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Antinutritional factors in faba bean. Tannin content in *Vicia faba*: possibilities for plant breeding

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SUMMARY - Condensed tannins are amongst the most important antinutritional factors in *Vicia faba*. Genetic variability for the amount of condensed tannins in the testa of faba bean, mainly linked to genes controlling flower or grain colour, is available in natural populations. A positive response to selection for low tannin content grains in faba bean lines with coloured flowers has been found. The role of tannins in the physiology of the plant in relation to plant breeding is discussed.

RESUME - "Facteurs antinutritionnels de la fève. Teneur en tannins chez *Vicia faba*: possibilités d'amélioration". Les tannins condensés sont un des facteurs antinutritionnels les plus importants de *Vicia faba*. La variabilité génétique pour la quantité de tannins condensés dans la testa de la fève, liée principalement à des gènes qui contrôlent la couleur de la fleur ou de la graine, est disponible dans des populations naturelles. Il a été trouvé une réponse positive à la sélection des graines à faible teneur en tannins dans des lignées de fève à fleurs colorées. Le rôle des tannins dans la physiologie de la plante en relation avec l'amélioration génétique est traité.

Introduction

Among the secondary products of plant metabolism, tannins have attracted the attention of plant breeders, mainly because of two contradictory reasons; their negative effect on the nutritional quality of the varieties produced, and the role they play in certain defence mechanisms against pathogens and pests.

The chemistry of tannins is complex and not well understood, and it is difficult to relate it with the tannin effects which are of interest to plant breeders. Tannins are classified into two groups: condensed tannins which are polymers of flavonols and yield anthocyanidins when heated in acid solution, and hydrolysable tannins which produce gallic acid as a degradation product. The latter are easily eliminated with the action of the gastric juices and do not represent a problem in animal feeding. For this reason they are not included in our analysis.

Flavan, the basic structural unit of condensed tannins is made up of two benzene rings linked together by a

three carbon atom fragment. This unit is also the basic structure of anthocyanidins and their glycosides (anthocyanins), the latter generally being responsible for the scarlet, red, mauve, purple and blue colours in plants organs. Methylation, hydroxylation or glycosidation are possible modifications of the basic unit of polymers resulting in a marked variability of the physiological and biochemical properties of these substances. These properties could be as basic as the auxin transport regulators (Jacobs and Rubery, 1988), protectors of inner tissues against visible and/or UV irradiation (Salatino *et al.*, 1988), or activators of nodulation genes in *Rhizobium* (Firmin *et al.*, 1986).

Kristiansen (1984) proposed a biosynthetic pathway for tannins (procyandin C2) in barley by adding leucocyanidin (flavan-3,4-diols) to procyandin B3 (dimer flavan-3,4-diols, flavan-3-ol). The degree of polymerization is a parameter of high biological and agronomical significance. Protein binding activity of tannins (a factor responsible for the decrease of protein digestibility) increases with size, and it peaks somewhere between 3 and

10 monomers (Goldstein and Swain, 1963). These relatively short oligomers seem to be responsible for agronomical characters such as the bird repellence of sorghum seeds (Butler, 1982) and the decrease in infestation of *Vicia faba* by *Callosobruchus maculatus* (Boughdad *et al.*, 1986). The polymerization reaction has been proposed to be a nonenzymatic process. Nevertheless, differences in the degree of polymerization in sorghum could be under genetic control as it is a characteristic of some varieties (Bullard *et al.*, 1981), although factors such as grain structure or other biochemical components of the seed could also be important. The analytical problems in the evaluation of tannin quality and quantity remain unsolved.

In this paper we present the results of our research on the study of tannins in the seeds of *Vicia faba*.

