

# Seed reserve mobilization and the partitioning of dry matter in barley seedlings prior to emergence.

M. Lieffering, M. Andrews and B. A. McKenzie

Department of Plant Science, Lincoln University, N.Z.

## Abstract

The effects of nitrate ( $\text{NO}_3^-$ ), ammonium ( $\text{NH}_4^+$ ) and chloride ( $\text{Cl}^-$ ) on rate of endosperm reserve mobilization and shoot to root dry weight ratio (S:R) were examined in barley (*Hordeum vulgare*) prior to emergence. Caryopsis dry weight (d.wt) decreased while shoot d.wt, S:R and shoot and root  $\text{NO}_3^-$  content increased with increased  $\text{NO}_3^-$  applied over the range 0 - 20 mol  $\text{m}^{-3}$ . At an external concentration of 5 mol  $\text{m}^{-3}$ , nitrogen uptake and assimilation were as great with  $\text{NH}_4^+$  as with  $\text{NO}_3^-$  but  $\text{NH}_4^+$  did not affect the rate of reserve mobilization or S:R. Addition of 5 mol  $\text{m}^{-3}$   $\text{Cl}^-$  increased the rate of reserve mobilization and S:R. Shoot fresh weight and percentage water of shoot and root increased with additional  $\text{NO}_3^-$  or  $\text{Cl}^-$  but did not change with additional  $\text{NH}_4^+$ . It is proposed that  $\text{NO}_3^-$  or  $\text{Cl}^-$  causes increased water uptake by seedlings which results in increased water entering the caryopsis hence a greater rate of reserve mobilization. Increased S:R with  $\text{NO}_3^-$  or  $\text{Cl}^-$  appears to be related to increased rate of mobilization of endosperm reserves.

**Additional key words:** *Hordeum vulgare* L., nitrate, ammonium, chloride, shoot:root

## Introduction

For barley (*Hordeum vulgare* L.) cultivated in darkness, application of either a full nutrient solution or 5 mol  $\text{m}^{-3}$  potassium nitrate ( $\text{KNO}_3$ ) plus 5 mol  $\text{m}^{-3}$  calcium nitrate instead of distilled water caused a 45 to 65% increase in shoot dry weight (d.wt) within 7 days of planting (Nátr, 1988a,b). Increased shoot growth was due to a greater rate of endosperm reserve mobilization and to a greater allocation of reserves to the shoot at the expense of the root. For barley sown at 70 mm depth, addition of 20 mol  $\text{m}^{-3}$  nitrate ( $\text{NO}_3^-$ ) as  $\text{KNO}_3$  to an otherwise complete nutrient solution caused increases in endosperm reserve mobilization and the proportion of reserves allocated to the shoot prior to emergence from the substrate (Andrews, Lieffering and McKenzie, 1991). The  $\text{NO}_3^-$  concentrations used in these studies are at the upper end of the range found in agricultural soils (Barber, 1984; Haynes *et al.*, 1986; Wild, 1988). In the present study, relationships between applied  $\text{NO}_3^-$  concentration, rate of reserve mobilization and the partitioning of dry matter between shoot and root were examined in barley prior to emergence from the substrate. In addition,  $\text{NO}_3^-$ , ammonium ( $\text{NH}_4^+$ ) and chloride ( $\text{Cl}^-$ ) were compared with regard to their effect on seedling growth.

## Materials and Methods

Seed of barley (*Hordeum vulgare* L. cv. Triumph) was obtained from the Crop Research Division of the Department of Scientific and Industrial Research, Lincoln, New Zealand. Individual seed weight was  $44 \pm 1$ ,  $46 \pm 1$  and  $48 \pm 1$  mg in Experiments 1, 2, and 3 respectively. Seed showed 98% germination and was not chemically treated.

All experiments were carried out in the dark at  $10 \pm 1^\circ\text{C}$  in a controlled environment chamber. In all experiments, seed was placed at 70 mm depth in 80 mm diameter, 180 mm tall pots (20 per pot) filled with a vermiculite/perlite (1:1) mixture soaked in basal nutrient solution (Andrews, Love and Sprent, 1989) containing the appropriate treatment. In all treatments, potassium was maintained at 23.6 mol  $\text{m}^{-3}$  using potassium sulphate as necessary. Pots were flushed with the appropriate nutrient solution every 2 days. Seedlings were harvested 21 days after sowing and fresh weight (f.wt) of the shoot and root determined. The shoot, root and caryopsis were then dried at  $70^\circ\text{C}$  for 4 days for d.wt determination.

