The Effect of Weathering on the Quality of Sorghum Grain

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Resumen: El grano de sorgo Sorghum bicolor (L.) Moénch durante su desarrollo es generalmente expuesto a la luz solar, lluvia, enfermedades y plagas. El enmohecimiento del grano se refiere a la deterioración del mismo debido a diversos mecanismos: químicos, enzimativos, acción de las bacterias, hongos e insectos. Los microorganismos invaden y colonizan los tejidos de la flor al momento de la antesis y durante el desarrollo del cariopsis (una semana a un mes). Cuando el grano madura fisiológicamente, el 100% del grano contiene colonias de hongos en el mesocarpo almidonoso y en las células cruzadas y tabulares del pericarpio. En el grano maduro, condiciones climáticas húmedas y una temprana colonización de hongos incrementa el deterioro de la semilla.

Las reacciones químicas son catalizadas por la luz solar y las reacciones enzimáticas (ej. pigmentación en respuesta al ataque de insectos contribuyen a que se deteriore la calidad del grano). Un exceso de humedad después de la madurez del mismo puede también iniciar los procesos de germinación en la panícula. La germinación ocasiona una extensiva hidrólisis enzimática dado a enzimas endógenas (alpha-beta amilasas y proteasas).

Los cambios en las propiedades físicas del grano incluyen: 1) un pericaripio descolorido, mohoso y obscuro; 2) endospermo suave o harinoso; 3) falta de llenado de grano y tamaño adecuado; 4) una parcial o completa germinación de la semilla (reducida tasa de germinación); 5) micotoxinas; 6) reducción en los valores de materia seca, densidad y peso hectolítrico, y; 7) una composición alterada de compuestos fenólicos.

Los cultivares de sorgo que muestran resistencia a deterioración tienen: 1) endospermo córneo; 2) presencia de ciertos compuestos fenólicos (taninos, flavan-4 ol etc.); 3) pericarpio delgado; 4) más cera en el pericarpio; 5) baja incidencia de fisuras y quebraduras en el grano y; 6) baja tasa de absorción de agua. Otros factores también contribuyen a una mayor resistencia, por ejemplo una panícula más abierta, panículas encorbadas donde las glumas sirven como techos de protección para el grano en lugar de que sirvan como recipientes para retener agua, glumas más largas, maduración durante la temporada donde no hay lluvias, y otros. Obviamente muchos de

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los mecanismos de resistencia están vinculados con la prevención del contacto del agua con el grano.

Los cultivares resistentes contienen menos hongos en la cariopsis y glumas que los cultivos susceptibles. Los cultivares resistentes también responden más rápidamente al ataque de hongos por medio de la producción de niveles más altos de compuestos fenólicos en los tejidos de las glumas. Los cultivos resistentes con pericarpio rojo usualmente tienen niveles significantes de flovan-4-ols.

Antes de la madurez fisiológica no ocurre un deterioro extenso del grano, dado a que éste tiene altos niveles de compuestos fenólicos. El cariopsis inmaduro contiene entre 3 y 10 veces más compuestos fenólicos libres y taninos que el cariopsis maduro. Una reducción significativa en la cantidad de fenoles libres ocurre al momento de madurez fisiológica.

Los cultivares resistentes generalmente contienen niveles menores de ácidos fenólicos libres cuando son cultivados en condiciones adversas comparados con los susceptibles. El nivel alto de ácidos fenólicos en granos dañados o en susceptibles probablemente provengan de metabolitos de hongos. El análisis de los compuestos fenólicos del grano puede predecir el nivel de resistencia a la deterioración en campo.

Muchos factores afectan la deterioración del grano (ver arriba). Los fitomejoradores han y siguen mejorando al sorgo comercial para que resista al deterioro en campo. Granos afectados levemente pueden ser decorticados para remover al pericarpio descolorido y para pulverizar los granos suaves y deteriorados antes de que el mismo llegue a los productos alimenticios. La calidad del sorgo para consumo animal o humano es mejor si el grano sin dañar es utilizado.

