

Applied nitrogen and seed nitrogen effects on growth of barley (*Hordeum vulgare* L.) seedlings prior to emergence: importance of water uptake

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Abstract

For barley (*Hordeum vulgare* L.) prior to emergence from the substrate, addition of 5 mol/m³ nitrate (NO₃⁻) or 5 mol/m³ chloride (Cl⁻) to a nitrogen (N) free but otherwise complete nutrient medium resulted in decreased residual seed dry weight (d.wt) and increased shoot + root d.wt 14 - 21 days after sowing (DAS). Increased seedling growth with additional NO₃⁻ or Cl⁻ was associated with increases in shoot + root and residual seed anion content, total plant water and residual seed water content (% water). Seedling growth rate was greater for high N content seed than for low N content seed. This effect was associated with greater seed water content in high N seed from 2 DAS onwards. Additional NO₃⁻ did not affect residual seed water content until 14 DAS. The effects of seed N and NO₃⁻ on seedling growth were detected 10 and 14 DAS respectively. It is proposed that the increased seedling growth rate with additional NO₃⁻ is due to an increased rate of mobilisation of seed reserves caused by increased seedling water uptake, while the seed N effect on seedling growth is due to increased water uptake by the seed directly.

Additional key words: germination, nitrate, osmoticum, water uptake, emergence

Introduction

Growth rate of temperate cereal seedlings has been reported to increase with increased seed nitrogen (N) content (Lowe and Ries, 1973; Metivier and Dale, 1977a,b; Rahman and Goodman, 1983). Additional nitrate (NO₃⁻) increased seedling growth rate of low N content seed but had little effect on high N content seed. It was concluded that additional NO₃⁻ relieved an inadequate supply of N from the endosperm of low N seed which limited early seedling growth (Metivier and Dale, 1977a,b). However, additional NO₃⁻ can also increase growth rate of barley seedlings grown in darkness (Lieffering *et al.*, 1992). Chloride (Cl⁻) was similar to NO₃⁻ in its effect on barley seedlings but ammonium (NH₄⁺) did not affect seedling growth. Uptake and assimilation of N were as great with additional NO₃⁻ or NH₄⁺ and Lieffering *et al.* (1992) concluded that the NO₃⁻ effect was not related to the products of N assimilation. Nevertheless, the seed N effect on barley seedling growth appears to be related to levels of organic N, as NO₃⁻ content of barley seed is negligible (Andrews *et al.*, 1992).

In the study of Lieffering *et al.* (1992), increased shoot + root dry weight (d.wt) with additional NO₃⁻ or Cl⁻ was matched by a proportionally similar increase in

shoot + root fresh weight (f.wt). Indeed, water content (% water) of root and shoot was often greater with additional NO₃⁻ or Cl⁻. In contrast, additional NH₄⁺ did not affect total seedling water or water content of shoot or root. It was proposed that the NO₃⁻ and Cl⁻ effects on seedling growth were due to an increased rate of water uptake by the seedling possibly acting via increased residual seed water content. The rate of imbibition has been reported to be greater for high N content barley and wheat seed (Lopez and Grabe, 1971). It is possible that the seed N effect on seedling growth is due to an increased rate of water uptake by the seed directly. The objectives of the present study on barley were to determine if NO₃⁻ and Cl⁻ accumulate in seedlings to levels which would be important osmotically, and if NO₃⁻ and seed N effects on seed d.wt coincide with increased seed/residual seed water content.

Materials and Methods

Barley (cv. Triumph) seed was obtained from the New Zealand Institute for Crop and Food Research Ltd, Lincoln, New Zealand. Seed lines with individual seed weights within a 2 mg range were selected for experiments 1 and 2. In experiments 3 and 4, two seed lots of barley with the same range of individual seed

weights (46 - 48 mg) but different seed N content (1.4 and 1.9%) were used. Experiment 5 used the high N seed lot only. Nitrate-N was < 0.1% of total N in both the low and high N seed lots. Seed N and NO_3^- content were determined as described in Andrews *et al.* (1992). These low and high N seed lots were obtained from a field experiment in which plants were supplied 0 fertiliser N (1.4% seed N) or 100 kg N/ha (1.9% seed N). All experiments which used low and high N seed lots were carried out within one year of harvesting seed. All seed lots showed > 95% germination and were not chemically treated. All experiments were carried out in the dark at 5-10°C in a controlled environment chamber.

