Proc. Indian Acad. Sci. (Plant Sci.), Vol. 99, No. 4, August 1989, pp. 301–306.

Seed coat and hypocotyl pigments in greengram and blackgram

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MS received 10 September 1988

Abstract. Three anthocyanins were found in *Vigna radiata* (greengram) accessions with black seed coat, but were absent in cultivars having green seed coat. *Vigna mungo* (blackgram) had only one of them. The same 3 anthocyanins were found in cultivars with purple red hypocotyl of *Vigna radiata* accessions but were absent in the ones with green hypocotyls. *Vigna mungo* with purple red hypocotyls showed the presence of two anthocyanins. One common to both was identified as delphinidin-3-glucoside and the other is probably cyanidin-3-glucoside. Among the two accessions of *Vigna radiata* var *sublobata* examined, one was similar to *Vigna radiata* accessions with black seed coat having delphinidin-3-glucoside and two other anthocyanins, while the other resembled *Vigna mungo* having only delphinidin-3-glucoside in seed coat and both delphinidin and cyanidin-3-glucosides in hypocotyl. Chlorophyll content of the seed coats was in the following decreasing order: green, black, brown and yellow. Browning of the seed coat of cv TAP-7 after storage over a year was associated with reduction in chlorophyll content.

Keywords. Anthocyanins; blackgram; greengram; *Vigna mungo*; *Vigna radiata*.

1. Introduction

Greengram (*Vigna radiata* (L) Wilczek) and blackgram (*Vigna mungo* (L) Hepper) show diversity of seed coat colour ranging from yellow, green, brown to black. Inheritance of seed coat colour in both species has been reported (Singh 1982; Dwivedi and Singh 1986a, b). However very little information is available concerning the biochemical basis of colour variation in these two crops. Ishikura *et al* (1981) observed the presence of anthocyanins in seed coat and hypocotyl of blackgram and wild accessions of *V. radiata* var *sublobata*. Anthocyanins were also found in greengram cultivars with purple red hypocotyl. Anthocyanins, chlorophylls and carotenoids are the major pigments that impart various shades of colour to plant parts. The intensity of pigmentation increases with the increase in concentration of anthocyanins and chlorophyll (Swain 1965). Seed coat colour and hypocotyl pigmentation are useful markers in genetic studies and cultivaral identification. The present study was undertaken to identify the variations in anthocyanins in seed coat and hypocotyl and in chlorophyll content of seed coats of greengram and blackgram.

2. Materials and methods

Greengram, blackgram and their wild relatives used in this study are listed in table 1. For extraction of anthocyanins, seed coats were separated from the seeds soaked overnight in water and 1–2 g fresh weight was then extracted in 1% HCl in methanol. The extract was filtered and concentrated under vacuum, washed with 25 ml petroleum ether to remove lipids and polyphenols. This partially purified

Table 1. Anthocyanins in greengram, blackgram and their wild relatives.

	Seed coat				Hypocotyl				
		Anthocyanins				Anthocyanins			
Accessions	Colour	A-I	A-II	A-III	Colour	A-I	A-II	A-III	A-IV
V. radiata (greengram)									
RUM-5	Black	+	+	+	Purple-red	+	+	+	−
M-872	Black	+	+	+	Purple-red	+	+	+	−
GP-67	Black	+	+	+	Purple-red	+	+	+	−
ML-5	Green	−	−	−	Purple-red	+	+	+	−
TAM-8	Green	−	−	−	Purple-red	+	+	+	−
TAP-7	Green	−	−	−	Purple-red	+	+	+	−
VM-33	Brown	−	−	−	Light green	−	−	−	−
PS 2/11	Yellow	−	−	−	Light green	−	−	−	−
V. mungo (blackgram)									
T-9	Black	+	−	−	Purple-red	+	−	−	+
V. radiata var *sublobata* accession from									
Trombay	Dark brown	+	−	−	Purple-red	+	−	−	+
Nagpur	Dark brown	+	+	+	Light green	−	−	−	−

+ and − indicate presence and absence respectively.

concentrate was used for paper chromatography. The anthocyanins from one week old hypocotyls were also extracted, concentrated and purified similarly.

