Use of the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique to analyse the Anthocyanidin Synthase (ANS) gene Locus in Zimbabwean sorghum landraces with different seed proanthocyanidin profiles

Ngonidzashe Mangoma* and Zephaniah Dhlamini

Department of Applied Biology and Biochemistry, National University of Science and Technology, Corner Gwanda Road/ Cecil Avenue, PO Box AC 939, Ascot, Bulawayo, Zimbabwe.

Received 26 March, 2014; Accepted 9 July, 2014

INTRODUCTION

Sorghum bicolor (cultivated sorghum) is a cereal crop of the hot, semi-arid tropics, believed to have originated from Africa (Taylor, 2006; Waniska, 2000). The crop can do well in a wide range of climatic conditions, including the arid and semi-arid tropics that characterise most of Africa. Despite this, sorghum has remained largely a subsistence crop in Africa, grown mainly by resource poor communal farmers for domestic consumption,
and quantities of flavonoids (flavonoid profile) that each
condensed tannins (Dykes and Rooney, 2006). The types
compounds in their grain, while only some contain
(Patel, 2008). All sorghum varieties contain phenolic
anthocyanins, 3-deoxyanthocyanidins and flavanols
related flavonoids, and these include condensed tannins,
nutrition.

- Developing sorghum grain, and in human and animal
resistance to bird depredation and grain mold in
by communal farmers, due to their roles in conferring
flavonoids play important roles in the uptake of the crop
and storability (Nagaraj et al., 2013). Sorghum grain
tolerance, drought tolerance, early maturity, palatability
would prefer varieties that show high pest and disease
affordability, due to their roles in conferring
flavonoids path
t-related factors. Thus, a
balance of flavonoids is required if sorghum is to fully
reach its potential.

There are efforts to try and manipulate the flavonoid
pathway in sorghum in order to identify and develop
sorghum flavonoid pathway mutants that have desirable
and nutritionally superior flavonoid profiles. Sorghum
varieties for different applications would need to have
flavonoid profiles specific to the application, e.g. flour
milling and baking. Different applications would require
the presence of different classes and quantities of
flavonoids in sorghum grain. Such varieties can be
obtained by screening a large number of mutants for
those with desired flavonoid profiles.

It is important to understand how mutations within
certain flavonoid pathway genes would affect the
flavonoid profile in a given sorghum variety. The flavonoid
pathway is a complicated pathway with over 10 known
structural and regulatory genes (Winkel-
Shirley, 2001). Studies on mutations at these different
loci are yet to be carried out in sorghum, even though a
lot of such work has been carried out in other plants (von
Wettstein, 2007; Abrahams et al., 2002; Debeaujon et al.,
2001). Due to the complex nature of the flavonoid
pathway, it is difficult to accurately predict how a mutation
in a given flavonoid pathway gene would affect the
flavonoid pathway in sorghum. However, it is worthwhile
to carry out studies that relate any genetic polymor-
phisms at different flavonoid pathway loci to different
flavonoid profiles, with a view to understanding how
mutations in a given flavonoid pathway gene would affect
flavonoid accumulation.

In this study, we aimed at studying how sequence
variations at the anthocyanidin synthase (ANS) gene
locus relate to observed differences in condensed tannin
profiles in different sorghum varieties. This gene, also
called leucoanthocyanidin dioxygenase (LDOX) catalyses
the conversion of leucoanthocyanidins into antho-
cyanidins and sits at the branch-point between the PA-
specific branch and the anthocyanin-specific branch of
the flavonoid pathway (Abrahams et al., 2002). Our
interest is to find out if any sequence polymorphisms at
this locus may explain observed differences in grain
proanthocyanidin profiles. A number of flavonoid ANS
gene mutants in Arabidopsis, namely tt 11, tt 17 and tt 18
(tds-4) have been identified and characterised in
Arabidopsis and alfalfa, both at the biochemical and
molecular level (Misyura et al., 2012; Bowerman et al.,
2012; Abrahams et al., 2002; Buer and Djordjevic, 2009).
All these mutants were characterised by altered flavonoid
profiles and deposition patterns in the grain.

MATERIALS AND METHODS

Sample selection

A total of 61 sorghum landraces were obtained from the
International Crops Research Institute for the Semi-Arid Tropics
(ICRISAT) Research Station stationed at Bulawayo, Zimbabwe.
Selection was based on availability and grain colour. Dwarf
Wonder, a brown, inbred medium tannin line was the reference
landrace in the study. Condensed tannins were assayed for in all 61
grain samples, while only 11 landraces, including Dwarf Wonder,
were selected for ANS gene sequence studies.

