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BIOCHEMICAL PROFILING OF GEOGRAPHICALLY DIVERSE COWPEA (*Vigna unguiculata* L.) GENOTYPES FOR NUTRITIONAL QUALITY AND PHOTOCHEMICAL AND ANTIOXIDANT POTENTIAL

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ABSTRACT

The seed coat color, in case of legumes, plays a major role for the presence of biological active components displaying free radical scavenging activities. The extensive variability of cowpea genotypes creates the need to characterize them with regard to their nutritional value as a tool in biofortification programs. The purpose of this study is to evaluate cowpea genotypes to identify superior genotypes with high nutritional quality and superior antioxidant potential. The average content of crude protein, crude fibre, ash and ether extract was found to be 23.8%, 6.9%, 3.4% and 1.4 % and sugar and starch contents were 60.4 mg/g and 313.9 mg/g, respectively. A significant negative correlation was found between color value (L*) and total phenol ($p<0.01$), tannin ($p<0.05$), saponin ($p<0.05$), DPPH activity ($p<0.05$), FRAP activity ($p<0.01$), total reducing power ($p<0.01$). Black colored Arka Garima possessed 3- fold more phenol, tannin and saponin content than cream colored Pusa Sampada genotype. The phenol and tannin contents were positively correlated with 2,2-diphenyl-1-picrylhydrazyl free radical scavenging activity ($p<0.05$), ferric reducing antioxidant power (FRAP) ($p<0.05$) and total reducing power ($p<0.01$). Instead of colored genotypes, hydroxyl radical scavenging activity was observed maximum in creamish white color genotype Pusa Sampada and nitric oxide radical scavenging activity (NORSA) in greyish brown genotype BL-1. The maximum average seed yield was found in PFC-39 genotype followed by UPC-628 genotype. In conclusion, wide variation for all the traits can be used to develop nutritionally important genotypes.

Keywords: Bioactive compounds, FRAP activity, Seed coat color, Seed yield, Starch

Legume seeds constitute an important component of human diet in the developing countries as the poverty, religion or ethnic preferences preclude the consumption of meat (Mahala *et al*., 2013) The majority of legumes contain phytochemicals/ bioactive compounds including enzyme inhibitors, phytohemagglutinins, phytoestrogens, saponins, and phenolic compounds, which serve as functional metabolites and influence human metabolism. Phytochemicals, in general, are free radical scavengers and help to maintain the balance between oxidants and antioxidants (Prakash and Gupta, 2011). Excessive generation of ROS has been implicated in multitude of disease states ranging from tissue injury to pathophysiological consequences (Anderson and Major, 2002; Kumaran and Karunakaran, 2007). The defense provided by antioxidant system is crucial in restoring the metabolism by removing free radical intermediates and inhibiting other oxidants. Both enzymatic and non-enzymatic antioxidant molecules alone or in combination mitigate oxidative stress in relieving the tissue from pathological conditions. Natural antioxidants have been gaining considerable interest among nutritionists, food manufacturers and consumers due to their presumed safety and potential therapeutic value (Kaur *et al*., 2014). Epidemiological studies provide convincing evidence of their role in relieving/ lowering the incidence of degenerative diseases (Flight and Cliffton, 2006).

Cowpea (*Vigna unguiculata* L.), an important *Kharif* legume crop, belongs to the *Fabaceae* family. It is broadly cultivated in the tropical, subtropical regions and is adapted well in environments of high temperature and low rainfall. Cowpea is considered as an incredible source of many other health-promoting components, such as soluble and insoluble dietary fiber, phenolic compounds, minerals, and many other functional compounds including B group vitamins (Khalid and Elhardallou, 2016). The epidemiological evidence indicates that the consumption of cowpea provides protective effects for several chronic diseases like, gastrointestinal disorders, cardiovascular diseases, hypercholesterolemia, obesity, diabetes and several types of cancer. In India area under cowpea cultivation is 3.9 million hectares with a production of 2.21 million tons grains (Singh *et al*., 2012). Cowpea can be categorized into purple, black, blue, red, coffee, maroon, pink, grey and cream based on the color of seed coat (Xu *et al*., 2007). Cream and red color

