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BEAN TANNIN: STATE-OF-THE-ART AND PERSPECTIVES OF RESEARCH

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Introduction

An enormous effort has been devoted to studies on the chemistry, functional and biochemical significance of a special group of the polyphenolic compounds family. This important group of compounds are called "tannins". The importance of vegetable tannins lies in their role in several processes: as repellents to predators, animal or microbial; in enzyme inhibition; in the formation of complexes with dietary proteins and other nutrients; in interaction with growth regulators; as potential mutagens; in post-storage hardening and darkening; and probably a large list of other function that still are unknown. In bean knowledge in depth is scarce but the implications of the tannin in the total quality of the bean has a fair amount of empirical evidence.

The present seminar is mainly devoted to show the many facets of tannin in quality of beans, and to pointing out practical problems that need a rapid solution to develop better bean.

Tannin definition

The word "tannin" cannot be precisely defined in the chemical sense and for this reason it has been misapplied and abused in both the botanical and biochemical literature. The more accurate description of tannins found in vegetative tissues appear in Table 1. This can be a broad group of compounds that are illustrated in Figure 1, where the index N can vary from 0 to 8-10, resulting in molecules from the size of dimers, with low reactivity to polymerus with high reactivity (Haslam, 1974).

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* Bean Nutrition and Quality Lab.

Tannin content if foods

Man consume a number of foods containing considerable amounts of dietary tannins, as is shown in Table 2, (Rickard, 1986; Rao et al., 1982; Maxon et al., 1972). And in legumes, common beans and faba beans present the higher limits in the range of content of tannins, these are illustrated in Table 3 (Price et al., 1980; Rao et al., 1982; Cabrera et al., 1986; Strickland, 1984).

Pathogens resistance

Among the several roles of tannins in the plants, an important aspect is that of plant protection. Plants produce protective chemical substances after infection. These compounds were named phytoalexins, not all are tannins but many of them form part of this group of compounds. Among these are pisatin isolated from pea pods (Perrin et al., 1962) and phaseollin isolated from bean (Cruickshak et al., 1963). The evidence with Fusarium solani F. phaseoli shows increments in total phenols for material with low initial levels, but small increments in materials with relatively high initial level as is shown in Table 4 (Statler, 1970).

Insects resistance

Also tannis are often cited as examples of substances than can inhibit the growth of insects (Feeny, 1968). In beans the relationship between tannin content and resistance still is not clear and is an open field of research.

Citology of specialized cells for tannin production

Recent studies have shown that specialized phenol-storing cells occur randomly in many tissues of a considerable number of plants. Following synthesis, the phenols are apparently stored in specialized compartments (vacuoles), and kept in a reduced form within the cells until some disturbance (injury or infection) occurs (Beckman et al., 1970, Esau, 1963).

Physiological effects

Several authors noted inhibitory effects of tannins on plant growth and development. In sorghum the preharvest seed germination has a high correlation ($r = -0.81$) with tannin level (Harris et al., 1970)(see Table 5), and in the seeds of sericea the effect of tannins in inhibiting germination is evident in studies with whole and dehulled seeds (Logan et al., 1969). This effect is apparently mediated by the inhibitory action of the tannins upon the gibberelins. Evidence exists of this in pea seedlings (Corcoran et al., 1972), and in rice (Harada et al., 1974). No clear evidence of this effect has been studies in beans.

Effects of agronomic factors on tannin contents

Plant phenolics appear to exhibit a variety of responses to water deficits. In sorghum the water stress during some periods post-anthesis increase the tannin content, as appear in Table 6 (Hoshino et al., 1982). Low soil fertility apparently increases these levels too. Addition of S to the soil decreased the tannin content in Lotus pedunculatus (Barry et al., 1983). Even the amount of light can effect a control on total phenol biosynthesis (Duke et al., 1976). No controlled studies of all these factors in beans were reported. Our own data show some relationship between tannin content and grain color, but the ranges for each color group are wide and it is possible to find the same tannin value in beans of very different colors (Table 7). That shows that the relationship mentioned in several publications between seed color and tannin content is only valid with the color group means and must be evaluated more carefully. Table 1 with seed testa brilliance show that brilliant seed has higher levels (Table 8) and high seed tannin levels also are associated with indeterminate bush growth habit (Table 9).

Heritability and inheritance

In a study of heritability and inheritance of the tannin content of seed in F_2 generations low tannin content was found to be dominant to high in the progenies of the most of crosses. But the genetic relation between color and tannin content is still not clear (Ma et al., 1978). For our data it appears that low tannin is associated with white seeds or is a material inherited character in level as well as in the structure of the tannin fraction composition as is shown by the similarities in the UV-spectra of the extracts.

