THE UNIVERSITY OF MANITOBA

THE INHERITANCE AND SELECTION OF TANNIN-FREE FABABEANS (*Vicia faba* L.)

A Thesis

Submitted to the Faculty of Graduate Studies

by

Heather J. Crofts

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of

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THE INHERITANCE AND SELECTION OF TANNIN-FREE FABABEANS (*Vicia faba* L.)

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HEATHER JANE CROFTS

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LIST OF ABBREVIATIONS USED IN TABLES

T-cont	-	Tannin-containing
T-free	-	Tannin-free
P	-	Purple-flowered
W	-	White-flowered
♂	-	Male-designated parent
♀	-	Female-designated parent
A	-	Ackerperle
D	-	Diana
H	-	Herz Freya
U	-	UMFB-9
B	-	Broad Windsor
F	-	Fidrim
K	-	Kodrim
T	-	Triple White
s.e.	-	Standard error

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ABSTRACT

Crofts, Heather Jane. M.Sc., The University of Manitoba, October, 1979. The Inheritance and Selection of Tannin-free Fababeans (*Vicia faba* L.). Major Professor; Laurie E. Evans.

The primary objective of this study was to determine the mode of inheritance of a tannin-free characteristic when crosses were made between tannin-free cultivars of the *major* subspecies (Fidrim, Kodrim and Triple White) and cultivars of the *minor* subspecies which are grown in Western Canada (Ackerperle, Diana and Herz Freya). Crosses were also made with an advanced line of the University of Manitoba's fababean breeding programme (UMFB-9) and with a tannin-containing cultivar of the *major* subspecies (Broad Windsor). Secondary objectives were to develop a method of screening for tannin-free seed and to study its relationship to other plant and seed characteristics.

The tannin-free characteristic was found to be conferred by the same single recessive gene in all three tannin-free cultivars. There was no maternal effect and segregation was independent of seed size and hilum colour. There was an absolute linkage with white flower colour and the absence of red pigment in the stem and reddish brown spots on the stipules. These characteristics are therefore useful in identifying plants which produce tannin-free seed.

The gene had other effects which need to be taken into consideration, namely a high incidence of seed marked by a dark grey-brown pigment,

reduced seed coat thickness, an apparent change in seed coat composition (a higher acid detergent fibre fraction) and possibly a greater susceptibility to Fusarium sp. Link and Rhizoctonia sp. D.C..

Three chemical tests were performed on seed coats. These were the vanillin-HCl test, the prussian blue test and the sodium hydroxide test. The simplest and most reliable test for distinguishing between tannin-free and tannin-containing seed was the sodium hydroxide test. Seed could also be classified as tannin-free if it was buff-coloured and failed to turn brown with age.

1. INTRODUCTION

The fababean (Vicia faba L.) is a grain legume grown in much of the north temperate zone and at higher altitudes in the cool season of some subtropical regions (10b). The crop was introduced into Western Canada in the early 1970's following trials in which it proved to have a high protein content (20 to 30%) and yields that equalled those of the high yielding utility wheat grown for comparative purposes. The small-seeded subspecies, Vicia faba var. minor Beck., is preferred because seed can be handled with conventional cereal equipment and seed costs are lower than for larger-seeded types.

Fababeans are known to contain antinutritional factors. Campbell and Marquardt (16) found that autoclaved or microwave-treated fababeans were utilized 10 to 15% more efficiently by chicks than were raw fababeans. They localized a major growth inhibitor in the seed coat and identified it as a condensed tannin.

Several fababean cultivars have been found to have no condensed tannin in their seed coats. These all belong to the large-seeded subspecies, Vicia faba var. major Herz.. Breeding work is now underway to incorporate the character into the minor subspecies. This study was made to determine the inheritance of the tannin-free characteristic when crosses are made between the two subspecies, to discover the best methods of screening for it in a breeding programme, and to note any detrimental effects associated with its introduction.

2. LITERATURE REVIEW

2.1 Condensed Tannins

2.1.1 Definitions

Tannins are well known constituents of many plants. The type, amount and distribution of tannin within plants is characteristic for each species. Within a particular species, the amount varies with the stage of growth, time of year, physiological condition of the plant and other factors (17,38).

Tannin refers to any naturally-occuring, water-soluble compound of high enough molecular weight (between 500 and 3,000) containing a sufficiently large number of phenolic hydroxyl or other suitable groups to enable it to form effective cross-links between proteins and other macromolecules (11). Tannins are technically categorized by their ability to precipitate gelatin from solution (28).

There are two classes of tannins - condensed and hydrolysable. Hydrolysable tannins are esters of sugars and gallic acid. Condensed tannins* have a complex, random structure in nature but are mostly polymers of flavanols, called flavolans. The various terminologies and the relationship of the condensed tannins to other flavonoid compounds are clarified in Figure 1.

*In the remaining text, 'condensed tannin' and 'tannin' will be used synonymously.

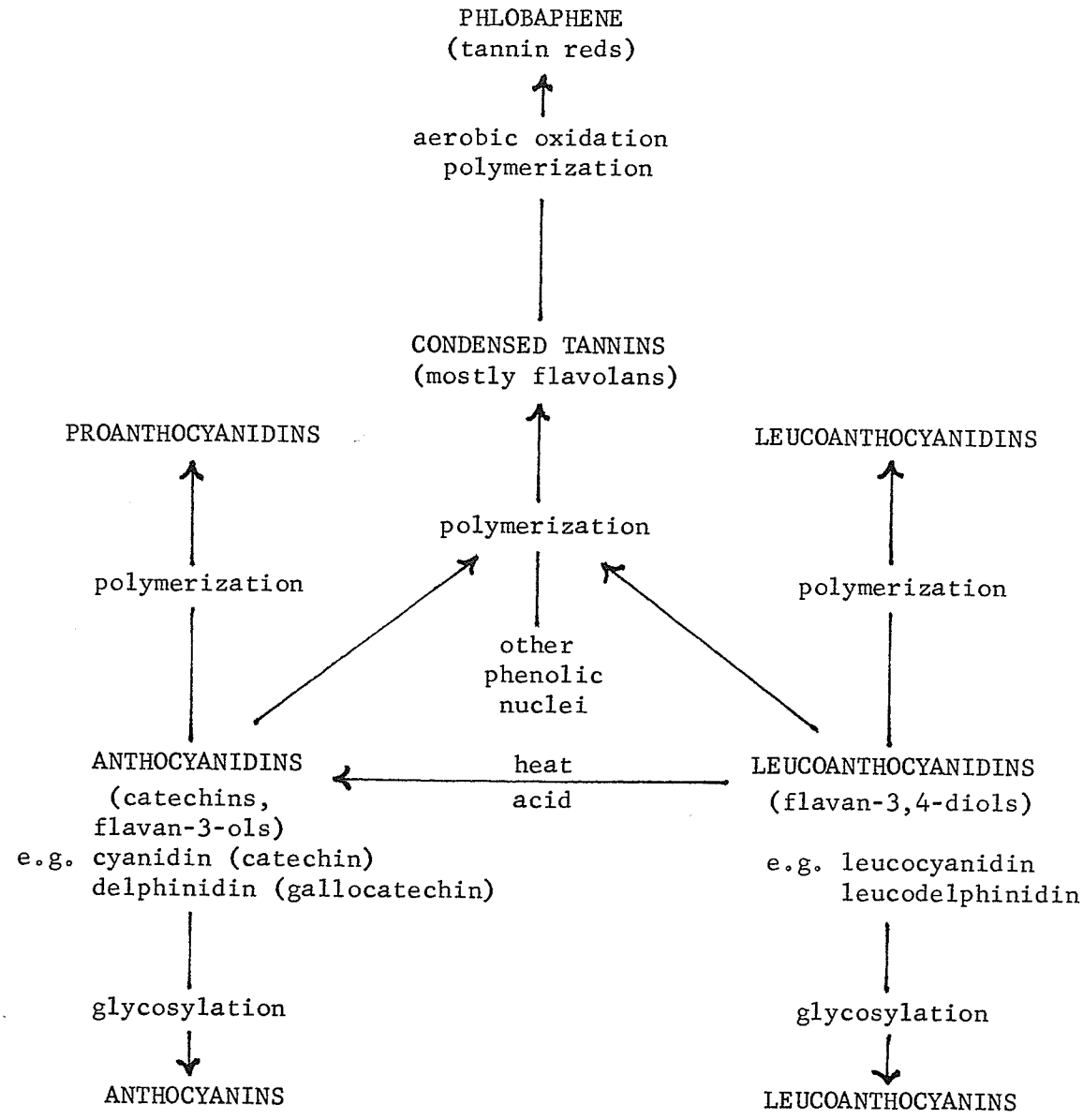

Figure 1. The relationship of condensed tannin to other flavonoid compounds

Condensed tannins are colourless compounds (57) but they are readily oxidized to dark red or reddish-brown phlobophene (38). They yield anthocyanidins as degradation products when they are heated (73). Gel electrophoresis has indicated that anthocyanidins and leucoanthocyanidins occur naturally in the presence of condensed tannins (39) and since they all have similar chemical properties, anthocyanidins and leucoanthocyanidins could be responsible for reactions in plant tissues that have been attributed to condensed tannins (6).

Anthocyanins and leucoanthocyanins are gylcosides of anthocyanidins and leucoanthocyanidins respectively. Anthocyanins are coloured compounds occuring in all orders of higher plants. They are closely linked to development and are responsible for the pigmentation of stems, leaves, fruits and flowers. Leucoanthocyanins are colourless and are suspected of acting as growth hormones in developing seeds (8). They are particularly widespread in plants containing condensed tannins and their presence appears to be associated with a woody habit in the plant (6).

Condensed tannin has been isolated and purified from the seed coats of fababeans (52,53,88). It is extractable in water and soluble in several organic solvents. Marquardt (46) found the condensed tannin to be a polymer of cyanidin, delphinidin and an unknown subunit. Martin-Tanguy *et al*. (53) found polymers consisting of molecules of both anthocyanidins (cyanidin and delphinidin) and leucoanthocyanidins (leucocyanidin and leucodelphinidin).

2.1.2 Chemical Estimation

Methods for estimation of tannin content were developed primarily for sorghum and forages and are based on the formation of coloured products. No method measures tannin content in clearly definable substances (54). Several standard methods are available which differ in their specificity. Some methods make no distinction between tannin and other phenolic compounds but this is not a problem when the concentration of nontannin phenolics is low, as it is in sorghum grain (66). Reactivity differs with the various reagents and polyphenols due to steric hindrance and the blocking of active sites by polymerization (68). Methods which depend on redox reactions (such as Folin-Denis reagent and the formation of the prussian blue complex) are less specific than others such as vanillin which substitutes in the A-ring of flavonoids when there is no polymerization at the active site. Vanillin will detect monomeric anthocyanidins and leucoanthocyanidins as well as condensed tannin.

Maxon and Rooney (54) evaluated many different methods for tannin analysis in sorghum grain and concluded that only three methods had potential - Batesmith and Rasper's method (7), urea extraction followed by reaction with ferric ammonium sulphate, and a modification of the vanillin-HCl method developed by Burns (13). Maxon and Rooney (54) modified the method by extracting with 1% HCl instead of pure methanol. While the reproducibility of this latter method was less than that of the other two, it was relatively simple and rapid. Recently, several other modifications have been suggested to further reduce extraction time and to improve sensitivity, accuracy and reproducibility (12,19,66,67,79). The vanillin-HCl method and its modifications have

been used for the analysis of condensed tannin in species such as sorghum (14,18,66), crownvetch and sericea lespedeza (15), fababean (50) and common bean (45).

Price and Butler (66) recommend the formation of a prussian blue complex as a method of enabling the polyphenol content of sorghum grains to be quantitatively compared. The test is based on the reduction by tannin and other phenols of ferric ion to ferrous ion followed by the formation of a ferri-cyanide - ferrous ion complex which can be measured spectrophotometrically. The advantages of this test over the vanillin-HCl test are its rapidity and its sensitivity. Price and Butler (66) found several sorghum cultivars considered to be low in tannin to contain tannin according to the prussian blue test but not the vanillin-HCl test. The disadvantages of the prussian blue test are that almost any reducing substance will give the reaction and the estimated amount of polyphenols changes rapidly with the time allowed for extraction. However, the proportion of nontannin phenolics can be accounted for by extraction of nontannins in aqueous salt solutions and, provided that the seed samples are roughly equivalent in size, the tannin extracted in a given time will be proportional to the total tannin content. There is also difficulty in making a colour assessment after addition of the reagents since all tannin-containing samples will eventually give a blue solution. It is therefore important to score the colour after a specific time interval.

