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Short Communication

CHARACTERISATION OF ANTHOCYANINS FROM RADIATION INDUCED BLACK AND GREY SEED COAT MUTANTS OF COWPEA

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The presence of natural compounds like chlorophyll, carotenoids, flavonoids and polyphenols determines the colour of plant parts including seed coat. A correlation is often found between the content of these compounds and the seed coat colour. Ishikura *et al.* [1] reported the presence of an anthocyanin, delphinidin-3-glucoside in the black seed coat of urdbean. Pandey *et al.* [2] found three anthocyanin compounds in the black seed coat of mungbean cv. RUM-5. Anthocyanins have also been extracted and separated from the seed coat of black soybean[3]. The germplasm collection of cowpea (*Vigna unguiculata* (L.) Walp) exhibits variations in seed coat colour ranging from pure white, yellow, green, brown, grey, stippled black to dark black. Variations in seed coat colour have also been brought about by induced mutations. Sharma[4] obtained stippled black, brown and other seed coat colour mutants from the white seeded cultivar Pusa Phalguni following mutagenic treatments owing to mutator system. The cowpea varieties V-16 and V-240, both with brown seed coat colour, are the mutants selected from the stable lines following treatment of white seeded Pusa Phalguni with 0.8% DMS [5, 6]. Pandey and Pawar[7] obtained several seed coat colour mutants including stippled black, grey, brown, yellow and light green in addition to partially coloured testa following irradiation of a white seeded cultivar V-130 of cowpea. The stippled black and grey mutants showed instability in seed coat colour in subsequent generations. The present investigation was undertaken to find out the presence of anthocyanins in the coloured testa of the mutants, especially in the stippled black and grey seed coat. A local cowpea genotype, Ratnagiri local (RL), characterised with jet-black seed coat was also included in the investigation.

The cowpea mutants and genotypes used in this study are listed in Table 1. For the extraction of anthocyanins, seed coats were separated from the seeds soaked overnight in water and 1-2 g fresh weight was then ground in a mortar and pestle with 15 ml of 1% HC1 in methanol. The extract was filtered and washed with 25

ml petroleum either to remove lipids and polyphenols. This partially purified extract was concentrated by evaporating overnight at room temperature and was used for paper chromatography. The compounds were separated on Whatman No. 1 filter paper by ascending paper chromatography for 4 h using *n*-butanol-acetic acid-water (BAW 3:1:1) as the solvent. For better separation of the bands, the chromatogram was run twice in the same solvent after drying for half an hour after the first run. The mobility (R_f value) of the individual anthocyanin was noted down after each run. The bands on the chromatogram were eluted with 0.1% methanolic Hcl and used for determining their absorption maxima with a UVKON 940 Spectrophotometer at 200-600 nm.

Table 1. Characteristics of anthocyanin bands separated by paper chromatography

Genotype/Mutant	Seed coat colour	Anthocyanin bands and their visible colour on chromatogram		
		Band	wet	Dry
Ratnagiri Local	Dark black	Ist	pink	blue
		IInd	red	purple
		IIIrd	red	purple red
		IV	red	purple
V-130 SCM-1	Stippled-black	Ist	pink	blue
		IInd	red	purple red
V-130 SCM-2	Grey	Ist	pink	blue
		IInd	red	purple red
V-130 SCM-3	Turkey-grey	Ist	pink	blue
		IInd	red	purple red

Two coloured bands were observed on the chromatogram after the first run in all the three mutants viz. stippled black (SCM-1), grey (SCM-2) and turkey grey (SCM-3). In case of the seed coat of RL, only one band was distinct and the other appeared as a long smear. After the second run, while no additional bands were observed in the mutants, four distinct bands were observed in the RL (Fig. 1). The characteristics of the anthocyanin bands observed on the chromatogram are given in Table 1. The three mutant samples showed only two bands, as observed in first run, even after the second run, albeit with changed R_f values. The R_f values and spectral characteristics of individual bands are given in Table 2. The absorption maxima of the eluted compounds from different bands were observed to be in the

