.3 a	46.3 b
P concentration ($g\,kg^{-1}$)	2.1 a	2.0 a
P content (mg per plant)	2.3 a	3.2 b
Leaf area (cm^2 per plant)	338.4 a	496.1 b

[a] Means followed by the same letter, within the same row, are not significantly different at $P \leq 0.05$.

3.2.1. Growth rate

Chilling treatments (temperature and duration) greatly affected growth of sorghum plants during chilling exposure. RGR of plants was affected by chilling temperature × duration and temperature × N level interactions (Table 1).

Chilling effect was severe after 1 day of exposure: plants lost 40–70 $mg\,g^{-1}$ per day dry weight at all chilling temperatures. The effect was pronounced after 4 and 8 days: growth of plants ceased completely at 2 and 5 °C, since RGR values were about zero or

Table 1
Significance of treatments effects on RGR, RLGR, N and P uptake rate during chilling and recovery periods and on N and P concentration after chilling and recovery periods

Source of variation	Character					
	RGR	RLGR	N uptake rate	P uptake rate	N concentration	P concentration
	During chilling				After chilling	
Chilling temperature (T)	**	**	n.s.	n.s.	*	n.s.
Chilling duration (D)	**	**	*	n.s.	n.s.	n.s.
N level (N)	**	**	**	n.s.	**	n.s.
$T \times D$ interaction	**	**	**	n.s.	n.s.	n.s.
$T \times N$ interaction	*	**	n.s.	n.s.	n.s.	n.s.
$D \times N$ interaction	n.s.	n.s.	**	n.s.	n.s.	n.s.
$T \times D \times N$ interaction	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	During recovery				After recovery	
Chilling temperature (T)	**	**	*	*	*	n.s.
Chilling duration (D)	**	**	*	n.s.	*	n.s.
N level (N)	n.s.	*	*	*	*	n.s.
$T \times D$ interaction	**	**	n.s.	n.s.	**	n.s.
$T \times N$ interaction	n.s.	**	**	*	n.s.	n.s.
$D \times N$ interaction	n.s.	**	**	n.s.	n.s.	n.s.
$T \times D \times N$ interaction	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

n.s. not significant.
* Significant at $P \leq 0.05$ probability level.
** Significant at $P \leq 0.01$ probability level.

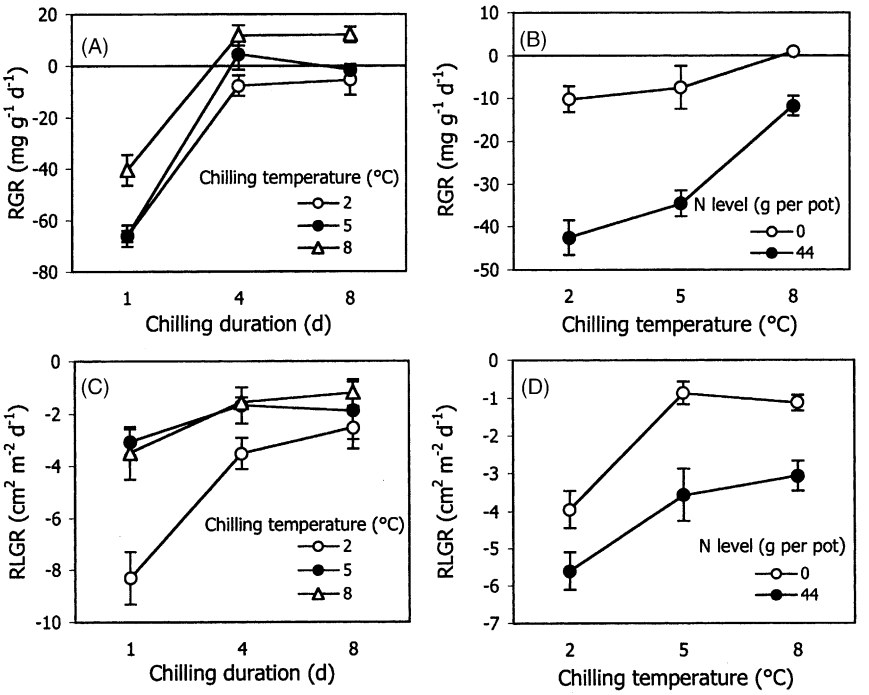

Fig. 1. Relative growth rate (RGR) and relative leaf growth rate (RLGR) of sorghum plants during the chilling period. For RGR, chilling temperature × chilling duration interaction (A) and chilling temperature × N level interaction (B). For RLGR, chilling temperature × chilling duration interaction (C) and chilling temperature × N level interaction (D). The vertical bars represent ±S.E. of the mean. When not indicated, error bar lies within the symbol.