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seeds are commercial cultivars in cowpea growing areas. Seed coat color is an important trait that affects nutritional, antinutritional composition and antioxidant potential of different cowpea genotypes (Drabu *et al*., 1988) . The legume seed color has been directly related with the presence of phytochemicals such as condensed tannins, phenols and flavanoids. The screening of cowpea genotypes with different seed coat color will be beneficial in identifying nutritionally superior genotypes with high nutritional composition, antioxidant potential and low antinutritional factors. This genotype can be used in local breeding programme for developing commercially important variety with high nutritional status. Cowpea contains a number of antinutritional components like phytic acid, protease inhibitors, phenolics, tannins, flavonoids, saponins and amylase inhibitors. The present study includes the phenolics, flavonoids, saponin and tannins.

MATERIALS AND METHODS

Seed material

The present investigation was carried out on ten different colored cowpea genotypes. The parentage and seed description of the genotypes are listed in Table 1. Broken and damaged seeds and foreign material were handpicked from the sample before the analytical studies. The CL-367 and CL-88 are the released varieties of Punjab Agricultural University, Ludhiana and PFC-12, PFC-39 and PFC-40 are advanced lines. The BL-1 and BL-2 are recommended varieties of Indian Grassland and Fodder Research Institute, Jhansi. The black color genotype Arka Garima is a release from IIMR, Bengaluru. Pusa Sampada and UPC-628 were developed by Indian Agricultural Research Institute, New Delhi and Gobind Ballabh Pant University of Agricultural Technology, Pantnagar , respectively.

Seed coat color

Seed coat color of cowpea genotypes was measured by using Hunter Lab mini Scan XE Plus colorimeter (Model 45/0-L, HAL, USA) having 25 mm port size. The parameters determined were L*, a* and b* values; L* value measures lightness ranging from black (L = 0) to white (L = 100), a* value measures greenness (-a*) and redness (+a*) and b* value assesses blueness (-b*) and yellowness (+b*) (Lu *et el*, 2018). The Hunter Lab colorimeter was calibrated using color standards (white and black) ceramic tiles supplied by the manufacturer and the observations were then made using D-65 illuminant and 10° viewing angle. Three determinations of each sample using three replicates were performed. From the L*, a* and b* values, hue angle (h^o) and chroma (C) were computed. Hue angle (h^o) is the dimension of the color we readily perceive when we look at the color.

$h^o = \tan^{-1} (b^*/a^*)$.

Chroma (C) is the attribute of color which indicates the degree of departure of the color from gray of the same lightness that means how close the color is to either gray or the pure hue, and is given as

$C= [a^{*2}+ b^{*2}]^{1/2}$.

Extraction and estimation of nutritional and antinutritional components

From grounded seed samples, crude protein, crude fibre, ash content and ether extract were estimated as per AOAC (2005). Total sugars were estimated as per Dubois *et al.* (1956). The dried sugar free residue was processed for the estimation of starch (Yoshida *et al.,* 1976). For total phenols, 0.5 mL folin-phenol reagent (1:1 v/v with H_2O) and 1 mL of saturated solution of sodium carbonate was used (Swain and Hills, 1959). The tannin was estimated using Folin-Denis reagent(

Table 1. Parentage and seed description of cowpea genotypes.

Genotype	Parentage	Seed Description
BL-1	Single plant selection from IL-515	Grayish brown, large, round seed
BL-2	Single plant selection from IL-978	Dark brown, small, oblong seed
C-88	C 74 × H2 through Irradiation	Brown seed, large, oblong seed
CL-367	C 74 × Strain 90	Light cream, small, D-shaped seed
PFC-40	C 88 × GFC 3	Dull brown with long black spots, small, oblong seed
PFC-39	C 88 x UPC 5286	Gray, large, round seed
Arka Garima	TUV 762 × *V. unguiculata var. sesquipedalis*	Black, large, oblong seed
PFC-12	CL367 × C74	Cream with brown spots, small, oblong seed
Pusa Sampada	Mutation breeding of *Pusa Phaguni*	Creamish white, large, round seed
UPC-628	Pure line selection from CK-76-4200	Light brown, large, oblong seed

Sadasivam and Manickam, 1992). The intensity of color developed was measured at 700 nm against reagent blank. Both phenol and tannin concentrations were expressed as tannic acid equivalents. Flavonoid was estimated as per Chang *et al.* (2002). The total flavonoids were calculated from the standard curve using rutin (10-50µg). For saponin content, 1 mL of reagent A (0.5 mL anisaldehyde mixed with 99.5 mL of ethyl acetate) and 1 mL concentrated sulphuric acid were used (Fenwick and Okanfull, 1958). The standard curve with saponin was run simultaneously.