Relationship with other nutrients

The relation with other nutrients is not clearly defined. With respect to fiber the data in Vicia faba show no significant relationships (Marquardt et al., 1978), but for sorghum the correlation between these are significant (Fuller et al., 1966). With total protein the situation is very difficult to interpretate. In sorghum some reports show a positive correlation and other a negative correlation. In beans the correlation is positive but not significant (Fuller et al., 1966; Fuller, 1964; Arora et al., 1974); Harris, 1973; Bressani et al., 1983). A very clear negative relationship is evident between the globulin fraction of protein and the tannin content, in sorghum. This negative relationship, if it exists in beans, is very important for the digestibility of protein problem (Jambunathan et al., 1973; Ramachandra et al., 1977; Landry et al., 1970).

Nutritional effects

The case of the "negative" characteristics of tannins in nutritional aspects is their capacity to interact with dietary proteins forming an insoluble an indigestible complex, and by inhibiting the action of the

digestible enzymes. Table 10 shows clearly the inhibitiory effects, but is also very clear that there are big differences among the different species evaluated. The similar tannin content in chickpea and pigeon pea inhibit the evaluated enzymes with a difference of a hundred percent. Apparently the chickpea tannins are more reactive than pigeon pea. This is an important fact which shows the need of specific studies for each type of genetic material. For the relationship between tannin content and digestibility a common feature is the wide variability for digestibility especially in beans (Bressani, 1982; Rodriguez de Mora, D., 1982). Less variability is noted for other grains such as finger millet (Ramachandra et al., 1977). The tannin ability to interact with other nutrients is clear from the data showing its interaction with starch, which reduces its digestibility to approximately 20% (Desphande, 1982). In other ratios between tannin and iron found in other foods, the tannins can reduce the amount of available iron to practically null values (Rao et al., 1982). Table 11. An important point to emphasize appears in Table 12, which is the small difference between the "in vitro" protein digestibility of some cooked bean cultivar. The maximum difference is near to 3% and the range of tannin content is similar. This data shows the role of more than one factor working simultaneously to affect digestibility. The tanins interacting with non-globular protein fractions reduced the digestibility of these proteins. The denaturalization by heat of the globular fraction part increases the digestibility of these proteins. These effects in the opposite sense produce the small difference found. But in general it is important to point out the relative low digestibility level of bean protein (80%) with respect to other foods (Elias et al., 1979).

Tannins play an important role in two acceptability characteristics that have strong effects on nutritive value. 1) the post-storages hard-seed development by the tannin polimerization. This is an accepted concept an there exists a fair amounts of incidental information, but these still does not exist a detailed controlled study on this important bean acceptability factor. 2) the same polimerisation causes the phenomena known as post-storage darkening and the only report with detailed data has been made in lentils (Nozolillo et al., 1984).

Methods of determination

Several analitical procedures have been developed and modified for analysing polyphenolic compounds in plant extracts. The methods can be classified as oxidation-reduction methods, colorimetric methods, gravimetric methods, "functional" methods, and separation methods as gas-liquid chromatography, high performance liquid chromatigraphic and others. But this still does not exist the "method" for tannin determination. The most common method is the vanillin assay, which was developed a century ago by Lindt, but actually recognized as the Burns method. This has low specificity (Sarkar et al., 1976) but is fast and cheap. The most modern assay method is the Dr. Lehel Telek (Telek, 1986) method which is very sensitive and specific but with high time, personal

and material cost. Our laboratory adapted and developed a modification to the vanillin assay with the goal of increasing its specificity and improving the quality of the data by use of "real" adequate standards for quantification. In Table 13 appear the data of some bean accessions by the different methods.

Goals and feasibility

The main goal of any work in tannins is to contribute to better beans production. This work can best be conducted at CIAT because CIAT has the world's bean germplasm collection, and the experience and knowledge of the wide range of variables that effect the tannin content to accurately carry out these types of studies.

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TABLE 1.

TANNINS ARE:

- VEGETABLE POLYPHENOLS.
- WATER SOLUBLE.
- HAVING MOLECULAR WEIGHTS BETWEEN 500–3000.
- WITH THE ABILITY TO PRECIPITATE PROTEINS FROM AQUEOUS SOLUTION.
- WITH 1 OR 2 HYDROXYPHENOLIC GROUPS FOR EACH 100 UNITS OF MOLECULAR WEIGHT.

FIGURE 1.