In Experiment 1, plants were supplied 0, 1.0, 5.0 or 20.0 mol  $\text{m}^{-3}$   $\text{NO}_3^-$  as  $\text{KNO}_3$ . Dried shoot and root material was ground and an aqueous extract of a 10 - 30 mg sample was analysed for  $\text{NO}_3^-$  content as described

by Mackereth, Heron and Talling (1978). There were three nitrogen (N) treatments in Experiment 2: 0 N, 5.0 mol m<sup>-3</sup> NO<sub>3</sub><sup>-</sup> and 5.0 mol m<sup>-3</sup> NH<sub>4</sub><sup>+</sup> added as ammonium sulphate. Nitrate, NH<sub>4</sub><sup>+</sup> (Mackereth *et al.*, 1978) and total N (Europa Scientific CN analyser) content of all plant parts were determined. In Experiment 3, plants were supplied 0 N, 5.0 mol m<sup>-3</sup> NO<sub>3</sub><sup>-</sup> or 5.0 mol m<sup>-3</sup> Cl as potassium chloride.

Each experiment was a randomised complete block design. Experiment 1 had five replicates while Experiments 2 and 3 had six replicates. An analysis of variance was carried out on all data. All effects discussed have an F ratio with a probability P<0.05 and were obtained in repeat experiments. Means stated as significantly different are on a basis of an LSD (P<0.05) test.

## Results and Discussion

Previously, application of 20 mol m<sup>-3</sup> NO<sub>3</sub><sup>-</sup> was shown to increase the rate of mobilization of endosperm reserves and the shoot to root d.wt ratio (S:R) of barley seedlings prior to emergence from the substrate (Andrews *et al.*, 1991). In Experiment 1, the magnitude of the NO<sub>3</sub><sup>-</sup> effect on mobilization of seed reserves was shown to be dependent on external NO<sub>3</sub><sup>-</sup> concentration as caryopsis d.wt decreased with increased applied NO<sub>3</sub><sup>-</sup> over the entire range used (Fig. 1a). Also, shoot d.wt

increased with decreases in caryopsis d.wt but root d.wt changed little thus S:R increased with increased applied NO<sub>3</sub><sup>-</sup> throughout. Shoot and root NO<sub>3</sub><sup>-</sup> content increased with increased applied NO<sub>3</sub><sup>-</sup> concentration over the entire range used (Fig. 1b). At applied NO<sub>3</sub><sup>-</sup> concentrations of 1 - 20 mol m<sup>-3</sup>, NO<sub>3</sub><sup>-</sup> content was greater in root than in shoot. Values for NO<sub>3</sub><sup>-</sup> content of shoot and root in the present study were greater than those obtained for mature plants grown on comparable NO<sub>3</sub><sup>-</sup> supply in a previous study (Andrews *et al.*, 1992).

No report was found of the extent of NO<sub>3</sub><sup>-</sup> assimilation in temperate cereals prior to emergence from the substrate. Barley seedlings grown in the dark have been shown to have nitrate reductase activity (Aslam and Huffaker, 1982) and therefore may assimilate NO<sub>3</sub><sup>-</sup> prior to emergence. In Experiment 2, the effect of NH<sub>4</sub><sup>+</sup> on seedling growth and the relationships between N uptake, N assimilation, mobilization of endosperm reserves and S:R were examined. Additional NO<sub>3</sub><sup>-</sup> caused a decrease in caryopsis d.wt and increases in shoot d.wt and S:R as in Experiment 1, but additional NH<sub>4</sub><sup>+</sup> did not affect d.wt of shoot, root or caryopsis (Table 1). However, N uptake was as great with NH<sub>4</sub><sup>+</sup> as with NO<sub>3</sub><sup>-</sup>. Also, as NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N constituted only a small proportion (<1%) of total N in seedlings supplied NH<sub>4</sub><sup>+</sup>, then N assimilation was as great with NH<sub>4</sub><sup>+</sup> as with NO<sub>3</sub><sup>-</sup>. The N containing products of NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup> assimilation are likely to be the same (Layzell, 1990). Thus, although NO<sub>3</sub><sup>-</sup> effects