The assessment of large numbers of lines in a breeding programme requires a quick qualitative test. A modification of the prussian blue test has been suggested (66) in which extraction time is reduced to one minute. Within a few seconds of adding the reagents, the solution will

vary from yellow through green to blue - colours which can be assessed by eye and samples graded accordingly. A qualitative estimation of tannin in legume pasture species was made by Jones et al. (39) using the 'vanillin-HCl spot test'. Leaves and stems were crushed between chromatography paper and vanillin-HCl solution applied to the imprints on the paper. They graded the plants into five groups according to the intensity of the colour (light pink to intense red-violet) over the imprint.

Marquardt et al. (50) found that tannin-containing seed coats of fababean cultivars formed a dark coloured complex when exposed to oxygen or incubated in basic solution whereas tannin-free seed coats did not. They suggested that this darkening may be associated with the formation of an altered form of condensed tannin, in which case it would be a valuable test for distinguishing between tannin-free and tannin-containing cultivars. Soaking whole grains in a 5% solution of sodium hydroxide in water has proved to be a completely reliable test for distinguishing between red and white wheats, though samples tend to be too variable to allow sub-classification according to shade. After 20 or 30 minutes the red grains appear brick red whilst the white grains turn a straw yellow (90). The red pigment of wheat grains is a phlobophene (57).

2.1.3 Effects

The significance of a high tannin content relates to the ability of tannin to form cross-links with soluble proteins and other macromolecules, thus enabling them to inhibit many enzyme systems (11). Condensed tannins have been implicated as inhibitors of plant enzymes involved in germination and in dead and dying cells, as factors in the resistance of plant cells to attack by fungi and other pathogens, as a cause of astringency when eaten by animals and as inactivators of digestive enzymes and intestinal epithelium in animals.

Various condensed tannins have been found to inhibit plant enzymes. They have been shown to retard preharvest seed germination in sorghum (33), wheat (84) and barley (37). Condensed tannins appear to act as gibberellin antagonists in germinating wheat and barley seeds and in the growth of pea and cucumber seedlings (17).

Phenolics are highly toxic to micro-organisms and are often localized in plants around points of infection where they may increase in concentration or undergo reactions to form toxic derivatives. In fababeans there is possibly an inhibitor of polyphenoloxidase. When this inhibitor is destroyed, the polyphenols are oxidized into compounds which inhibit the growth of pathogenic fungi (44). Phenolics present in seed coats have been found to be responsible for aiding the resistance of sorghum to moulding (caused mainly by Fusarium sp. Link) (35), the resistance of common beans to Fusarium solani f. sp. phaseoli (Burkh.) Snyd. & Hans. (82) and Rhizoctonia solani Kühn (65), and peas to Fusarium solani f. sp. pisi (F.R. Jones) Snyd. & Hans. (42) and to Pythium ultimum Trow (55). However, total quantities of phenols in pea seedling exudates are probably not related to Fusarium and Pythium root

rot resistance (41). In 1978, P. Kharbanda (personal communication) planted three cultivars of fababeans with tannin-free seed coats and several with tannin-containing seed coats on land in which soil pathogens had built up over six consecutive years of legume crops. The tannin-free cultivars were amongst those most susceptible to root rot (mainly Fusarium sp. Link and Rhizoctonia sp. D.C.)

The ability of tannins to complex with proteins can be advantageous in preventing bloat in ruminants feeding on certain tannin-containing herbaceous legumes. The insoluble complexes are able to prevent foaming in the rumen (39). Tannin might be acting as an inhibitor of pectin esterase which causes the gelling of pectin (60). Sarkar et al. (73) tested a number of herbaceous legumes. All tannin-free legumes caused bloat and all but one of the eight tannin-containing legumes did not cause bloat.

On the other hand, tannin-containing plants can be a disadvantage because of their astringency resulting from binding of glycoproteins in saliva, thus discouraging feeding (11). Chemical analysis of developing grain of bird-resistant and susceptible sorghum hybrids showed that the resistant hybrids had four to eight times the amount of total astringents (87). Blessin et al. (9) suggested that leucoanthocyanins may have resulted in some of the astringency ascribed to condensed tannins. The low palatability of various herbage plants such as sericea lespedeza has been attributed to their condensed tannin content (22).

More importantly, condensed tannins in feed have been reported to depress growth rates of cattle, pigs, rabbits, chicks and rats. There is general agreement that very high levels of tannin in feeds such as

sericea lespedeza forage, carobs and grain sorghum exert toxic effects (30). Condensed tannins from carobs primarily inhibit α-amylase (86). Condensed tannin in sorghum have also been identified as inhibitors of amylase (54).

Schaffert et al. (74) suggest that sorghum tannins do not affect digestibility directly but indirectly by reducing the amount of nitrogen available for bacterial growth in ruminants. In poultry, supplementation with methionine overcomes growth depression of birds fed tannin-containing sorghum (3,27). Armstrong et al. (4) has given three possible explanations for this, the most probable being that the sorghum tannins adversely affect methionine utilization. Through amino acid supplementation of chick diets, Elkin et al. (24) found this to be true. Lysine was the first limiting amino acid in their low tannin sorghum-soybean meal-safflower meal diet but methionine and lysine became equally limiting in the equivalent high tannin sorghum-soybean meal-safflower meal diet. The overall result of feeding high compared with low tannin sorghum to poultry was reduced dry matter utilization and nitrogen retention which gave depressed chick growth, bowed legs and swollen hock joints (23,24).

Condensed tannin in fababeans are said to have similar effects to those in sorghum. Nitzan (58) found that rats fed tannin-containing fababean meal had lower enzymic activity, significantly reduced amylase levels and poorer nitrogen utilization and retention than rats fed heated soybean meal. Wilson et al. (89b) demonstrated in vitro the presence of trypsin inhibitor activity which was eliminated by autoclaving. Autoclaving (47,48,51,89b) and removal of the seed coat

(88) have both been shown to improve the utilization of fababeans by poultry. Marquardt et al. (52) fed chicks a diet containing 3.9% purified condensed tannin isolated from fababean seed coats and showed reduced feed intake, negative weight gains, larger feed:gain ratios and reduced dry matter, amino acid and crude fibre retentions. Martin-Tanguy et al. (53) also found that condensed tannins in fababeans had an inhibitory effect on protein digestion by poultry. Bond (10a) found the in vitro digestibility of fababean seed coats by rumen bacteria to be about three times greater when they contained no tannin. It seems likely that the tannin inhibits enzyme systems responsible for fibre digestion either by inhibiting cellulases or other carbohydrases, or by inducing nitrogen deficiency in the in vitro system due to protein binding, or both (32).

However, not all agree that condensed tannin is an important antinutritional factor in fababeans. Sjödin (82) found no difference in the feed intake of mice fed fababeans with and without the seed coat, Wilson et al. (89a) found no response in the growth of chicks to the removal of tannin from fababeans, and work at the Rowett Research Institute (56) has tended to indicate that the level of tannin in fababeans does not affect poultry. Ivan and Bowland (36) fed raw and autoclaved fababeans to pigs but failed to detect any improvement in digestibility.

Despite some disagreement as to the effects of condensed tannin and its importance in animal feeding, breeding is being undertaken to produce more tannin-free cultivars.

2.1.4 Inheritance

Specific phenolics in a plant species are generally simply inherited (44). Since condensed tannins are composed of a number of monomeric flavonoids, single genes affecting polymerization or the production of a major precursor would be expected to result in simple inheritance of a low-tannin or tannin-free characteristic.

Ma and Bliss (45) analysed four F_2 populations of common bean resulting from crosses between lines differing in tannin content. The continuous pattern of distribution for three of the populations suggested a quantitative inheritance pattern, but the forth population had an F_2 segregation which did not deviate significantly from that expected for one incompletely dominant gene for tannin, indicating that a few genes are probably responsible for genetic differences. Low tannin was dominant to high tannin in the other three populations and broadsense heritability was high (H_b= 0.84 to 0.97). Paroda *et al.* (59) also found low tannin to be dominant to high tannin in sorghum and the narrowsense heritability was high (H_n= 0.61).

In fababeans, several sources of a gene for tannin-free seed coats have been found amongst cultivars with white flowers and white (or buff) seeds. There are at least two different complementary genes that confer the tannin-free characteristic suggesting that the synthesis of condensed tannin can be blocked in at least two different stages (62). Amongst the tannin-containing cultivars, there is a range of tannin concentrations and a considerable amount of this is environmental variation (50).

2.2 Plant Characteristics

2.2.1 Seed Weight

The seed weight of a legume species is commonly used as an indicator of seed size. Vicia faba L. has been divided into four subspecies accordingly - paucijuga (less than 0.2 g per seed), minor (0.2 to 0.5 g), equina (0.5 to 0.8 g) and major (over 0.8 g). The larger-seeded cultivars are grown mainly for human consumption and all sources of the tannin-free characteristic have been large-seeded types (Vicia faba var. major Herz.). The major disadvantage of the major cultivars when grown on a large scale is the high cost of seed to the producer. Smaller seed size is also favoured on the Canadian prairies because the crop can then be handled by cereal equipment (78).

Seed weight together with seeds per pod are the most highly heritable of the yield components of fababeans and are the only components unaffected by virus infection (29) but they are not very closely correlated with yield (64). In crosses between major and minor subspecies, seed weight mostly reflects the genotype of the maternal parent. Erith (25) crossed major and minor cultivars and showed that the character was quantitatively inherited with the mean weight of seed from F_1 and F_2 plants falling closer toward that of the minor parent.

2.2.2. Seed Coat

In legumes, the seed coat is tissue of the maternal parent. When the plant dies, phenolic monomers are transformed into immobile polymers such as condensed tannin and phlobophene (28). Since condensed tannins are largely formed post-mortally, it is not surprising that they are often found in high concentrations in seed coats (5).

Condensed tannins are generally associated with woody tissue. In herbaceous plants, the presence or absence of tannin or leucoanthocyanin in the leaves appears to be connected with a woody habit in the plant (6). As well as containing about 90% of the tannin, the fababean seed coat contains about 90% of the seed's crude fibre (70).

The legume seed coat is comprised of three distinct layers beneath the cuticle. The outermost is the palisade layer (epidermis) composed of sclereids with unevenly thickened walls. The subepidermal layer is composed of hourglass cells having an uneven deposition of secondary wall, and the tissue below this is parenchyma (26). The palisade and hourglass cells are primarily responsible for the thickness of the seed coat and it is in the cell walls of the palisade cells and the lumina of the hourglass cells that the condensed tannin is deposited in the fababean (25).

Seed coat thickness is related to seed coat colour in rapeseed. Yellow-seeded rapeseed has a thinner seed coat than the normal brown-seeded cultivars. In this case, the reduction in seed coat thickness is mostly due to thinner palisade cells (only one half to two thirds the normal size). As in fababeans, the palisade cells are normally thick-walled and pigmented (85).

Bond (10a) found that rumen bacteria were able to utilize much more tannin-free than tannin-containing seed coat of fababean but he did not relate this to seed coat composition. Griffith and Jones (32) found similar differences in digestibility and attributed them primarily to the inhibition of cellulase by condensed tannin. The possibility that the cell wall of tannin-containing seed coat is inherently less digestible due to a greater degree of lignification

was not supported by their preliminary data which indicated very little difference in lignin content. However, Marquardt et al. (49) analysed the seed coats of six tannin-containing and three tannin-free cultivars and found that the tannin-containing seed coats had about 50% more lignin and a significantly lower proportion of cellulose and acid detergent fibre. Condensed tannin is able to complex with the components of crude fibre. It should be noted that lignin and condensed tannin are related in that they are both polyphenolic compounds synthesized via the shikimic acid pathway (72,77).

Seed coat thickness is an important consideration in breeding since a decrease in thickness reduces the proportion of crude fibre and allows easier drying and grinding. It also increases susceptibility to damage at harvest. Cracking and splitting of the seed coat reduces market value and allows readier entry of micro-organisms and the onset of moulding and rotting. Susceptibility to some root rot and damping-off diseases has been associated with thin seed coats. Thin seed coats allow a greater sugar exudation from the developing seeds, thus promoting fungal growth (41,42,43,75,76).

