THE STRUCTURE OF THE SEED COAT OF LUPINS (*LUPINUS* SP.) AS AN INDICATOR OF POTENTIAL CHANGES IN NUTRITIVE VALUE

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SUMMARY

The chemical components and structures of seed coats of 19 lines of *Lupinus angustifolius, L. pilosus, L. atlanticus and L. cosentinii* were determined. The results showed that neutral and acid detergent fibre contents in *L. pilosus, L. atlanticus and L. cosentinii* were significantly higher ($P<0.01$) than in *L. angustifolius.* There were no differences in lignin and protein contents among *L. pilosus, L. atlanticus and L. cosentinii. The* thickness of seed coats had large variation among the species. *L. pilosus* had the thickest coat (611.6 ± 34.7 μm), and *L. angustifolius* had the thinnest (302.9 ± 5.3 μm). The correlation between the chemical components and the thickness of layers is discussed.

Keywords: seed coat structure, chemical component, lupin

INTRODUCTION

The seed coat is an important structure because it is the protective barrier between the embryo and the external environment. Seed coats have effects on seed performance such as interference with water uptake and gas exchange, mechanical resistance, prevention of the exit of inhibitors from the embryo and supply of inhibitors to the embryo (Esau 1977; Bewley and Black 1985). These functions may be changed with breeding and selection because the modification of 1 or more characteristics could cause a chain of events where each change is accompanied by or associated with other changes (Lush and Evans 1980; Plitmann and Kislev 1989). For example, reduction of branches of lupins *(L. angustifolius)* was associated with a decrease of seed size and an increase in the number of seeds (Delane and Hamblin 1986).

The most obvious physical change in legumes with breeding and selection was a decrease in seed coat thickness (Lush and Evans 1980). Anatomically, such a decrease is caused by a reduction in the palisade layer. Generally, domesticated legumes have thinner seed coats than their wild relatives. Changes in seed coat thickness may have 2 effects that influence animals: i) altered distribution and content of nutrient components, and ii) altered utilisation of those components of the seed coat. With past and ongoing plant breeding efforts with *L. angustifolius,* it would seem likely that a range of changes may have occurred in the seed coat. If these show a predictable pattern, the direction and rate of change that might occur through domestication of other lupin species currently being investigated (Buirchell and Cowling 1992) such *as L. pilosus* and *L. atlanticus* might be estimated. Therefore this work examined seed structure and seed coat chemical composition among different species, and lines in *L. angustifolius* to determine the range of variation.

MATERIALS AND METHODS

The lupin lines

The seeds of 19 lines of lupins including 13 lines of *Lupinus angustifolius, 2* lines of *L. pilosus,* 3 lines of *L. atlanticus and* 1 line of *L. cosentinii* came from Western Australia. Six hundred seeds in each line were separated into coat and kernel by hand and the seed coat thickness, seed coat percentage of whole seed weight and the chemical components of the seed coat were determined.

The coat thickness analysis

Three seed coat pieces (5 mm in any dimension) of each line were embedded in GMA monomer (GMA, polyethylene glycol and benzoyl perioxide) for 2 days. Slides were prepared for the thickness analysis using the Sorvall JB4 microtome with glass knives. The 2.0 micrometer thick tissue sections were observed with a x10 objective lens. The thickness of 4 tissue layers (palisade, sub-palisade, hourglass and parenchymatous) was measured by images collected with a Bio-Rad computer imaging system MRC1000 connected to a Nikon Diaphot 300 microscope and using CoMOS image analysis software.

The chemical component analysis

Neutral detergent fibre (NDF), acid detergent fibre (ADF) and lignin (2 replicates) in 19 lines were determined using the method of Harris (1984). Protein (2 replicates) of 19 lines was determined using the Kjeltec Auto 1030 (Kjeldahl method).

RESULTS

The seed coat proportion of seed weight and seed coat chemical components

The seed coat proportion in whole seed and seed coat chemical components showed great variation among the species. A higher seed coat proportion of a whole seed and higher ADF and NDF ($p<0.01$) were found in wild lupins (Table 1). The coat percentage ranged from 28.4% to 32.7% in *L. pilosus, L. atlanticus* and *L. consentinii,* but *was* only 23.8% in *L. angustzjblius.* NDF and ADF levels in *L. consentinii, L. atlanticus* and *L. pilosus* were significantly higher than in *L. angustifolius*.