Extraction and estimation of antioxidant activity

Powdered seed was extracted in methanol thrice. The extracts were pooled and diluted to a final volume. DPPH activity was measured for capacity of seed extract to scavenge 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radical as per the method of Blois (1958). In the known amount of methanolic extract, 0.5 mL methanol and 3 mL 0.1mM DPPH reagent was added. After incubation in dark for 30 minutes at room temperature, the discoloration of .the purple color solution was measured against reagent blank at 515 nm.

The ferric reducing antioxidant power (FRAP) is based on the ability of the sample to reduce Fe^{3+} to Fe^{2+} ions in the presence of TPTZ (2, 4, 6-tripyridyl-s-triazine) (Benzie and Strain, 1996). The 0.1 mL sample was added to 1.9 mL FRAP reagent. After incubating the reaction mixture at 37ºC for 30 minutes, absorbance was measured at 593 nm. The standard curve of iron sulphate heptahydrate (5–30 µg) was run simultaneously.

Total reducing power was determined by the method of Sreeramulu *et al*. (2009). To the 0.2 mL of the methanolic extract was added 800 µl 0.2 M sodium phosphate buffer to make its final volume 1 mL. After addition of 0.5 mL potassium ferricyanide, incubation at 50ºC for 20 minutes was carried out. To terminate the reaction, 0.5 mL TCA was added and centrifuged at 1000 rpm for 10 minutes. To the supernatant, 1.9 mL distilled water and 0.1 mL ferric chloride solution were added and absorbance of reaction mixture was measured spectrophotometrically at 700 nm against reagent blank. Ascorbic acid (5–40 µg) was used as a standard.

The hydroxyl ion radical scavenging activity determination was based on the Fenton-type reaction system generating hydroxyl radical(Li. *el al.,* 2008) . The reaction mixture consists of 1 mL of 1,10-phenanthroline (0.75 mM) and 1 mL of ferrous sulphate (0.75 mM), 1.5 mL of 0.2 M phosphate buffer (pH 7.4) and 0.5 mL of sample extract. The reaction mixture without sample was termed as control. The reaction was started by adding 1 mL of hydrogen peroxide (0.01 %) to each tube. The absorbance was measured at 536 nm after incubating the reaction mixture at 37 ºC for 60 min.

Nitric oxide radical scavenging activity was estimated by the method of Marcocci *et al*. (1994). Aqueous solution of sodium nitroprusside at physiological pH generates nitric oxide spontaneously which interacts with oxygen to produce nitrite ions that can be estimated by use of Griess reagent. The 0.1 mL aqueous sodium nitroprusside and 0.2 mL of 0.2 M sodium phosphate buffer (pH 7.8) were added to sample. The mixture was incubated at room temperature for 2.5 h. Then 0.12 mL of Griess reagent was added and absorbance was measured at 540 nm against blank. Free radical scavenging activity was calculated as follows:

$$\text{Scavenging activity (\%)} = \frac{\text{Absorbance (control)} - \text{Absorbance (test)}}{\text{Absorbance (control)}} \times 100$$

Statistical analysis

Statistical analysis was performed by using SAS package (Version 9.3). Mean and standard deviation were calculated and Turkey's test was used to identify the significant differences among the genotypes. Pearson correlation test was conducted to determine correlation among variables. Significant levels were defined using ($p \leq 0.05$).