Within a tannin-containing cultivar, if conditions are such that there is a reduction in the proportion of seed which is seed coat, there is an associated increase in the percentage of tannin in the seed coat (50). There is a large variation in seed coat thickness within cultivars that is not related to seed weight (70). However, between cultivars, seed coat thickness is correlated with seed weight ($r = 0.66$) and with percentage of crude fibre ($r = -0.52$) (69).

2.2.3 Seed Colour

Seed colour is not important in animal feeding but is important to consumer acceptability. Dried fababean seeds may be various shades of buff (white to grey), green, red, purple, brown, marbled or black. Buff seeds are preferred for canning since they are the only ones which contain no leucoanthocyanidin which causes discolouration in processing (21). Green colour is due to chloroplasts in the palisade and parenchyma cells. It develops first along with red. Red and purple are both due to anthocyanic pigments. Purple pigment is deposited in the upper half of the cell cavities of the palisade layer and does not develop until late in the ripening period. The brown pigment develops after this in the palisade and hourglass cells. A dark pigment distributed in various patterns extending only to parts of the seed or over the entire seed gives rise to marbled or black. Seed colour appears first around the hilum and spreads irregularly throughout the seed coat as ripening proceeds (25,61). Each colour is controlled by a single gene which exerts its own independent action. Several pigments can co-exist in a single seed so the phenotype at maturity may indicate epistasis when in fact there is none. Green and red are recessive to buff. Purple, brown, marbled and black are dominant to buff (61,81). Genetic linkages occur only between the green and the red loci and between the marbled and the black loci (61).

Brown seed coat colour in fababeans is associated with the presence of condensed tannin in the seed coat. This is also true for the brown-coloured sorghums (34,87). No brown-coloured fababean seeds are tannin-free though there are some buff-seeded fababean cultivars that have higher concentrations of condensed tannin than brown-seeded

ones (50).

Tannin-free fababean seed does not darken with time. Tannin-containing seed tends to darken more rapidly in some cultivars than others (1). There is also a decline in condensed tannin concentration with storage (50). These two phenomena may be related. Colouration due to oxidation to phlobophene and/or interaction with other compounds might be making the condensed tannin less extractable.

2.2.4 Hilum Colour

Hilum colour is more important to canning than seed coat colour. The pale (colourless) hilum is due to the presence of melanin which, though chemically related to the anthocyanins and leucoanthocyanins, is under the control of different genes (71). Erith (25) records populations in which black hilum colour is dominant to non-black and cites Sirks (80) who found the same in most of his populations but also found a few cases in which black was not completely dominant, and Kaznowski (40) who found a population in which black hilum colour depended on a single recessive gene. It is difficult to distinguish between black and non-black hilum colour at maturity in seeds of a very dark colour.

2.2.5 Plant Colour

The stems of fababean plants are sometimes reddish in colour, particularly near the base of the stem in seedlings. This colour is due to the presence of anthocyanins. In several species, anthocyanin concentration is known to be influenced by light, temperature and the nutritional status of the plant (especially available phosphate) (72). Condensed tannins are present in the leaves and stems of certain legume

forages (73). In sorghum, tannin of the leaves and stems appears to be different to that of the seed (18). There are no reports of condensed tannin being found in fababean leaves and stems, but tannin-containing cultivars are marked by the presence of stipule spots - reddish-brown nectaries on the stipules (10a,62).

2.2.6 Flower Colour

Most fababean cultivars contain anthocyanin and melanin in their flowers. The 'normal' flower colour is white with two black blotches on the wings and with dark brown or purple lines on the standard. At least four loci have been found so far that affect flower colour (81). In addition to the basic genes for colour development, there are others which change the shade of anthocyanin pigmentation and still others which change the distribution of both anthocyanin and melanin (61).

It is generally known that flower colour affects the degree of cross-pollination by insects. De Vries (20) demonstrated a preference of the pollinating insects of fababeans for coloured over white-flowered plants. Insect pollination is important in fababeans since it maximizes heterozygosity and results in improved seed set and earlier maturity (63). However, greater seed set and earlier maturity in white-flowered cultivars can be achieved by the incorporation of genes for autofertility (2).

2.2.7 The 'White Flower Characteristic'

The tannin-free characteristic of fababeans conferred by the presence of one recessive gene is referred to as the 'white flower characteristic' because of its pleiotropic effect on flower colour. White flower colour was studied for the first time by Erith (25) and

has since been found to have an absolute link with the absence of leucoanthocyanidin or tannin-like compounds in the seed coat, and the absence of stipule spots (10a,62,71). It appears that each of several genes which produce the 'white flower characteristic' act at different levels of the synthesis of flavonoids for the whole plant. However, melanin in the hilum, the dark pigment which mottles seed coats, and green seed coat colour are under the control of different genes.

3. MATERIALS AND METHODS

3.1 Description of Cultivars

Crosses were made between eight cultivars of fababeans. Four of these were of the *minor* subspecies (Ackerperle, Diana, Herz Freya and UMFB-9) and four were of the *major* subspecies (Fidrim, Kodrim, Triple White and Broad Windsor) (Figure 2).

Ackerperle, Diana and Herz Freya were the currently recommended cultivars for Manitoba. They were developed in the Federal Republic of Germany at Oberlimpurg, Memmingen and Saatzuchtwirtschaft respectively. The other *minor* cultivar, UMFB-9, was an advanced line from the fababean improvement programme at the University of Manitoba. It was a single plant selection designated as PI 251231/2 which segregated from PI 251231 (Pullman, Washington) or resulted from cross-pollination at Winnipeg, male unknown. UMFB-9 was supported for licensing in 1977 but was not released to growers as originally scheduled due to contamination of breeders lines. All four *minor* cultivars have an average height between 120 and 130 cm and a protein content of about 30%. Ackerperle is smaller-seeded than the others and Herz Freya and UMFB-9 are the earliest maturing.

Cultivars Fidrim, Kodrim and Triple White were obtained from D. van der Ploegs, Barendrecht, Holland. They all have white flowers, seeds and hilums and were found by Marquardt *et al*. (50) to be tannin-free. Triple White has been used as a parent in the production of

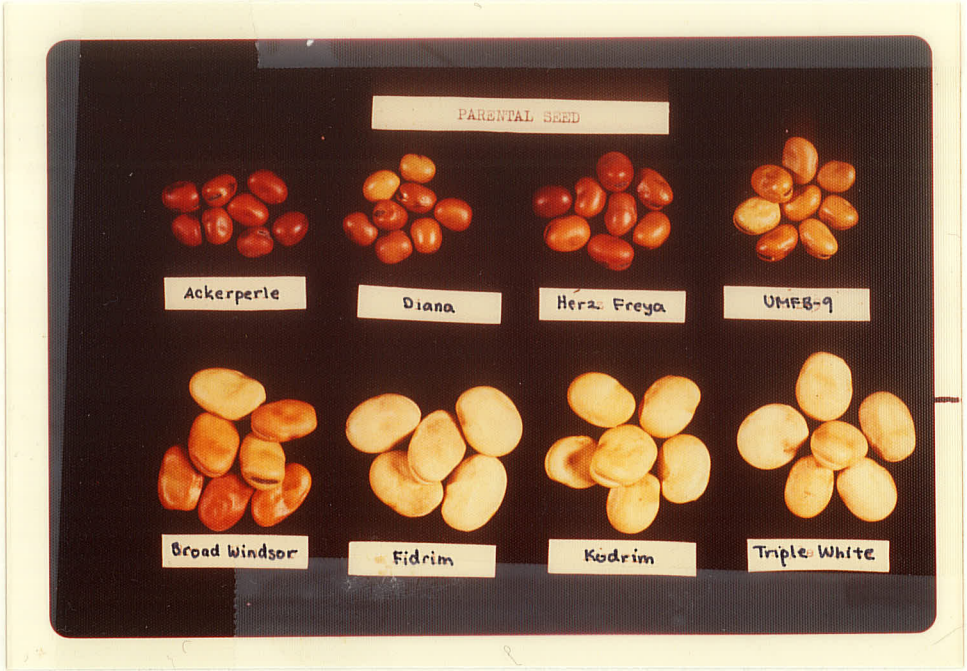

Figure 2. Seed of parental fababean cultivars

tannin-free lines in the University of Manitoba breeding programme. The studies of Rowlands and Corner (71) and Bond (10a) show that the tannin-free characteristic of Triple White is conferred by a single recessive gene. Fidrim and Kodrim are smaller-seeded than Triple White. Kodrim is the earliest maturing and the shortest but all three are earlier maturing than the four minor cultivars. Broad Windsor is the major cultivar sold commercially as a garden vegetable. The seed was obtained from McKenzie Steele Briggs Seeds. It is purple-flowered, tannin-containing, heterogeneous for hilum colour and has an average seed size below that of the other three major cultivars.

3.2 Growing and Harvesting Conditions

3.2.1 Growthroom

Plants grown in the growthroom were given a constant 16 hour day with a temperature of 19°C and 15°C during the night. Plants were grown in eight inch pots with up to four plants per pot. Hand-tripping of selfed plants every second day during the flowering stage seemed to improve seed set and hasten maturity. The time required per generation was almost four months and plants grew very tall, possibly due to insufficient light intensity. Considerably better yields were obtained when only one plant was grown per pot (as was done for the backcrosses). After flowering had ceased, plants were moved into the greenhouse in order to increase light intensity and thus hasten maturity. Seed was harvested and threshed by hand.

3.2.2 Greenhouse

Plants were sown in two greenhouse benches in July and harvested in September. Families were grown in double rows 30 cm apart with 15 cm between plants. Yields averaged only one or two pods per plant but plants matured earlier than those grown in the growthroom. Seed was harvested and threshed by hand.

3.2.3 Field

Approximately 4,000 individual plants were sown at 'The Point' (University of Manitoba, Winnipeg) on May 1st, 1978. Plants were sown in double rows 60 cm apart with 30 cm between plants. They were harvested individually as they matured over a three week period from mid August. Threshing was done mechanically. Seed was dried and stored for six months before being assessed for weight, colour and tannin content.

3.3 Crossing Proceedure

Fababeans are a partially cross-pollinated crop, the degree of cross-pollination depending largely upon the size of the insect population. Hence, populations can be highly heterogeneous. Parental plants were individually labelled and each female-designated plant was pollinated by a single male-designated plant so that progeny from crosses showing segregation for a trait in the F_1 generation due to parental heterozygosity could be excluded from the analysis for that trait.

Plants were cross-pollinated by hand in a growthroom where insects were excluded. All petals were removed at the time of emasculation as

this made pollination easier. The same proceedure is not recommended where temperature is not controlled because the exposed pistil is liable to desiccation. Pollinations were found to be as effective when made at the same time as emasculation as when a day later, provided that the stigma has reached the receptive stage. No bagging of flowers was necessary. Supposed crosses between major and minor plants that were accidently selfed could be easily identified by F_1 flower colour if the female was white-flowered since selfed seed produces white rather than purple-flowered F_1 plants. If the female was purple-flowered, accidental selfing could not be detected until seed was obtained from F_1 plants. Seed from crosses between major and minor plants has a distinctive oval shape and a size almost midway between the two subspecies. The proportion of accidentally selfed seed was less than 1%.

3.3.1 Single Crosses

All possible crosses, including reciprocals, were made between the eight cultivars using four individual pairs of plants per cross. Flowers of male-designated plants that were not used for pollen were left to produce selfed seed which was subsequently grown out along with the generations of crossed seed for comparative purposes. The parental and F_1 generations were grown in a growthroom and most of the F_2 generation was planted in the field. Only progeny from two F_1 plants from each of the crosses Triple White x UMFB-9, Triple White x Ackerperle and their reciprocals were grown in a growthroom and allowed to self and produce F_2 seed. Twenty seeds from each of eight Ackerperle/Triple White F_2 plants were subsequently grown out as F_2 families in the greenhouse.

3.3.2 Backcrosses

All possible backcrosses between Triple White and Ackerperle and between Triple White and UMFB-9 (including reciprocals) were made in a growthroom using two pairs of plants for each cross. The progeny were then allowed to self in the growthroom. Four BC_1F_1 plants were selected from each of the backcrosses involving Triple White and Ackerperle and their progeny (20 from each plant), grown in families in the greenhouse and allowed to self for yet another generation to produce BC_1F_2 seed.