even negative, while at 8 °C plants were able to grow and RGR values were higher than 10 mg g^{-1} per day (Fig. 1A). The extent of the decline of RGR was greater as temperature decreased: after 1 day of exposure, RGR of plants grown at 2 and 5 °C was always lower compared to those grown at 8 °C. These data indicate that after 1-day chilling plants suffered injury at all temperatures but when the duration of chilling was prolonged to 4 days the plant was able to react to chilling. However, the ability of the plant to adapt to chilling decreased with the decrease of temperature treatments, at 8 °C the plant succeeded to produce positive growth rates after 4 and 8 days of exposure, while at the lower temperatures of 2 and 5 °C the plant ceased growth and maintained the pre-chilling dry weight.

Relative growth rate of plants differed also with chilling temperature and N level (Fig. 1B). Averaged over chilling durations, RGR of N-fertilised and non-fertilised plants were negative at all temperatures, with markedly lower values for fertilised ones. The RGR of both fertilised and non-fertilised plants progressively increased between 2 and 8 °C temperature treatments, but the increase was higher for the former (91%) than for the latter (72%).

Similarly to RGR, also RLGR was affected by chilling temperature × duration and temperature × N level interactions (Table 1). Plants exposed to all chilling temperatures suffered photosynthetic area reductions since RLGR values were always negative (Fig. 1C and D). Averaged over N levels, RLGR showed lower decreases at higher temperatures and longer chilling durations; at 2 °C values were significantly lower than those at 5 and 8 °C at any duration, but this difference decreased when time of exposure increased (Fig. 1C).

Relative leaf growth rate of both N-fertilised and non-fertilised plants showed lower decreases with higher temperatures of treatment and N-fertilised plants had lower values than non-fertilised ones at all temperatures (Fig. 1D). At the lowest temperature the difference between fertilised and non-fertilised plants

was lowest, so the disadvantage of fertilised plants decreased with more drastic temperatures.

3.2.2. Nitrogen and phosphorus uptake

Nitrogen uptake rate during chilling exposure was affected by chilling temperature × duration and chilling duration × N level interactions. Averaged over N levels, N uptake rate was negligible at all temperatures after 1-day chilling exposure. When chilling was prolonged to 4 days, N uptake increased and the increase was higher at the higher temperatures, but if chilling persisted (8 days), N uptake decreased again at the two higher temperatures, showing that adaptation mechanisms were not able to ensure high rates of uptake when cold exposure was prolonged (Fig. 2A).

Averaged over chilling temperatures, N uptake rate was about zero after 1-day exposure and increased with the increase of duration of exposure to 4 days, the rates of increase being higher for non-fertilised plants than for fertilised plants (Fig. 2B). When chilling was prolonged from 4 to 8 days, N uptake rate of non-fertilised plants decreased by about 30%, while that of N-fertilised plants did not change significantly.

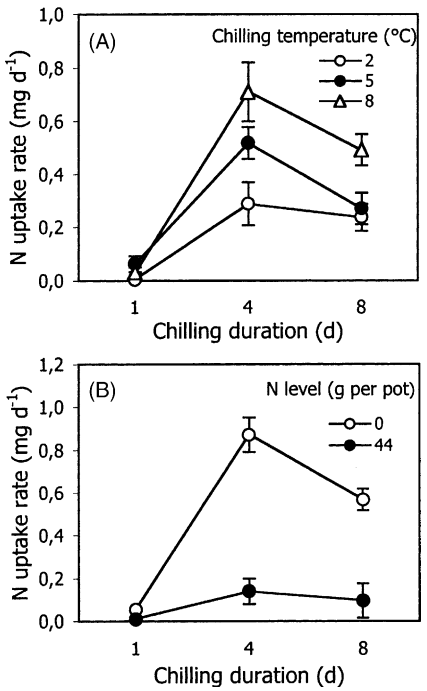

Fig. 2. Nitrogen uptake rate of sorghum plants (mg per day) during the chilling period. Chilling temperature × chilling duration interaction (A) and chilling duration × N level interaction (B).