DMACA screening assay for sorghum grain proanthocyanidins

The aldehyde dye, dimethylaminocinnamaldehyde (DMACA) reacts
with proanthocyanidins (PAs) and their immediate precursors,
flavan-3-ols and flavan-3,4-diols, producing a blue coloured
complex. It does not react with anthocyanidins and anthocyanins,
making it suitable for use in tracing condensed tannin biosynthetic
activity in fruits, seeds and tissues (Bogs et al., 2005; Abrahams et

*Corresponding author. E-mail: ngonidzashemangoma@nust.ac.zw.

Author(s) agree that this article remain permanently open access under the terms of the Creative Commons Attribution License 4.0
International License
Quantification of proanthocyanidins in sorghum grain samples

Sorghum grain PAs were quantified in a grain total flavonoid extract. To obtain the extract, exactly 1.0 g of each sorghum grain sample was ground to a fine meal using a mortar and pestle, and then total flavonoids in the meal were extracted in 10 ml of 1% (v/v) HCl in methanol, with shaking. After extraction, the mixtures were centrifuged at 5000 rpm for 30 min and the clear supernatants refrigerated at 4°C until they were analysed. Total condensed tannins in each extract were then quantified by the butanol-HCl assay as described by Hagerman (2000). All samples were analysed in triplicate, and the results expressed as absorbance units at 550 nm per gram of sample. This assay is based on the acid-catalysed hydrolysis of proanthocyanidins in a mineral acid to produce pink/red coloured anthocyanidins that absorb maximally at 550 nm. An iron reagent is normally included in the reagent to enhance colour formation and stability.

Quantification of proanthocyanidins in sorghum grain samples

Sorghum grain PAs were quantified in a grain total flavonoid extract. To obtain the extract, exactly 1.0 g of each sorghum grain sample was ground to a fine meal using a mortar and pestle, and then total flavonoids in the meal were extracted in 10 ml of 1% (v/v) HCl in methanol, with shaking. After extraction, the mixtures were centrifuged at 5000 rpm for 30 min and the clear supernatants refrigerated at 4°C until they were analysed. Total condensed tannins in each extract were then quantified by the butanol-HCl assay as described by Hagerman (2000). All samples were analysed in triplicate, and the results expressed as absorbance units at 550 nm per gram of sample. This assay is based on the acid-catalysed hydrolysis of proanthocyanidins in a mineral acid to produce pink/red coloured anthocyanidins that absorb maximally at 550 nm. An iron reagent is normally included in the reagent to enhance colour formation and stability.

PCR amplification of the sorghum ANS gene in selected landraces

The ANS gene primers used in this study were designed for use in Arabidopsis complementation studies (Liu et al., 2010). The primer sequences are ANS 1 (5’ - CGCGCGATAGTGAATTAGT) and ANS 2 (5’ - ATTTAGTGGATGGACGAAAGC). PCR amplification was performed in 50 µl reactions with the Phire Hot Start II DNA Polymerase (Thermo Scientific Inc., Pretoria, South Africa), using the following program: initial denaturation at 98°C (30 s); 30 cycles consisting of denaturation at 98°C (5 s), annealing at 55°C (5 s), and extension at 72°C (30 s); and then a final extension at 72°C (60 s), in a GENEAMP® PCR System 9700 thermocycler machine (Life Technologies). After cycling, a 10 µl aliquot of each reaction was electrophoresed on a 1% agarose gel for 1 h and PCR products were confirmed by viewing the gel under ultraviolet irradiation.

RFLP analysis of ANS gene amplicons

Restriction digestion analysis was performed directly on PCR amplicons as a preliminary screen for polymorphisms at the ANS gene locus in the selected landraces. The PCR amplicons were digested using the restriction enzymes Hinfl, Mbol, TaqI and AluI (Thermo Scientific Inc, Pretoria, South Africa), following the manufacturer’s instructions. Enzyme selection was based on a virtual digest of the ANS gene sequence using several enzymes.Only those enzymes producing a sufficient number of well-spaced cuts were selected for use (Table 1).

RESULTS

Variation in seed PA content

The sorghum landraces used in this study showed significant variability in their seed proanthocyanidin profiles. Both qualitative and quantitative assays for proanthocyanidins were carried out, using the DMACA stain assay and the butanol-HCl assay, respectively.