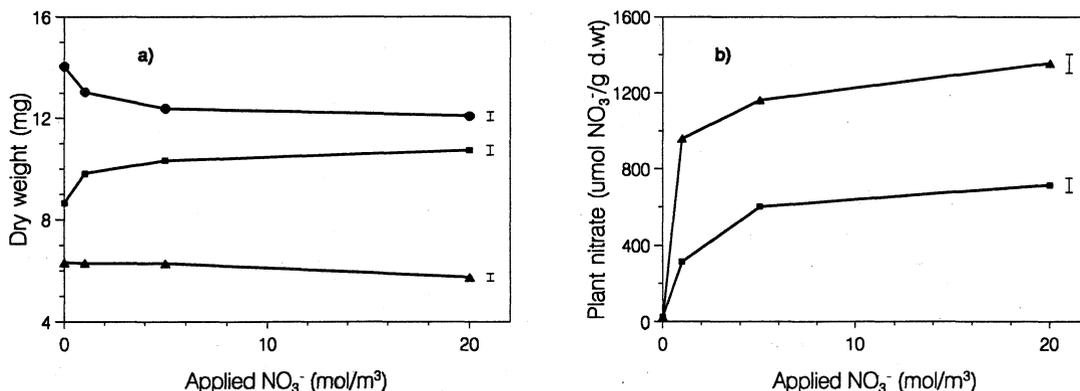


Figure 1. Effect of different concentrations of applied NO<sub>3</sub><sup>-</sup> on a) shoot (■), caryopsis (●) and root (▲) d.wt and b) NO<sub>3</sub><sup>-</sup> content of the shoot and root of barley prior to emergence from the substrate. Vertical lines indicate SEM.

**Table 1.** Effect of 5 mol m<sup>-3</sup> applied NO<sub>3</sub><sup>-</sup> or NH<sub>4</sub><sup>+</sup> on shoot (S) and root (R) f.wt and d.wt, caryopsis (C) d.wt, S:R, shoot and total N, NO<sub>3</sub><sup>-</sup>-N and NH<sub>4</sub><sup>+</sup>-N content of barley prior to emergence from the substrate.

Applied N	D.wt (mg)				F.wt (mg)		N (µg seedling <sup>-1</sup> )		
	S	C	R	S:R	S	R	Total N	NO <sub>3</sub> <sup>-</sup> -N	NH <sub>4</sub> <sup>+</sup> -N
nil	8.67	14.06	6.38	1.36	94.4	97.6	610.5	5.1	0.5
NH <sub>4</sub> <sup>+</sup>	8.36	14.51	6.02	1.39	99.1	90.3	920.2	4.6	1.4
NO <sub>3</sub> <sup>-</sup>	10.31	12.46	6.33	1.63	138.5	107.8	976.9	185.2	0.7
SEM	0.37	0.41	0.14	0.06	4.3	5.2	15.4	10.2	0.5

on barley seedlings appear to be related to the amount of NO<sub>3</sub><sup>-</sup> taken up (Fig. 1a,b), they do not appear to be related to products of NO<sub>3</sub><sup>-</sup> assimilation such as proteins/enzymes, as is the case with mature plants (Khamis and Lamaze, 1990; Zhen and Leigh, 1990).

In Experiment 2, shoot f.wt and percentage water of shoot and root increased with additional NO<sub>3</sub><sup>-</sup> but did not change with NH<sub>4</sub><sup>+</sup> (Table 1). It is possible that the NO<sub>3</sub><sup>-</sup> effects on reserve mobilization and S:R ratio are related to water uptake. Chloride is an ion which is readily taken up by plants but which is not assimilated (Clarkson and Hanson, 1980). Addition of Cl<sup>-</sup> at concentrations of 5 or 20 mol m<sup>-3</sup> can result in substantial increases in percentage water of shoots (Andrews *et al.*, 1989). In Experiment 3, addition of Cl<sup>-</sup> caused increases in shoot f.wt and percentage water in shoot and root (Table 2). Chloride also caused increases in the rate of mobilization of endosperm reserves and S:R. These data, in conjunction with those obtained in Experiments 1 and 2, indicate that NO<sub>3</sub><sup>-</sup> effects on seedlings prior to emergence are osmotic effects. It is proposed that NO<sub>3</sub><sup>-</sup> causes increased water uptake by seedlings which results in increased water entering the caryopsis and hence a greater rate of reserve mobilization. If NO<sub>3</sub><sup>-</sup> accumulates

in the endosperm reserves then this would have a more direct effect on water uptake by the caryopsis. The increase in S:R with additional NO<sub>3</sub><sup>-</sup> or Cl<sup>-</sup> appears to be related to the increased rate of reserve mobilization. Studies are currently under way to determine the relationships between rate of reserve mobilization, and NO<sub>3</sub><sup>-</sup> and water content of the caryopsis.

