Available online at www.sciencedirect.com
SCIENCE DIRECT
Plant Science 169 (2005) 542–552
PLANT SCIENCE
www.elsevier.com/locate/plantsci

# Characterization of a deletion allele of a sorghum Myb gene *yellow seed1* showing loss of 3-deoxyflavonoids

Jayanand Boddu [a,1], Catherine Svabek [a], Farag Ibraheem [a], A. Daniel Jones [b], Surinder Chopra [a,*]

[a] *Department of Crop & Soil Sciences, 252 Agricultural Sciences & Industries Building, The Pennsylvania State University, University Park, PA 16802, USA*
[b] *Department of Chemistry, Pennsylvania State University, University Park, PA 16802, USA*

Received 24 April 2005
Available online 31 May 2005

**Abstract**

In sorghum, 3-deoxyflavonoid pigments or phlobaphenes observed in the pericarp of mature seed are derived from flavan-4-ols through the flavonoid biosynthetic pathway. We show here that phlobaphenes accumulation in pericarp, glumes and leaves is genetically linked with the functional *yellow seed1* (*y1*) gene. Molecular and genetic analysis was performed on a loss of function allele of *y1* present in the line BTx623. This sorghum line does not accumulate any detectable levels of flavan-4-ols or visible phlobaphenes in pericarp, glumes and leaves. Molecular structure of the *y1*[BTx623] showed a deletion of 3218 bp which removes 5′ non-coding, putative promoter, exon1, intron1, exon2, and part of the intron2 sequences. The null *y1* allele designated as *y1-ww* (**w**hite pericarp, **w**hite glume) is not transcribed and this results in a loss of Y1-regulated expression of structural genes needed for the biosynthesis of flavan-4-ols. Further LC–MS analysis of seed extracts of a functional *y1* allele detected the presence of positively charged compounds known as 3-deoxyanthocyanidins. Compounds identified were apigeninidin, luteolinidin, and a methoxylated derivative of apigeninidin. These compounds were not detected in BTx623 seed extracts. Previous studies have shown that 3-deoxyanthocyanidins are induced in sorghum leaves challenged with *Colletotrichum sublineolum*, a fungus that causes anthracnose in sorghum. Our results now provide an evidence for a common flavonoid pathway that may lead to the biosynthesis of flavan-4-ols and 3-deoxyanthocyanidins in sorghum.


*Keywords:* Anthracnose; Flavan-4-ols; MYB; Phytoalexins; *Sorghum*; 3-Deoxyanthocyanidins

## 1. Introduction

Flavonoid biosynthesis in plants is an extensively characterized branch of the phenylpropanoid pathway and a number of genes encoding enzymatic and regulatory functions have been identified [1,2]. Biological importance of flavonoids has been implicated in plant growth and developmental processes including pollen tube germination [3,4], resistance to insect feeding [5], formation of pollinator attractant pigments and UV radiation filters [6,7]. The importance of de novo accumulation of flavonoids has been shown in plant defense responses against pathogens [8,9]. In sorghum, flavonoids belonging to the 3-deoxyflavonoid category have been shown to be the major determinants of grain mold resistance [10]. These compounds arise through a series of enzymatic steps beginning with the conversion of phenylalanine into naringenin, which then enters the flavonoid biosynthetic pathway (Fig. 1). The fate of naringenin depends upon the genetics of the plant, which determines the formation of 3-deoxyflavonoids (phlobaphenes) or 3-hydroxyflavonoid (anthocyanins) pigments. In maize, duplicated loci have been identified as regulators of anthocyanin biosynthesis in kernel aleurone and vegetative plant parts [11,12]. The alternate branch leads to the biosynthesis of two flavan-4-ols, apiferol and luteoferol, that

* Corresponding author. Tel.: +1 81 48651159; fax: +1 81 48637043.
*E-mail address:* sic3@psu.edu (S. Chopra).
[1] Present address: Department of Agronomy and Plant Genetics, University of Minnesota, St Paul, MN 55108, USA.

0168-9452/$ – see front matter © 2005 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.plantsci.2005.05.007