Qualitative assay for PAs in sorghum grain samples

The DMACA assay was used to characterise available sorghum landraces on the basis of the presence or absence of PAs in their grain. It is a qualitative assay that detects PAs or their immediate precursors. With the DMACA assay, 89% of the brown lines, 4% of the red lines and none of the white lines tested positive for PAs and/or their precursors (Figure 1). A few samples gave inconclusive results (Figure 2). Being a qualitative assay, the DMACA assay was used in combination with the butanol-HCl, a quantitative assay for PAs in order to get more insight about each sorghum landrace.

Quantitative assay for PAs in sorghum grain samples

The butanol-HCl assay is based on the acid-catalysed depolymerisation of PAs into anthocyanidins. Due to the absence of a tannin standard, butanol-HCl assay results were expressed as absorbance units at 550 nm per gram of sample (AU@550 nm/g of sample). All 27 brown landraces had detectable grain PA levels, except for only

Table 1. Expected ANS gene PCR amplicon restriction profiles.

| Restriction enzyme | Frequency of cuts | Cut positions | Expected sizes of restriction products (bp) |
|--------------------|------------------|--------------|------------------------------------------|
| Hinfl              | 1                | 1218         | 52, 1218                                 |
| Mbol               | 4                | 167, 740, 929, 1231 | 39, 167, 189, 302, 573 |
| TaqI              | 6                | 312, 576, 774, 1059, 1125 | 48, 54, 69, 77, 138, 344, 540 |
| AluI               | 5                | 77, 125, 179, 719, 788, 926 | 66, 145, 198, 264, 285, 312 |

al., 2002). In this study, DMACA was used to screen 61 sorghum landraces on the basis of PA accumulation in the grain samples available. The DMACA assay was performed as described by Abrahams et al. (2002), with minor modifications. About 20 healthy, dry seeds from each landrace were soaked in DMACA solution (2% [w/v] DMACA in 3M HCl/ 50% methanol (v/v)) in a microcentrifuge tube for seven days, followed by washing thrice with 70% ethanol. Washed seeds were dried and then analysed for the development of a blue/blue-black colour, indicating the presence of PAs and/or their precursors.
Figure 1. Number of tannin positive, negative and inconclusive sorghum landraces as determined by the DMACA assay.

Figure 2. Sorghum seed stained with DMACA dye. [+] - DMACA positive; [-] - DMACA negative; [+/−] - inconclusive result.
the three landraces IS 22331, IS 1207 and IS 21683 (Figure 3). The reference landrace, Dwarf Wonder, is a medium tannin line, thus in this study all landraces showing lower tannin levels were designated low tannin, while those accumulating higher quantities were designated high tannin lines. All but three red lines assayed for did not accumulate grain PAs. Of the three that did, IS 13900 accumulated very low levels while IS 14387 and IS 9254 accumulated high quantities (Figure 4). Meanwhile, all white lines tested PA negative by the butanol-
Figure 5. Levels of proanthocyanidins in white sorghum grain samples.

Figure 6. PCR amplification of the sorghum ANS gene. Lane marked M contains a 1 kb DNA ladder, lanes 1 - 10 contain the samples as follows: 1 - IS 22320; 2 - IS 13402; 3 - IS 21487; 4 - IS 22318; 5 - IS 13483; 6 - IS 22294; 7 - IS 1196; 8 - IS 22335; 9 - IS 21456; 10 - IS 21457; D - dwarf wonder.

HCl assay (Figure 5).

PCR amplification of the sorghum ANS gene

Good quality ANS gene PCR amplification products were obtained in all samples (Figure 6). The ANS amplicon obtained was 1270 bp in size, as expected. This includes the whole of the gene’s coding sequence (CDS) as well as 33 and 36 bp of the 5’ and 3’ UTR, respectively. The ANS gene has been cloned in sorghum and the sequence filed under accession number XM_002451291.
(www.ncbi.nih.nlm.gov). It is important to produce amplicons that include the whole of the CDS if any determinations involving how changes in a gene may affect its functionality are to be made. Also, high quality PCR amplicons were recovered and purified using the Zymoclean™ Gel DNA Recovery kit (Zymo-Research, Pretoria, South Africa). Only 2.5 µl of the recovered and purified PCR amplicons were electrophoresed on 1% agarose gel, producing good images (Figure 7).