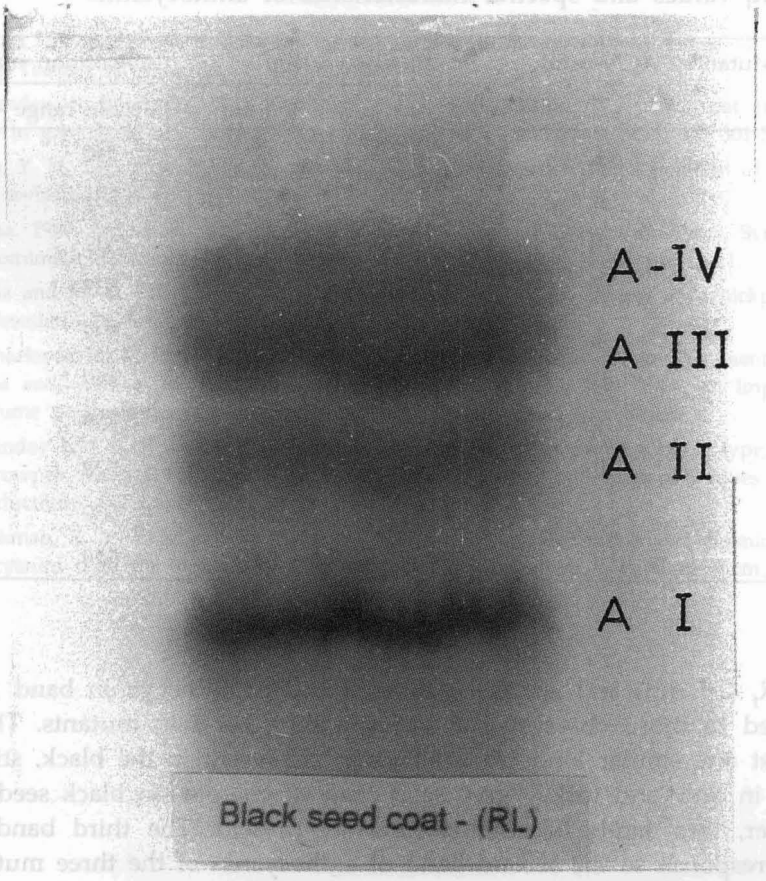

Fig. 1

range of 528-540 nm and 274-286 nm in visible and UV spectra respectively. Both anthocyanins and their aglycones are known to show two characteristic absorption maxima when in acid solution; -a strong one in the visible region between 465 and 550 nm and a smaller one in the ultra violet at about 275 nm [8]. The observed spectral values thus confirmed that the separated compounds were different kinds of anthocyanins. The R_f values (1st run) and maximum absorption in visible and UV range of the two anthocyanins extracted and separated from the mutants (SPM1, SPM2, SPM3) were found to be almost the same indicating the same kinds of anthocyanins present in them. The only difference was found in respect of the UV absorption of the second anthocyanin of the sample SCM-2.

Table 2. R_f values and spectral characteristics of anthocyanins

Genotype/Mutant	ACN-band	R_f value × 100		Absorption maxima (nm)	
		1st run	2nd run	Visible range	UV range
RL	I	23.0	47	540	278
	II	-	63	535	281
	III	-	75	532	280
	IV	-	77	535	286
SCM-1	I	24.0	38	539	280
	II	39.0	59	530	281
SCM-2	I	24.0	38	539	281
	II	39.0	59	528	274
SCM-3	I	23.5	38	539	281
	II	39.0	59	529	281

The R_f (1^{st} run) and spectral values of the 1^{st} anthocyanin band (A-1) of the RL, matched to those observed for corresponding band in mutants. This indicates that at least one similar kind of anthocyanin is present in the black, stippled black as well as in grey and turkey grey seed coats of cowpea. In black seed coat of the RL however, four kinds of anthocyanins are present. The third band of the RL sample corresponds to the second band of anthocyanin of the three mutant samples in respect of spectral characteristics. It is therefore inferred that RL black seed coat contains four kinds of anthocyanins two of which are similar to those present in stippled black, grey and turkey grey seed coats. Further, in the RL seed coat, the second and the fourth bands show the same absorption maxima of 535 nm in visible range indicating close resemblance in their structure.

While the identification of individual anthocyanins needs further tests, the first band present in all the samples could be inferred as delphinidin-3-glucoside on the basis of its R_f and spectral values. Pandey *et al.* [2] in their studies on seed coat and hypocotyl pigments in greengram and blackgram had observed the R_f value of the 1^{st} band (A-1) in BAW to be 0.25 and its spectral value in visible range to be 540 nm and identified it as delphinidin-3-glucoside. These values correspond to those of the 1st band of anthocyanins in the present investigation. The present finding indicates that delphindin-3-glucuside is ubiquitous as a pigment in *Vigna* species.

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