RESULTS AND DISCUSSION

Color value

Significant differences ($p<0.05$) were found in color values of different cowpea genotypes. The luminosity (L*) varied from 5.8 to 56.1 with a mean value of 36.8 (Table 2). The L* value was observed maximum in creamish white *Pusa Sampada* genotype and minimum in black *Arka Garima* genotype. Similarly, the bean accessions grouped into red, black, white, purple, pink, yellow, and brown tones varied in luminosity from 69.43 % to15.30 % (Alcazar-valle *et al.*, 2020). Luminosity (*L) is the radiant intensity per unit area and is associated with the color (black to white). The a* and b* values significantly varied from 5.5 to 18.6 and 4.9 to 26.3, respectively. The a* and b* values were observed maximum in brown UPC -628 genotype. Similar observations were found in other legumes with different seed coat color (Segev *et al.*, 2010). Significant negative correlation between total phenol, tannin, DPPH activity, FRAP activity, total reducing power (TRP) with lightness (L*) was found. However, there was no significant correlation between flavonoid, nitric oxide radical scavenging activity, hydroxyl radical scavenging activity with color values (Table 6).Our results point that tannins and phenol serve as functional metabolites in determining

Table 2. Color values of different colored cowpea genotypes.

Genotype	L*	a*	b*	hº	Chroma
BL-1	33.6±0.22^{d}	8.4±0.07^{d}	6.8±1.25^{g}	0.66±00.02^{d}	10.8±0.21^{f}
BL-2	36.3±0.43^{cd}	13.5±0.78^{b}	15.4±0.59^{e}	0.81±0.03^{abc}	20.4±0.18^{e}
C-88	29.5±1.91^{e}	17.3±0.74^{a}	19.0±1.84^{d}	0.79±0.04^{abc}	25.6±0.53^{b}
CL-367	52.1±1.12^{b}	5.0±0.31^{f}	21.8±0.53^{c}	0.99±0.01^{a}	22.3±1.12^{d}
PFC-40	34.5±1.76^{cd}	14.1±1.12^{b}	20.7±1.28^{cd}	0.89±0.04^{ab}	25.0±0.63^{b}
PFC-39	29.3±0.97^{e}	8.4±0.15^{d}	11.7±1.17^{f}	0.88±0.06^{ab}	14.4±0.75^{f}
Arka Garima	7.8±0.75^{f}	10.5±0.20^{c}	4.9±0.76^{g}	0.43±0.02^{e}	11.5±0.42^{g}
PFC-12	54.9±0.36^{ab}	6.3±0.31^{e}	23.6±0.61^{bc}	0.99±0.01^{a}	24.4±0.37^{c}
Pusa Sampada	56.1±1.64^{a}	5.5 ±0.43^{ef}	24.8±0.52^{ab}	0.99±0.02^{a}	25.4±0.29^{b}
UPC-628	36.6±0.30^{c}	18.6±0.18^{a}	26.3±0.75^{a}	0.88±0.04^{ab}	32.2±0.18^{a}
Mean	37.07	10.76	17.5	0.76	21.2

Values are mean ± SD of triplicates
Values with different letters in the same column are significantly different (p<0.05)

DPPH activity, FRAP activity and total reducing power (TRP). The previous work also indicated variation in the content of tannins with seed coat color in legumes (Wu *et al.*, 2004).

Nutritional composition

A significant (p<0.05) difference was observed among different colored cowpea genotypes with regard to nutritional traits (Table 3). Protein, carbohydrates and fats are mostly concentrated in cotyledon portion. Therefore, seed coat color may not influence the nutritional composition of seed. Pulses are gaining more interest in the field of developing healthy foods because they are 2.5-3 times rich in proteins than cereals. Proteins in legume seeds represent from about 20% (dry weight) in pea and beans, up to 38–40% in soybean and lupin. The crude protein content (%) among different cowpea genotypes varied from 20.5 to 26.6 with an average value 23.7. The maximum crude protein content was found in cream colored PFC-12 genotype which was at par with grey colored PFC-39. Our results were in accordance with previous studies on cowpea having crude protein ranging from 22.9 to 25.4% (Liyange *et al.,* 2014). Carbohydrates account for the major component of legume seed and varied from 65-72%. Legume carbohydrates are generally digested slowly and due to this reason legumes are considered as low glycemic index foods that prevent many degenerative diseases (Guillon and Champ, 2002). Starch content (mg/g) lay in the range from 229.6 to 359.5 with mean value 313.9. The significantly high starch content was observed in decorticated cowpeas as compared to whole seeds (Sasanam *et al.*, 2011). Sugars play a crucial role in maintaining the

