THE RELATIONSHIP BETWEEN LIGNIFICATION AND FLAVONOID PRODUCTION IN SUBTERRANEAN CLOVER

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[*Manuscript received August* 8, 1970]

Summary

The lignin content of both seed coat and burr of a white-seeded mutant of the Geraldton variety of subterranean clover was significantly less than the parent. Reduced lignification occurred in plants homozygous for the simple recessive gene c^i which also inhibited flavonoid production. This dual effect is explained by a genetic blockage in that part of the biochemical pathway common to the synthesis of both lignin and flavonoid compounds.

The presence of gene c^i was also associated with an increased permeability of the seed coat, which could detract from the agronomic potential of varieties homozygous for this gene.

I. Introduction

Chemically produced isoflavone mutants of the Geraldton cultivar of subterranean clover (*Trifolium subterraneum*) have been described by Francis and Millington (1965*a*, 1965*b*). Wong and Francis (1968) subsequently reported on the total flavonoid content of these and an additional white-seeded mutant (N4285). This latter mutant was deficient in all flavonoids which apparently represented an early block in the pathway of flavonoid biosynthesis.

Kannenberg and Allard (1964) showed that a simply inherited recessive gene c^i, when present in the homozygous state, substantially reduced the production of both pigment and lignin in white-seeded lima beans (*Phaseolus lunatus*). Since it seemed possible that the white-seeded clover mutant could be analogous to that in the lima beans, a comparison was made of the lignin contents of the seed coats of both the parent and the mutant varieties. The dry mature herbage was also analysed for lignin because of the possible influence of lignin on the digestibility of dry pastures by sheep (Hume, Somers, and McKeown 1968).

Studies on the inheritance of the white-seeded character and of its effect on seed coat permeability are also reported because of the importance of hard-seededness for survival of annuals in the Mediterranean environment of southern Australia (Quinlivan 1968).

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II. Methods

(a) *Genetic Analysis*

Genetic analysis was carried out on the F_1 and F_2 populations derived from reciprocal crosses between N4285 and the Geraldton parent. Plants were individually tested for isoflavone content by the methods of Francis and Millington (1965*a*).

(b) *Lignin Analysis*

The Geraldton and N4285 cultivars were grown as replicated (2) rows on a loam-based soil at Shenton Park Research Station, near Perth, W.A. Samples (2 g) of material collected from each replication about 2 weeks after maturity were analysed for lignin content by the method of Van Soest (1963). For comparison a third clover, mutant L858 (now cv. Uniwager) was also analysed for lignin content. Like N4285, mutant L858 is deficient in all flavonoids (Wong and Francis 1968), but is distinguishable from N4285 by black seeds. It is homozygous for the recessive genes $b^i b^i$ (Francis and Millington 1965*b*). No lignin contents have previously been recorded for mutant L858.

(c) *Tests for Hard Seeds*

Tests for hard (impermeable) seeds were made on two replications of 100 seeds at four test periods over 3 months during which the seeds were stored in a daily fluctuating temperature of 15–60°C. The details of the method were described by Quinlivan (1968). A "hard" seed is defined as one which does not imbibe water after 10 days on a moisture pad.

III. Results

(a) *Isoflavone Content of Geraldton and N4285 Seeds*

The isoflavone contents of parent and first-generation seeds are given in Table 1 and show that the white-seeded segregates were all low in isoflavones. The F_2 segregation ratio (864 like Geraldton, 301 like N4285) approximated to a 3 : 1 mendelian ratio (χ^2 for deviation from an expected 3 : 1 ratio = 0·435, which is not significant) for segregation of a simple recessive gene which we have designated c^i.

Table 1

Isoflavone content of Geraldton and N4285 seeds and of seeds from the F_1 generation (Geraldton × N4285)

Generation	Isoflavone Content (as % of dry wt.)*		
	Formononetin	Genistein	Biochanin A
Parents			
Geraldton (black seeds)	1·01	0·54	1·05
N4285 (white seeds)	0·24	0·02	0·11
F_1 (black seeds, all like Geraldton)	1·04	0·62	1·10

* Percentage average standard error of mean = 9·4.

(b) *Lignin Content of Test Varieties*

N4285 seed coat and burr contained less lignin ($P < 0·05$) than Geraldton but there was no difference in the lignin content of L858 and Geraldton seed coat and burr.

N4285 also contained less lignin ($P < 0{\cdot}05$) than Geraldton. Results are summarized in Table 2.