In experiments 1 and 2, seed was placed at 70 mm depth in 80 mm diameter, 180 mm tall pots (10 per pot) filled with a vermiculite/perlite (1:1, v/v) mixture soaked in a N-free but otherwise complete nutrient solution (basal nutrient solution; Andrews *et al.*, 1989) containing the appropriate treatment. Potassium was maintained constant in all treatments by adding potassium sulphate as necessary. Pots were flushed with 250 ml of the appropriate nutrient solution every 3 days. In experiment 1, plants were supplied basal nutrient solution alone or with 5 mol/m³ NO_3^- as potassium nitrate or 5 mol/m³ Cl^- (potassium chloride) added. Plants were harvested 21 days after sowing (DAS) and separated into shoot + root and residual seed for fresh weight (f.wt) determination. All plant parts were then dried at 70°C for 4 days and re-weighed. Dried material was ground and aqueous extracts of 10-30 mg samples were analysed for NO_3^- , Cl^- , phosphate (PO_4^{3-}) and sulphate (SO_4^{2-}) concentration using a Waters (Massachusetts, USA) 712 WISP ion exchange column. The sum of these anions is given. In experiment 2, barley was supplied basal nutrient solution alone or with 5 mol/m³ NO_3^- added. Plants were harvested 4, 8, 12, 16, 20 or 24 DAS and residual seed f.wt and d.wt determined.

In experiment 3, low and high N content seed was placed in petri dishes (10 per dish) containing 10 Whatman no. 1 filter papers maintained saturated with basal nutrient solution. Plants were sampled 2, 4, 8, 12 or 16 DAS and seed f.wt and d.wt determined. Experiments 4 and 5 were carried out in pots as for experiments 1 and 2. In experiment 4, low and high N content barley seed was supplied basal nutrient solution alone or with 5 mol/m³ NO_3^- added. Plants were harvested 10 or 14 DAS and shoot + root and residual seed f.wt and d.wt determined. In experiment 5, high N content barley seed was supplied with basal nutrient solution alone or with 5 mol/m³ NO_3^- added. Plants were harvested 21 DAS and shoot + root and residual seed d.wt determined.

All experiments were of a randomised complete block design and were repeated once as described. There were six replicate pots per treatment at all harvests in all experiments. An analysis of variance was carried out on all data. All effects discussed have a probability $P < 0.01$ and were obtained in the repeat experiments. Means stated as significantly different are on the basis of an LSD ($P < 0.05$) test. Variability quoted in the text is standard error of mean (SEM).

Results

At harvest (21 DAS) in experiment 1, shoot + root f.wt, d.wt and anion ($\text{NO}_3^- + \text{Cl}^- + \text{PO}_4^{3-} + \text{SO}_4^{2-}$) content were greater with additional NO_3^- or Cl^- than with basal nutrient solution (Table 1). Residual seed water content and anion content were also greater with NO_3^- or Cl^- than with basal nutrient solution. Residual seed d.wt was less with additional NO_3^- or Cl^- than with basal nutrient solution. Nitrate and Cl^- comprised around 70% of the total anions measured in seedlings supplied NO_3^- and Cl^- respectively (results not shown).

Table 1. Effect of additional nitrate (NO_3^-) and chloride (Cl^-) on dry weight (d.wt), water content and anion ($\text{NO}_3^- + \text{Cl}^- + \text{phosphate} + \text{sulphate}$) content of shoot plus root (S+R) and residual seed (RS) of barley 21 days after sowing.