Table 1. The structure of lupin seeds and composition of seed coats - the values are means ± SE

Species	Seed weight (mg)	Seed coat (% of seed weight)	Crude protein (% of DM)	NDF (% of DM)	ADF (% of DM)	Lignin (% of DM)	n[A]
L. consentinii	178±1	32.7±0.01	4.4±0.2	75.5±1.0	69.6±1.4	4.9±1.4	1
L. atlanticus	238±9	31.0±0.02	2.3±0.2	78.9±0.5	67.7±0.7	1.5±0.2	3
L. pilosus	419±5	28.4±0.01	3.0±0.4	81.1±0.6	72.7±0.8	2.0±0.3	2
L. angustifolius	155±3	23.8±0.01	3.1±048	72.1±0.2	60.3±0.3	2.0±0.2	13

[A] number of lines.

Lignin content of coats showed no significant difference among *L. pilosus, L. atlanticus* and *L. angustifolius*, but *L. consentinii* contained more than twice the amount of lignin than the others ($P<0.01$). Crude protein content in seed coats ranged from 2.3% in *L. atlanticus* to 4.4% in *L. consentinii.*

The thickness of seed coats

The total thickness of the seed coat varied among species (Table *2). L. pilosus* had the thickest coat, and *L. angustifolius* had the thinnest. Among wild species, the coat of *L. pilosus* was thicker than the others ($P<0.01$). There was no significant difference between coat thicknesses of *L. consentinii* and *L. atlanticus* ($P>0.05$). The thickness of different layers of the seed coat also varied among species. *L. pilosus* had the thickest palisade layer and a relatively thin sub-palisade layer. Compared with the domesticated lupin *(L. angustifolius*), the wild lupins had a much thicker palisade layer ($P<0.01$).

Table 2. The depth of the 4 cell types (μm) forming distinctive layers in the seed coats of different lupin species - the values are means ± SE

Species	Palisade	Sub-palisade	Hourglass	Parenchymatous	Total	n[B]
L. consentinii	179 ± 14^{a}[A]	121 ± 5^{a}	41 ± 10^{bc}	114 ± 8^{bc}	455 ± 28^{a}	1
L. atlanticus	147 ± 8^{a}	124 ± 13^{a}	69 ± 5^{a}	145 ± 6^{a}	484 ± 14^{a}	3
L. pilosus	339 ± 25^{b}	85 ± 5^{b}	59 ± 8^{ab}	130 ± 4^{ab}	612 ± 35^{b}	2
L. angustifolius	74 ± 2^{c}	83 ± 2^{b}	37 ± 1^{c}	109 ± 2^{c}	303 ± 5^{c}	13

[A] Within columns values with different letters are significantly different ($P<0.05$).
[B] number of lines.

Within species *(L. angustifolius*), the thickness of layers of seed coat in lines that were released in different years is shown in Table 3. The coat total thickness of lines showed no significant difference ($P>0.05$), but some difference existed in different layers. Uniharvest (released in 197 1) had the thinnest palisade and sub-palisade layers which were significantly different from those of Geebung, Danja, Wandoo (released in 1987) and Yorrel, Gungurru and Warrah (released in 1988). There were no significant differences in hourglass and parenchymatous layers among lines.

Table 3 Seed weight (mg), seed coat content (%) and layer depth (μm) of the 4 cell types forming distinctive layers in the seed coats of different lines of *L. angustifolius* - the values are means ± SE