**DISCUSSION**

Selecting or breeding for flavonoids in sorghum is a fairly unexplored area. Unlike in other plants like *Medicago truncatula* (alfalfa), *Camellia sinensis* (the tea tree) and *Arabidopsis thaliana* where the flavonoid pathway is fully understood, a lot is still unknown in sorghum. This study is part of efforts aimed at the identification and characterisation of flavonoid pathway mutants in sorghum. In this study, 61 sorghum landraces which are part of the sorghum genebank, made up of Zimbabwean and other Southern African sorghum lines were used. The study showed that 44% of these landraces contained quantifiable condensed tannins in their grain. Another study which focused on the use of RAPD markers to determine the genetic relatedness of Zimbabwean sorghum varieties with different seed proanthocyanidin

**ANTS gene locus sequence polymorphism analysis**

The aim of this project was to investigate the relationship between differences in seed PA profiles in local sorghum landraces and sequence variations at a key flavonoid pathway locus, the *anthocyanidin synthase* (ANS) gene. This gene is vital for the biosynthesis of condensed tannins in sorghum. The method used in this study is the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique. This combines the DNA amplifying prowess of the PCR to a specific gene and the specific cleavage of the DNA amplicons by the action of a group of nucleases called restriction endonucleases.

Restriction digestion analysis of DNA is used to detect polymorphisms that cause the disappearance of existing, or emergence of new, restriction enzyme sites. In this study, the following restriction enzymes were used: *HinfI, MboI, TaqI* and *AluI*. The enzymes *MboI, TaqI* and *AluI* are all 4-base cutters, while *HinfI* is a 5-base cutter. Each ANS gene fragment was cut sixteen times by all the enzymes combined. High resolution restriction data was generated for the enzymes used. However, with all these restriction enzymes, no differences in restriction patterns were detected, implying that the restriction maps for the amplicons under study using the available restriction enzymes were the same (Figures 8, 9 and 10).
Figure 8. Sorghum ANS gene PCR product restricted by the enzyme MboI. Lane marked M contain a 1 kb DNA ladder, lanes 1 - 10 contain the samples as follows: 1 - IS 22320; 2 - IS 13402; 3 - IS 21487; 4 - IS 22318; 5 - IS 13483; 6 - IS 22294; 7 - IS 1196; 8 - IS 22335; 9 - IS 21456; 10 - IS 21457; D - dwarf wonder.

Figure 9. Sorghum ANS gene PCR product restricted by the enzyme HinfI. Lane M contains a 1 kb DNA ladder, while: 1 - IS 22320; 2 - IS 13402; 3 - IS 21487; 4 - IS 22318; 5 - IS 13483; 6 - IS 22294; 7 - IS 1196; 8 - IS 22335; 9 - IS 21456; 10 - IS 21457; D - dwarf wonder.
levels concluded that 35% of the landraces used in the study had detectable proanthocyanidins in their grain (Dhlamini and Niang-Sithole, 2014). These results agree with the results obtained in this study, even though different batches of local landraces were used.

The DMACA assay was used not only as a preliminary screen for PAs in the selected sorghum landraces, but also to reveal those sorghum lines that have an altered PA distribution pattern. In other plants, altered seed DMACA staining patterns have been successfully used to identify flavonoid pathway mutants. In studies in Arabidopsis, several transparent testa (tt) and tannin deficient seed (tds) mutant lines were identified through the use of DMACA staining (Abrahams et al., 2002). Further studies on these lines indicated that they were indeed flavonoid pathway mutants at several loci vital for PA biosynthesis and accumulation (Abrahams et al., 2002). Mutations in some flavonoid pathway genes, e.g. ANS and TT12, have been shown to not only affect presence and quantities of certain flavonoids, but also final PA deposition patterns (Abrahams et al., 2002; Debeaujon et al., 2001).

Using the DMACA assay, five landraces showing unusual staining patterns were identified. These are IS 22331 (brown non-tannin), IS 13446 (chalky-white), IS 9254 (red high-tannin), IS 13900 (red low-tannin) and IS 9361 (brown high-tannin). All showed a patchy appearance after DMACA staining, typical of some flavonoid pathway mutants. The butanol-HCl assay was used to further characterise these lines, showing in the process that of those lines showing unusual DMACA results; IS 22331 (pale brown) and IS 13446 (chalky white) did not contain any seed PAs, while the 2 red lines IS 9254 and IS 13900 contained PAs. The black and white DMACA colouration given in IS 13446 is typical with many other chalky white grain samples. The chalky-white appearance of the grain of some sorghum varieties is due to either the presence of starch grains in the mesocarp or the presence of a pigmented testa layer containing insoluble tannins underneath a colourless pericarp (Rooney and Murty, 1982). A sectioning examination has shown IS 13446 to indeed possess a pigmented testa underneath a clear pericarp.