Treatment	Dry weight (mg)		Water (%)		Anions ($\mu\text{mol/g}$ d.wt)	
	S+R	RS	S+R	RS	S+R	RS
Control	16.7	12.8	92.1	69.5	960	90
NO_3^-	19.3	9.7	92.5	72.4	1884	134
Cl^-	18.6	10.5	92.6	72.4	1495	122
SEM (n=6)	0.52	0.51	0.14	0.24	43.6	10.5

From 0 to 12 DAS in experiment 2, residual seed d.wt and residual seed water content were similar with additional NO₃ or basal nutrient solution (Fig. 1). Sixteen DAS, residual seed d.wt was lower and residual seed water content greater with additional NO₃. Differences in residual seed d.wt and water content

between the two treatments increased from 16 DAS to 20-24 DAS.

Two DAS in experiment 3, seed water content was 25.8 ± 0.26% and 27.4 ± 0.23% for low and high N content seed respectively (Fig. 2A). The difference in water content between low and high N content seed

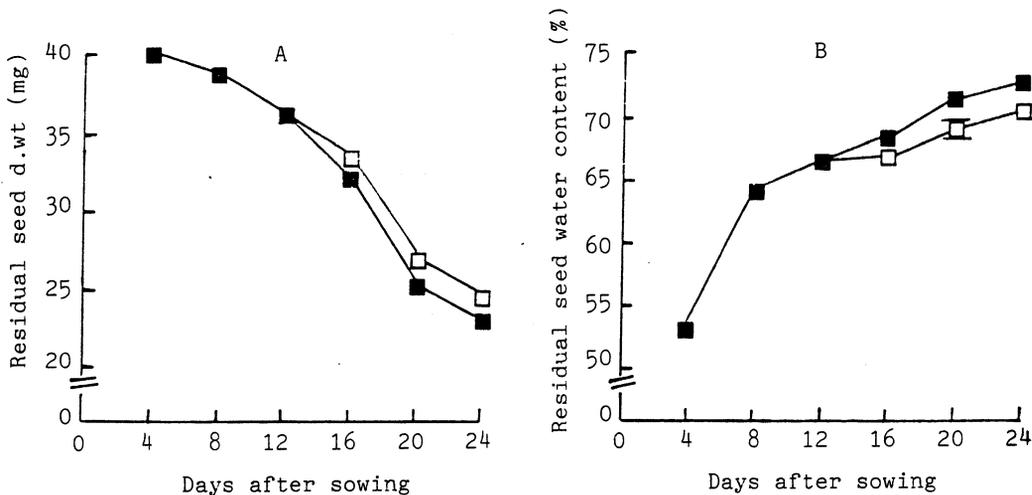


Figure 1. Effect of additional nitrate (■) on dry weight and water content of barley seed 0-24 days after sowing. Bars indicate ± SEM where larger than symbol.

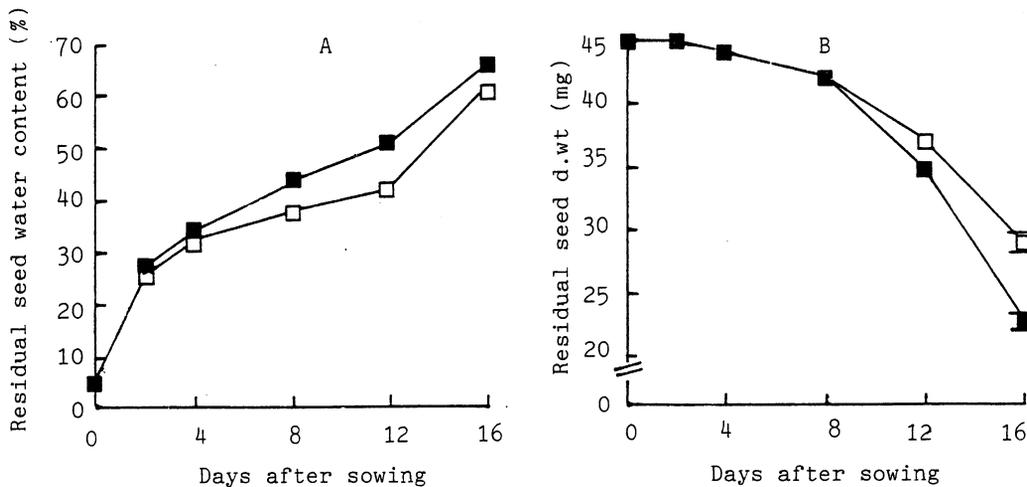


Figure 2. Effect of low (□) and high (■) seed nitrogen content on dry weight and water content of barley seed 0 - 16 days after sowing. Bars indicate ± SEM where larger than symbol.

increased with harvest date until 12 DAS. Residual seed d.wt was less for high N content seed than for low N content seed 12 DAS (Fig. 2B).