Table 3. Study of nutritional composition of different colored cowpea seeds

Genotype	Crude protein (%)	Crude fibre (%)	Ash (%)	Ether extract (%)	TSS (mg/g)	Starch (mg/g)
BL-1	23.15±0.50^{de}	6.0±0.10^{h}	3.52±0.04^{cd}	1.11±0.01^{f}	52.21±1.27^{ef}	229.63±0.75^{i}
BL-2	22.45±0.32^{e}	8.1±0.15^{a}	3.83±0.04^{a}	0.85±0.03^{g}	74.55± 1.63^{a}	302.88±0.29^{f}
C-88	24.55±0.24^{bc}	7.1±0.15^{cd}	3.50±0.01^{d}	1.31±0.02^{d}	71.59±0.73^{b}	321.66±1.49^{d}
CL-367	23.25±0.24^{de}	7.6±0.20^{b}	3.21±0.03^{f}	1.51±0.02^{c}	50.57±0.75^{f}	299.63±0.66^{g}
PFC-40	24.54±0.72^{bc}	6.9±0.10^{def}	3.40±0.02^{e}	1.81±0.02^{a}	57.55±0.96^{d}	309.24±1.20^{e}
PFC-39	25.76±0.25^{ab}	6.6±0.15^{ef}	3.70±0.02^{b}	1.33±0.03^{d}	59.74±0.36^{cd}	325.33±1.19^{c}
Arka Garima	23.95±0.13^{cd}	6.2±0.10^{gh}	3.61±0.03^{bc}	1.23±0.01^{e}	61.68±0.82^{c}	359.58±1.13^{a}
PFC-12	26.62±0.77^{a}	7.3±0.10^{bc}	3.36±0.05^{e}	1.71±0.04^{b}	61.81±0.89^{c}	348.49±1.29^{b}
Pusa Sampada	22.97±0.30^{de}	7.0±0.10^{cde}	3.00±0.02^{g}	1.68±0.03^{b}	53.62±1.17^{e}	346.70±1.16^{b}
UPC-628	20.56±0.25^{f}	6.6±0.15^{fg}	3.24±0.03^{f}	1.07±0.03^{f}	61.01±0.95^{c}	296.33±0.47^{h}
Mean	23.78	6.9	3.44	1.36	60.43	313.95

Values are mean ± SD of triplicates
Values with different letters in the same column are significantly different (p<0.05)

Table 4. Study of total phenols, tannins, flavonoids and saponins in colored cowpea genotypes.

Genotype	Total phenols (mg/g)	Tannins (mg/g)	Flavonoid (mg/g)	Saponin (mg/g)
BL-1	2.16±0.15^{bc}	1.47±0.03^{b}	0.55±0.01^{e}	2.87±0.05^{f}
BL-2	2.52±0.20^{ab}	1.42±0.02^{b}	0.78±0.04^{b}	3.83±0.26^{e}
C-88	2.14±0.15^{bc}	1.40±0.03^{c}	0.65±0.01^{d}	6.15±0.13^{c}
CL-367	0.88±0.09^{e}	0.68±0.09^{e}	0.75±0.01^{bc}	3.82±0.20^{e}
PFC-40	2.23±0.11^{bc}	1.45±0.06^{b}	0.68±0.01^{cd}	7.55±0.42^{b}
PFC-39	2.10±0.17^{c}	1.48±0.07^{b}	0.41±0.02^{f}	5.57±0.36^{c}
Arka Garima	2.70±0.10^{a}	1.60±0.04^{a}	0.98±0.01^{a}	9.85±0.15^{a}
PFC-12	1.49±0.14^{d}	1.29±0.02^{d}	0.69±0.03^{cd}	4.33±0.13^{de}
Pusa Sampada	0.74±0.04^{e}	0.56±0.04^{f}	0.70±0.05^{bcd}	4.85±0.15^{d}
UPC-628	2.53±0.10^{ab}	1.39±0.08^{c}	0.72±0.02^{bcd}	5.66±0.13^{c}
Mean	1.95	1.27	0.69	5.44

