

FINAL REPORT

Development of a low bloat risk lucerne cultivar

Daarstelling van 'n lae opblaasrisiko lusern kultivar

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Executive Summary

Title: Development of a low bloat risk cultivar

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Aim: To determine selection criteria and to study the environmental influence on these factors.

The main aim of the study conducted the past four years was to determine selection criteria which could be used in a breeding program for developing a low bloat risk lucerne cultivar. A study was done to determine if Random Amplified Polymorphic DNA (RAPD analysis) patterns characteristic of the cultivars could be obtained. The report of Burger indicated that by using bulk groupings of genomic DNA from several individuals of a heterogeneous population in PCR reactions with random primers, RAPD patterns can be obtained. This indicates that lucerne cultivars may be characterised using this technique. However very few significant differences were found between the cultivars in the other studies.

Bekker found a significant difference in the immediately soluble protein fraction of WL 321 (31.4%) and CUF101 (34.9%). However, the difference in mean degradation rate of the insoluble but potentially degradable fraction of the two cultivars was not significant as was the difference in the mean maximum extent of protein degradation. The difference in the *in vivo* digestion study was also far more significant for growth stage than for cultivar effects. The electron microscopy study of Bekker also revealed little intercultivar variation for cuticle and mesophyll cell wall thickness. Robbertse's electron microscopy study of the anatomy of *in vitro* digested leaves showed very little difference between cultivars and even between species. The study indicated that digestion of the protoplast takes place before any deterioration of the cell wall could be detected.

Results of the % cell rupture and % tissue damage screening method of Venter revealed no difference between lucerne cultivars. Because of the small variation between cultivars within the lucerne species, it was decided to study known bloat causing and non-bloat causing legume species. A significant difference was measured between bloat causing and non-bloat causing species in terms of % cell rupture with the Additive Main effects and Multiplicative Interaction (AMMI) statistical model. These results correlate well with those obtained by Paulsmeier in an *in vitro* foam formation study in which significant differences were obtained between the non-bloat causing and bloat causing cultivars. The lucerne and clover species formed larger quantities of stable foam than the non bloat causing species, Lotus and Pink Serradella (results: Progress report 1995).

The anatomical study in support of this phenomenon must be considered bearing in mind the results of Bekker and Robbertse. Their results place a question mark on the whole cell rupture hypothesis in terms of cell wall thickness and digestion rate. The results from the screening method indicate that the anatomy of the leaf is

significant. Anatomical features which determine the total strength of the leaf are important. Such features are the venation pattern of the leaf, the percentage strengthening tissue and the ratio of palisade to spongy mesophyll. Other features such as cuticle thickness, epidermis cell wall thickness, percentage intercellular spaces are important in terms of the penetration and digestion rate of the rumen microbes. Percentage tissue damage may appear to be insignificant in terms of the release of intracellular components, but is in terms of the tempo the cell walls of the inner leaf tissue are exposed to microbe digestion. It was therefore decided to study the leaf anatomy using a stereological and plant biomechanical approach. This means the entire leaf structure must be studied and the different features related to its importance for tempo of biodegradation and mechanical damage. This study has been presented, and accepted, as a PhD proposal at the department of Botany at the University of Pretoria. All the material needed for the study was collected and prepared for electron- and light microscopy study during the growing season August to December 1996. It is foreseen that, with the academic support from the university, useful data in support of the screening method will be obtained. The final results will be presented as a PhD thesis at the end of 1998.

Results of the screening method have been presented as a paper, and the development of the method was also presented as a poster, at the Annual Grassland congress during January 1997.

No additional funds will be requested for the completion of the anatomical study as this is a final report. However, a motivation will be submitted for the use of funds left over from the 1996 financial year, as some of the practical actions could not be completed until the PhD proposal was accepted.