From this study, it can be concluded that most red (92%) and all white (100%) sorghum grain samples tested negative for PAs, while 89% of the brown lines tested positive for grain PAs (Figures 1, 3, 4 and 5). Many white sorghum varieties are mutated at loci higher than the flavonoid pathway at or before points when coloured flavonoids begin to appear in the grain, thus

---

**Figure 10.** Sorghum ANS gene PCR product restricted by the enzyme TaqI. Lane marked M contain a 1 kb DNA ladder, lanes 1 - 10 contain the samples as follows: 1 - IS 22320; 2 - IS 13402; 3 - IS 21487; 4 - IS 22318; 5 - IS 13483; 6 - IS 22294; 7 - IS 11196; 8 - IS 22335; 9 - IS 21456; 10 - IS 21457; D - dwarf wonder.
explaining why they stain negative for tannins and do not accumulate any assayable tannins. In tannin sorghum, tannins accumulate either in the pigmented testa only (type II) or in both the pigmented testa and pericarp (type III), meaning the presence of a pigmented testa is vital for tannin accumulation (Earp et al., 2004).

The DMACA assay was used in combination with the butanol-HCl assay. This is because use of the DMACA assay alone in the biochemical characterisation of tannin-producing mutants in sorghum is insufficient. Firstly, DMACA not only reacts with condensed tannins, but also with their immediate precursors, that is, flavan-3-ols and flavan-3,4-diols (Abrahams et al., 2002). This means any mutant expressing any one of the precursors will stain positive, giving a false positive result for the presence of tannins when in actual fact the mutant will be accumulating only the precursors. Secondly, studies have shown that those mutants that accumulate minute quantities of the soluble PA precursors tend to leach those precursors into the DMACA staining solution before the reaction is complete, leading to a false negative result (Koupai-Abyazani et al., 1993). As a consequence, the butanol-HCl assay was used to confirm the results of the DMACA assay.

Even though the flavonoid pathway has several structural and regulatory genes, any mutation on any one of these many loci has the potential effect of altering the flavonoid profile of the mutant line carrying the mutation. It is important to identify such mutations and establish their exact effects on the flavonoid pathway and on flavonoid profiles. Such studies have been carried out in other plants like arabidopsis, alfalfa and barley (Misyura et al., 2012; Abrahams et al., 2002; Bowerman et al., 2012; Buer and Djordjevic, 2009; von Wettstein 2007). However, similar studies are yet to be carried out in sorghum, hence this study is aimed at attempting to establish a link between sequence polymorphisms at the ANS gene locus and differences in proanthocyanidin profiles in sorghum.

Sequence polymorphisms at the ANS locus were determined using a combination of PCR and restriction digestion analysis (PCR-RFLP). Each ANS gene fragment was cut sixteen times by all the enzymes combined, translating to a DNA fragment of 65 bp in length. This covers the actual position of the cut and the other bases that make up the restriction site. Any mutation within this 65 bp region of the amplicon under study would have been detected by this method. However, with all these restriction enzymes no differences in restriction patterns were detected (Figures 8, 9 and 10).

This potentially means either of two things, firstly that there are no polymorphisms at this locus in the landraces under study, and as a result any observed differences in PA profiles may be due to mutations at other loci or due to environmental factors, or secondly that the RFLP technique probably missed some potential SNPs due to its inherent shortcomings.

The RFLP technique has one major handicap when used in the detection of sequence variations among similar sequences. The method targets only those mutations that occur within restriction sites ad ignores the rest of the gene. In this case, this method only focused on a 65-bp region in a PCR amplicon, 1270 bp in size. This translates to only 5% of the gene. Inasmuch as the method may be a very useful and cheap rapid screening tool for DNA mutations, it suffers from low coverage. The method can however be made more effective if more restriction endonucleases are used. This way the portion of the gene directly screened for any mutations significantly increased.

Conflict of Interests

The author(s) have not declared any conflict of interests.

ACKNOWLEDGEMENTS

This work was made possible through funding provided by the National University of Science and Technology Research Board. Acknowledgements also go to ICRISAT-Bulawayo, Zimbabwe, who provided the germplasm and other forms of technical support.

REFERENCES

Abrahams S, Tanner GJ, Larkin PJ, Anthony AR (2002). Identification and Biochemical Characterisation of Mutants in the Proanthocyanidin Pathway in Arabidopsis. J. Plant Physiol. 159:561-576.