TANNIN GENERAL STRUCTURE

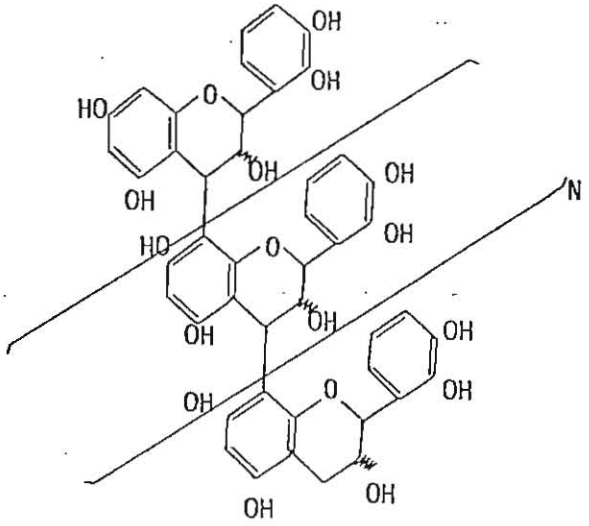

TABLE 2. TANNIN CONTENT* OF SOME FOODS

FOOD	TANNIN CONTENT %, C.E.(1)	REF.
CASSAVA (Manihot esculenta)		Rickard (1986)
FRESH	0.36	
FARINHA	0.027	
WHEAT (Triticum aestivum)	0.041	Rao et al (1982)
SORGHUM (Sorghum vulgare)		Maxson et al (1972)
WHITE	0.077	
RED	3.25	
RAGI (Eleusine coracana)	0.360	Rao et al (1982)
CONDIMENTS		
CORIANDER (Coriandrum sativum)	0.311	Rao et al (1982)
TAMARIND (Tamarindus indica)	0.600	Rao et al (1982)
TURMERIC (Cucurma domestica)	3.350	Rao et al (1982)
CHILLI POWDER (Capsicum annum)	0.980	Rao et al (1982)

* MV-HCl

(1) Catechin equivalents

TABLE 3. TANNIN CONTENT* OF LEGUMES

LEGUME	TANNIN CONTENT % (C.E.)[1]	REF.
ADZUKI BEAN (Vigna angularis)	0.290	Price et al (1980)
BLACK GRAM (Ph. mungo)	0.540–1.197	Rao et al (1982)
CHICK PEA (Cicer arietinum)	0.078–0.272	Rao et al (1982)
COWPEA (Vigna sinensis)	0.175–0.590	Rao et al (1982a)
FABA BEAN (Vicia faba)	0 –3.540	Cabrera et al (1986)
GREEN GRAM (Ph. aureus)	0.437–0.799	Rao et al (1982)
KIDNEY BEAN (Dolichos lablab)	1.024	Rao et al (1982a)
PIGEON PEA (Cajanus cajan)	0.380–1.710	Rao et al (1982a)
COMMON BEAN (Ph. vulgaris)	0.17 –3.500	
PEA (Pisum spp.)	0.026–0.530	Stickland (1984)

* MV-HCL

(1) Catechin equivalents

TABLE 4.

RESISTANCE OF BEAN PLANTS TO FUSARIUM SOLANI F. PHASEOLI

TOTAL PHENOLS OF PLANS EXTRACTS EXPRESSED AS MG EQUIVALENTS OF CATECHOL PER G OF PLANT TISSUE

	TOTAL PHENOL VALUES (mg/g)[a]			
	1 WEEK		3 WEEKS	
	HEALTHY	INFECTED	HEALTHY	INFECTED
GOLDEN GEM	0.14	0.23	0.11	0.31
VI 123	0.15	0.22	0.09	0.57
VI 111	0.13	0.25	0.08	0.35
VI 114	0.15	0.24	0.08	0.55
R-275	0.18	0.21	0.17	0.20
PI-203958	0.17	0.20	0.19	0.19

(a) Averages of 12 plants.

From Statler, (1970)

TABLE 5.

THE RELATIONSHIP OF TANNIN CONTENT OF SORGHUM SEED TO PREHARVEST SEED GERMINATION

NUMBER OF HYBRIDS	TANNIN CONTENT C.E.(%) MEAN	GERMINATION INDEX (a) MEAN
21	10.2	1.3
21	3.6	3.7

(a) 0=none 1=Trace 2=2 to 10% 3=11 to 25%
4 =26 to 40% 5=above 40%

From Harris, H. B.; R. E. Burns, (1970).

TABLE 6.

CHANGES IN TANNIN CONTENT AFTER ANTHESIS IN SORGHUM (FUNK'S BR-79) FOLLOWING WATER STRESS DURING DIFFERENT PERIODS

STRESS DAYS POST-ANTHESIS	TANNIN CONTENT MEAN (mg/seed) (C.E.)
Control	7.9
20-45	9.3
0-20	7.3
0-45	7.3

From Hoshino, T.; R. D. Duncan, (1982)

TABLE 7.

