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The Relationships of Sorghum Kernel Pericarp and Testa Characteristics with Tannin Content

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Abstract: A practical approach for determining tannin contents is very useful for sorghum breeders. Tannin contents were determined with vanillin-HCl method for 24 sorghum entries varying in origin, pericarp color, presence or absence of testa and testa color. The relations of these kernel characteristics with their tannin contents were investigated. It was revealed that dark color pericarp produces trace amount of tannins, while the tannin contents in the sorghum cultivars with purple testa were much lower than those with brown testa. Subdivision of type 1 and type 2 sorghum was proposed to provide sorghum breeders more practical classification system for selecting cultivars with suitable tannin contents.

Key words: *Sorghum bicolor*, kernel, tannin, breeding, pericarp, testa

INTRODUCTION

Sorghum [*Sorghum bicolor* (L.) Moench] is the fifth most important cereal crop grown on over 44 million hectares in both temperate and tropical regions. Sorghum is mainly grown as rainfed crop by subsistence farmers in the semi-arid tropical regions of Africa and Asia as well as by other farmers in the USA, China and Latin America. About 88% of sorghum production is used as human food and animal feed. Nearly 150 insect species have been reported as pests on sorghum. Sorghum is a host for more than 100 plant pathogens, including fungi, bacteria, virus and nematodes. Some of these pathogens cause more serious damage and are more widespread than others (ICRISAT, 2007).

Phenols (mainly condensed tannins) in sorghum kernel are considered a desirable agronomic trait since it can protect sorghum from being damaged by birds, insect pests and diseases (Waniska *et al*., 2001). But in the view of nutritive value, tannins are considered undesirable due to its capacity of binding proteins and make them less digestive as well as producing astringent taste (Ambnla *et al*., 2003). Therefore, debates on the necessity and amount of tannins in sorghum kernels exist between nutritionists and agronomists and consumers and growers. Sorghum breeders have been wandering between the two extremes. How to take the advantages of tannins in fields and to minimize their disadvantages of reducing nutritive value of sorghum grain is a knotty task for sorghum breeders.

However, in ruminants, tannins can induce beneficial effects. For example, higher retention of nitrogen has been observed in sheep and cattle with low to moderate levels of tannins in forages. In these cases, the lower digestibility of nitrogen was compensated for by reduced urinary loss of hydrogen. Moderate levels of tannins (The preferred tannin content is between 0.1-0.6%) in forage legumes can have beneficial responses in ruminants, resulting in higher growth rates and milk yield (Hagerman and Klucher, 1986). Less than 1% tannin in sorghum grain is generally considered moderate amount (Hancock, 2000). Mild amount of tannins is also necessary for high quality liquors in many regions in China (Lu and Sun, 2005). Therefore, mild amount of tannins in sorghum grain is needed in many cases even in view of feed nutrition and grain quality.

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Sorghum containing condensed tannins has dominant B1B2 genes that produce a thick, pigmented testa layer in the kernel upon maturation (Earp and Rooney, 1986). This layer varies in thickness, intensity and color. Peircarp color is determined by R-Y-genes whether the pericarp is red (R-Y-), colorless or white (R-yy, rryy), or lemon yellow (rrY-).

The most widely accepted method of determining condensed tannins in sorghum is the vanillin-HCl method (Price *et al*., 1978) when the blanks are subtracted to eliminate background of non-tannin materials. However, it requires significant time and is not readily applied in routine grading of sorghum. The kernels can also be dissected and visually evaluated for the presence of a testa layer which can be difficult without sufficient experience.

In the present study, we investigated the relationships between sorghum kernel pericarp and testa colors with tannin contents and their relevance in breeding programs. The study was initiated at Carlsberg Research Center, Demark and completed at The Agri-Biotechnology Research Center of Shanxi Province, China in 2006.

MATERIALS AND METHODS

Twenty-four sorghum entries, varying in pericarp color, absence or presence of testa and color of testa, were from China (Shanxi Academy of Agricultural Science), Africa (Ford Research Center at Katum, Sudan and a few local varieties from Tanzania and Rwanda) and USA (Purdue University and Texas A and M University). Tannin content analysis was conducted with modified Vanilin-HCl method (Hahn and Rooney, 1986). The method is based on the ability of condensed tannins to react with vanilin in the presence of mineral acids to produce a red color. Grain samples were ground at same day with tannin content analysis. Ground samples (1 g was extracted with 20 mL of 1% HCl in methanol) for 20 min at 30°C in a water bath. The samples were centrifuged at 2000 rpm for 4 min. The supernatant (1.0 mL) was reacted with 5 mL vanillin solution (0.5% vanillin + 2% HCl in methanol) for 20 min at 30°C. Blanks were run with 4% HCl in methanol in place of vanillin reagent and subtracted. Each sample was assayed for 3 replications and the mean was calculated to represent its tannin content. The entries were grouped according to the official United States Standards for Grain (UMSDA, 1987). Genotypes of entries were visually determined by the description of Rooney and Miller (1981).