Anthocyanins were separated by ascending paper chromatography for 4 h on Whatman No. 1 filter paper. The solvent used was n-butanol-acetic acid-water (BAW, 3:1:1). The spots on the chromatogram were eluted with 1% methanolic HCl; pooled, concentrated under vacuum and used for identification following the procedures of Harborne (1967) and Jackman *et al* (1987). Anthocyanins of hypocotyls were co-chromatographed with seed coat anthocyanins in two solvents BAW (3:1:1) and amylalcohol-acetic acid-water (AmOH-A-W, 2:1:1).

The mobility of the individual anthocyanins was determined by descending chromatography in 4 solvents, BAW (n-butanol-acetic acid-water, 4:1:5 top layer); BuHCl (n-butanol-2N HCl, 1:1); 1% HCl (water-conc. HCl, 97:3) and AAH (acetic acid-conc. HCl-water, 15:3:82).

The absorption maxima of each anthocyanin dissolved in 0·01 methanolic HCl were determined with a Shimadzu UV-visible recording spectrophotometer UV–240 at 200–600 nm. Based on the chromatographic and spectral values, anthocyanin A-I was tested for the sugar group and the aglycone by acid hydrolysis following the procedure of Ishikura and Shibata (1973).

Seed coats (500 mg fresh weight) separated from the soaked seeds as described previously, were dried between tissue paper folds and chlorophyll was extracted in 30 ml of 80% acetone. Chlorophyll content was estimated by the method of Arnon (1949). Anthocyanins were found to interfere in chlorophyll estimation from black seed coats, hence chlorophylls from the acetone extracts were solublised in diethyl ether for spectrophotometric measurements and chlorophyll content was calculated using the equations of Comar and Zscheile (1942).

3. Results

3.1 *Anthocyanins in seed coat*

Three anthocyanins designated as A-I, A-II and A-III were found in *V. radiata* accessions with black seed coats (table 1 and figure 1). These were also present in the *sublobata* accession from Nagpur with dark brown seed coat. Anthocyanins were absent in *V. radiata* accessions with yellow, green and brown seed coats. *V. mungo* cv T–9 with black seed coat and *sublobata* accession from Trombay with dark brown seed coat showed the presence of A-I only.

3.2 *Anthocyanins in hypocotyl*

All *V. radiata* accessions with purple red hypocotyls showed the presence of A-I, A-II and A-III (table 1). The R_f values of these when co-chromatographed with the seed coat anthocyanins in two different solvents, BAW (3 : 1 : 1) and AmOH-A-W (2 : 1 : 1) were similar. Anthocyanins were absent in the accessions with green hypocotyls. *V. mungo* and *V. radiata* var *sublobata* accession from Trombay also had purple red hypocotyls but showed only the presence of A-I and another spot designated A-IV.

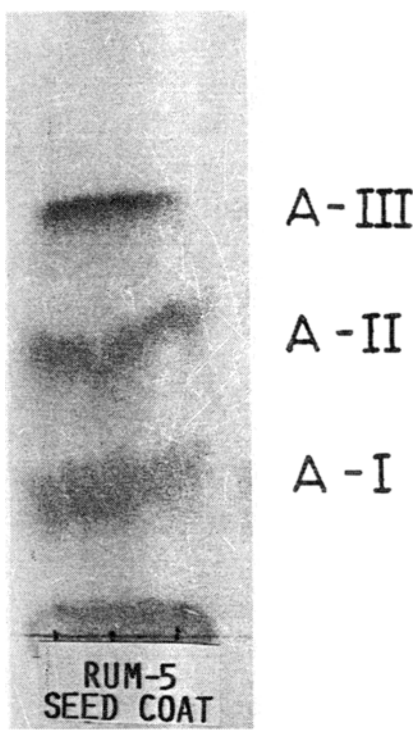

Figure 1. Paper chromatogram showing 3 bands, A-I, A-II and A-III of anthocyanins separated from black seed coat of greengram cv RUM 5.