DESCRIPTIVE STATISTICS FOR TANNIN CONTENT[1] IN COMMON BEANS BY PRIMARY SEED TESTA COLOR

COLOR	N	MEAN* C.E.	MIN	MAX
Pink	16	8.25[de]	4.74	11.57
Red	26	14.00[a]	5.87	30.20
White	28	0.34[g]	0.17	0.77
Cream-Beige	26	8.45[d]	0.34	17.94
Yellow	5	6.51[f]	2.87	12.10
Purple	5	11.17[b]	4.13	16.17
Black	44	10.01[c]	2.33	16.10
TOTAL	155	8.35	0.17	30.20

* Means with the same letter are not significantly different by the Duncans multiple range test at 0.05

(1) Catechin equivalents, mg/g of flour, of grain.

TABLE 8.

TANNIN CONTENT[1] OF COMMON BEAN BY GROUPS OF BRILLIANCE OF THE SEED COAT

BRILLIANCE	N	MEAN*
OPAQUE	29	8.25[b]
INTERMEDIATE	78	7.70[c]
BRILLIANT	9	10.13[a]

1 Catechin equivalents mg/g the flour of grain.

* Means with the same letter are not significantly different by the Duncans multiple range test at 0.05.

TABLE 9.

TANNIN CONTENT(1) OF COMMON BEANS BY GROWTH HABIT OF THE PLANT

GROWTH HABIT	N	MEAN*
Bush determined	27	6.97[c]
Bush indetermined		
With guide	40	9.05[a]
Indetermined		
No climbing	43	7.91[b]
Climber	5	8.05[b]

(1) Catechin equivalents, mg/g. flour of grain.

* Means with the same letter are not significantly different by the Duncans multiple range test at 0.05.

TABLE 10.

VARIETAL DIFFERENCES IN THE ENZYME INHIBITORY PROPERTY OF POLYPHENOLS OF CHICK PEA AND PIGEON PEA

CULTIVAR	TANNINS (mg/g)	ENZYME INHIBITION (%)			
		TRYPSIN	CHYMO-TRYPSIN	HUMAN SALIVA	PANCREAS
CHICKPEA					
RABAT	1.9	33.6	26.3	29.8	17.5
L-550	2.3	34.5	25.7	31.5	20.8
PANT G-114	5.3	86.3	72.5	73.4	56.9
G-130	5.8	88.7	79.0	80.3	64.5
USA-613	6.1	81.6	70.9	78.6	61.0
PIGEON PEA					
HY-3C	3.7	37.9	36.0	34.5	21.8
NP(WR)-15	6.0	40.5	38.6	32.7	19.7
C-11	14.2	91.5	90.3	86.0	80.9
BDN-1	15.2	90.3	91.6	79.4	69.3
No-148	14.9	88.0	85.9	75.8	68.5

From Singh, U., (1984)

TABLE 11.

EFFECT OF THE ADDITION OF TANNIN ON THE IONISABLE IRON IN RED GRAM (Cajanus cajan) FOOD

TANNIC ACID (mg/100 g)	MOLAR RATIO FE/TANNIN	IONISABLE (%)
0	0	10.6
12.8	10:1	10.6
25.5	10:2	10.6
38.3	10:3	10.6
63.8	10:5	7.7
102.0	10:8	7.7
127.5	10:10	4.8
255.0	10:20	3.8
382.5	10:30	0.0

From Narasinga Rao, B.S.;
T. Prabhavati, (1982)

TABLE 12.

TANNIN CONTENT EFFECT ON THE "IN VITRO" DIGESTIBILITY OF COOKED BEAN OF DIFFERENT CULTIVARS

BEAN CULTIVARS	"IN VITRO" DIGESTIBILITY (%)	TANNIN CONTENT (mg TANNIC ACID EQUIV/G SAMPLE)
White	81.3 ± 1.8	1.9
Red	78.7 ± 2.8	4.1
Black	77.9 ± 2.5	5.3

Abridge from Elias, L. G. et al, (1979)

TABLE 13. TANNIN CONTENT BY DIFFERENT METHODS

SAMPLE	VANILLIN ASSAY(1)	PVP MODIFIED VANILLIN ASSAY(1)	TELEK'S METHOD(2)	CIAT'S METHOD(2)
G-1459 (B)	0.728	0.337 (46)*	1.388	0.748
G-3715 (B)	1.294	0.540 (42)	2.472	1.343
G-7034 (Y)	1.560	0.742 (48)	1.620	2.497
G-13679 (Y-W)	0.318	0.066 (21)	0.860	1.898
G-2270 (C-P)	1.794	0.924 (51)	1.588	2.725
RAB-035 (R)	2.530	1.165 (46)	0.679	0.360
G-4090 (R)	1.434	0.897 (63)	0.452	0.481

(1) CATECHIN EQUIVALENTS, %

(2) TANNIN EQUIVALENTS, %

* % OF THE MV-HCl DETERMINATION