Absorbance was read at 500 um and determined by subtracting blank readings from sample readings. The reference was developed by using commercial catechin to prepare a standard curve.

Photos of kernels were taken to represent the various types of pericarp and testa.

RESULTS AND DISCUSSION

The tannin contents of group I entries varied between 0-0.2% with the average of 0.08% CE; those of group II varied between 0.1-5.8% CE with the average of 1.8% CE and those of group III varied between 3.6-12.8 CE with the average of 5.9% CE. The differences among the three groups were obvious but without a clear-cut (Table 1).

Some entries in group I did not have detectable tannin by the present test and the others in the same group did contain trace amount of tannins. The two entries in the group I (without testa) with brown or red pericarps all produced faint positive reaction, an indication of the existence of minor amount of tannins. The results implied that the dark pericarp per se was also responsible for producing a little amount of tannins.

The entries in group I were most variable in tannin contents. Some entries, e.g., Feterita and Kafinam B, with obvious testa contained very little amount of tannins, implying the presence of testa

Table 1: Kernel characteristics, their genotypes, grouping and tannin contents

Entry	Source	Pericarp color	Testa[a]	Testa color[b]	Group[c]	Tannin (%[d])	Pericarp genotype[e]	Testa genotype[e]
Jinfu No1	China	Red	A	–	I (S)	0.2	RRYY	b1b1b2b2SS
BTX 378	USA	Red	A	–	I (S)	0.1	RRYY	b1b1b2b2SS
MR 724	ICRISAT	White	A	–	I	0.0	RRyy	b1b1b2b2ss
MR 723	ICRISAT	White	A	–	I	0.0	RRyy	b1b1b2b2ss
2219A	ICRISAT	White	A	–	I	0.0	RRyy	b1b1b2b2ss
CK60B	USA	White	A	–	I	0.1	RRyy	b1b1b2b2ss
TX623B	USA	White	A	–	I	0.1	RRyy	b1b1b2b2ss
Daber	Africa	White	A	–	I	0.1	RRyy	b1b1b2b2ss
Hegri/durra	USA	Yellow	A	–	I	0.1	rryy	b1b1b2b2ss
P721N	USA	White	A	–	I	0.1	RRyy	b1b1b2b2ss
KafinamB	ICRISAT	White	P	p	II (P)	0.1	RRyy	B1B1B2B2tptp
Feterita	Africa	White	P	P	II (P)	0.2	RRyy	B1B1B2B2tptp
IS0135	USA	White	P	B	II (B)	1.2	RRyy	B1B1B2B2TpTp
IS2319	USA	White	P	B	II (B)	1.4	RRyy	B1B1B2B2TpTp
IS8544	USA	White	P	B	II (B)	5.8	RRyy	B1B1B2B2TpTp
IS8768	USA	White	P	B	II (B)	2.9	RRyy	B1B1B2B2TpTp
Lishihuang	China	Yellow	P	B	III	4.2	rrYY	B1B1B2B2TpTp
Sanchisan	China	Red	P	B	III	4.4	RRyy	B1B1B2B2TpTp
Xin No. 7	China	Red	P	B	III	4.4	RRyy	B1B1B2B2TpTp
Jin No. 5	China	Red	P	B	III	5.1	RRYY	B1B1B2B2TpTp
HC356	China	Red	P	B	III	3.6	RRYY	B1B1B2B2TpTp
HM65	China	Red	P	B	III	4.1	RRYY	B1B1B2B2TpTp
S-37	Africa	Red	P	B	III	4.1	RRYY	B1B1B2B2TpTp
BR64	USA	Red	P	B	III	12.8	RRYY	B1B1B2B2TpTp

(a) A: Absent; P: Present; (b) Testa color was determined by visual observation of kernels with pericarp scratched off with a pocket knife; (c) According to Rooney and Miller (1981) and (d) CE

Fig. 1: Testa of sorghum kernels: Top: purple testa; bottom left: brown testa; bottom right: no testa

did not necessarily result in high amount of tannins. We noticed that the testa color of both varieties was purple instead of being brown as in most other type 2 sorghum varieties (Fig. 1). The purple testa is controlled by a pair of recessive gene, tptp and its dominant counterpart, Tp, produces brown testa (Rooney and Miller, 1981). Thus, sorghum breeders intending to select for low tannin varieties should not discard materials solely by the presence of testa, but also observe the color of the testa. On the contrary, some cultivars without testa, e.g., P954063 and BTX 623, did contain detectable tannins, implying that tannins do not ouly locate in testa, but also in pericarp to certain extent. Therefore, breeders should not select tannin-free lines solely by the absence of testa.