Genetics of testa tannin content and its relation with flower and grain colour

As previously mentioned, tannins share some precursors with anthocyanin pigments in their biosynthetic pathway. This is the reason for the well known relationship between white flowered plants and absence of tannins in their seeds (Dickinson *et al.*, 1957). Pigmentation mutants have played an important role in the elucidation of the biosynthetic pathway of anthocyanidins and for this reason we decided to study the relationships between flower, testa colour, and tannin content in *Vicia faba*. In Table 1 we have summarised the results of the analysis of 55 lines with different generations of selfing. The relationship of flower colour with tannin content of the whole seed is significant. As found by other researches, white flowered lines lack tannin in their seeds. The spotted lines have less tannin in the testa than lines with diffuse pigmentation in the flower and similar trends are

found in plants with yellow flowers. Differences in lines with different grain colour are less evident, probably because the genetics of seed colour is more complex than that of flower colour (Cabrera, 1988). Nevertheless, plants with red seed coat and spotted flowers have very low tannin content.

In Table 2 we show result from a similar study but instead of pure lines, F₂ plants of parentals differing in tannin content, flower and/or grain pigmentation were used (Cabrera and Martín, 1989b). The data on tannin content are given as percent of testa weight. An approximate comparison of both studies can be made by assuming that the seed coat was constant at 15% of total weight of the seed. Even after this adjustment, the values of tannin content in both tables are not similar, because of different analytical procedures used in each study. A modified Folin method (Singleton and Rossi, 1965), used in the first study gives an overestimation of tannin content as compared to the Vanillin procedure (more specific method for tannins and its precursors) used in the second (Broadhurst and Jones, 1978). The relationship between flower colour and tannin content is also manifested in the second study. However, the influence of the gene for red colour (r), located in chromosome IV (Cabrera *et al.*, 1989), on tannin content became evident, probably because of the more homogeneous genetic background of the material analysed.

Despite the analytical problems, the relation between flowers and seed coat colours and tannin content of the seed is obvious. More importantly, when studying the genetic basis of tannin content in the testa we found that although additiveness was the main component, dominant variance was also significant in some crosses. Furthermore, if the plant with red grains or diffuse pigmentation in flowers are eliminated, the dominant component of the variance disappears and the additive component is reduced (Cabrera and Martín, 1989a).

Table 1. Mean tannin content (%) in grain of faba bean pure lines in relation to flower and testa colours^a.

Flower colour	Testa colour						Total
	Grey	Green	Red	Normal	Brown	Yellow	
White	0.1 ± 0.0	0.1 ± 0.0	—	—	—	—	0.1 ± 0.0a
Yellow-spot	—	—	1.5 ± 0.2	—	—	1.5 ± 0.2b	—
Normal	—	1.5 ± 0.1	1.7 ± 0.3	2.2 ± 0.0	2.3 ± 0.2	2.3 ± 0.0	1.8 ± 0.1b
Diffuse yellow	—	—	—	2.3 ± 0.1	—	—	2.3 ± 0.2c
Diffuse brown	—	2.3 ± 0.2	2.8 ± 0.1	2.5 ± 0.2	2.5 ± 0.4	—	2.5 ± 0.1c
Total	0.1 ± 0.0a	1.5 ± 0.2b	2.1 ± 0.2c	2.2 ± 0.1c	2.4 ± 0.3c	2.3 ± 0.0c	—

^aMeans with different letters in the same line or column are statistically different (p < 0.01)

Table 2. Mean values for tannin content in the testa of F₂ plants of faba bean in relation to flower and testa colour^a.

Flower colour	Testa colour					Total Yellow
	Grey	Green	Red	Normal	Brown	
White	0.4 ± 0.0	–	–	–	–	0.4 ± 0.0 a
Yellow-spot	–	1.7 ± 0.1	–	3.6 ± 0.1	3.9 ± 0.1	4.5 ± 0.1 b
Normal	–	2.7 ± 0.1	3.0 ± 0.0	4.2 ± 0.0	4.2 ± 0.0	3.6 ± 0.0 b
Diffuse yellow	–	–	–	4.4 ± 0.1	–	4.4 ± 0.2 c
Diffuse brown	–	2.6 ± 0.1	3.2 ± 0.1	4.8 ± 0.1	5.1 ± 0.4	4.5 ± 0.1 c
Total	0.4 ± 0.0 a	2.5 ± 0.1 b	3.0 ± 0.0 c	4.1 ± 0.0 c	4.2 ± 0.0 c	

^aMeans with different letters in the same line or column are statistically different ($p < 0.01$)

Our conclusion is that the main part of the quantitative variability detected on tannin content of the testa in *Vicia faba* grain is a reflection of major genes, mainly red seed coat (*R/r*), diffuse pigmentation (*Sdp/sdp*) and yellow flower (*Yf/yf*) genes, the expression of their being influenced by environmental factors.