In experiment 4, shoot + root d.wt was greater for high N seed than for low N seed 10 DAS but was not affected by additional NO_3^- until 14 DAS (Table 2). Also, residual seed d.wt was less and residual seed water content greater for high N seed than for low N seed 10 DAS, but neither were affected by additional NO_3^- until 14 DAS (Table 2). There was a seed N x NO_3^- interaction 14 DAS in that residual seed d.wt was less and residual seed water content and shoot plus root d.wt greater with additional NO_3^- for high N seed only. In a separate experiment, additional NO_3^- was found to affect seedling growth of high N seed in the longer term (21 DAS). Without NO_3^- , shoot + root d.wt and residual seed d.wt were 18.1 ± 0.38 mg and 12.7 ± 0.24 mg respectively; comparable values with NO_3^- were 20.5 ± 0.11 mg and 9.8 ± 0.19 mg.

Discussion

In experiment 1, additional NO_3^- and Cl^- caused an increase in shoot + root growth rate of barley seedlings grown in darkness, a result also reported by Lieffering *et al.* (1992). Increased shoot + root d.wt was associated with a concomitant decrease in residual seed d.wt. Thus it seems certain that both NO_3^- and Cl^- increased the rate of mobilisation of seed reserves, but the possibility that effects were due to differences in seed leachate or rate of dark respiration should be considered. Firstly, if increased shoot + root d.wt with additional NO_3^- or Cl^- was a result of increased carbon availability for growth due to decreased seed leachate, then total seedling (root

+ shoot + seed) d.wt when NO_3^- had given an effect should have been greater with additional NO_3^- . This was not the case (Table 1; see also Andrews *et al.*, 1994). The second possibility that increased shoot + root d.wt with additional NO_3^- was caused by increased carbon availability for growth due to a decreased rate of dark respiration cannot be discounted on the basis of the work done here. However, this possibility seems highly unlikely because NO_3^- uptake and assimilation are energy requiring processes which are likely to increase, not decrease, the rate of dark respiration (Andrews, 1993). Nevertheless, it is acknowledged that further work is required to confirm this.

Previously, increases in shoot + root d.wt with additional NO_3^- or Cl^- were associated with increases in total seedling water and water content of root and shoot. It was proposed that the NO_3^- and Cl^- effects on seedling growth were due to an increased rate of water uptake by the seedling, possibly acting via increased residual seed water content (Lieffering *et al.*, 1992). In experiment 1, additional NO_3^- and Cl^- were shown to increase residual seed water content also. In darkness, within the regularly flushed substrate, water loss by seedlings is likely to have been small (N.B. seedlings were within a substrate which was maintained at water holding capacity) therefore an increase in total seedling water is likely to have been due to increased water uptake rather than decreased water loss. A substantial increase in total seedling water indicates a substantial increase in total seedling osmoticum. It seems likely that increased osmoticum was at least partly due to increased solute derived from seed reserves. However, in experiment 1, total anion ($\text{NO}_3^- + \text{Cl}^- + \text{SO}_4^{2-} + \text{PO}_4^{3-}$) content averaged over the root and shoot increased from $960 \mu\text{mol/g}$ d.wt

Table 2. Effect of seed nitrogen (N) content and additional nitrate (NO_3^-) on dry weight of shoot plus root (S+R) and dry weight and water content of residual seed (RS) of barley 10 and 14 days after sowing (DAS).

