Molecular manipulation of urea metabolism in potato

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Urea is the most frequently used N fertilizer globally. For example, in China and India, urea accounted for 53% and 83% respectively, of total N fertilizer consumption in 1998. Together, both countries consumed 41% of all N fertilizer used worldwide in that year[1]. Urea is a cheap source of fertiliser with a high N content, it can be applied as a foliar application thus eliminating the groundwater pollution associated with nitrates, and potentially allowing targeted fertiliser applications to meet only the demands of a growing crop. Potential disadvantages include leaf damage at high concentrations and losses due to volatilisation. This article outlines a transgenic approach to more fully assess the potential to improve the efficacy of foliar applied urea as a plant and environment friendly nitrogen source.

In the plant, urease, which is a nickel requiring enzyme, catalyses the hydrolysis of urea to carbamate and ammonia (NH_3). Carbamate is unstable and yields a second molecule of ammonia and carbonic acid (Fig. 1). The release of ammonia during the urease reaction leads to a pH rise, since at neutral pH most NH_3 becomes protonated (NH_3 (aq.) + $H^+ \leftrightarrow NH_4^+$). Ammonia may escape from the system (volatilise). The action of urease requires several accessory proteins for activation

Potato urease and urease accessory protein genes Prior to transformation, potato urease and urease accessory protein (*UreG*) genes were isolated and extensive genomic sequence data generated. One allele of the gene has been sequenced completely and a second allele partially. Potato urease was shown to be a single copy gene present on chromosome 5 with a truncated Ty1-*copia* retrotransposon located in an intron of one of the alleles[1]. In addition, five different cDNAs encoding isoforms of urease accessory protein G (*ureG*) were cloned. The 5'-coding region of these cDNAs is highly polymorphic within *Solanum tuberosum* ssp. *tuberosum*, containing mainly a simple sequence repeat encoding histidine and aspartate. All ureG isoforms contained a P-loop motive and were similar to other plant and bacterial ureG sequences. Mapping on an ultra high density map of the potato genome and Southern blot analysis showed that the isoforms arise from allelic differences of a single copy gene located on chromosome 2[2].

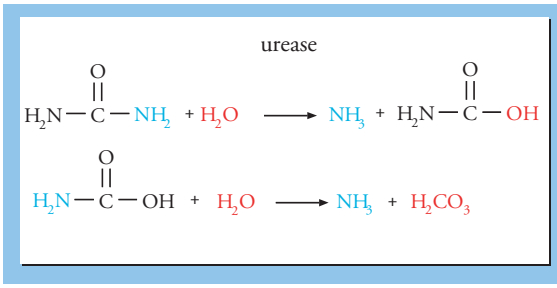


Figure 1 Reaction catalysed by urease.

Distribution of urease activity in the plant Urease activity was detected in all potato tissues. A detailed analysis of urease activity in the potato canopy during plant development showed that urease is a classic housekeeping enzyme, maintained at constant levels throughout the plant's life. Messenger RNA coding for urease accessory protein G was also found in all tissues tested and at fairly constant levels. In Western blot analysis, ureG could be detected in most tissues. These findings are consistent with the ubiquitous expression of potato urease. An attempt to correlate

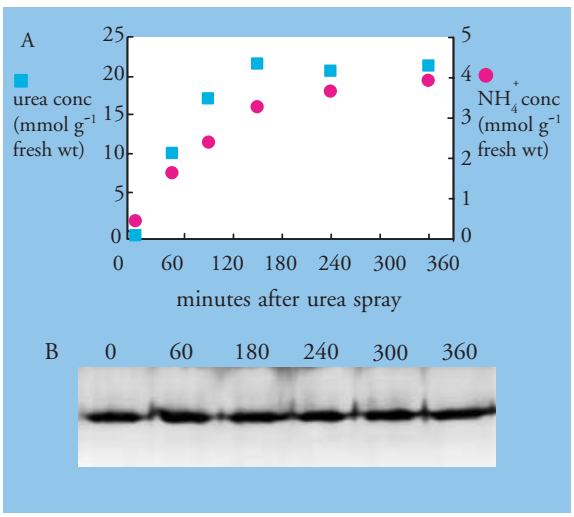
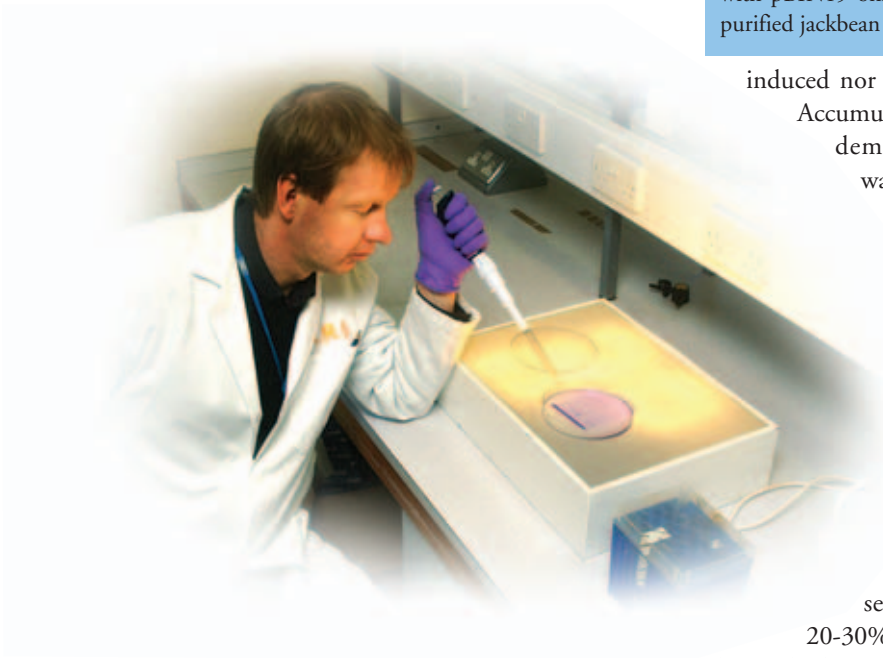