ABSTRACT

Grain of *Sorghum bicolor* (L.) Moench is exposed to sunshine, rain, diseases and pests during its development. Grain molding or weathering refers to deterioration of the grain by several mechanisms, i.e. chemical, enzymatic, bacterial, fungal and insects. Microbes invade and colonize spikelet tissues at anthesis and the developing caryopsis during the next week to month. By physiological maturity 100% of the grain contain colonies of fungi in the starchy mesocarp and in the cross and tube cells of the pericarp. Rain and warm temperatures after anthesis increase fungal colonization of developing caryopses.

In mature grain, wet weather and early fungal colonization increase the amount of deterioration. Chemical reactions are catalyzed by sunlight; and enzymatic reactions, e.g. pigmentation in response to insects, contribute to deteriorate grain quality. Excessive dampness after rain maturity also initiates grain germination in the panicle which causes extensive enzymatic hydrolysis of the grain in endogenous enzymes, i.e. alpha--and beta--amylases and proteases.

Changes in the physical properties of grain include: 1) moldy, dark and discolored pericarp; 2) a soft and chalky endosperm; 3) decreased grain filling and size; 4) partial or complete sprouting (reduced germination of seed); 5) mycotoxins; 6) decreased dry matter, density and test weight and, 7) altered composition of phenolic compounds.

Sorghum cultivars that exhibit some resistance to deterioration have: 1) a more coreous endosperm; 2) presence of specific phenolic compunds (tannins, flavan-ol, etc.); 3) thin pericarp thickness; 4) more surface wax; 5) decreased fissuring or cracking in the field and, 6) decreased rate of water uptake. Other factors also contribute to increased resistance, i.e. a more open panicle, drooping panicles where the glumes form little roofs over the grain (instead of cups to hold the water), longer glumes, maturation during the nonrainy season, and others. Obviously, many resistance mechanisms involve keeping the water away from the grain.

Resistant cultivars contain less fungi in the caryopsis and glumes than susceptible cultivars. Resistant cultivars also respond more quickly to fungal invasior via increases levels of phenolic compounds in glume tissues than in susceptible cultivars. Resistant cultivars with a red pericarp usually have significant levels of flavan-4-ols.

Extensive deterioration of the grain does not occur before physiological maturity because the grain contains higher levels of phenolic compounds. The immature cayopsis contains between 3 to 10 times more free phenolic compounds and tannins than mature caryopsis. A significant reduction in the amount of free phenols occur at physiological maturity.

Resistant cultivars generally contained lower levels of free phenolic acids when grown under weathering conditions compared to susceptible cultivars. The higher levels of phenolic acids in weathered, susceptible cultivars probably resulted from fungal metabolites. Analysis of phenols in the grain can predict the level of resistance to weathering.

Many factors affect grain deterioration (see above). Plant breeders have and are continuing to improve the ability of commercial sorghums to resist grain deterioration. Slightly molded grain can be processed by decortication to remove the discolored pericarp and to pulverize the soft, deteriorated kernels befores the damaged grain gets into the food product. The fodd/feed quality of sorghum is better if sound (not damaged), clean grain is utilized.

Grain of *Sorghum bicolor* (L.) Moench is exposed to sunshine, rain, diseases and pests during its development. Grain molding or weathering refers to deterioration of the grain by several mechanisms (Glueck and Rooney, 1980; Williams and Rao, 1981), i.e. chemical, enzymatic, bacterial, fundal and insects. Regardless of the cause, changes in the physical properties of grain include (Glueck and Rooney, 1980; Williams and Rao, 1981; Jambunathan, et al, 1986; Waniska, et al, in press): 1) moldy, dark and discolored pericarp; 2) a soft and chalky endosperm; 3) decreased grain filling and size; 4) partial or complete sprouting (reduced germination of seed); 5) mycotoxins; 6) decreased dry matter, density and test weight and, 7) altered composition of phenolic compunds.

The purpose of this paper is to review some of the current findings regarding changes that occur in the kernel when sorghum deteriorates in the field as a result of attack by molds and other associated reactions. The terms 'molding' and 'weathering', will be used interchangeably.