The role of tannins in faba bean

The condensed tannins in faba bean have similar biological effects and biochemical properties to those present in sorghum grains (Marquardt *et al.*, 1977). The changes in the degree of polymerization of condensed tannins of developing seeds in sorghum (Butler, 1982), and their relationship with bird repellence are well known. Likewise, in sorghum the genetic variation have been classified into three groups according to the degree of polymerization of tannins contained in them. In *Vicia faba*, as far as we know, no such study has been published; however, Tena and Menéndez-Romo (1987) have studied the evolution of tannin with grain development in our material and found the same pattern as in sorghum. The pattern shows an increase of condensed tannins (and monomers) in the seed coat with time which peaks around 30 days after flowering and is followed by a decline in tannin content associated with an increase in the degree of polymerization. They have also studied the changes of polymerization of tannins in the seed coat wilt time of three faba bean lines having diffuse pigmentation, VF91 (yellow), VF118 (violet) and VF123 (brown) using two dimension thin-layer chromatography. The evolution of tannin content and polymerization level were as previously found, although VF91 kept a noticeable amount of monomers (mainly catechin) and oligomers for six months, indicating a variation in the level of

polymerization linked to genetic differences on pigment synthesis. Yellow flowered plants and some white flowered plants share a character linked to changes in pigmentation. Pods and branches in both groups do not turn black at ripening, and this may be interpreted to be because of the differences in the levels of enzymes oxidising phenols or more plausibly as a change in the substrate of these enzymes common to both genetic variants. Moreover, plants with yellow flowers never show brown pigmentation in the seed (Cabrera and Martín, 1989a). Nevertheless, some white flowered plants do turn black at ripening and we have found that these variants carry the allele *Yf* for brown flower pigmentation while plants that kept a light colour carry the *yf* for yellow pigmentation. This means that anthocyanidins and related compounds are not involved in this change of colour.

There is another change in seed colour associated to ripening and storage of the seed coat of *Vicia faba*, linked with flower colour and with a clear biological impact. It is well known that the testa colour changes with time after ripening probably due to the oxidation of phenolic compounds. More importantly, in plants with coloured flowers, the seed colour in individual plants is not homogeneous, two groups, light and dark can always be obtained. The percentage of each is not maintained in the next generation after selfing. Marbach and Mayer (1974) have studied the relationship between seed colour, phenolic compounds, levels of enzymes oxidising phenolics compounds, and the permeability of the seed coat in the genus *Pisum*. They concluded that seed coat permeability to water is related to phenolic content and its level of oxidation and differences in permeability depended on the position of the seed in the mother plant. We also found differences in permeability related to seed colour; the grains of white flowered plants took up water amounting to 80% of its weight after 10 hours while dark seeds from coloured flowered plants increased their

weight by 46% and light seeds from the same plants only 20%. However 24 hours later there were no differences in the amount of water absorbed. The production of seeds with different germination requirements is a widely extended survival mechanism among wild plants, this has not been fully eliminated after domestication in *Vicia faba*.

However, the most important character related to this change of colour was the absence of infestation by *Bruchus rufimanus* of light coloured grains while dark ones and grains from white flowered plants were infested in 100% of the cases under field conditions. We tried to find a relation between colour and situation in the mother plant of the grain, but unlike in *Pisum* there was no relationship.