Figure 2 Urea, ammonium and urease levels in leaves at different times after a 2% foliar urea treatment. A, urea (■) and ammonium (●) concentrations in the leaves. B, native PAGE gel stained for urease extracted at different times after a 2% urea application. Samples were loaded on a constant fresh weight basis.

the amounts of urease and ureG from different tissues showed that there are likely to be other factors important for the activation of urease and/or the maintenance of urease activity.

Metabolism of foliar applied urea The levels of urea and ammonium increase drastically after foliar urea application, showing that urea readily penetrates the leaf surface and enters the leaf cells where it is hydrolysed by urease (Fig. 2). However, urea neither induced nor reduced the amounts of leaf urease. Accumulation of ammonium after urea spray demonstrated that the urease reaction was, at least initially, not rate limiting for the assimilation of urea.



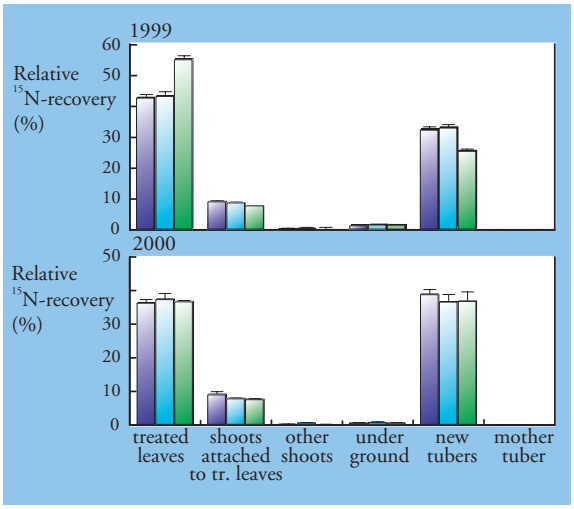


Figure 3 Urea ^{15}N recovery from field-grown plants. Plants in 1999 (upper panel) were harvested 36-48 hours after urea application. In 2000 (lower panel) plants were harvested 8 days after. Results are shown for three replicate plants indicated by different column shading. Error bars indicate the confidence interval (P = 95%) for the mass-spectrometric determination of ^{15}N.

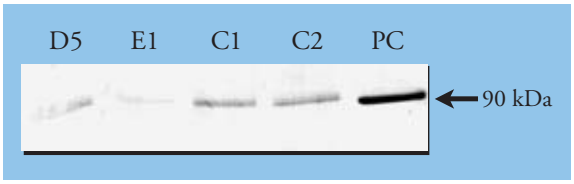


Figure 4 Western blot of protein extracts from leaves of urease antisense transgenics and controls. D5 and E1 are antisense lines. C1 and C2 are control lines, transformed with pBIN19 only. PC is a positive control containing purified jackbean urease (Boehringer).

Nitrogen losses by volatilisation from foliar applied urea did not exceed 18% in recovery experiments on field-grown potato plants. These relatively high recoveries are in accordance with findings from other studies in which the amounts actually intercepted by the leaves of the plant were known. Based on this data, it seems likely that nitrogen losses over 20-30% are generally caused by factors other than volatilisation. Urea nitrogen is initially quickly distributed throughout the plant, possibly because urea itself has access to the vascular system. Once the urea is hydrolysed and the nitrogen assimilated, the redistribution slows down. A significant proportion of the nitrogen remains in the treated leaf and is slowly mobilised for transport into the sink tissues (tubers) during leaf senescence (Fig. 3).

Urease and Ure G transgenics Transgenic potatoes have been generated with urease activity down-regulated (antisense urease or *Ure G*) and up-regulated (over-expression of potato urease). Plants with a range of leaf urease activities from *c.* 20 mU per gram fresh weight up to approximately 650 mU per gram fresh weight were produced. A reduction of urease antigen in the best antisense lines was demonstrated for both the urease (Fig. 4) and *ureG* antisense plants. RT-PCR results for the *ureG* antisense lines showed a reduction in *ureG* specific mRNA in lines with low urease activity. The results for *ureG* antisense lines confirm that this protein is involved in urease activation in plants.