Invasion by Fungi:

Fungi are responsible for mucho of the damage during early and later stages of deterioration (Glueck and Rooney, 1980; Williams and Rao, 1981). Bacteria and fungi invade and colonize spikelet tissues at anthesis (Castor and Frederiksen, 1980). After the glumes are opened at anthesis, the lodicles and related tissues start to be reabsorbed by the plant of they decompose (Castor and Frederiksen, 1980). This provides a source of nutrients for saprophytes including many species of Curvularia, Fusarium, and Colletotrichum (Castor and Frederiksen, 1980; Glueck and Rooney, 1980). These fungi colonize glume tissues and the developing caryopsis during the next week to month (Castor and Frederiksen, 1980); Forbes, et al., in press). By physiological maturity 100% of the grain contain colonies of fungi in the starchy mesocarp and in the cross and tube cells of the pericarp (Glueck and Rooney, 1980; Bandyopadhyay, et at., in press).

Rain and warm temperatures after anthesis increase fungal colonization of developing caryopses (Castor and Frederiksen, 1980; Williams and Rao, 1981). In mature grain, wet weather and early fungal colonization increase the amount of deterioration (Glueck and Rooney, 1980; Williams and Rao, 1981). One measure of fungal metabolism is the presence of ergosterol, a mycotoxin (Seitz, et al., 1983). Deterioration of mature grain increased during rainy conditions and the ergosterol content of grain increased geometrically compared to the weathering rating (Fig. 1). Hence, considerablely more damage occurs to the grain than indicated by visual ratings.

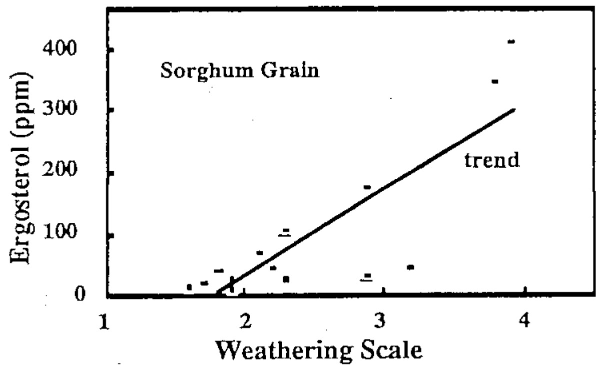

Figura 1. The relationship of ergosterol (a micotoxin) content in sorghum grain to a visual rating of weathering.

Inhibition by Phenolic Compounds:

Resistant cultivars contain less fungi in the caryopsis and glumes than susceptible cultivars (Castor and Frederiksen, 1980). Resistant cultivars also respond more quickly to fungal invasion via increased levels of phenolic compounds and pigmentation spikelet tissues than in susceptible cultivars (Forbes, et al., in press). Apparently, specific phenolic compounds in spikelet tissues limit the growth of fungi.

Most cultivars with a pigmented testa containing polymers of flavan-3-ols (tannins) exhibit resistance to weathering (Rooney and Miller, 1981; Bandyopadhyay, et al., in press). These cultivars contain higher levels of phenolic compounds, phenolic acids and tannins than cultivars without a pigmented testa, ej. sorghum classified as 'US#2 Yellow".

Apparently, tannins and or precursors of tannins impart antifungal and antibacterial activity in the developing and mature caryopsis. Cultivars with a red pericarp (with or without a pigmented testa) that contain significant levels of flavan-4-ols exhibit resistance to weathering (Jambunathan, et al., 1986). Cultivars with a white pericarp without a pigmented testa do not contain significant levels of flavan-4-ols; but some of these cultivars exhibit resistance to weathering. Apparently flavan-4-ols and related compounds are involved in some way with the resistance to grain weathering.

The content of free phenolic compounds ;and tannins in developing caryopses increased significantly during development (Doherty, et al., 1987). Maximun levels of phenolic compounds and tannins occur between 7 to 25 days after anthesis (Fig. 2 and 3). The maximun levels of free phenolic compunds and tannins were 2 to 8 times higher than observed in the mature grain. A significant decrease in these compounds was observed at physiological maturity (about 30 days after anthesis). Apparently, phenolic compounds and tannins are being bound to cellular tissues, and thereby, not extractable for analysis. The incidence of high levels of free phenolic compounds and tannins during development occurs during the period of early invasion and colonization of fungi of the caryopsis. Hence, it is likely than these compounds or specific components of these compounds are involved in the resistance mechanisms of grain weathering.