In Table 3 some results comparing dark and light seeds from the same plant are presented. Pure lines and F₃ plants were analysed. Differences in percentage of seed coat or ADF were not significant. No structural differences were found between both seed groups in sections of seed coats. Nevertheless, the tannin index (TI) of both groups was significantly different which seems to indicate a difference in phenols content. On the contrary, the reaction to vanillin was not significant, although a correspondence between values of TI and vanillin is evident. The higher values of the vanillin test could be interpreted as a lower level of polymerization of tannins in light colour testas, because this test only takes into account the terminal unit of the polymers and the present difference in colour could reflect two different patterns of polymerization or phenols oxidation, neither of which were under genetic control during the development of the seed. In sorghum grains, Glennie (1981) has studied the preharvest changes in polyphenols content, and in peroxidase and polyphenol oxidase (PPO) activities. He found a parallel behaviour between tannin evolution and the activity of both enzymes, with the decline of peroxidase not as rapid as that of tannins or PPO. These enzymes and oligomers have been associated with defence mechanisms. There appears to be a definite period of grain development in which the grain could be infested and also a period in which the high level of peroxidase and PPO or short tannin oligomers prevent such infestation. White flower varieties do not change testa colour and are susceptible to *Bruchus*. Coloured flower varieties have two types of grains; those with darker testa colour are sensitive to *Bruchus* infestation because they pass quickly to high polymerization levels and low peroxidase and PPO activities, and others with lighter testa colour take more time in reaching the same polymerization level and are immune to *Bruchus* infestation.

Breeding strategies

Production of tannin-free faba bean cultivars using the white flower genes is the easiest way of solving the

problem of these antinutritional factors. Nevertheless, Bond (1986) reported lower yield and poorer establishment of tannin-free cultivars and near-isogenic lines than their tannin-containing relatives. These results are not unusual and the decrease of yield could be a consequence of susceptibility of tannin-free cultivars to pathogens and pests. In Table 4 we present data on mean seed weight, number of seed per plant and yield per plant of F₂ plants from crosses of tannin-free by tannin-containing lines. The five lines used are of intermediate seed size (seed weight of line 14=0.41, 15=0.50, 23=0.41, and 44=0.33, 59=0.40 g). The only parameter that seems to be lower in tannin-free plants is seed weight and in terms of yield it is counterbalanced by a higher number of seeds per white plant. It is well known that seeds from white flowered plants have a lower percentage of testa than tannin containing plants (Cabrera and Martín, 1986). This fact could account for the difference. The poorer establishment could also be a result of a weaker microbial protection from a thinner and tannin-free testa. If white flowers prove to have a pleiotropic effect over characters of agronomical interest, other breeding strategies have to be tested.

Experiments of selection for low condensed tannin content in coloured flower faba bean populations are scanty. Sjödin *et al.* (1981) selected for low and high content of total phenolics, four different populations with normal testa colour during two cycles. In the first cycle a difference of 0.33 units was found between high and low tannin containing plants while in the second cycle there was no significant difference between both groups. The thousand-grain weight was strongly affected by selection, and the testa percentage which was significantly higher in the population with high content of total phenolics was also affected. The change in testa percentage could account for the change in phenolics content. It seems that selection for low tannin content in populations with homogeneous testa colour and standard pigmentation in flower is not effective. In contrast, the tannin

Table 3. Characteristics of seed coat of individual *Vicia faba* plants differing in darkening.

	Light colour X ± SE	Dark colour X ± SE	t
Seed coat %	15.38 ± 0.55	16.25 ± 0.47	1.21 ns
ADF	13.43 ± 0.37	13.99 ± 0.63	0.69 ns
T.I. ^a	6.97 ± 0.55	5.49 ± 0.31	2.34 *
T.I. ^b	6.66 ± 0.15	5.41 ± 0.11	6.58 ***
T.I. ^{a+b}	6.79 ± 0.24	5.44 ± 0.14	4.74 ***
Vanillin	3.65 ± 0.25	3.14 ± 0.20	1.58 ns

^aPure lines

^bF₃ plants

Table 4. Yield of F₂ plants differing in flower colour. (a) Seed weight (g), (b) seeds per plant, (c) yield per plant (g).