Bogs J, Downey MO, Harvey JS, Ashton AR, Tanner GJ, Robinson SP (2005). Proanthocyanidin Synthesis and Expression of Genes Encoding Leucoanthocyanidin Reductase and Anthocyanidin Reductase in Developing Grape Berries and Grapevine Leaves. Plant Physiol. 139:652-663.

Bowerman PA, Ramirez MV, Price MB, Helm RF, Winkel BS (2012). Analysis of T-DNA alleles of flavonoid biosynthesis genes in Arabidopsis ecotype Columbia. BMC Research Notes 5:485-493.

Buer CS, Djordjevic MA (2009). Architectural phenotypes in the transparent testa mutants of Arabidopsis thaliana. J. Exp. Bot. 60(3):751-763.

Debeaujon I, Peeters AJM, Léon-Kloosterziel KM, Koornneef M (2001). The TRANSPARENT TESTA12 Gene of Arabidopsis Encodes a Multidrug Secondary Transporter-like Protein Required for Flavonoid Sequestration in Vacuoles of the Seed Coat Endothelium. Plant Cell 13:853-871.

Dhlamini Z, Sithole-Niang, I (2014). Random amplified polymorphic DNA (RAPD) based assessment of genetic relationships among some Zimbabwean sorghum landraces with different seed proanthocyanidin levels. Afr. J. Biotechnol. 13(19):1943-1951.

Dykes L, Rooney, LW (2006). Sorghum and Millet phenols and antioxidants. J. Cereal Sci 44:236-251.

Earp CF, McDonough CM, Rooney LW (2004). Microscopy of pericarp development in the caryopsis of Sorghum bicolor (L) Moench. J. Cereal Sci. 39:21-27.

Hagerman AE (2002). The Tannin Handbook. Ohio University Press.
Koupai-Abyazani MR, McCallum J, Muir AD, Lees GL, Bohm BA, Towers GHN, Gruber MY (1993). Purification and characterization of a proanthocyanidin polymer from seed of alfalfa (Medicago sativa cv. Beaver). J. Agric. Food Chem 41:565-569.

Kumar AA, Reddy BVS, Sharma HC, Hash CT, Rao PS, Ramaiah B, Reddy PS (2011). Recent advances in sorghum genetic enhancement research at ICRISAT. J Plant Sci. 2:589-600.

Liu H, Yegang D, Hung C, Chun HS, Yu WW, Mingfu W, Chu IK, Yuezhi T, Lo C (2010). Molecular Dissection of the Pathogen-Inducible 3-Deoxyanthocyanidin Biosynthesis Pathway in Sorghum. Plant Cell Physiol. 51(7):1173-1185.

Misyura M, Colasanti J, Rothstein SJ (2012). Physiological and genetic analyses of Arabidopsis thaliana anthocyanin biosynthesis mutants under chronic adverse environmental conditions. J. Exp. Bot. pp.1-12.

Nagaraj N, Sachin M, Surajit H, Ban MCS, Chandrakanth MG (2013). Baseline Scenario of Post rainy Season Sorghum Economy in Marathwada Region of Maharashtra. Working Paper Series No. 38. Patancheru 502 324, Andhra Pradesh, India: Int. Crops Res. Institute Semi-Arid Tropics. 28pp.

Patel JM (2008). A Review of Potential Health Benefits of Flavonoids. Lethbridge Undergraduate Research Journal.

Rooney LW, Murty DS (1982). Evaluation of Sorghum food quality. Pages 571-588 in: Sorghum in the Eighties: Proceedings of an International Symbosium on Sorghum. L.R. House, L.K. Mughogho, J.M. Peacock, and J.V. Mertin, eds. Int. Crops Res. Inst. Semi-Arid Tropics (ICRISAT), Patancheru, India.

Taylor JRN (2006). Overview: Importance of sorghum in Africa. Depart of Food Science. University of Pretoria. Pretoria. South Africa. pp:1-21.

von Wettstein D (2007). From analysis of mutants to genetic engineering. Annu. Rev. Plant Biol. 58:1-19.

Wanioka RD (2000). Structure, phenolic compounds, and antifungal proteins of sorghum caryopses. Pages 72-106 in Technical and Institutional Options for Sorghum Grain Mold Management: Proceedings of an International Consultation, 18-19 May 2000, ICRISAT, Patancheru, India.

Winkel-Shirley B (2001). Flavonoid Biosynthesis: A colourful model for genetics, biochemistry, cell biology and plant biotechnology. Plant Physiol. 1126:485-493.

www.phytozome.net
www.ebi.ac.uk