The entries in group III contained much higher amount of tannins than the previous two groups, implying the presence of dark pericarp [designed as spreader(SS) by Rooney and Miller (1981), will increase tannin amount drastically when testa is present.

Tannin content in sorghum kernel varies in a great range, which could be related to its pericarp and testa characteristics. Sorghum varieties with dark pericarp but without testa contain a little amount of tannins and may have certain resistance to bird, insects and diseases in the regions where bird damage and grain diseases are not severe, thus to maintain the advantages of tannins in the fields and do not produce significant adverse effects on nutritive value of sorghum grain.

Bird damages and grain molds are very severe in experimental plots in Liaoning and Hebei provinces in northern China. But two group I hybrids with dark pericarp, i.e., Jinza No. 1 (TX3197A X Jinfu No. 1) and Liaoza No. 1 (TX622A X Jinfu No. 1) have been successively grown in millions of ha sorghum production fields for more than 20 years (Lu and Sun, 2005). These facts indicated that dark pericarp varieties (without testa) do possess certain resistances to birds and grain molds. Although there is ouly trace amount, tannins in pericarp are more effective in protecting sorghum grains compared with those in testa since it is in the out-most defense layer. Still, tannins in the pericarp are also easier to be eliminated simply by removing the pericarp. The range of tannin contents in sorghum pericarp was narrow (0-0.2%) in the present study. But we may find some varieties with higher tannin content for higher protection effects by screening through the germplasm and breeding efforts.

It is very interesting in our study to find that two group II varieties, Feterita and Kafinam B, contained tiny amount of tannins. This result was not consistent with the common view that the presence of testa is related with high tannin content. We assayed samples of 6 more Feterita type varieties with purple testa in the following test and got very similar results. Their tannin contents varied between 0.2-0.5% CE, implying that purple testa did relate with low tannin content. Purple testa mostly exists in Feterita and Hegri type sorghum varieties. Selecting for purple testa cultivars (hybrids) may be one of the solutions to cop with the contradiction between the nutritive value and field resistance of grain sorghum.

The trace amount of phenols detected in the group I entries in present study might not be tannins, but flavanols and dihydrochalcones (Price *et al*., 1978). These phenolic compounds are also effective in protecting sorghum grain form bird and disease damages (Lu and Sun, 2005).

Based on the above results, we propose to subdivide the present group I into group I (L) and group I (D) and the group II into group II (P) and group II (B). The group I (L) includes testa-free entries with colorless, white and yellow pericarp (most probably with 0.1% CE tannins or below) and group I (D) contains the testa-free entries with darker pericarps (most likely with tannin content of around 0.2%). The group II (P) includes the sorghum entries with purple testa (most probably with tannin contents of 0.5% CE or below), while the group II (B) is consisted of sorghum entries with brown testa with light pericarp (most likely with 1.0-6.0% CE of tannin). These subgroups are easily to be distinguished from another one in the same group. And the subdivision of the two groups makes the entries within the subgroup more uniform not only in pericarp and testa color, but in their tannin content as well. For instance, in the present study, the subgroup I (D) contained 0.2% CE tannins in average, while those in subgroup I (L) contained ouly 0.07% and subgroup II (P) contained averagely 0.15% CE tannins whereas the subgroup II (B) contained 2.8% tannins in average. The subdivision of the two groups will facilitate sorghum breeders in their efforts to manage their breeding stocks and to use germplasm. Sorghum breeders will be able to confidently select their breeding stocks for a narrow range of tannin content simply by observing the grain color and scratching the kernels with a pocket knife in fields. All the subgroup II (P) entries in the present study had light pericarp. We may also combine the dark pericarp with purple testa traits to breed for group II (DP) type cultivars (hybrids), which should possess acceptable resistances to birds, diseases and insects in fields and have little negative effects on sorghum grain nutritive value. It would be helpful to further characterize phenols present in the purple testa and study their biological effects to both human and animal nutrition and sorghum pathogens.

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