Cross		Coloured flower X ± SE	N	White flower X ± SE	N	t
59 X 14	(a)	0.44 ± 0.01	70	0.24 ± 0.01	43	15.38 ***
	(b)	58.01 ± 5.06		61.98 ± 6.08		0.50 ns
	(c)	26.28 ± 2.32		26.06 ± 2.75		0.06 ns
59 X 15	(a)	0.50 ± 0.05	162	0.47 ± 0.01	59	3.16 **
	(b)	56.17 ± 3.20		65.47 ± 3.47		2.19 *
	(c)	28.16 ± 1.23		30.49 ± 1.57		1.17 ns
44 X 14	(a)	0.44 ± 0.01	62	0.42 ± 0.01	34	1.05 ns
	(b)	45.34 ± 3.91		48.03 ± 5.79		0.38 ns
	(c)	20.34 ± 1.86		20.21 ± 2.42		0.04 ns
44 X 15	(a)	0.44 ± 0.06	156	0.41 ± 0.01	55	3.44 ***
	(b)	59.28 ± 2.78		65.69 ± 5.03		1.11 ns
	(c)	25.98 ± 1.25		26.83 ± 2.00		0.36 ns
23 X 14	(a)	0.45 ± 0.01	38	0.43 ± 0.01	35	1.71 ns
	(b)	66.82 ± 8.05		68.54 ± 5.76		0.17 ns
	(c)	30.29 ± 3.67		29.97 ± 2.81		0.07 ns
23 X 15	(a)	0.48 ± 0.06	127	0.45 ± 0.01	44	3.19 **
	(b)	52.13 ± 2.63		56.73 ± 5.02		0.79 ns
	(c)	25.04 ± 1.24		25.76 ± 2.32		0.27 ns
TOTAL	(a)	0.47 ± 0.03	615	0.43 ± 0.04	270	8.33 ***
	(b)	55.90 ± 1.40		61.74 ± 2.09		2.31 *
	(c)	26.09 ± 0.66		26.91 ± 0.93		0.72 ns

content estimated by the vanillin-HCl method was clearly reduced in one cycle of selection when major genes for grain and flower colour were present in the population under selection (Cabrera *et al.*, 1989). A composite population was created by mixing F₂ plants from crosses among lines differing in flower and seed coat colour. Selection for low and high tannin content was done. The two populations were grown and their progeny assessed for tannin content using near infrared reflectance (NIRS) spectroscopy. Two clearly distinctive populations were obtained. The two selected populations with high and low content of tannins were tested for flower and seed coat colour. It was observed that the two populations differed for these characters. The low tannin population had 96.5% plants with spotted flowers (normal black or yellow spots) and 3.5% with solid pigmentation, whereas in the high tannin population the latter percentage rose to 40%. With respect to seed coat colour, we found that 64% of the low tannin content population had brown and 15% red or red-brown seed coat colour. The rest of the plants had normal or yellow seed coat colour. However, in the high tannin content population 92.75% of the plants had normal and yellow seed coat colour; red or

red-brown seeds were not found. These results are as expected according to the genetics of tannin presented in the previous paragraph.

In addition to the production of tannin-free cultivars using the recessive white flower genes, others genes that reduce tannin content, as described previously, should be tested. A plant with red grain and lemon spot on flower could be the basis of low tannin content cultivars without the negative effect of white flower genes.

It is well established that phenol related compounds play an important role in plant metabolism and in the interaction of plants with the external environment (Zucker, 1983). In the previous section we presented some observations on the variability for some characters at plant level remaining for primitive forms of *Vicia faba*, like variability for water absorption of grain of the same plant associated with different testa colour and susceptibility to infestation by *Bruchus rufimanus*. Response to selection for lighter grains as expected was unsuccessful. We were not able to find a relationship between seed colour and situation in the mother plant, but this does not mean it does not exist. If a plant with only light colour

grain could be obtained it would be resistant to *Bruchus* infestation with only a small increment in tannin content.

Once elucidated the relation of colour genes with the tannin content of the testa, our main objective will be the study of tannin content in the vegetative part of the plant, with the aim of obtaining faba bean with the highest level of tannin in the vegetative part and the lowest in the grains. The study of the environmental influence in the tannin content of the seed and the elucidation of the genetic basis of the differences in polymerisation levels, if they do exist, will be the goals for our future work.

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