SUMMARY

The present study was undertaken to investigate the mechanism of ammonium regulation of NR in shoot tips of 7 day old dark grown wheat (*Triticum aestivum* HD 2204) seedlings. The results obtained are summarised as follows:

1. Nitrate induced NR and the kinetic and inhibitor studies confirmed that the induction was due to *de novo* synthesis of the enzyme.
2. When ammonium was given along with nitrate as NH_4NO_3, 90% more NR was induced in light and 142% more in dark compared to KNO_3 grown shoot tips.
3. The age of the shoot had an effect on the ammonium mediated stimulation of NR. In the 7 day old shoots the ammonium effect was the most pronounced.
4. It was observed that ammonium provides thermostability to NR *in vivo*. The decrease in the activity at higher temperatures was less in the presence of ammonium and also it was less susceptible to cold lability *in vivo*.
5. The nitrate uptake was more in light than in dark grown shoot tips and the readings corresponded well with the NR activities in light and dark.
6. Ammonium did not affect the nitrate uptake, nor did it affect the intracellular nitrate distribution. In fact,

the nitrate uptake in light, in KNO_3 induced shoot tips was three folds higher than in NH_4NO_3 induced shoot tips. The nitrate pool sizes in KNO_3 induced shoot tips were bigger than the nitrate pools in NH_4NO_3 induced shoot tips.

7. Of all the amino acids tested, none could mimick the ammonium effect. Cystine enhanced the NR activity by 27%, whereas aspartate and glutamate inhibited NR activity by 35% and 42% respectively.

8. When shoot tips were preincubated in KNO_3 for 18 h and then transferred to $NH_4H_2PO_4$ alone, the NR activity was stimulated slightly and then stabilized. This effect was not observed when shoot tips were transferred to KH_2PO_4 or water.

9. Ammonium stabilized NR <u>in vivo</u> but not <u>in vitro</u>. The <u>in vivo</u> half life of NR increased from 6 h in KNO_3 to 20 h in NH_4H_2PO .

10. The ammonium mediated stabilization of NR was inhibited by 6-methyl purine and cycloheximide, indicating that ammonium might be acting via the synthesis of a new protein.

11. Tungstate too, inhibited the stabilization of NR. This shows that when shoot tips, preincubated in KNO_3, were transferred to $NH_4H_2PO_4$ alone, in the absence of external supply of nitrate, some NR synthesis took place. Since $NH_4H_2PO_4$ alone could not induce NR and since this stabilization occurred only when the shoot tips were

transferred to $NH_4H_2PO_4$, after KNO_3 preincubation, it could be assumed that there may be stable mRNA's for NR which are produced in KNO_3 and are translated in the presence of ammonium. Thus ammonium could be acting at the post-transcriptional or at the translational stage.

12. Incorporation studies done with ^3H-uridine, ^3H-adenine and ^3H-leucine showed that ammonium did not affect the general RNA synthesis. However, more adenine was incorporated in NH_4NO_3 induced shoot tips. This could be indicating an increased polyadenylation in the presence of ammonium, thus further suggesting that ammonium could be acting at the post-transcriptional stage. The general protein synthesis was partially increased in the presence of ammonium.

13. The ammonium effect was not non-specific as it did not affect the activity of GS. The NiR activity was unaffected by ammonium at the concentrations at which NR showed the maximum stimulation but at lower concentrations of ammonium the NiR activity was stimulated slightly. The GDH activity increased in the presence of ammonium.

14. The ammonium effect of stimulation and stabilization of NR <u>in vivo</u> was simulated by NADH <u>in vitro</u>. Ferricyanide, an oxidising agent, inactivated NR, suggesting thereby that NR exists in an active and an inactive form.

15. Though the ammonium was toxic for chlorophyll synthesis, sucrose, a photosynthate, was found to enhance the NR activity in light and in dark. DCMU and chloramphenicol inhibited the NR activity upto 74% and 39% respectively.

16. Ammonium did not have any effect on the oxygen uptake but certain respiratory inhibitors like 2,4 DNP and antimycin inhibited the NR activity. Certain respiratory intermediates, enhanced NR activity *in vivo*. Succinate showed a slight stimulation in NR activity when given along with KNO_3 but not when given along with NH_4NO_3. Oxaloacetate and α-ketoglutarate increased the NR activity by 86% and 67% respectively when given with KNO_3. With NH_4NO_3, at low concentrations they showed slight increase but with increasing concentrations the NR activity increased. Citrate inhibited NR activity slightly, whereas malate had no effect. Interestingly, glycolate, the substrate for photorespiration, was found to stimulate the NR activity in light and dark. These intermediates of respiration and photorespiration probably act by increasing the NADH concentrations which in turn could be acting by stimulating and stabilizing NR.

17. Based on the results obtained a working model is proposed to explain the mechanism of ammonium action.