TABLE 2

LIGNIN CONTENT OF PARENT AND MUTANT VARIETIES

	Lignin Content (as % of dry wt.)			Least Significant Differences		
	Geraldton	N4285	L858	5% Level	1% Level	0·1% Level
Seed coat	4·2	0·9	4·6	0·42	0·70	0·84
Leaf	7·1	5·8	7·0	1·20	2·21	2·91
Burr	12·4	3·8	11·8	2·14	3·74	4·98

(c) *Hard Seed Content*

N4285 had a markedly lower hard seed content than the Geraldton parent, and after 3 months very few hard seeds remained. There was a very highly significant

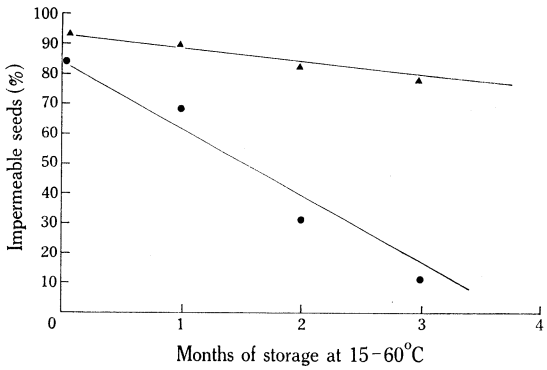

Fig. 1.—Effect of storage on hard seed content of Geraldton parent (▲) and N4285 mutant (●). The regression equations are as follows:

Geraldton: $y = 93{\cdot}4 - 4{\cdot}6x$

N4285: $y = 86{\cdot}3 - 22{\cdot}1x$

difference ($P < 0{\cdot}001$) between the Geraldton parent and the N4285 mutant in the slope of the mean regression of percentage hard seeds on time (Fig. 1).

IV. DISCUSSION

A simply inherited recessive mutant, designated c^i, is the third in the series capable of inhibiting isoflavone production. Other recessive genes a^i and b^i have already been described by Francis and Millington (1965*a*), and b^i and c^i have been shown to cause substantial reduction in all flavonoids when present as the homozygous recessive (Wong and Francis 1968). Unlike b^i (in mutant L858) the c^i gene appears to markedly reduce the lignin content of the seed coat in addition to the anthocyanin content.

These effects can be explained by a partial block early in the chain of biosynthesis having elements in common for both flavonoids and lignins (see Fig. 2). To inhibit both flavonoids and lignins, gene c^i would logically have to exert its effect prior to the cinnamic acid stage which would be common to both pathways (as indicated in the figure). The finding that many white-seeded clovers (e.g. Yarloop) have a lignin content similar to those with pigmented seeds (Hume, Somers, and

McKeown 1968) can be explained by a genetic block in the flavanonol ——→ anthocyanin sequence in the biosynthetic pathway. The suggested positions of the genetic blocks for N4285 are nevertheless by no means unequivocable, since the mutagen used (ethyl methanesulphonate) commonly causes minute deletions (Heslot and Ferrary 1958) and it is possible that a sequence of closely linked genes is involved affecting lignin, anthocyanins, and other flavonoids selectively. Such a block of genes could be expected to behave as a simple recessive if the deletion was small. However, irrespective of the underlying biochemical genetic mechanism involved, the white-seeded clover phenotype appears similar to the lima bean phenotype described by Kannenberg and Allard (1964).

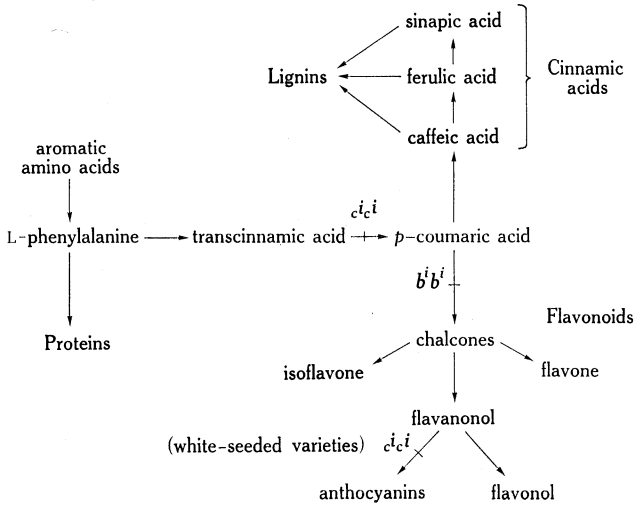

Fig. 2.—Probable pathway for interrelationships of flavonoids and lignins. Probable sites of action of mutant genes c^ic^i and b^ib^i are indicated. After Wong and Francis (1968) and Neish (1960).

In our experiments leaf lignification was less affected by the mutation than was seed coat lignification. This may mean that, in the presence of restricted quantities of precursor, vegetative plant parts (e.g. leaf) are preferentially lignified before reproductive parts (seed coat and burr), or that an alternative biosynthetic pathway exists for leaf lignins.

Mutant N4285, being essentially isogenic with the Geraldton parent, provides ideal material for comparisons involving *in vivo* and *in vitro* digestibility to animals, and the possible effects of lignin and fibre thereon. However, any nutritional advantage associated with the lower lignin content of the mutant N4285 is likely to be negated by the lack of hard-seededness, a severe agronomic disadvantage as a reasonable level of hard seeds is held to be an important prerequisite for continued survival in the drier areas of Western Australia (Quinlivan 1968).

V. Acknowledgments

The financial assistance of the Australian Wool Board and the advice and encouragement of Dr. A. J. Millington, then Reader in Agronomy, University of Western Australia, is gratefully acknowledged.

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