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Executive Summary

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# Role of Lectins in the Peanut Nodule

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This project investigated two lectins found in the nodules of peanut (*Arachis hypogaea* L.). One is a galactose-binding lectin (GL), the other a mannose-binding lectin (ML). While certain legume lectins have been implicated in the recognition of infective rhizobia, the peanut nodule lectins may function, *inter alia*, in protein storage or packaging and in this way contribute to the efficient performance of peanut nodules. The nodule lectins were previously found to differ in certain properties from related peanut seed lectins and may have belonged to a group of symbiotically important nodule-specific proteins termed nodulins. The present study provided evidence that the individual lectins are produced by different genes and examined their expression during nodule development.

GL- and ML-encoding cDNA clones were isolated using cDNA libraries from the seed and nodules of peanut. Sequence analysis indicated that GL clones from seed corresponded closely to a selected clone, SB2, whereas nodules yielded two different clones NG#24 and NG#30. Nucleotide and amino acid homologies between the lectin-encoding regions of SB2 and NG#30 were relatively high (92.0 and 89.2 %, respectively) whereas those between SB2 and NG#24 were lower (78.1 and 72.3 %, respectively). Clone NG#30 contained a glycosylation site that was absent in clones NG#24 and NG#30. Specific antisense RNA probes for NG#24 and NG#30, respectively, were synthesized. The two nodule ML clones that were isolated appeared to originate from the same gene and their nucleotide and amino acid homology with seed ML clones was at least 95 %. Nodule ML is therefore unlikely to be nodule specific.

During an initial plant growth experiment, synchronously formed peanut nodules emerged 10 d after inoculation with rhizobia. Nodule GL NG#24 and NG#30 transcripts were first detected at 14 d, when nodules turned pink due to the presence of leghaemoglobin. ML expression in nodules was only detected at 21 d. Late expression of GL and ML further indicated that neither are involved in rhizobial infection processes. Interestingly, there was evidence of differential gene expression. Maximum lectin expression occurred at 28 d, but levels of NG#24 were higher and declined more slowly than those of NG#30 over 42 d. Nodule GL may not be a true nodulin as low levels of NG#24 were detected at 21-35 d in roots of inoculated plants (NG#30 was also detected

in roots in a subsequent test). However, expression appeared to be governed by nodule-related processes as neither clone was detected in roots and shoots of uninoculated N-control plants, or shoots of inoculated plants. ML transcripts were, however, expressed in shoots and roots of both nodulated and N-control plants from 21 d.

In a second test, NG#24 and NG#30 transcripts were not detected in nodules formed by an ineffective strain, while ML transcripts remained at a resting level. Exposure of effectively nodulated peanut roots to combined nitrogen (14 mM nitrate) for 6 d induced an 8.3-fold reduction in the concentration of total RNA in this tissue together with lowered GL and ML transcript levels. Measurement of the acetylene reduction activity of nitrate-treated plants, however, showed that their $N_2$-fixing activity was similar to that of untreated control plants. This was unexpected as high nitrate is a powerful and rapid inhibitor of nitrogen fixation in other legumes. A possible explanation is that accumulated food reserves in the nodule, in the form of lipid, starch and lectin protein overcame nitrate-induced depletion of energy supply to the nodule. The effect of nitrate on the nodule has not previously been studied in peanut and is an aspect that will be further investigated.

In conclusion, this study shows that synthesis of nodule GL is governed by this structure. In contrast, peanut ML does not appear to be tissue specific. Although an effective nodule is required to stimulate lectin synthesis, GL and ML expression may not be directly linked to $N_2$ fixation activity; both genes were inhibited by nitrate under conditions that did not directly affect fixation. The localization of lectin gene expression sites in nodule tissues and the determination of gene structures responsible for tisssue-specific nodule GL gene expression are immediate targets for further analysis.

Aspects of this work were presented at the VIth Conference of the African Association for Biological Nitrogen Fixation, 12-17th September 1994, Harare, Zimbabwe and at the 10th International Congress on Nitrogen Fixation, 28th June - 3rd May 1995, St Petersburg, Russian Federation.

The nucleotide sequence data reported in this study appears in the Genbank, EMBL and DDBJ Nucleotide Sequence Databases under the accession numbers U22468 (NG#30), U22470 (NG#24), U22471 (SB2), U22469 (NMLa), U22472 (SMBa), U22473 (SMBb).

Two articles are in preparation for submission to an international journal: Cloning and expression of cDNA for galactose-binding lectin from peanut nodules & Cloning and expression of cDNA for mannose-binding lectin of peanut.

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