The differences of N uptake rate, however, were not sufficient to produce significant variations of N concentration in plants at the end of the chilling exposure. Nitrogen concentration of plants was therefore affected only by chilling temperature and N level mean effects. Treatment at a less drastic temperature (8 °C) resulted in a 6% lower N concentration than plants exposed to 2 and 5 °C. Nitrogen level increased N concentration by about 23% as compared to non-fertilised control (results not shown).

Phosphorus uptake rate during chilling exposure was not affected by chilling temperature, duration and N level. Values were always very low, even when positive growth rate was recorded. As a consequence, plant P concentration at the end of the chilling period was not affected by treatments (results not shown).

3.3. Effects of chilling injury during and after the recovery period

3.3.1. Growth rate

During the 10 days of post chilling recovery period at 27 °C, sorghum plants were able to restore growth processes showing positive RGR values, but symptoms of injury like necrotic areas appeared soon on leaves; the damaged area increased with the lower temperature treatments and with the increase of duration of chilling exposure. The damage of photosynthetic area was documented by a decrease of RLGR of plants, which was even negative in some treatments.

Relative growth rate of plants during recovery was affected by chilling temperature × duration interaction (Fig. 3A). The recovery of growth rate following chilling stress was different according to the severity of chilling exposure (temperature and duration). Relative growth rate of plants was high with 1-day exposure at all temperatures; when exposure was prolonged the growth rate decreased and the rate of decrease was inversely correlated with temperature. Plants exposed to 2 °C for 8 day were not able to recover any growth after 10 days, so chilling stress resulted in a permanent damage to this plants.

Relative leaf growth rate of plants was affected by temperature × duration, temperature × nitrogen and duration × nitrogen interactions. Averaged over N

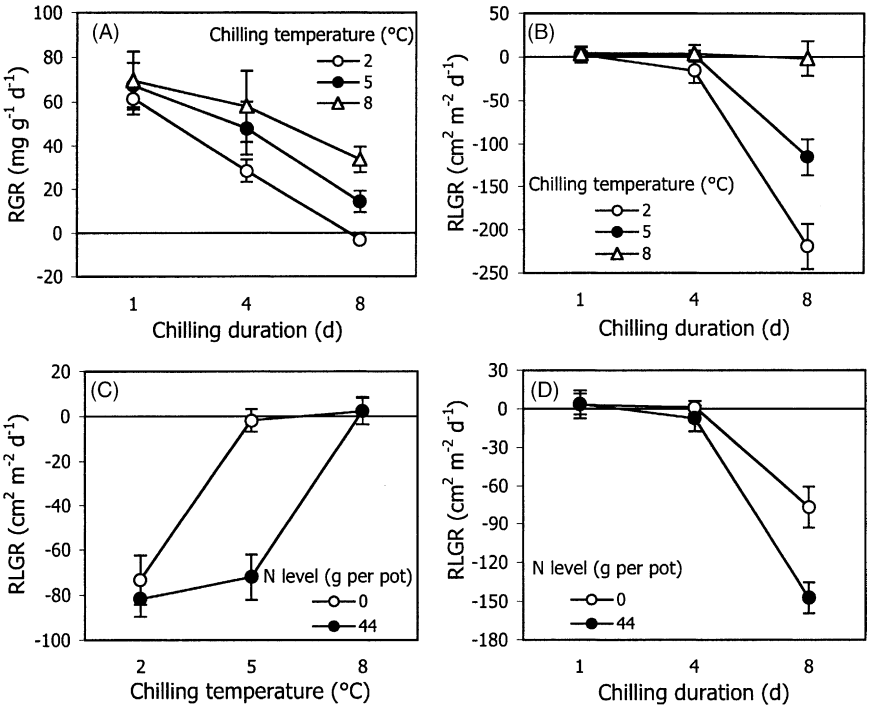

Fig. 3. Relative growth rate (RGR) and relative leaf growth rate (RLGR) of sorghum plants during the recovery period. For RGR, chilling temperature × chilling duration interaction (A) and for RLGR, chilling temperature × chilling duration interaction (B), chilling temperature × N level interaction (C), and chilling duration × N level interaction (D).

levels, RLGR of plants treated at 8 °C was inconsistent and not affected by chilling duration, whereas plants treated at 5 and 2 °C even showed negative RLGR values that decreased with the decrease of temperature and the increase of chilling duration (Fig. 3B).