Treatment	Seed N	NO_3^-	10 DAS		14 DAS			
			Dry weight (mg)	Water (%)	Dry weight (mg)	Water (%)	RS	
			S+R	RS	S+R	RS	RS	
Low	-	-	3.12	37.7	48.7	14.1	22.6	69.3
Low	+	+	3.21	37.5	48.9	16.3	18.9	72.2
High	-	-	4.08	35.3	57.5	17.1	18.7	72.2
High	+	+	4.13	35.6	57.2	17.7	17.7	73.9
SEM (n=6)			0.09	0.54	0.75	0.24	0.44	0.51

with basal nutrient solution to around 1900 and 1500 $\mu\text{mol/g}$ d.wt with additional NO_3^- and Cl^- respectively. Increases in anion concentration averaged over the root and shoot were around 70 and 40 mol/m^3 with additional NO_3^- and Cl^- respectively. Such increases in anion concentration together with counter ions would generate around 0.2 MPa or more osmotic potential (Wyn-Jones and Gorham, 1982). This would make a substantial contribution to the total osmotic potential of cells which under non stressed conditions is usually around 1 MPa or less (Wyn-Jones and Gorham, 1982; Dale, 1988; Kamisaka *et al.*, 1988).

The objective of experiment 2 was to determine if NO_3^- effects on seed d.wt coincided with increased seed/residual seed water content. Previously, additional NO_3^- was found not to increase shoot and root f.wt or d.wt of wheat (*Triticum aestivum* L.) until 10-14 DAS (Andrews *et al.*, 1991). Similarly, in experiment 2, NO_3^- did not affect d.wt or water content of the residual seed of barley until 16 DAS. The finding that the increase in the rate of mobilisation of seed reserves with additional NO_3^- coincided with increased seed water content is consistent with the proposal that the NO_3^- effect on shoot + root growth is caused by an increased rate of mobilisation of seed reserves due to increased residual seed water content (Lieferring *et al.*, 1992).

Proteins are the main form of seed N and represent the major colloidal constituent of seeds (Cardwell, 1984). The rate and degree of seed imbibition are closely related to the colloidal properties of the seed and rates of water uptake have been reported to be greater for high N content barley and wheat seed (Lopez and Grabe, 1971). The objective of experiment 3 was to determine if seed N effects on seed d.wt coincided with increased seed/residual seed water content. For barley 2 DAS in experiment 3, seed water content was $25.8 \pm 0.26\%$ and $27.4 \pm 0.23\%$ for low and high N content seed respectively. The difference in water content between low and high N seed increased with harvest date until 12 DAS. Residual seed d.wt was less for high N seed than for low N seed 12 DAS. If the increased rate of water uptake by the seed is the cause of the seed N effect on mobilisation of seed reserves, and if the NO_3^- effect on mobilisation of seed reserves is due to increased residual seed water content, then the seed N effect should occur before the NO_3^- effect as additional NO_3^- did not affect residual seed water content until 10-16 DAS. This was found to be the case in experiment 4. There was a seed N x NO_3^- interaction 14 DAS in experiment 4. Additional NO_3^- caused a decrease in residual seed d.wt and an increase in shoot + root d.wt of low N seed only. In experiment 5, additional NO_3^- was found to stimulate

mobilisation of seed reserves of high N seed in the longer term (21 DAS). This emphasises that the NO_3^- effect on the mobilisation of seed reserves occurs later than the seed N effect on mobilisation of seed reserves.

Conclusions

1. Additional NO_3^- and Cl^- can cause an increase in shoot + root d.wt and a decrease in seed d.wt of barley 14-21 DAS.
2. Nitrate and Cl^- can accumulate in barley seedlings to levels which are important osmotically.
3. Decreased seed weight of barley with additional NO_3^- coincides with increased residual seed water content.
4. Seedling growth of barley is greater for high N content seed than for low N content seed.
5. Increased seedling growth with high N content seed is associated with increased seed water content.
6. Findings are consistent with the proposal that NO_3^- , Cl^- and seed N content effects on seedling growth are due to an increased rate of mobilisation of seed reserves caused by an increased rate of water uptake by the seedling or seed.

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