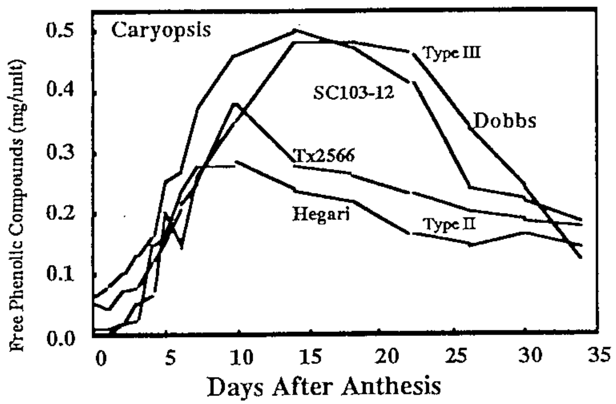

Figura 2. Effect of pericarp color and spreader gene on free phenolic compunds of type II and III sorghums (Reprinted from Doherty, et al., 1987).

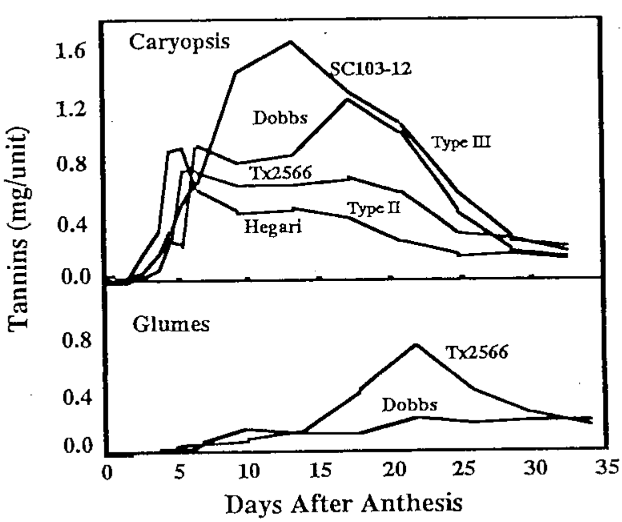

Figura 3. Effect of pericarp color and spreader gene on tannins in caryopsis (top) and glume tissues (bottom) of type II and III sorghums (reprinted from Doherty, et al., 1987).

Phenolic compounds and acids were quantitated in sorghum grown under conditions that yielded clean and weathered grain (Waniska, et al., in press). Higher levels of free phenolic acids, specially para-coumaric and caffeic acids, were observed in susceptible cultivars grown in the wet environmet. Bound phenolic acids were present at higher levels than free phenolic acids in all cultivars; however, bound phenolic acids did not significantly correlate with weathering resistance. Resistant and susceptible cultivars without a pigmented testa were properly grouped using a statistical procedure called the principal component analysis (Fig. 4). When the cultivars were grouped by pericarp color, weathering resistance, and environmental conditions, a scatter plot of free para-coumaric acid vs. free phenolic compounds separated the resistant and susceptible groups into the left and into the right halves of the scatter plot. The higher levels of phenolic acids in weathered, susceptible cultivars probably resulted from fungal metabolites. Hence, analysis of phenols in the grain can predict the level of resistance to weathering. This is currently being verified in projects at Texas A&M University and ICRISAT.

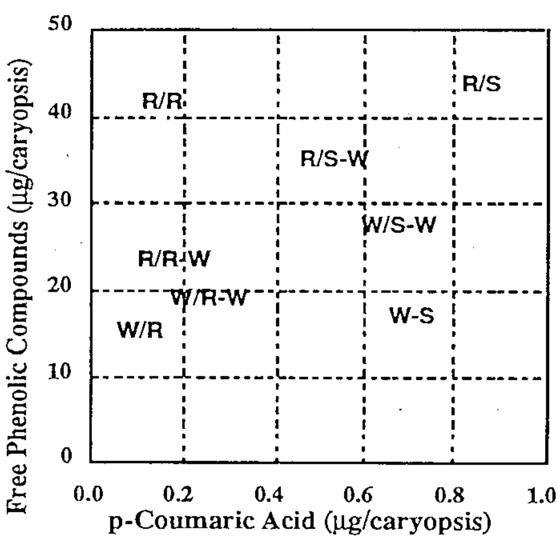

Figura 4. Classification of sorghum cultivars into resistant and susceptible groups according to their phenolic acid composition.