The generations produced and the numbers of plants involved are summarized in Appendices 1, 2 and 3.

3.4 Condensed Tannin Analyses

3.4.1 Vanillin-HCl Spot Test

Leaves, stems and roots of plants of the eight parental cultivars at the seedling and flowering stages were crushed between Whatman 3M chromatography paper and tested for the presence of condensed tannin as described by Jones et al. (39). Sainfoin plants (Onobrychis viciaefolia Scop.) known to have condensed tannin in their foliage were used for comparison.

3.4.2 Vanillin-HCl Spectrophotometric Estimation

A quantitative estimation of the condensed tannin present in the seed coats of seed from each of the parental cultivars and from some randomly chosen Triple White/Ackerperle F_2 and backcrossed plants was made with a modification of Burn's vanillin-HCl method including suggestions by Price et al. (67) to reduce the time required for the test and to increase precision. A seed coat sample weighing 0.2 g was placed in 20 ml of methanol and ground by a Polytron homogenizer for one minute. The suspension was then rotated for 20 minutes at 2500 rpm. To 1.0 ml of the supernatant was added 5 ml of a solution of 1% vanillin and 8% HCl mixed 1:1. The reaction took place in a water bath at 30°C. Standards were set up having a known concentration of condensed tannin isolated from fababeans. The optical density of the test and standard solutions was read at 500 nm after 20 minutes.

The test was repeated for each of three plants of the five tannin-containing parental cultivars to determine its reproducibility. It was found to be sufficiently reproducible to detect significant differences between plants (CV = 16.5%).

3.4.3 Qualitative Tests

Two qualitative tests were used to estimate the tannin content of seed coats - the prussian blue test and the sodium hydroxide test.

Seed from all F_2 plants and backcrosses was tested for the presence of phenolic compounds using the highly sensitive prussian blue test described by Price and Butler (66). A small piece of seed coat (approximately 0.01 g) was placed in 2 ml of water and swirled for one minute on a vortex mixer. The seed coat was then removed. To the extract was added 1 ml of 0.008M $Fe(NO_3)_3$ in 0.008M HCl followed by 1 ml of 0.003M $K_3Fe(CN)_6$. All test tubes and reagents were kept in a water bath at 50°C. The colour of the solution was recorded after five seconds. The complete range of colours was observed but reproducibility was not good so the test could only reliably distinguish between tannin-free and tannin-containing seed coats. Tannin-free seed coats gave a solution that was more yellow than the $K_3Fe(CN)_6$ reagent. Tannin-containing seed coats gave a solution that was more green than this yellow-green reagent. Samples could be tested at the rate of 25 to 30 in an hour. Only a few samples lay midway between the two classifications. For these, the test was repeated.

The sodium hydroxide test was that suggested by Marquardt *et al.* (50) for the identification of tannin-free seed coats. Approximately 0.1 g of seed coat was placed in 5 ml of 0.1M NaOH and left for an hour in the light. A brown solution indicated the presence of tannin. The test was used to verify the results of the prussian blue test.

3.5 Plant Observations

3.5.1 Disease

The incidence of disease and the proportion of plants reaching the flowering stage and producing seed was noted to see whether there was any major difference in susceptibility between tannin-free and tannin-containing segregants growing under field conditions.

3.5.2 Stem Colour

Notes were taken on the presence or absence of reddish pigment in the stems of seedlings of segregating populations of Triple White/Ackerperle and Triple White/UMFB-9 in the growthroom. Plants were classified one and two weeks after germination. The colour tends to disappear after this time. A plant was classed as having the pigment when even the slightest trace of red could be observed on the stem. Wet weather and mud made it impossible to classify populations in the field.

3.5.3 Stipule Spots

The presence or absence of stipule spots was recorded for segregating populations in the field and for Triple White/Ackerperle and Triple White/UMFB-9 populations in the growthroom at two and three weeks after germination. Stipule spots usually became visible at the third or forth node above ground.

3.5.4 Flower Colour

Every plant was classed as having either a 'normal' (referred to as purple) flower colour or a white flower colour (complete absence of pigmentation). All parental cultivars were indeterminate so there was

a long period of time over which flower colours could be recorded.

3.5.5 Seed Colour

Seed coat colour could not be accurately assessed until brown-coloured seeds had turned dark enough to be distinguishable from buff-coloured seeds. Within a plant having brown seed, there was generally a range of shades depending upon the position of a particular seed on the plant. Plants were also assessed for the extent and intensity of dark brown or grey to black pigment covering their seed. They were classified into two groups for their basic seed colour (brown and buff) and into three groups for the extent and intensity of the dark pigment:

0 - Very slight or no dark areas.

1 - Some darkening, mostly around the edges.

2 - Very dark pigment covering most of the seed.

There was some difficulty in distinguishing between genetically-determined discolouration and that caused by disease.

3.5.6 Hilum Colour

Hilums exhibited a complete range of colours from buff to black. Since non-black hilums tend to reflect the colour of the seed coat, plants were classified into only two groups - black hilum and non-black hilum.

3.5.7 Seed Weight

Seed from each plant grown in the growthroom or greenhouse was weighed to 0.01 g and divided by the total number of seeds. Plants grown in the field produced much higher yields so for these, the mean seed weight was estimated from a ten seed sample. Ten seeds were

sufficient to give a coefficient of variation below 5% (Appendix 4).

3.5.8 Seed Coat

All seed from F_2 plants grown in the field was examined for the presence of split or cracked seed coats. The Triple White x UMFB-9 cross was chosen for closer examination.

Thirty each of tannin-free and tannin-containing segregants were randomly selected from the population. Five seeds were taken from each plant and weighed individually. The seed was then cracked in a garlic press and the pieces of seed coat removed and weighed. The percentage of seed weight due to the seed coat and the difference between seed weight and seed coat weight were calculated for each seed and averaged for plants.

Nine plants (five seeds from each) were also measured for seed coat thickness using a micrometer which could measure curved surfaces to 0.0001 inches. Three measurements were made per seed at different places on the shell but away from the thickened hilum area. Seed coat thicknesses were then compared with percentage seed coat measurements.

Differences in the percentage of seed coat were examined between:

1. Tannin-free and tannin-containing plants.
2. Tannin-free plants having some damaged seed and tannin-free plants having no damaged seed.
3. Damaged seed and undamaged seed within a tannin-free plant.

Seeds were also tested to see whether there was any difference in the seed coat composition of tannin-free versus tannin-containing plants. Samples consisted of an equal combination of seed from each of the 30 tannin-free segregants, an equal combination of seed from each of the 30

tannin-containing segregants and seed from plants of each parental line grown in the field at the same time. The four samples were analysed for permanganate lignin, cellulose and acid detergent fibre using the method of Göering and Van Söest (31).

4. RESULTS AND DISCUSSION

4.1 Condensed Tannin Analyses

4.1.1 Vanillin-HCl Spot Test

This test failed to detect the presence of condensed tannin in the leaves or roots of any of the eight parental cultivars but there was a very slight reddening of the imprint of the stem where it was pigmented. The nectaries of the stipules were clearly visible as dark red spots on the imprints of the tannin-containing cultivars indicating the accumulation of condensed tannin or monomeric anthocyanidins or leucoanthocyanidins in these cells. Condensed tannin was either not present in the rest of the plant or present in insufficient quantities to be detected by this test (Figure 3). Since stipule spots and stem pigments visible on the imprints are also visible on the actual plants, this test is of no further value as a screening procedure.

4.1.2 Vanillin-HCl Spectrophotometric Estimation

Quantitative estimates of the tannin content of cultivars varied widely from plant to plant within S_2 lines grown in the field, indicating a low heritability for the trait (Table 1).

No significant differences in tannin content could be detected between the five tannin-containing cultivars. The overall mean was 5.0 mg of condensed tannin per g of seed coat. Marquardt *et al.* (50) obtained higher values using a longer extraction period. No

TABLE 1. Nested analysis of variance for the condensed tannin content[a] of cultivars grown at the Point (Winnipeg, 1978)

Source of variation	Df	SS	MS	F
Between cultivars[b]	4	28.39	7.10	0.98
Plants within cultivars	10	72.24	7.22	10.78[c]
Samples within plants	15	10.05	0.67	
Total	29	110.68		

[a]Vanillin-HCl method of estimation

[b]Cultivars were Ackerperle, Diana, Herz Freya, UMFB-9 and Triple White

[c]Significant at 0.01 level

significant amount of tannin was detected in the seed coat of any plant of the tannin-free cultivars.

Further analyses were made on seed from plants randomly selected from Triple White/Ackerperle F_2 populations grown in the field (Table 2) and from Triple White/Ackerperle lines that were backcrossed to Triple White and grown out in the greenhouse (Table 3).

In a few of the white-flowered plants of segregating lines, a very slight amount of tannin was detected. However, values below 0.1 mg/g and above 10 mg/g are not very reliable as the standard curve was not linear beyond these extremes.

4.1.3 Qualitative Tests

More than 2,000 F_2 and backcrossed plants of lines segregating for flower colour were classified as either tannin-free or tannin-containing by means of the prussian blue test. Seed from all of the white-flowered plants was classified as tannin-free and seed from all the purple-flowered plants was classified as tannin-containing with a few exceptions. Some of these apparent exceptions were eliminated from the list when a repetition of the test revealed that they were previously recorded as tannin-containing when they were actually tannin-free. Initial false positives probably arose when care had not been taken to completely remove all traces of cotyledon (which contains reducing substances) from the tannin-free piece of seed coat.

Those plants that were still found to have seeds with a tannin content not expected according to their flower colour, were tested again by placing their seed coats in 0.1M NaOH and leaving them to stand overnight. This test confirmed the result of the prussian blue

TABLE 2. Condensed tannin content of seed from Triple White, Ackerperle and Triple White/Ackerperle F_2 plants grown at the Point (Winnipeg, 1978)

Population	Flower colour	Number of plants analysed	Tannin content[a] mg/g
Triple White	W	3	0.005 ± 0.005
Ackerperle	P	3	4.119 ± 0.371
T x A	W	4	0.025 ± 0.011
T x A	P	4	5.250 ± 2.987
A x T	W	4	0.007 ± 0.004
A x T	P	4	4.379 ± 1.693

[a]Vanillin-HCl method of estimation

TABLE 3. Condensed tannin content of seed from some BC_1F_2 plants of Triple White/Ackerperle backcrossed to Triple White grown in the greenhouse

Line	Flower colour of BC_1F_1 parent	Flower colour of plant	Tannin content[a] mg/g
T x (T x A)3	W	W	0.000
T x (T x A)5	P	P	0.171
T x (T x A)5	P	W	0.143
T x (A x T)16	W	W	0.000
T x (A x T)13	P	P	5.971
T x (A x T)13	P	W	0.000
(T x A) x T16	W	W	0.000
(T x A) x T48	P	P	12.286
(T x A) x T48	P	W	0.000
(A x T) x T5	W	W	0.000
(A x T) x T3	P	P	8.743
(A x T) x T3	P	W	0.071

[a] Vanillin-HCl method of estimation

test in every case except for seed from purple-flowered plants that was obviously disease infected. For this seed, the prussian blue test showed an absence of phenolic compounds but the NaOH test was positive.

Only 15 plants remained that could be exceptions to the theory that the genes causing the seed coat to be free of tannin have a pleiotropic effect on flower colour. Ten seeds from each of these plants were grown out in the greenhouse to see whether the discrepancy was due to a misclassification of the flower colour of F_2 plants in the field. There was some cross-pollination in the field so the test was not entirely satisfactory. It was assumed that if at least 80% of the progeny had the same flower colour, this was the flower colour of the F_2 plant. Under this assumption, 11 F_2 flower colours had been wrongly recorded. Data for the four remaining plants is listed in Table 4.

The colour of the seed from the F_2 plants was that which was expected on the basis of the tannin analyses, not the F_2 flower colour. Their purple-flowered progeny all produced brown-coloured seed and their white-flowered progeny all produced buff-coloured seed. Therefore, these exceptions most likely had their F_2 flower colour wrongly recorded though there was too much cross-pollination in the field to allow confirmation. Since flower colour appears to be directly related to tannin content, the terms tannin-containing and tannin-free will be used synonymously with white-flowered and purple-flowered respectively.