3.3 *Identification of anthocyanins*

R_f values of A-I of the seed coats and hypocotyls of all the *Vigna* accessions in the 4 solvents—BAW, BuHCl, 1% HCl and AAH were 0·25, 0·11, 0·04 and 0·17 respectively, which were in conformity with the values for delphinidin-3-glucoside as reported by Harborne (1967). The spectral values for A-I and its aglycone in the visible region were 540 and 546 nm respectively. The visible maximum of the aglycone at 546 confirms the identity of delphinidin (Shrikhande 1976). The values for delphinidin-3-glucoside in visible spectra have been reported to be 534 or more (Harborne 1967, Ishikura *et al* 1981). The test for sugar liberated on hydrolysis of A-I confirmed that it was identical to glucose. Thus, A-I was identified as delphinidin-3-glucoside. A-II, A-III and A-IV are yet to be identified.

3.4 *Chlorophylls in seed coats*

Chlorophyll content of 4 varieties of *V. radiata* with green seed coat ranged between 307–364 μg per g fresh weight (table 2). The cultivars with black, brown and yellow seed coats showed lesser amount of chlorophyll than the green cultivars, except cv GP–67 with black seed coat whose chlorophyll content was numerically equal to that of cultivars with green seed coats. TAP–7 seeds stored for over a year, which had turned brown showed 50% reduction in total chlorophyll; the reduction being greater in chlorophyll *a* than in chlorophyll *b*.

4. Discussion

From the seed coats and hypocotyls of the *Vigna* species, 4 anthocyanins A-I, A-II,

Table 2. Chlorophyll content of seed coats of greengram and blackgram.

Accessions	Seed coat colour	Chlorophyll content (μg/g fresh weight) Total	a	b	Ratio a/b
V. radiata					
ML-5	Green	334	212	122	1·73
K-851	Green	364	234	130	1·80
TAM-8	Green	307	199	108	1·84
TAP-7	Green	348	221	127	1·66
TAP-7*	Brown	169	87	82	1·06
GP-67	Black	341	217	124	1·75
RUM-5	Black	294	171	123	1·39
M-872	Black	271	173	98	1·76
VM-33	Brown	255	150	105	1·42
PS 2/11	Yellow	88	50	38	1·31
V. mungo					
T-9	Black	233	141	92	1·53
C D at	1%	43	26	20	
	5%	32	19	15	

*After 1 year storage.

A-III and A-IV could be separated. Ishikura *et al* (1981) had reported the presence of two anthocyanins delphinidin-3-glucoside and cyanidin-3-glucoside in the purple red hypocotyls of *V. mungo* which correspond respectively to A-I and A-IV in the present study. Thus A-I could be identified as delphinidin-3-glucoside. Based on similar R_f, A-IV was inferred to be cyanidin-3-glucoside. However this needs to be confirmed by spectral and hydrolysis studies. The other two anthocyanins A-II and A-III need to be chemically identified. The presence of anthocyanins in the seed coats and hypocotyls was independent of each other. Such expression of anthocyanins in different plant tissues is known (Harborne 1967).

Genetic differences and phylogenetic relationships of greengram, blackgram, and their wild relative *V. sublobata* have been examined previously using different approaches (Jain and Mehra 1980; Miyazaki *et al* 1984; Shanmugam and Sree Rangasamy 1984; Smartt 1985). Though the cultivated species, greengram and blackgram are morphologically similar, their gene pools are not identical (Smartt 1985) and they do not readily produce stable hybrids. The present study shows that additional two anthocyanins are present in *V. radiata* accessions with black seed coats which are not found in blackgram.

V. sublobata is widely accepted as the ancestral type both for greengram and blackgram. On the basis of morphological diversity observed in *V. sublobata* accessions from India Arora *et al* (1973) identified two forms, one which easily crossed with *V. radiata* and the other with *V. mungo* (Smartt 1985). Lukoki *et al* (1980) reclassified the wild forms as *V. radiata* var *sublobata* and *V. mungo* var *silvestris*. Among the two accessions of *V. sublobata* examined for the seed coat anthocyanins, one resembled *V. radiata* and the other *V. mungo*. The anthocyanin similarities were in agreement with those observed in seed protein polypeptides (Thakare *et al* 1987). These data support the idea (Lukoki 1980; Smartt 1985) that *V. mungo* and *V. radiata* have evolved from two distinct forms of *V. sublobata*.

Acknowledgement

The authors are thankful to Dr A Banerji for helpful suggestions.

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