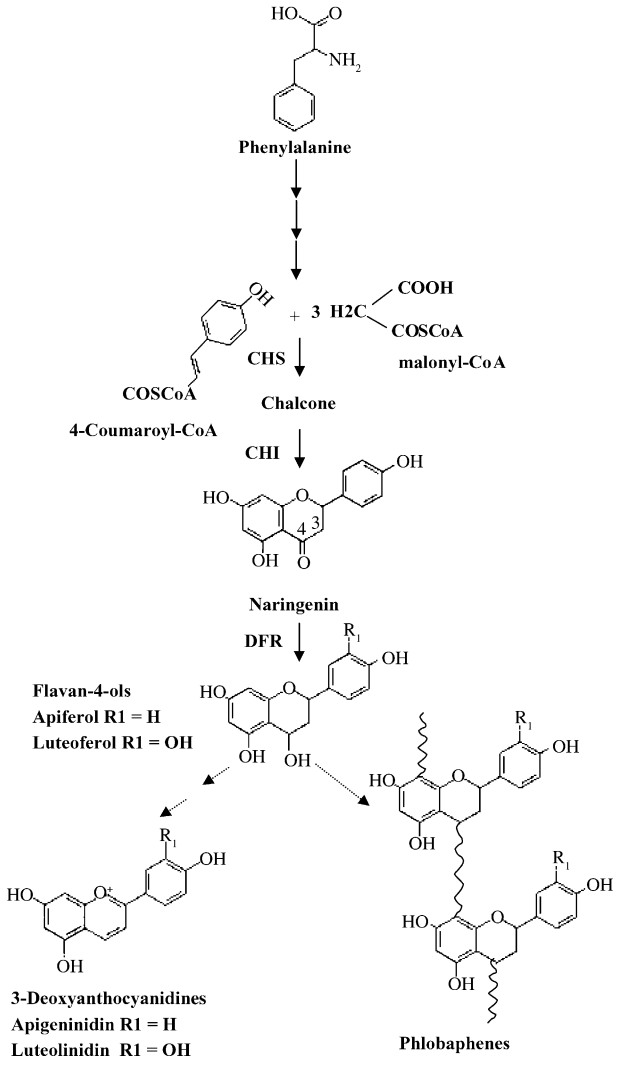


Fig. 1. The sorghum biosynthetic pathway leading to flavan-4-ols, phlobaphenes and 3-deoxyanthocyanidin phytoalexins. Enzymes shown are: CHS, chalcone synthase; CHI, chalcone isomerase; DFR, dihydroflavonol reductase. Pathway modeled after Styles and Ceska [13], Kambal and Bate-Smith [20] and Lo and Nicholson [28].

are precursors of the red phlobaphenes (Fig. 1) [13]. The structure of these pigments is not well defined, but they are believed to arise by non-enzymatic polymerization of flavan-4-ols [13,14]. In maize, these pigments are most obvious in the floral tissues including the kernel pericarp (the outermost layer of the kernel derived from the ovary wall) and glumes (floral bracts that subtend the seed) [13]. The *p1* (*pericarp color1*) gene encodes a protein with a Myb-homologous DNA binding domain and regulates phlobaphene biosynthesis in maize [15]. The P1 protein functions as a regulator of transcription of at least three maize genes required for flavonoid biosynthesis: *c2*, *chi1*, and *a1*, which encode chalcone synthase, chalcone isomerase, and dihydroflavonol reductase, respectively [15–17]. Although anthocyanins and phlobaphenes biosynthesis originate from a common precursor (naringenin) and share a subset of biosynthetic genes, their regulators have no epistatic interaction in maize [13,15].

We are interested in the genetics of biosynthetic route of sorghum flavan-4-ols and phlobaphenes. Previous studies indicated that the sorghum *y1* (*yellow seed1*) locus may determine the seed pericarp pigmentation [18–20]. A genetic evidence for the involvement of *y1* locus came from the analysis of a mutable *Y1-cs* (*Y1-candystripe*) allele [21,22]. The *Y1-cs* allele is associated with variegated pigmentation of grain pericarp and leaf tissues. The leaf expression becomes obvious at the time of maturity or by bleaching young leaves [21–23]. We have previously shown that the *Y1-cs* allele contains the *Candystripe1* transposon which disrupts the function of *y1* gene [22]. However, the *Y1-cs* allele can spontaneously revert to a functional state leading to the appearance of frequent red sectors (somatic reversions) and full red seeded heads (germinal reversions). Germinal reversions lead to heritable and functional alleles designated as *Y1-rr* (red pericarp, red glume). Our recent molecular characterization of the *y1* gene showed that it is homologous to the maize *p1* gene (J. Boddu, S. Chopra, unpublished). To define the precise role of *y1* gene, we have now compared and characterized an *Y1-rr*, a mutable (*Y1-cs*) and a loss of function allele of *y1* for their ability to accumulate flavan-4-ols and phlobaphenes. In addition to the study of regulation of gene expression required for flavan-4-ols, our goal is to understand the biosynthetic route of 3-deoxyanthocyaninidin phytoalexins in sorghum. Compounds belonging to the 3-deoxyanthocyanidin class have structural similarities with flavan-4-ols and these include luteolinidin, apigeninidin and their derivatives [24–26]. These compounds have been shown to accumulate as a site-specific response to the ingress of anthracnose fungus (*Colletotrichum sublineolum*) in sorghum leaves [26]. The role and importance of sorghum 3-deoxyanthocyanidins in plant disease resistance has been well established but their genetics and regulation of biosynthesis is not very clear [27]. Our results here show that sorghum genotypes carrying functional and non-functional alleles of the *y1* gene differ in their ability to synthesize flavan-4-ols and phlobaphenes. Interestingly, we found that 3-deoxyanthocyanidins are also present in seeds of functional *y1* line, suggesting a common role of Y1 transcription factor in the regulation of flavan-4-ols, phlobaphenes and 3-deoxyanthocyanidins.

## 2. Materials and methods

### 2.1. Sorghum genetic stocks

We have used here gene and allele nomenclature as used in maize genetics. All genetic stocks and crosses were grown at the Agronomy Farm, The Pennsylvania State University. The RR-30 stock carries a functional *Y1-rr* allele of the *y1* locus. This stock originated from the spontaneous excision of the *Cs1* (*Candystripe1*) transposable element from a line carrying the *Y1*-cs allele (stock name CS-30). The CS-30 line is a spontaneous derivative of CS8110419, and it has a