Of the two qualitative tests used to distinguish between tannin-

TABLE 4. Data for four possible exceptions to the 'white flower characteristic'

Population	Line	Flower colour		Seed colour	Tannin analysis[a]	
		F_2	F_3		Prussian blue	NaOH
U x T	2000	W	6P:4W	brown	+	+
F x D	2645	W	1P:9W	brown	+	+
F x B	2818	P	7P:3W	buff	0	0
T x H	3241	P	6P:4W	buff	0	0

[a]Symbols: + = tannin; 0 = no tannin

free and tannin-containing seed, the sodium hydroxide test proved to be preferable to the prussian blue test because the classification was clearer and the test did not suffer the possibility of error due to either incomplete removal of the cotyledon or the occurrence of disease infected seed coats. It was thought that the prussian blue test would have the advantage of being more sensitive to small amounts of tannin but this would require greater standardization than was possible for a quick test. Slight differences could be observed between the tannin-free seed coats using the sodium hydroxide test if they were left to soak for three or four days (Figure 4) but it could not be determined whether these differences were related to the presence of slight amounts of tannin.

Figure 3. Seedling imprints developed with vanillin-HCl

Figure 4. Seed coats soaked in sodium hydroxide solution for 48 hours

4.2 Plant Observations

4.2.1 Disease

Loss of plants grown in the field was mainly due to Fusarium sp. Link and Rhizoctonia sp. D.C. (as found in P. Kharbanda's nearby plots) and a severe strain of bean yellow mosaic virus (BYMV) which attacked the plants from about the flowering stage and often resulted in a complete loss of yield. Plant survival rates are given in Table 5.

The relatively low survival rate (66%) of the tannin-free S_2 lines up until flowering could have resulted from a greater susceptibility of these lines to Fusarium sp. Link and Rhizoctonia sp. D.C. However, the tannin-free x tannin-free F_2 lines had only a slightly lower survival rate than the other F_2 lines. It should be noted that though the tannin-free x tannin-containing F_2 lines segregate for tannin content, all the F_2 seed planted had a tannin-containing seed coat since the F_1 plants were all tannin-containing. These lines had a good seedling survival rate. There was little difference between lines in the survival rate of plants after they reached the flowering stage, suggesting that viral attack was not related to the presence or absence of tannin in the plant. This suggestion is supported by comparing the survival rates of tannin-free and tannin-containing plants within segregating lines after attack by BYMV (Table 6).

4.2.2 Stem Colour

The three tannin-free cultivars showed no sign of reddish pigmentation on the stem whereas it was present in a large proportion of plants of the five tannin-containing cultivars. The characteristic

TABLE 5. Survival of F_2 and S_2 plants grown at the Point (Winnipeg, 1978)

Population	Number of seeds planted	Survival at the flowering stage	Survival from flowering till seed set
T-cont S_2	140	85%	86%
T-free S_2	74	66%	92%
T-cont x T-cont F_2	2256	82%	95%
T-free x T-free F_2	117	79%	92%
T-cont x T-free F_2	890	84%	97%
T-free x T-cont F_2	1001	93%	94%
Total	4478	85%	95%

TABLE 6. Survival of tannin-free versus tannin-containing segregants of F_2 lines grown at the Point (Winnipeg, 1978)[2]

Description of plants	Number of plants	Survival from flowering till seed set
T-free F_2	394	94%
T-cont F_2	1291	95%
Total	1685	95%

was more pronounced in some cultivars than others and also varied greatly in its expression on plants within a cultivar. None of the stems of seedlings of the segregating lines of Triple White/UMFB-9 grown in the growthroom showed the characteristic but it was obvious on certain plants of the Triple White/Ackerperle lines. Not all of the plants without the pigment were tannin-free but all of those plants which had an observable reddish pigment were found later to develop stipule spots and have purple flowers. Of the 69 tannin-containing segregants of Triple White/Ackerperle lines, 10 could be predicted to produce tannin-containing seed one week after germination and 44 could be predicted two weeks after germination. The proportion of tannin-containing plants that could be detected early by this method may be improved if seedlings are grown under conditions of high light intensity, low temperature or poor nutritional status.

4.2.3 Stipule Spots

The absence of stipule spots is believed to be a pleiotropic effect of the 'white flower characteristic' (10a,62). This conclusion was supported by data from all the plants grown in the field. Dark reddish-brown spots were visible on all tannin-containing plants. The spots were often not visible on stipules at the lowest nodes of the plant. They generally appeared by the third or fourth node above ground but in a few cases they did not become obvious until the time of flowering. The segregating Triple White/Ackerperle and Triple White/UMFB-9 lines grown in the growthroom were examined for the presence of stipule spots two and three weeks after germination. Approximately 75% of the tannin-containing plants could be identified

after two weeks and 99% could be identified after three weeks. The presence of stipule spots as illustrated in Figure 5 and/or red stem pigmentation (which is not clear in the figure) could be used as early selection criteria for the 'white flower characteristic'.

4.2.4 Flower Colour

The range of flower colours found within tannin-free x tannin-containing lines is illustrated in Figure 6.

Type (a) is the flower of a tannin-free plant. Type (c) is the normal flower colour type. Types (b) and (d) were found more rarely, mostly amongst F_3 plants grown in the greenhouse. Types (b), (c) and (d) come from tannin-containing plants and were classified as having purple flowers. Since type (b) could easily be misclassified as white-flowered, the presence or absence of stipule spots on the plant should be noted at the same time.

The 'white flower characteristic' has been found to be conferred by a single recessive gene in Tripe White (10a,71) and the white flower colour of the other two tannin-free cultivars, Fidrim and Kodrim, could be expected to show a similar pattern of inheritance. Crosses between the three tannin-free cultivars produced only white-flowered F_2 progeny so the same gene for the 'white flower characteristic' was present in each. F_2 progeny of all tannin-free x tannin-containing crosses and reciprocals were tested against a 3:1 ratio for flower colour segregation (Appendices 5 and 6). The data from the 15 different crosses and their reciprocals was tested for homogeneity. The heterogeneity chi-square value (χ^2_{29}=14.57) was not

Figure 5. Fababean seedlings ten days after germination

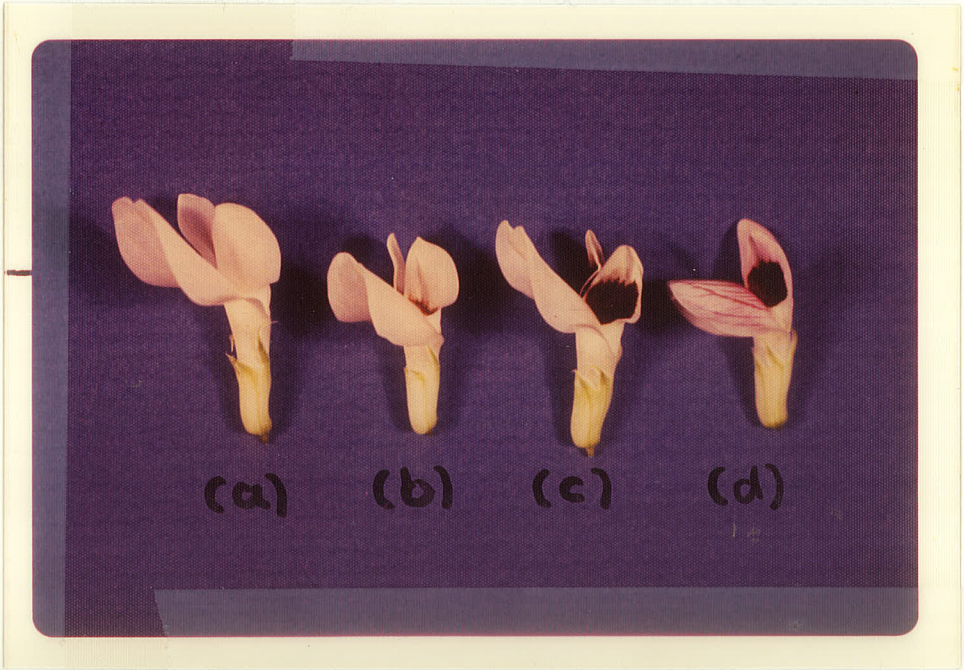

Figure 6. Flower types

significant (P>0.90) so data was combined and is summarized in Table 7.

Backcrossed populations exhibited the expected 1:1 segregation for flower colour (χ^2_1=2.27, P>0.10) with the exception of one line and this was likely due to chance (Appendix 7). Backcross data could be combined (χ^2_{15}=11.16, P>0.50) and is summarized in Table 8.

The fact that all F_2 populations and all but one of the backcrossed populations did not differ significantly from a 3:1 and 1:1 ratio respectively, confirms that Triple White, Fidrim and Kodrim all have the same single recessive gene conferring the 'white flower characteristic', that its expression is the same in the minor as in the major subspecies, and that there is no significant maternal effect.

The cultivars were each homogeneous for flower colour with the exception of one Ackerperle plant in an S_2 line in the field which had white flowers. This could have been a spontaneous mutant.

Forty seeds from each of the eight white-flowered segregants in the field were planted in the greenhouse. Eleven percent of the progeny were purple-flowered indicating that the degree of cross-pollination was at least this high. Introduction of the 'white flower characteristic' into the minor types may not have too severe an effect on the degree of heterozygosity provided that a sufficient population of pollinating insects is present.

4.2.5 Seed Colour

The seed colour of segregating populations ranged from almost pure white through to dark brown. After six months of storage, it

TABLE 7. Chi-square values for flower colour segregation in F_2 populations

Population	P	W	X^2 value for 3:1	Probability
T-free x T-cont F_2	714	221	0.93	0.25 - 0.50
T-cont x T-free F_2	577	173	1.50	0.10 - 0.25
Total	1291	394	2.35	0.10 - 0.25

TABLE 8. Chi-square values for flower colour segregation in backcrossed populations

Population	P	W	X^2 value for 1:1	Probability
T-free x (T-cont x T-free)	10	15	1.00	0.25 - 0.50
T-free x (T-free x T-cont)	12	8	0.80	0.25 - 0.50
(T-cont x T-free) x T-free	51	64	1.47	0.10 - 0.25
(T-free x T-cont) x T-free	74	87	1.05	0.25 - 0.50
Total	147	174	2.27	0.10 - 0.25

was not difficult to classify the seeds of most plants as either buff (white to grey) or brown-coloured. The buff-coloured seeds came from tannin-free plants and the brown-coloured seeds from tannin-containing plants. Seed of some plants darkened at a much slower rate than others (Figure 7).

The shade of brown of the seed was also quite varied within plants depending upon the position of the seed on the plant. Seed colour could be determined by examining the seed on a plant basis or by harvesting in bulk and sorting the seed electronically. The latter method could also be used to exclude infected or otherwise discoloured seed such as the large number covered to some extent by a dark brown or grey to black pigment. F_2 families grown in the greenhouse revealed that this pigment was genetically determined. It was only visible on otherwise buff-coloured seed of tannin-free plants (Figure 8).

The dark pigment manifest itself in different patterns on seed from different F_2 plants within a cross, though most commonly it is found around the edge of the seed. It may be what Rowlands and Corner (71) and Picard (61) referred to as 'marbled'. Picard (61) found the marbled phenotype to be monofactorially inherited and dominant to buff. Because of the variability in the degree of expression of the characteristic and the presence of other factors causing similar discolouration, classification into the three groups (0,1 and 2) was fairly subjective so no conclusions regarding the number of genes involved could be made from this study (Appendix 8). The result is summarized in Table 9.

None of the parental cultivars or F_2 progeny from the tannin-free x tannin-free or tannin-containing x tannin-containing crosses had

Figure 7. Seed from tannin-containing versus tannin-free F_2 plants of the cross Triple White x UMFB-9

Figure 8. Seed from tannin-free F_2 plants of the cross Triple White x UMFB-9 showing different shades of buff and extent of dark pigmentation

TABLE 9. Pigmentation scores for white-flowered F_2 segregants

Population	Number of plants with score:		
	0	1	2
T-free x T-cont F_2	78	61	60
T-cont x T-free F_2	70	44	42
Total	148	105	102

darkly pigmented seed. Neither did any tannin-containing F_2 segregant of the other crosses express the characteristic. It was restricted to seed from tannin-free segregants and was present within all F_2 populations from crosses between tannin-free and tannin-containing cultivars. The one white-flowered Ackerperle S_2 plant that appeared in the field had small buff-coloured seed with a dark grey pigment around the edges. The above data leads to the conclusion that the gene or genes determining the presence of the dark pigment are not present in Triple White, Fidrim or Kodrim or that these cultivars have some mechanism by which the genes are not expressed. The genes or the means of expression of the characteristic are inherited from each of the five tannin-containing cultivars but only function when combined with the 'white flower characteristic'. In Picard's study, 'marbled' phenotype was also brought in by the minor parent but only when it was the female parent (61). In this study, the dark pigment appeared regardless of the direction of the cross. In every population there were at least some pure buff-coloured segregants so it should be possible to select for this combination of genes.