During the recovery period, no change in photosynthetic area was recorded in non-fertilised plants at 5 and 8 °C treatments, and in N-fertilised plants only at 8 °C. Marked losses of photosynthetic area was recorded on both N treatments at 2 °C and in N-fertilised plants at 5 °C (Fig. 3C).

Significant differences were also observed also in chilling-induced the RLGR of N-fertilised and non-fertilised plants as affected by chilling duration (Fig. 3D). The increase of chilling duration from 1 to 4 days did not change significantly RLGR values of plants, that were near to 0, while 8 days of exposure reduced it markedly. The extent of the chilling-induce decline in leaf growth rate was greater in N-fertilised plants compared to non-fertilised ones.

3.3.2. Nitrogen and phosphorus uptake

During the recovery period, N uptake rate was affected by chilling temperature × N level and chilling duration × N level interactions. Averaged over chilling durations, N uptake of non-fertilised plants was very low and did not change significantly among temperatures. In contrast, with exposure to more drastic cold temperature, N uptake rate of N-fertilised plants decreased about 5–4-fold when comparing treatment temperatures of 8, 5, and 2 °C (Fig. 4A). Thus, following chilling stress, N fertilised plants had higher ability to recover N uptake compared to non-fertilised ones.

Nitrogen uptake rate during the recovery period was different according to N level and duration of chilling exposure (Fig. 4B). Fertilised plants exposed to 1-day chilling had N uptake rate twice as large as non-fertilised plants, while at longer exposures, no significant difference was determined according to N level. The increase of chilling duration decreased N uptake rate of both fertilised and unfertilised plants, but the rate of decrease was higher for the former than for the latter.

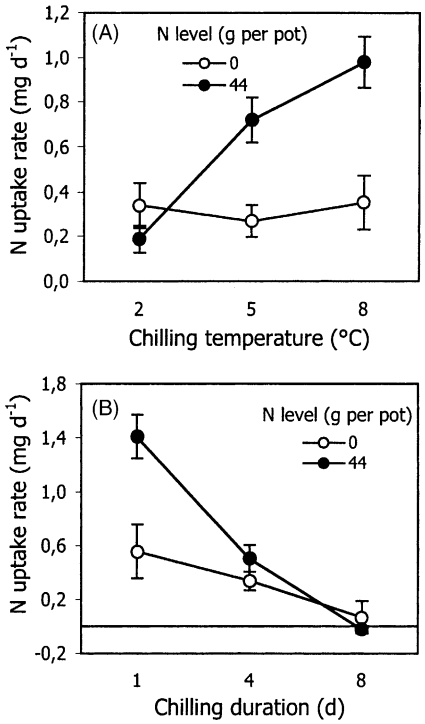

Fig. 4. Nitrogen uptake rate of sorghum plants (mg per day) during the recovery period. Chilling temperature × N level interaction (A) and chilling duration × N level interaction (B).

With the 8-day exposure treatment, N uptake rate of plants was about zero. Thus, a high ability to recover N uptake rate was evidenced only for N-fertilised plants when the exposure to chilling did not exceed 1 day, whereas with the longer exposures both fertilised and non-fertilised plants were unable to recover.

Nitrogen concentration of plants, determined at the end of the period of recovery, was affected by chilling temperature × duration interaction and N level mean effect. Nitrogen concentration increased with the increase of duration and the rate of increase was higher at 2 °C than at 5 °C, while at 8 °C the increase was negligible (Table 3). Thus, N concentration did not vary significantly after 1 and 4 days of exposure among temperatures, while after 8 days N concentration was 25% higher at 2 °C and 49% higher at 5 °C as compared to 8 °C. Nitrogen level increased N concentration of plants by about 27% as compared to the control (results not shown).