Other Resistance Mechanims

Several structural characteristics of the sorghum kernel correspond to improved resistance to weathering; endosperm texture, pericarp thickness, surface wax, and kernels with a larger proportion of corneous to fluory endosperm usually exhibit less weathering (Glueck and Rooney, 1980). Apparently, they mycelia of fungal colonies are not able to penetrate the dense, tightly packed aleurone and peripherial endosperm layers.

A thin pericarp on sorghum caryopses normally corresponds to less weathering (Glueck and Rooney, 1980). Since fungal colonies are observed inside the pericarp in 100% of sorghum cultivars, nutrients in the pericarp must be relatively readily available to fungi. Hence, a thick mesocarp that contains starch and protein should support more fungal colonies than sorghums with a thin mesocarp. Also, most of the free phenolic compounds are located in the pericarp and the adjoining testa layer. These apparently bioactive compunds would be diluted with starch, etc. in a thick pericarp.

The wax on the surface of the grain is thicker and continous in weathering resistant cultivars (Glueck and Rooney, 1980). Fungi was not able to penetrate trough a wax-covered, epicarp cell surface. Water was also not able to penetrate the epicarp. Hence, water and fungi probably enter the kernerl trough holes on the surface of the epicarp, i.e. through the hylar and stylar areas.

Kernels that retain their integrity, without cracking have improved weathering resistance (Glueck and Rooney, 1980). Mature Kernels of some cultivars crack or break apart when exposed to rain after a dry period. Apparently the rapid rehydration caused considerable internal stresses.

Water Uptake and Movement in the Grain:

The rate of absorption of water by the grain affects its rate of deterioration (Glueck and Rooney, 1980). Water normally enters the disrupted connective tissue between the pericarp and the rachis branch. Water enters the cross and tube cells of the pericarp and rapidly moves around the kernel. Concurrenlty water movement appears to be through the hilum (black layer) into the germ. After 30 min., water moved into the endosperm in the void area where the endosperm, germ and pericarp meet. Some water also enters the kernel through the style, and moves around the kernel in the cross and tube cells. Once water was in the endosperm, it moved readily through the less organized floury endosperm. After 3 hr the water began to move into the corneous endosperm.

Breaks in the kernel increased the rate and altered the pattern of water uptake. Cultivars with more breakage or with a more rapid rate of water uptake (intact kernels) exhibited less resistance to weathering. The leachate from these cultivars was also greater, e.g. more nutrients were easily extacted from the kernel. A thicker mesocarp thickness and a softer endosperm texture usually corresponded to more water uptake and more leachate.

Other factors also contribute to increased resistance (Glueck and Rooney, 1980), i.e. panicle shape, glume character, season avoidance, seed size, etc. A more open panicle does not permit the harboring of insects, fungi, etc. compared to a compact panicle shape. Drooping panicles where the glumes form little roofs over the grain (instead of cups to hold the water) decrease the exposure of the grain to moisture (which is required for fungal growth). Longer glumes are considered to protect the grain from fungi; but only if the glumes do not trap water. Grain maturation during the nonrainy season avoids the environmental conditions required for better fungal growth. Grain size does not seem to be important, except for very large kernels.

Practical Approaches

The problem of grain weathering is being approached from three direction: plant breeding, biochemical mechanisms and utilization. Sorghum plant breeders have been and continue to identify and improve the weathering resistance of elite cultivars. Several biochemical approaches were delineated in this report. Utilization of weathered grain is often necessary because the problem is not resolved yet. Field applications of fungicides decrease the extent of weathering, improve germination, but are expensive. Decortication of weathered grain es a practical method to improve its food quality. The abrasive decortication process will remove the moldy and discolored pericarp and shatter the soft, chalky kernels. Thus, the remaining grits will contain only a small percentage of the deteriorated grain.

Processing the grits into food products should result in products with acceptable quality.

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