4.2.6 Hilum Colour

The three tannin-free major types (Triple White, Fidrim and Kodrim) have seed with buff-coloured hilums. The minor types (Ackerperle, Diana, Herz Freya and UMFB-9) have black-coloured hilums with the exception of a small proportion of seed with brown hilums. Hilum colour data from the seed of F_2 populations derived from crosses between the tannin-free major and minor cultivars excluded results

from any crosses where the minor parent had a brown hilum or where the F_1 family was segregating for hilum colour due to parental heterozygosity for the character. Black was dominant to non-black. The populations were tested for their goodness-of-fit to the simple 3:1 ratio which Erith (25) found in most of her populations (Appendices 9 and 10). Results could be combined for tannin-free x tannin-containing populations ($\chi^2_{14}=5.51$, $P>0.95$) and for tannin-containing x tannin-free populations ($\chi^2_{14}=5.93$, $P>0.95$) but the two groups of populations could not be combined ($\chi^2_1=8.02$, $P<0.01$). The results are summarized in Table 10.

Although the overall result indicates that black hilum colour is caused by a single dominant gene, there is a significant deviation from the expected 3:1 ratio if the female parent has a black hilum. There is also more than the expected number of non-black hilums when the female parent has a non-black hilum. The fact that non-black hilum can appear black when the seed coat is blackened around the hilum area may explain why the maternal effect did not reach significance in both directions. The opposite error - that of scoring black hilums as non-black because the seed is immature is less likely since hilum colour develops early.

Broad Windsor had a larger proportion of seeds with brown hilum than did the other tannin-containing cultivars. Data from crosses between plants of Broad Windsor (having black hilums) and the tannin-free cultivars showed an inheritance pattern that was different from that of the other tannin-free x tannin-containing and tannin-containing x tannin-free populations (Table 11).

TABLE 10. Chi-square values for hilum colour segregation in F_2 populations

Population	Black	Non-black	X^2 value for 3:1	Probability
T-free x T-cont F_2	520	190	1.17	0.25 - 0.50
T-cont x T-free F_2	467	116	8.10	0.00 - 0.01[a]
Total	987	306	1.25	0.25 - 0.50

[a]Significant at 0.01 level

TABLE 11. Hilum colour of F_1 and F_2 generations of crosses between Broad Windsor[a] and the other major type cultivars

Population	Hilum of seed from F_1 plants	Number of F_2 plants having seed with:	
		Black hilum	Non-black hilum
B x F	segregating	13	9
B x K	non-black	0	10
B x T	non-black	3	27
F x B	non-black	0	36
K x B	black	25	8
T x B	non-black	0	11

[a]Only Broad Windsor seed with black hilum

In four of the six crosses, non-black was dominant to black. If the Broad Windsor parent in these crosses was heterozygous, it could not be detected by a segregating F_1 family. In the other two crosses, black appeared to be dominant to non-black. The fact that the pattern of dominance was not the same for all pairs of crossed plants suggests that the Broad Windsor population is highly heterogeneous and probably contains several genes and/or alleles for hilum colour.

Chi-square tests for independence were performed to determine whether there was any significant association between flower colour and hilum colour. F_2 segregants of the crosses Triple White x Ackerperle, Triple White x UMFB-9 and their reciprocals were chosen for the analysis since these populations were the largest (Table 12).

This data supports the conclusion of Rowlands and Corner (71) that the dark melanic pigment of the flowers is not chemically related to the melanic hilum pigment.

4.2.7 Seed Weight

Seed weight was found to be a maternal characteristic. In other words, the average weight of seeds from a plant of a particular cultivar was characteristic of that cultivar irrespective of whether it had been crossed to a *major* or *minor* cultivar (Appendix 11). F_2 seed weight was a characteristic of the F_1 plant (Figures 9 and 10) and there was no significant difference in mean seed weight between progeny of reciprocal crosses. Similarly, F_3 seed was characteristic of the F_2 plant from which it was obtained.

The seed weight of seed from F_2 plants of *major* x *minor* and *minor* x *major* crosses grown in the field along with parental lines

TABLE 12. Chi-square test for flower colour - hilum colour association in *major* x *minor* and *minor* x *major* crosses

Population	P Black	P Non-black	W Black	W Non-black	X^2 value for independence	Probability
A x T	63	16	12	5	0.69	0.25 - 0.50
T x A	33	11	10	3	0.02	0.75 - 0.90
U x T	165	42	49	11	0.11	0.50 - 0.75
T x U	91	37	11	10	0.16	0.50 - 0.75

Figure 9. Seed from F_1 plants of crosses made with Ackerperle as the female parent

Figure 10. Seed from F_1 plants of crosses made with Triple White as the female parent

tended to be smaller than the mid-parental value despite the fact that the seed weight of the parental lines may have decreased owing to two generations of inbreeding (Table 13).

The mean weight of F_2 plants was either equivalent to or less than the mid-parental value. Erith (25) found that this was true for the crosses she made between *major* and *minor* cultivars.

The relationship between seed weight and tannin content (as indicated by flower colour) was investigated by means of t-tests (Tables 14, 15 and 16).

If seed weight and flower colour were linked, seeds from the white-flowered segregants would be expected to be heavier. This was not the case. In fact, in a few populations the reverse relationship was significant, so it should be relatively easy to select for a tannin-free *minor* type. There was a tendency for the purple-flowered segregants to have heavier seed in backcrossed and F_2 populations both in the field and the greenhouse, though the difference was usually not great enough to reach significance. This unexpected result could be due to a greater susceptibility of white-flowered segregants to disease (though this would be more likely to affect yield than seed weight) or to some pleiotropic effect of the 'white flower characteristic'.

4.2.8 Seed Coat

The seed of approximately 1400 F_2 plants from tannin-free x tannin-containing and tannin-containing x tannin-free populations were examined for seed coat damage. Of the tannin-containing segregants, less than 5% had any seed with split or cracked seed coats

TABLE 13. Seed weight of F_2 and parental lines grown at the Point (Winnipeg, 1978)

Population	Minor parent g/seed	Major parent g/seed	MP[a] g/seed	Seed from F_2 plants g/seed
A x F and F x A	0.39 ± 0.02	1.28 ± 0.05	0.84	0.70 ± 0.02
A x K and K x A	0.39 ± 0.02	1.34 ± 0.05	0.87	0.70 ± 0.02
A x T and T x A	0.39 ± 0.02	1.00 ± 0.07	0.70	0.68 ± 0.02
D x F and F x D	0.45 ± 0.02	1.28 ± 0.05	0.87	0.70 ± 0.02
D x K and K x D	0.45 ± 0.02	1.34 ± 0.05	0.90	0.73 ± 0.01
D x T and T x D	0.45 ± 0.02	1.00 ± 0.07	0.73	0.73 ± 0.01
H x F and F x H	0.46 ± 0.02	1.28 ± 0.05	0.87	0.72 ± 0.02
H x K and K x H	0.46 ± 0.02	1.34 ± 0.05	0.90	0.75 ± 0.02
H x T and T x H	0.46 ± 0.02	1.00 ± 0.07	0.73	0.72 ± 0.01
U x F and F x U	0.44 ± 0.02	1.28 ± 0.05	0.86	0.75 ± 0.02
U x K and K x U	0.44 ± 0.02	1.34 ± 0.05	0.89	0.85 ± 0.02
U x T and T x U	0.44 ± 0.02	1.00 ± 0.07	0.72	0.67 ± 0.01

[a]Mid-parental value

TABLE 14. Differences between the seed weight of seed from purple-flowered and white-flowered F_2 plants grown at the Point (Winnipeg, 1978)

Population	$\bar{x}_p$ g/seed	$\bar{x}_W$ g/seed	$\bar{x}_p - \bar{x}_W$	Df	t
A x K	0.70	0.69	+0.01	27	0.19
A x T	0.72	0.65	+0.07	73	1.09
D x F	0.71	0.74	-0.03	27	0.49
D x K	0.76	0.76	0.00	39	0.02
D x T	0.74	0.65	+0.09	28	2.04
H x F	0.69	0.71	-0.02	35	0.24
H x K	0.81	0.79	+0.02	18	0.22
H x T	0.81	0.69	+0.12	33	2.43[a]
U x F	0.85	0.71	+0.14	25	3.10[b]
U x K	0.86	0.89	-0.03	4	0.79
U x T	0.65	0.64	+0.01	240	0.20
F x A	0.69	0.62	+0.07	29	1.74
F x D	0.71	0.64	+0.07	65	1.88
F x H	0.76	0.66	+0.10	47	1.93
F x U	0.75	0.58	+0.17	42	4.24[b]
K x A	0.72	0.62	+0.10	27	1.69
K x D	0.72	0.69	+0.03	73	0.79
K x H	0.73	0.73	0.00	57	0.02
K x U	0.87	0.76	+0.11	9	1.39

(Continued)

TABLE 14. (Continued)

Population	$\bar{x}_p$ g/seed	$\bar{x}_W$ g/seed	$\bar{x}_p - \bar{x}_W$	Df	t
T x A	0.61	0.59	+0.02	28	0.42
T x D	0.74	0.71	+0.03	55	0.74
T x H	0.71	0.68	+0.03	92	0.89
T x U	0.71	0.72	-0.01	153	0.08

[a]Significant at 0.05 level

[b]Significant at 0.01 level

TABLE 15. Differences between the seed weight of seed from purple-flowered and white-flowered F_2 plants grown in the growthroom

Population	$\bar{x}_p$ g/seed	$\bar{x}_W$ g/seed	$\bar{x}_p - \bar{x}_W$	Df	t
A x T	0.86	0.69	+0.17	17	2.56[a]
U x T	0.68	0.66	+0.02	23	0.45
T x A	0.81	0.63	+0.18	23	3.70[b]
T x U	0.80	0.81	-0.01	27	0.36

[a]Significant at 0.05 level
[b]Significant at 0.01 level

TABLE 16. Differences between the seed weight of seed from purple-flowered and white-flowered backcrossed plants in the growthroom

Population	$\bar{x}_p$ g/seed	$\bar{x}_W$ g/seed	$\bar{x}_p - \bar{x}_W$	Df	t
T x (A x T)	0.83	0.77	+0.06	13	0.71
T x (U x T)	0.99	0.78	+0.21	6	0.80
T x (T x A)	0.77	0.74	+0.03	10	0.46
T x (T x U)	1.04	0.87	+0.17	6	1.23
(A x T) x T	1.11	0.87	+0.24	15	2.69[a]
(U x T) x T	0.91	0.82	+0.09	35	1.41
(T x A) x T	0.82	0.78	+0.04	43	1.41
(T x U) x T	0.91	0.96	-0.05	51	0.99

[a]Significant at 0.05 level

whereas more than 40% of the tannin-free segregants had some damaged seeds. A higher proportion of damaged seed amongst tannin-free segregants was also noted for greenhouse-grown plants that were threshed by hand. Splitting began near the place where the radicle would break through (the thinnest part of the seed coat). Cracking generally began as a result of seed coat wrinkling (Figure 11). The damaged seed coats did not appear to be caused by disease, so it was thought that it may be due to the tannin-free segregants having thinner seed coats.

Seed coat thickness is independent of the position of a seed on the plant but is very variable within seed coats so a large number of tedious measurements need to be made to obtain a good estimation of the trait (70). Alternatively, the percentage of seed weight due to the seed coat could be measured. This trait was found to be fairly well correlated with seed coat thickness (Table 17). Measurements were made for tannin-free and tannin-containing seed over seed weights ranging from 0.5 to 0.9 g (Appendix 12).

There was little variation in percent seed coat measurements within plants, so this trait rather than seed coat thickness was measured to test for differences between tannin-free and tannin-containing plants (Table 18).

A highly significant difference in percent seed coat existed between the 30 tannin-free and 30 tannin-containing plants. There was also a highly significant difference between plants within these two groups.