Phosphorus uptake rate during the recovery period was affected only by chilling temperature mean effect.

Table 3
Nitrogen concentration of sorghum plants ($g\,kg^{-1}$) at the end of the recovery period (±S.E.)[a]

Chilling temperature	Chilling duration		
	1	4	8
2	18.2 ± 2.1	22.3 ± 2.4	29.6 ± 2.5
5	18.0 ± 1.5	19.1 ± 1.9	24.8 ± 1.5
8	17.4 ± 1.0	17.8 ± 0.8	19.9 ± 1.9

[a] Chilling temperature × chilling duration interaction.

It increased with the increase of temperature, but values were always very low. However, variations in P uptake rate during the recovery period owing to treatments were not sufficient to determine differences in P concentration at the end of chilling exposure.

Expressing plant dry weight and leaf area as a percent of the non-chilled control allowed an estimate of the growth reduction resulting from chilling treatments. Plant dry weight and leaf area were affected by chilling temperature × duration of exposure × N level interaction. The exposure to chilling reduced plant dry weight at all temperatures and durations with the exception of non-fertilised plants exposed for 1 day to 8 °C (Fig. 5A and C). Nitrogen-fertilised plants showed greater sensitivity to chilling than non-fertilised plants: values of plant dry weight were from 87 to 39% of the controls for the former and from 99 to 43% for the latter. Values of N-fertilised plants dry weight decreased when chilling duration was greater than 1 day, while values of non-fertilised plants decreased only when duration was over 4 days.

Chilling exposure reduced also leaf area per plant and the effect was greater than that on plant dry weight, especially when chilling was prolonged to 8 days, and greater on N-fertilised plants than unfertilised control (Fig. 5B and D). Thus, chilling effects on plants were better evidenced with measurements of photosynthetic area rather than of plant dry weight, mainly due to the onset of necrotic area on leaves during recovery which decreased photosynthetic area without affecting leaf dry weight.

4. Discussion and conclusion

Chilling exposure, i.e. the treatment involving both the duration and the temperature of exposure, severely

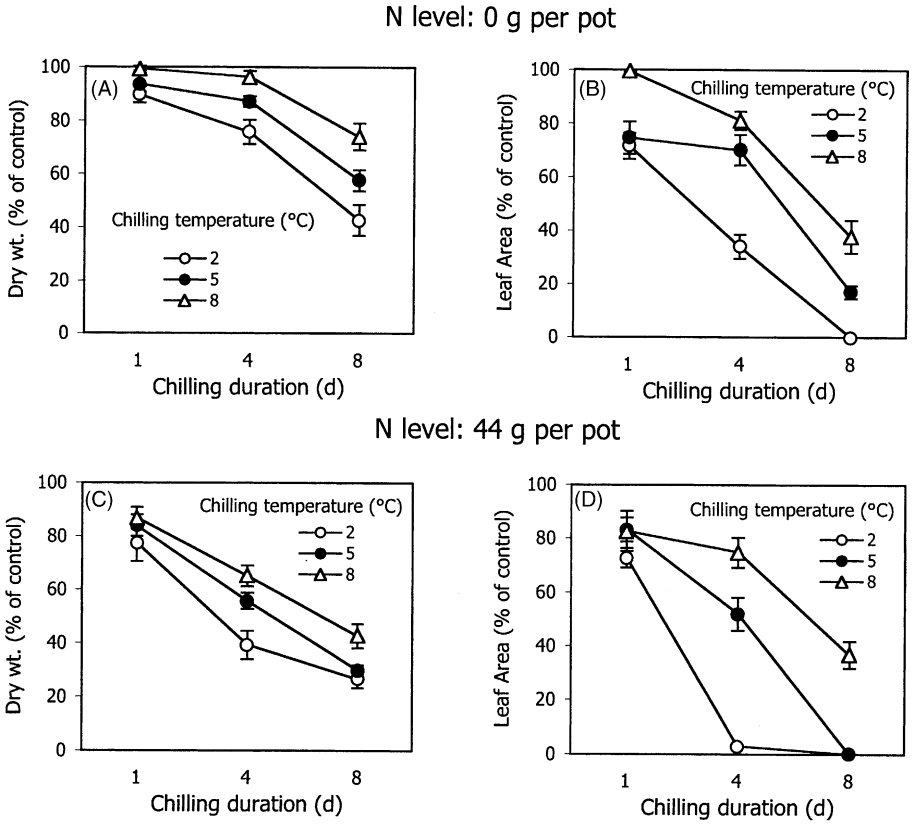

Fig. 5. Dry weight (A and C) and leaf area (B and D) of sorghum plants (percentage of control plants) at the end of the recovery period. Chilling temperature × chilling duration × N level interactions.