Year[A]	Seed weight (mg)	Seed coat content	Layer depth					n[B]
			Palisade	Sub-palisade	Hourglass	Parenchym-atous	Total	
1967	158±3^{ab}	24.4±0.1^{ab}	67±2^{ab}	77±3^{b}	34±3.5^{a}	115±5^{a}	293±9^{a}	6
1971	187±1^{a}	22.8±0.1^{b}	55±2^{b}	61±2^{b}	36±2.7^{a}	105±2^{a}	257±3^{a}	6
1976	176±1^{ab}	22.9±0.1^{b}	67±3^{ab}	73±4^{ab}	30±4.2^{a}	106±5^{a}	275±12^{a}	6
1979	162±2^{ab}	23.7±0.1^{ab}	66±3^{ab}	75±2^{ab}	36±1.7^{a}	111±4^{a}	288±6^{a}	6
1980	148±1^{b}	24.9±0.2^{a}	74±5^{ab}	104±8^{a}	35±1.4^{a}	99±3^{a}	313±5^{a}	4
1982	155±1^{ab}	24.5±0.1^{ab}	70±5^{ab}	94±9^{a}	31±1.3^{a}	109±4^{a}	304±14^{a}	6
1987	146±1^{b}	23.5±0.2^{ab}	80±5^{a}	90±4^{a}	40±2.7^{a}	105±5^{a}	316±13^{a}	18
1988	147±7^{b}	24.2±0.3^{ab}	79±4^{a}	85±3^{a}	38±3.5^{a}	115±5^{a}	317±11^{a}	23

Within columns values with different letters are significantly different (P<0.05).
[A] line released time: 1967 (Uniwhite), 1971 (Uniharvest), 1976 (Marri), 1979 (Illyarrie), 1980 (Yandee), 1982 (Chittick), 1987 (Geebung, Danja and Wandoo) and 1988 (Yorrel, Gungurru and Warrah).
[B] number of samples for tested for each year.

The correlation between fibre content and thickness of coat layers

NDF and ADF contents in seed coats of lupins are significantly related to the palisade, hourglass, parenchymatous and the total thickness of seed coat (P<0.01) (R= 0.75, 0.73, 0.77 and 0.87 respectively for NDF; R=0.81, 0.64, 0.63 and 0.86 respectively for ADF). Lignin concentration was not related to the thickness of layers (P>0.05).

DISCUSSION

The results showed the thickness of the seed coat layers varied considerably among the species. Generally, domesticated lines had a much thinner palisade layer and total seed coat thickness. For example, the palisade in *L. angustifolius* is only 29%, 33% and 18% of that of *L. cosentinii, L. atlanticus* and *L. pilosus* respectively. Within species, the *seed* coat thickness of *L. angustifolius* of different lines was not different (P>0.05), but the palisade and the sub-palisade thickness were significantly different (P<0.05) between lines. For example, from 1971 to 1988, the palisade of lines have increased (55.3 pm in 1971 to 78.9 μm in 1988). These changes would seem to indicate that they may be correlated with the selection strategies employed by the plant breeders over this time period.

NDF and ADF contents in seed coats of lupins are significantly related to the layer depths of seed coats (P<0.01). Generally, wild lupin species had a thick seed coat with more ADF and NDF than *L. angustifolius*. For example, *L. pilosus* had the thickest palisade layer; it contained 8 1.1% NDF and 72.7% ADF, whereas only 72.1% NDF and 60.3% ADF were found in *L. angustifolius* which had the thinnest palisade layer.

Lignin concentration was not related to the thickness of layers (P>0.05). This means the content of lignin in lupins does not appear to be linked with the change of the thickness of layers. However, the relatively high lignin in *the L. cosentinii* could present problems with availability of the protein, ADF and NDF for digestion as these components can be fermented by rumen bacteria, but their digestibility depends on the extent of lignification (Van Soest 1982). Compared with the cell wall content of the seed coats of other grains, the lupin coat had a lower lignin which resulted in a relatively higher digestibility, 62.5% for NDF, 62.1% for ADF in lupin coats (Rowe and Hargreave 1988). This suggests that lignin in lupins should not generally be a limiting factor for the utilisation of seed coat fibre by ruminants.

General changes in structural and chemical properties of the seed coat of various lupin lines (including commercial cultivars) indicate the need for characterising species used in nutritional studies for ruminants. Also, the developments that have occurred with sweet white lupins *(L. angustifolius)* over the past 30-40 years of breeding serve as a strong reminder that changes in seed coat composition will occur with selection for other characteristics that are important to the agronomic success of a species. If these changes are systematic, we may be able to predict the changes that might occur with the domestication of new species (e.g. *L. pilosus),* and how these may impact on animal production.

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