Within the tannin-free group of plants, differences in percent seed coat corresponded with the presence of some damaged seed but

Figure 11. Split and cracked seed from F_2 plants of the cross UMFB-9 x Triple White

TABLE 17. Correlation of seed coat percentage with seed coat thickness of seed from F_2 plants of Triple White x UMFB-9 grown at the Point (Winnipeg, 1978)

	Df	r
Individual seeds	43	0.71[a]
Averaged over plants	7	0.76[b]

[a]Significant at 0.01 level

[b]Significant at 0.05 level

TABLE 18. Nested analysis of variance for seed coat percentage of seed of Triple White x UMFB-9 F_2 plants grown at the Point (Winnipeg, 1978)

Source of variation	Df	SS	MS	F
Between T-free and T-cont groups	1	430.47	430.47	48.67[a]
Plants within groups	58	513.01	8.85	29.84[a]
Seeds within plants	240	71.14	0.03	
Total	299	1014.61		

[a]Significant at 0.01 level

there was no significant difference within plants between seed with and without a damaged seed coat (Table 19).

Differences in seed coat thickness between plants can be related to differences in seed weight.

In their study, Rowland and Fowler (70) found a significant correlation between seed weight and seed coat thickness between cultivars ($P<0.01$) but not within cultivars ($P>0.05$). This implies that within cultivars the smaller seeds had a higher percentage of seed coat. In this study, a higher correlation of seed coat percentage with seed weight existed within the F_2 population for plants of the tannin-free group than for plants of the tannin-containing group (Table 20). The smaller-seeded tannin-containing segregants maintained a fairly similar percentage of seed coat compared with the larger-seeded ones because of reduced thickness of their seed coats, whereas the smaller-seeded, tannin-free segregants had a higher percentage of seed coat than the larger-seeded tannin-free segregants. Therefore, it should not be difficult to select small-seeded, tannin-free segregants that have seed coats thick enough to prevent them from splitting or cracking.

Little seed coat damage was found amongst the large-seeded, tannin-free parental lines. This could be explained by their flattened shape which gives them a greater surface:volume ratio or a thicker seed coat. Both would give Triple White a higher percentage of seed coat than the average of the tannin-free progeny derived from crosses with the small-seeded, tannin-containing cultivars (Table 21).

The average percentage of seed coat of the tannin-free F_2

TABLE 19. Tests for differences in seed coat percentage within and between Triple White x UMFB-9 F_2 plants grown at the Point (Winnipeg, 1978)

Comparison	Df	t
T-free vs. T-cont plants	58	6.98[a]
T-free plants with vs. T-free plants without damaged seed	28	2.55[b]
Damaged vs. undamaged seed within T-free plants (paired t-test)	3	0.64

[a]Significant at 0.001 level

[b]Significant at 0.05 level

TABLE 20. Correlation of seed coat percentage with seed weight - seed coat weight for seed from Triple White x UMFB-9 F_2 plants grown at the Point (Winnipeg, 1978)

Description of plants	Df	r
T-free	28	-0.66[a]
T-cont	28	-0.22

[a]Significant at 0.01 level

TABLE 21. Mean seed coat percentage of seed from Triple White, UMFB-9 and Triple White x UMFB-9 plants grown at the Point (Winnipeg, 1978)

Population	Number of plants	% Seed coat
Triple White	15	11.61 ± 0.01
UMFB-9	15	12.10 ± 0.26
Triple White x UMFB-9		
(a) T-free plants	30	10.23 ± 0.27
(b) T-cont plants	30	12.63 ± 0.21

segregants was well below that of the parental tannin-free cultivar. There may be genes for a thicker seed coat in the absence of tannin that have been selected for in Triple White.

The difference between the percentage of seed coat of the tannin-free versus the tannin-containing segregants was probably not affected much by the correlation with seed weight since the average seed weight for each of the two groups was about equal (Table 22). Table 22 also indicates a smaller seed coat weight as being a possible explanation for tannin-free segregants tending to have lower seed weights than tannin-containing segregants (Tables 14, 15 and 16).

Further tests were carried out to see whether differences in seed coat thickness were related to differences in seed coat composition (Table 23).

It was thought that the thicker, heavier seed coats of the tannin-containing lines compared with the tannin-free lines might be caused by greater lignification of the tannin-containing cells of the seed coat, namely the palisade and hour-glass cells. These cells possess unevenly thickened cell walls and make a significant contribution to variations in seed coat thickness (26). There was a significant difference between the permanganate lignin content of the tannin-free versus the tannin-containing parental cultivar as expected. The tannin-containing F_2 lines had a greater lignin content than the tannin-free F_2 lines though significance was not established owing to a large standard error. Further testing is required. The low lignin content of Triple White did not allow seed coat damage like that of the tannin-free F_2 lines, but this may be a result of the flattened shape and thicker seed coat of this tannin-free cultivar.

TABLE 22. Comparison of tannin-free with tannin-containing seed from Triple White x UMFB-9 plants grown at the Point (Winnipeg, 1978)

Parameter	T-free	T-cont	Df	t
Seed weight (g)	0.77	0.75	59	0.41
Seed weight - Seed coat weight (g)	0.67	0.68	58	0.25

13 9.3

TABLE 23. Comparative chemical composition of seed coats of Triple White, UMFB-9 and bulked samples of seed from F_2 progeny of Triple White x UMFB-9[a]

Population	Acid-detergent fibre %	Cellulose and cutin %	Permanganate lignin %
UMFB-9	62.51 C	58.06 C	4.23 A
T-cont F_2 lines	62.07 C	58.22 C	3.48 AB
T-free F_2 lines	68.64 A	62.28 A	2.98 AB
Triple White	65.30 B	62.37 B	2.65 B
Standard error	0.42	0.49	0.31

[a]Values are expressed on a dry matter basis. All values represent duplicate samples. Means not sharing a common letter within each column are significantly different at P=0.05 (Duncan's Multiple Range Test)

Values from acid-detergent fibre and cellulose and cutin analyses are similar to those that Marquardt et al. (49) found for tannin-containing versus tannin-free cultivars. It is interesting to note that the values for the parental tannin-free cultivar, Triple White, are significantly different from those of the tannin-free F_2 lines. This was also true of seed coat percentage and suggests that genes are present in Triple White that act to minimize the effect of the 'white flower characteristic' on the seed coat.

Although the tannin-free lines tend to contain less lignin in their seed coats, they have a higher acid-detergent fibre value, indicating that absence of tannin corresponds with a greater percentage of cellulose and/or cutin and a correspondingly lower percentage of hemicellulose and protein. Cellulose fibrils and the incrustation of cell walls with cutin would be expected to give the seed coat greater rigidity but it was the tannin-free seed coats having the high cellulose and/or cutin content that were thinner and more prone to splitting and cracking.

The relationship of tannin to seed coat composition may actually be caused by an interference of tannin with the analytical procedures or an involvement in one fraction and not another. It would be interesting to determine the concentration of tannin in the cellulose versus the hemicellulose fraction. This might help to resolve the problem of how a single gene could have such a broad range of effects.

When introducing the 'white flower characteristic' into the commercial minor cultivars, seed coat structure should be considered as a selection criterion. Selection could be based on seed coat

percentage or simply on the absence of any visible cracks. Variability in seed coat percentage appears to be sufficient to permit the production of a tannin-free minor cultivar with seed coat properties at least as good as those of the parental tannin-free cultivar.

5. SUMMARY AND CONCLUSIONS

Fidrim, Kodrim and Triple White all possess the same single gene for the 'white flower characteristic' which causes them to have seed coats free of condensed tannin. The 'white flower characteristic' has a number of pleitropic effects resulting from the blockage of the production of certain phenolic compounds in the stem, stipules, flowers and seed coat. Tannin-free plants of segregating lines were found to lack any reddish pigment in their stems or spots on their stipules. They had white flowers and buff-coloured seeds that were often marked by a dark brown or grey to black pigment that was not expressed in the parental cultivars. Black hilum colour, caused by the deposition of melanin, was determined by a single dominant gene in the minor cultivars and was found in crosses with Triple White to segregate independent of the 'white flower characteristic'.

Because of the pleiotropic effects of the 'white flower characteristic', selection for a tannin-free minor cultivar could be made more easily on the basis of visible plant and seed characteristics than by chemical tests. Tannin-containing segregants in a growthroom could be rogued soon after germination on the basis of reddish stems and/or stipule spots so that the remaining tannin-free segregants could benefit from the increased space for growth and available light. Selection against tannin-containing segregants in the field should be based on the presence of stipule spots and coloured flowers and could be made at any

time up until the end of the flowering stage.

If there is any uncertainty as to the presence or absence of tannin in the seed of a particular line, the clearest indication is obtained by placing a seed coat sample in a basic solution and leaving it overnight. The prussian blue test is difficult to standardize for rapid qualitative testing, but if the development of a cultivar with a low tannin content is found to be preferable to the introduction of the tannin-free 'white flower characteristic', it may be possible to refine this test so that it can be used to give a rapid indication of the level of phenolic compounds in the seed coat. For quantitative analyses, the vanillin-HCl test with certain modifications proved to have good reproducibility but was not reliable for detecting very slight amounts of tannin that might have been present in some lines possessing the 'white flower characteristic'.

It should not be difficult to reduce seed size to that enabling the employment of cereal equipment since the trait does not appear to be linked with the 'white flower characteristic' and the F_2 mean lies closer to the size of the minor than the major parent. There was no maternal effect on the inheritance of either trait. The only significant maternal effect was found in the inheritance of hilum colour. If a cultivar suitable for canning were required, it would be preferable to use Fidrim, Kodrim or Triple White as the female parent.

Seed coat colour is not so important if the grain is to be used solely for animal rations. A black hilum colour should cause no problem. However, for marketing purposes it would be better to select against the dark pigmentation which occurs over most of the tannin-free seed since it tends to give the seed the appearance of being infected or damaged in

some way. It is possible to select for a pure buff-coloured seed. Approximately 40% of the tannin-free F_2 segregants were free or almost free of the dark pigment. Further study is required to determine the inheritance of the trait.

Two other deleterious effects were found to be associated with the 'white flower characteristic'. These were the tendency of tannin-free seed coats to split or crack and possibly a greater suseptibility to disease, noteably Fusarium sp. Link and Rhizoctonia sp. D.C.. The former may affect the latter to some extent.

Tannin-free F_2 plants within a cross exhibited a high degree of variability in seed coat percentage and seed coat damage. If facilities are not available to prevent cross-pollination by insects, near isogenic lines could be obtained by growing out selected tannin-free lines in isolated plots and roguing any purple-flowered plants that appear as a result of crossing with one of the currently grown commercial cultivars. Sufficient seed could be generated within one or two more generations to allow replicated field testing of tannin-free lines. In this way, some estimate of the optimum seed coat thickness could be obtained - one which has the minimum fibre percentage required to prevent seed coat damage. Seed coat characteristics may also be related to the disease resistance of these lines. The selection of lines resistant to Fusarium sp. Link and Rhizoctonia sp. D.C. appears to be of particular importance in the development of a tannin-free minor cultivar for Western Canada.