affected sorghum growth, evidencing that photosynthetic rate is more affected than respiratory rate. The nature and severity of chilling damage was a function of the severity and duration of the exposure. Short chilling exposure caused marked losses in plant dry weight, which is consistent with the hypothesis of Steffen et al. (1989) that photosynthesis is immediately inhibited after exposure to low temperature, whereas respiration proceed. When the duration of chilling was prolonged the plant was able to react to chilling, probably restoring photosynthesis, but the ability of the plant to adapt decreased with the decrease of temperature, and only at 8 °C the plants succeeded to produce positive growth rates, while at the lower temperatures they ceased growth.

Leaf area was decreased by chilling exposure at all temperature and duration treatments, probably due to losses of leaf turgor, that were more severe at the lowest temperature and at the longest duration of chilling exposure.

Nitrogen availability modified the response of the plant to chilling temperature and duration, with N-fertilised plants being more sensitive to chilling, showing higher reductions both in dry weight and leaf area and lower also N uptake. Reports about relationships between chilling and mineral nutrition are very scanty. However, Starck et al. (2000) reported that in tomato plants chilling caused reductions in both net photosynthesis and stomatal conductance that were higher in nutrient deficient plants. In our research, a lower metabolic activity and a higher stomatal conductance may have reduced carbohydrate and water losses due to respiration in non-fertilised plants compared to fertilized ones.

Chilling also affected N uptake rate with marked differences due to temperature and duration of

exposure. Though N uptake was higher at the higher temperature of treatment, plants showed lower N concentration as compared to plants exposed to lower temperatures, suggesting that plant growth was affected less than N uptake, causing a dilution effect.

Chilling exposure did not cause visible stress lesions to plants during or at the end of the chilling period, so plants could be held at all chilling temperatures without apparent injury, although growth was reduced. Visual symptoms of injury appeared indeed during the recovery period after chilling. Plants exhibited necrotic areas on leaves. Damage was temperature and time dependent, since the injury was more intense with the increase of chilling duration and the decrease of chilling temperature. Nitrogen fertilised plants were more sensitive than non-fertilised ones, showing large necrotic areas if exposed at 2 and 5 °C, while non-fertilised plants suffered only if exposed to 2 °C, which could suggest a different plasma membrane composition of plants in response to N supply (Hällgreen and Öquist, 1990).

Chilling resistance may be estimated not only by the ability of the plant to maintain a relatively high growth rate during chilling exposure but also by the ability of the plant to recover after the interruption of exposure to chilling temperature. In our research the ability to recover was a function of the severity and duration of the exposure and of N availability. The recovery of growth rate following chilling stress decreased with more drastic temperature treatments and longer exposures. Non-fertilised plants were able to recover higher growth rates following chilling stress than N-fertilised plants when the temperature was raised, while for N uptake the reverse was true, with N-fertilised plants having higher N uptake rates than unfertilised plants. Thus, N-fertilised plants could take advantage of the high N uptake rate to produce new leaves, necessary for the replacement of chill injured leaves.

Critical conditions found in this research cannot be directly referred to field conditions as experiments were carried out in pots and at low photon flux density. However, these results demonstrate considerable capacity of sorghum plants to recover from short chilling treatments during the early stage of growth, even though significant growth reduction occurred during chilling, whereas with longer exposures plants were not able to fully recover. Plant size and N concentration in tissues modified plant response to chilling in that non-fertilised plants showed higher ability to maintain high growth rate during chilling exposure and recovered to a greater extent following chilling than N-fertilised plants. Further research is therefore needed to confirm results in field conditions and to elucidate interactive effects of chilling and radiation level.

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