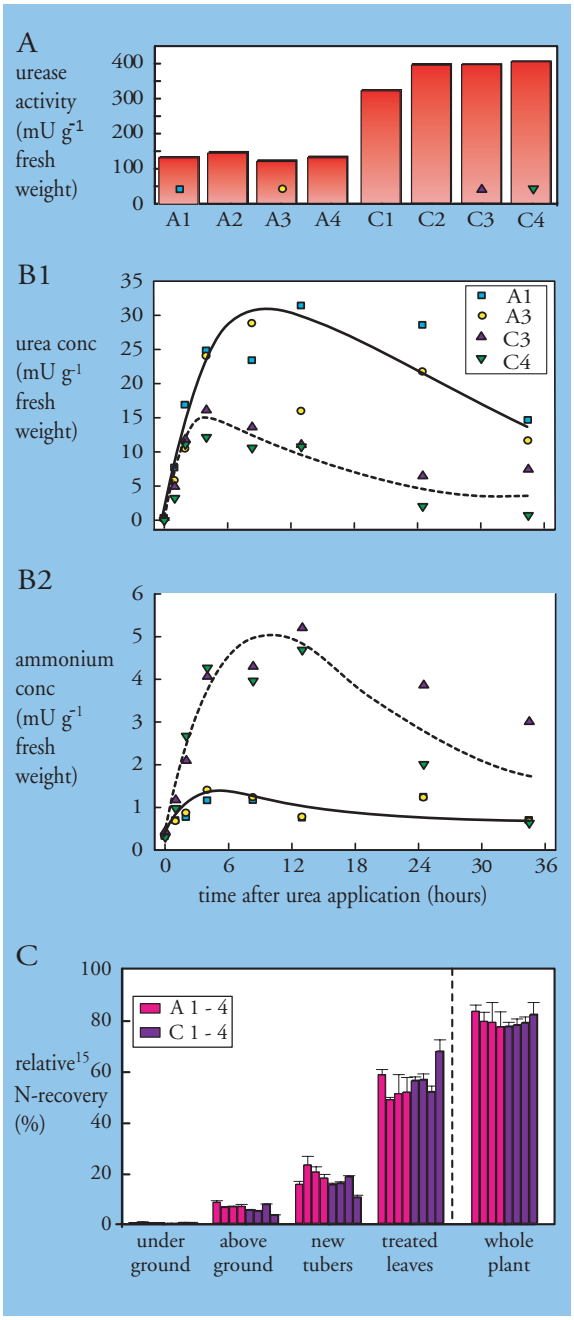


Figure 5 Urea/ammonium accumulation and N distribution/losses following urea application in control plants and transgenic plants with urease activity down-regulated. A, urease activities in leaves of transgenic plants (A1-A4), vector-only control plants (C1-C3) and wild type (C4). B1, urea concentration in leaves after urea application. B2, ammonium concentration in leaves after urea application. C, 15N distribution and recovery 8 days after application of non-labelled urea followed by 15N urea. Percentages refer to total amount of 15N applied. The error bars indicate the confidence interval (P = 95%) for the mass-spectrometric determination of 15N.

Using the transgenic plants generated the influence of urease activity on the losses of foliar applied urea fertiliser was investigated (Fig. 5). Results from ^{15}N pulse-chase experiments using a simplified leaf disc model system indicated that urease activity was not correlated with nitrogen losses. However, clear differences in leaf urea and ammonium content were demonstrated which were associated with urease activity. Experiments with intact plants confirmed these results. Despite the higher levels of endogenous ammonium after urea application in plants with low urease activity, there were no increased nitrogen losses from these plants. Ammonia volatilisation was not correlated with leaf urease activity. Apoplastic ammonium concentration, which is a major determinant of the plant's ammonia compensation point, might be unaffected by modifications to symplastic ammonia concentration due to the compensating action of NH_4^+ transport systems in the cell membrane.

Conclusions Urea has long been known as an alternative nitrogen source to nitrate for crop production. It is advantageous in avoiding nitrate losses when used in foliar applications as it is taken up by the plant directly and should rarely come into contact with the soil. Urea is readily assimilated via the leaves and incorporated into the plant's nitrogen pool. Although urea hydrolysis leads to greatly increased ammonium concentrations in the canopy there are no obvious harmful effects for the plant if the urea concentrations in applications are not too high. Importantly, there does not seem to be a connection between the level of urease activity in the leaf cells and volatilisation losses from the canopy after urea spray. Therefore, it appears that urea metabolism by the potato leaves does not lead to any additional nitrogen loss to the environment.

Acknowledgements

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References

[1] Witte, C-P., Le, Q.H., Bureau, T. & Kumar, A. (2001). *Proceedings of the National Academy of Science,* USA **98**, 13778-13783.

[2] Witte, C-P., Isidore, E., Tiller, S.A., Davies, H.V. & Taylor, M.A. (2001). *Plant Molecular Biology* **45**, 169-179.