## References

- Andrews, M., Lieferring, M. and McKenzie, B.A., 1991. Nitrate effects on mobilisation of seed reserves in temperate cereals. New Zealand Society of Plant Physiologists; Seed Physiology Symposium (in press).
- Andrews, M., Love, B.G. and Sprent, J.I., 1989. The effect of different external nitrate concentrations on the growth of *Phaseolus vulgaris* cv. Seafarer at chilling temperatures. *Annals of Applied Biology* 114, 195-204.
- Andrews, M., Morton, J.D., Lieferring, M. and Bisset, L., 1992. The partitioning of nitrate assimilation between root and shoot of a range of temperate cereals and pasture grasses. *Annals of Botany* 70, 271-276.
- Aslam, M. and Huffaker, R.C., 1982. *In vivo* nitrate reduction in roots and shoots of barley (*Hordeum vulgare* L.) seedlings in light and darkness. *Plant Physiology* 70, 1009-1013.

**Table 2.** Effect of 5 mol m<sup>-3</sup> applied NO<sub>3</sub><sup>-</sup> or Cl<sup>-</sup> on shoot (S) and root (R) f.wt and d.wt, caryopsis (C) d.wt and S:R of barley prior to emergence from the substrate.

Treatment	D.wt (mg)			S:R	F.wt (mg)	
	S	C	R		S	R
nil	9.98	17.57	6.41	1.55	105.6	121.5
NO <sub>3</sub> <sup>-</sup>	12.67	14.37	6.34	1.99	148.5	127.5
Cl <sup>-</sup>	11.93	15.36	6.24	1.91	139.5	128.9
SEM	0.52	0.25	0.20	0.07	4.8	4.5

- Barber, S.A., 1984. Soil Nutrient Bioavailability. A Mechanistic Approach. 398 pp. John Wiley. New York, U.S.A.
- Clarkson, D.T. and Hanson, J.B., 1980. The mineral nutrition of higher plants. *Annual Review of Plant Physiology* **31**, 239-298.
- Haynes, R.J., Cameron, K.C., Goh, K.M. and Sherlock, R.R., 1986. Mineral Nitrogen in the Plant-Soil System. 483 pp. Academic Press. Orlando, Florida, U.S.A.
- Khamis, S. and Lamaze, T., 1990. Maximal biomass can occur in corn (*Zea mays*) in the absence of  $\text{NO}_3^-$  accumulation in either leaves or roots. *Physiologia Plantarum* **78**, 388-394.
- Layzell, D.B., 1990.  $\text{N}_2$  fixation,  $\text{NO}_3^-$  reduction and  $\text{NH}_4^+$  assimilation. In: Plant Physiology, Biochemistry and Molecular Biology. 389-406. (eds. D.T. Dennis and D.H. Turpin). Longman Scientific and Technical. Essex, United Kingdom.
- Mackereth, F.J.H., Heron, J. and Talling, J.F., 1978. Water Analysis: Some Revised Methods for Limnologists. 120 pp. Scientific Publications of the Freshwater Biological Association no. 36.
- Nátr, L., 1988a. Shoot/root ratio during the early heterotrophic growth of barley as influenced by mineral nutrition. *Plant and Soil* **111**, 237-240.
- Nátr, L., 1988b. Vliv genotypu a minerálních živin na heterotrofní růst mladých rostlin ječmene. *Rostlinná Výroba* **34**, 69-76.
- Wild, A., 1988. Plant nutrients in soil: nitrogen. In Russell's Soil Conditions and Plant Growth. 11<sup>th</sup> edition. (ed. A. Wild). pp. 652-694. Longman Scientific and Technical. Essex, United Kingdom.
- Zhen, R.G. and Leigh R.A., 1990. Nitrate accumulation by wheat (*Triticum aestivum*) in relation to growth and tissue N concentrations. *Plant and Soil* **124**, 157-160.