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LIST OF APPENDICES

APPENDIX TABLE 1. Numbers of crossed plants

T-cont x T-free population	Generation			T-free x T-cont population	Generation		
	P	F_1	F_2		P	F_1	F_2
A x F	4	2	9	F x A	4	7	46
A x K	4	9	42	K x A	4	11	48
A x T	4	6	97	T x A	4	3	62
D x F	4	12	37	F x D	4	10	70
D x K	4	13	38	K x D	4	15	82
D x T	4	6	32	T x D	4	11	61
H x F	4	8	40	F x H	4	7	51
H x K	4	6	20	K x H	4	11	60
H x T	4	9	38	T x H	4	13	101
U x F	4	8	27	F x U	4	11	44
U x K	4	1	6	K x U	4	2	15
U x T	4	21	269	T x U	4	15	190
B x F	4	8	22	F x B	4	10	45
B x K	4	3	10	K x B	4	8	34
B x T	4	12	63	T x B	4	7	26
Total	60	124	750	Total	60	141	935

(Continued)

APPENDIX TABLE 1. (Continued)

T-cont x T-cont population	Generation			T-free x T-free population	Generation		
	P	F_1	F_2		P	F_1	F_2
A x D	4	19	120	F x K	4	4	14
A x H	4	23	85	F x T	4	2	0
A x U	4	15	72	K x F	4	7	15
A x B	4	13	64	K x T	4	7	29
D x A	4	30	148	T x F	4	3	13
D x H	4	30	196	T x K	4	7	25
D x U	4	22	149				
D x B	4	10	75				
H x A	4	15	84				
H x D	4	19	95				
H x U	4	10	62				
H x B	4	12	53				
U x A	4	14	106				
U x D	4	24	204				
U x H	4	8	44				
U x B	4	12	54				
B x A	4	8	47				
B x D	4	17	82				
B x H	4	6	42				
B x U	4	7	76				
Total	80	314	1858	Total	24	30	96

APPENDIX TABLE 2. Numbers of backcrossed plants

Population	Generation			Population	Generation		
	F_1	BC_1	BC_1F_1		F_1	BC_1	BC_1F_1
A x (A x T)	2	58	79	U x (U x T)	2	5	-
(A x T) x A	2	25	80	(U x T) x U	2	15	-
A x (T x A)	2	44	80	U x (T x U)	2	26	-
(T x A) x A	2	39	80	(T x U) x U	2	37	-
T x (A x T)	2	16	57	T x (U x T)	2	9	-
(A x T) x T	2	21	41	(U x T) x T	2	37	-
T x (T x A)	2	12	71	T x (T x U)	2	8	-
(T x A) x T	2	48	64	(T x U) x T	2	53	-
(A x F) x A	2	12	-	(U x F) x U	2	23	-
(F x A) x A	2	27	-	(F x U) x U	2	16	-
(A x F) x F	2	11	-	(U x F) x F	2	22	-
(F x A) x F	2	16	-	(F x U) x F	2	18	-
(A x K) x A	2	15	-	(U x K) x U	2	16	-
(K x A) x A	2	16	-	(K x U) x U	2	14	-
(A x K) x K	2	12	-	(U x K) x K	2	12	-
(K x A) x K	2	13	-	(K x U) x K	2	13	-
Total	32	445	552	Total	32	324	-

APPENDIX TABLE 3. Numbers of selfed plants

T-cont cultivars	Generation			T-free cultivars	Generation		
	P	S_1	S_2		P	S_1	S_2
A	31	31	39	F	31	13	18
D	13	10	21	K	14	8	14
H	42	24	22	T	17	10	17
U	31	23	23				
B	30	16	23				
Total	147	104	119	Total	62	31	49

APPENDIX TABLE 4. Variability in seed weight estimates with different sample sizes

Line	Sample size	Weight per seed[a] g	CV
382	100	0.75	0.6%
	50	0.76	0.9%
	20	0.76	0.7%
	10	0.77	2.4%
1918	100	0.69	0.3%
	50	0.68	1.7%
	20	0.69	1.6%
	10	0.68	2.7%
2372	100	0.54	2.9%
	50	0.54	4.9%
	20	0.55	4.1%
	10	0.57	3.5%
3001	100	0.73	0.8%
	50	0.74	1.2%
	20	0.74	2.0%
	10	0.73	1.9%
3458	100	0.76	1.3%
	50	0.76	3.2%
	20	0.76	3.9%
	10	0.75	3.6%

[a]Mean of three samples

APPENDIX TABLE 5. Chi-square values for flower colour segregation in F_2 tannin-containing x tannin-free lines

Population	P	W	X^2 Value for 3:1	Probability
A x F	5	4	1.81	0.10 - 0.25
A x K	29	13	0.79	0.25 - 0.50
A x T	79	18	2.15	0.10 - 0.25
Total	113	35	0.14	0.50 - 0.75
D x F	28	9	0.01	0.90 - 1.00
D x K	30	8	0.32	0.50 - 0.75
D x T	25	7	0.17	0.50 - 0.75
Total	83	24	0.38	0.50 - 0.75
H x F	31	9	0.13	0.50 - 0.75
H x K	16	4	0.27	0.50 - 0.75
H x T	29	9	0.04	0.75 - 0.90
Total	76	22	0.34	0.50 - 0.75
U x F	21	6	0.11	0.50 - 0.75
U x K	5	1	0.22	0.50 - 0.75
U x T	209	60	1.04	0.25 - 0.50
Total	235	67	1.28	0.25 - 0.50
B x F	15	7	0.55	0.25 - 0.50
B x K	7	3	0.13	0.50 - 0.75
B x T	48	15	0.05	0.75 - 0.90
Total	70	25	0.09	0.75 - 0.90
TOTAL	577	173	1.50	0.10 - 0.25

APPENDIX TABLE 6. Chi-square values for flower colour segregation in F_2 tannin-free x tannin-containing lines

Population	P	W	X^2 Value for 3:1	Probability
F x A	35	11	0.03	0.75 - 0.90
F x D	51	19	0.17	0.50 - 0.75
F x H	40	11	0.32	0.50 - 0.75
F x U	32	12	0.27	0.50 - 0.75
F x B	30	15	1.67	0.10 - 0.25
Total	188	68	0.33	0.50 - 0.75
K x A	38	10	0.44	0.50 - 0.75
K x D	65	17	0.80	0.25 - 0.50
K x H	50	10	2.22	0.10 - 0.25
K x U	11	4	0.02	0.75 - 0.90
K x B	27	7	0.35	0.50 - 0.75
Total	191	48	3.88	0.03 - 0.05[a]
T x A	46	16	0.02	0.75 - 0.90
T x D	49	12	0.92	0.25 - 0.50
T x H	79	22	0.56	0.25 - 0.50
T x U	144	46	0.06	0.75 - 0.90
T x B	17	9	1.28	0.25 - 0.50
Total	335	105	0.30	0.50 - 0.75
TOTAL	714	221	0.93	0.25 - 0.50

[a]Significant at 0.05 level

APPENDIX TABLE 7. Chi-square values for flower colour segregation in backcrossed lines

Population	P	W	X^2 Value for 1:1	Probability
T x (A x T)	6	10	1.00	0.25 - 0.50
T x (T x A)	7	5	0.67	0.25 - 0.50
(A x T) x T	6	15	3.86	0.03 - 0.05[a]
(T x A) x T	19	29	2.08	0.10 - 0.25
Total	38	59	4.55	0.03 - 0.05[a]
(A x F) x F	6	5	0.09	0.75 - 0.90
(F x A) x F	9	7	0.25	0.50 - 0.75
Total	15	12	0.33	0.50 - 0.75
(A x K) x K	6	6	0.00	0.90 - 1.00
(K x A) x K	6	7	0.08	0.75 - 0.90
Total	12	13	0.04	0.75 - 0.90
T x (U x T)	4	5	0.12	0.50 - 0.75
T x (T x U)	5	3	0.50	0.25 - 0.50
(U x T) x T	18	19	0.03	0.75 - 0.90
(T x U) x T	25	28	0.17	0.50 - 0.75
Total	52	55	0.08	0.75 - 0.90
(U x F) x F	9	13	0.73	0.25 - 0.50
(F x U) x F	7	11	0.89	0.25 - 0.50
Total	16	24	1.60	0.10 - 0.25
(U x K) x K	6	6	0.00	0.90 - 1.00
(K x U) x K	8	5	0.69	0.25 - 0.50
Total	14	11	0.36	0.50 - 0.75
TOTAL	147	174	2.27	0.10 - 0.25

[a]Significant at 0.05 level

APPENDIX TABLE 8. Pigmentation scores of seed from tannin-free F_2 segregants

T-cont x T-free population	Number of plants			T-free x T-cont population	Number of plants		
	0	1	2		0	1	2
A x F	0	1	2	F x A	1	4	4
A x K	3	2	8	K x A	1	2	7
A x T	3	4	10	T x A	6	3	5
D x F	3	3	1	F x D	7	6	5
D x K	2	5	1	K x D	7	4	5
D x T	2	4	1	T x D	4	5	2
H x F	2	2	0	F x H	2	5	3
H x K	3	4	0	K x H	5	1	4
H x T	3	4	0	T x H	7	7	8
U x F	1	2	3	F x U	6	3	2
U x K	0	0	1	K x U	1	2	0
U x T	34	8	8	T x U	11	12	13
B x F	6	1	0	F x B	10	2	2
B x K	2	1	0	K x B	7	0	0
B x T	6	4	5	T x B	3	5	0
Total	70	44	42	Total	78	61	60

APPENDIX TABLE 9. Chi-square values for hilum colour segregation in F_2 tannin-containing x tannin-free lines

Population	Black	Non-black	X^2 Value for 3:1	Probability
A x F	5	2	0.05	0.75 - 0.90
A x K	34	9	0.38	0.50 - 0.75
A x T	75	21	0.50	0.25 - 0.50
Total	114	32	0.74	0.25 - 0.50
D x F	26	6	0.67	0.25 - 0.50
D x K	27	3	3.60	0.05 - 0.10
D x T	24	6	0.40	0.50 - 0.75
Total	77	15	3.71	0.05 - 0.10
H x F	13	0	4.33	0.03 - 0.05[a]
H x K	15	5	0.00	0.90 - 1.00
H x T	30	6	1.33	0.10 - 0.25
Total	58	11	8.59	0.00 - 0.01[b]
U x F	20	7	0.01	0.90 - 1.00
U x K	5	1	0.22	0.75 - 0.90
U x T	193	50	2.54	0.10 - 0.25
Total	218	58	2.34	0.10 - 0.25
TOTAL	467	116	8.10	0.00 - 0.01[b]

[a]Significant at 0.05 level

[b]Significant at 0.01 level

APPENDIX TABLE 10. Chi-square values for hilum colour segregation in F_2 tannin-free x tannin-containing lines

Population	Black	Non-black	X^2 Value for 3:1	Probability
F x A	26	7	0.25	0.50 - 0.75
F x D	52	15	0.24	0.50 - 0.75
F x H	37	13	0.03	0.75 - 0.90
F x U	6	4	1.20	0.25 - 0.50
Total	121	39	0.03	0.75 - 0.90
K x A	34	11	0.01	0.90 - 1.00
K x D	57	24	0.93	0.25 - 0.50
K x H	44	16	0.09	0.75 - 0.90
K x U	10	3	0.03	0.75 - 0.90
Total	145	54	0.48	0.75 - 0.90
T x A	44	13	0.15	0.50 - 0.75
T x D	45	14	0.05	0.75 - 0.90
T x H	64	23	0.10	0.75 - 0.90
T x U	101	47	3.60	0.05 - 0.10
Total	554	97	1.30	0.25 - 0.50
TOTAL	520	190	1.17	0.25 - 0.50

APPENDIX TABLE 11. Seed weight of seed from parental plants selfed or cross-pollinated in the growthroom

♂ \ ♀	A	D	H	U	B	F	K	T
A	0.45	0.43	0.58	0.42	0.68	1.02	1.19	1.07
D	0.48	0.54	0.52	0.57	0.81	1.14	1.09	1.12
H	0.45	0.47	0.46	0.57	0.81	1.14	1.09	1.12
U	0.45	0.50	0.41	0.48	0.83	1.07	1.06	1.08
B	0.48	0.48	0.41	0.58	0.79	1.16	1.07	0.93
F	0.45	0.55	0.45	0.59	0.78	1.44	1.14	1.08
K	0.50	0.52	0.44	0.56	0.72	1.37	1.37	1.36
T	0.46	0.48	0.52	0.48	0.89	1.01	0.91	1.20
Mean	0.47	0.50	0.47	0.53	0.78	1.19	1.13	1.10
s.e.	0.01	0.01	0.02	0.02	0.02	0.06	0.05	0.05

APPENDIX TABLE 12. Measurements of seed coat percentage and seed coat thickness[a] of seed from F_2 plants of Triple White x UMFB-9

Line	Tannin analysis[b]	Seed coat percentage	Seed coat thickness x 10^{-3} inches
3299	0	9.89 ± 0.17	6.06 ± 0.25
3302	+	14.24 ± 0.48	6.24 ± 0.10
3305	0	9.00 ± 0.10	5.08 ± 0.11
3330	+	11.99 ± 0.08	5.92 ± 0.07
3338	0	9.89 ± 0.11	5.66 ± 0.09
3342	0	10.13 ± 0.14	5.86 ± 0.06
3372	+	14.11 ± 0.34	6.64 ± 0.08
3376	0	12.84 ± 0.22	5.74 ± 0.10
3392	0	8.99 ± 0.04	4.58 ± 0.07
Mean		10.13 ± 0.06	5.86 ± 0.06

[a]Made three measurements per seed for five seeds of each plant

[b]Symbols: + = tannin; 0 = no tannin