Values are mean ± SD of triplicates
Values with different letters in the same column are significantly different (p<0.05)

structural integrity of bio-membranes and proteins. The total soluble sugar content varied significantly from 50.6 to 74.5 mg/g. The dark brown color genotype BL-2 exhibited maximum and light creamish genotype CL-367 had exhibited minimum total sugar. Dietary fiber contains a unique blend of bioactive components including resistant starches, vitamins, minerals, phytochemicals and antioxidants. Dietary fiber resists digestion in the small intestine, thereby allowing it to enter the large intestines. The crude fibre content in our study varied from 6.0% to 8.1% with an average value of 6.9%.The crude fibre content was observed maximum in dark brown BL-2 genotype and minimum in greyish brown BL-1 genotype. Ash or minerals are inorganic substances present in all body tissues and fluids and their presence is necessary for the maintenance of certain physicochemical processes which are essential to life. Ash content was observed maximum in dark brown color genotype BL-2. In four different cowpea cultivars ash content ranged from 3.7-5.1%. Ether extract includes free fatty acids, oil-soluble pigments, wax, unsaturated fats, cholesterol and phospholipids. Ether extract was high in dull brown genotype PFC-40 and low in dark brown genotype BL-2. In a previous study, crude fat was observed maximum in white cowpea (1.8%) and minimum in red cowpea genotype (0.4%) (Segev *et al*., 2010)

Anti-nutritional /compounds

The antinutritional compounds in cowpea varied significantly (p<0.05) with seed coat color and ranged from 0.7 to 2.7mg/g (Table 4). A strong positive

Table 5. Study of yield (q/ha) data of colored cowpea genotypes.

Genotype	Seed Yield		Average
	2015-16	2016-17	
BL-1	12.5±0.56^{b}	12.1± 0.35^{c}	12.3±0.45^{b}
BL-2	9.9±0.47^{d}	9.6±0.11^{d}	9.7±0.33^{d}
C-88	12.8±0.32^{b}	11.0±0.30^{cd}	11.9±0.31^{bc}
CL-367	11.4±0.51^{c}	10.6±0.41^{cd}	11±0.45^{c}
PFC-40	9.4±0.21^{de}	13.9±0.47^{b}	11.6±35^{c}
PFC-39	15.8±0.28^{a}	17.3±0.33^{a}	16.5±0.30^{a}
Arka Garima	6.9±0.45^{f}	8.2±0.04^{e}	7.5±0.31^{f}
PFC-12	8.4±0.33^{e}	8.0±0.03^{e}	8.2±0.21^{e}
Pusa Sampada	10.2±0.12^{d}	9.2±0.59^{d}	9.7±0.24^{d}
UPC-628	12.6±09^{b}	13.2±0.40^{b}	12.9±0.18^{b}
Mean	10.9	11.3	11.1

Values are mean ± SD of triplicates
Values with different letters in the same column are significantly different (p<0.05)

Table 6. Correlation coefficients between nutritional and antinutritional factors, antioxidant activity and seed yield of cowpea genotypes

	CP	ADF	NDF	CF	Ash	EE	TSS	Starch	NFE	TP	Tannins	Flavonoid	Saponin	DPPH	FRAP	TRP	HRSA	NORSA	SY
CP	1																		
ADF	-0.679*	1																	
NDF	-0.611	.846**	1																
CF	0.008	0.424	0.248	1															
Ash	0.312	-0.42	-0.23	0.034	1														
EE	0.539	-0.3	-0.35	0.014	-0.54	1													
TSS	0.038	0.219	0.322	0.421	0.613	-0.486	1												
Starch	0.412	0.118	0.279	0.195	-0.08	0.386	0.247	1											
NFE	-.907**	0.487	0.488	-0.402	-0.39	-0.44	-0.245	-0.448	1										
TP	-0.145	-0.24	0.091	-0.344	.699*	-0.595	0.549	-0.176	0.186	1									
Tannins	0.176	-0.5	-0.16	-0.398	0.768**	-0.43	0.478	-0.174	-0.065	0.928**	1								
Flavonoid	-0.248	0.134	0.287	0.182	-0.04	-0.071	0.223	0.468	0.105	0.163	-0.03	1							
Saponin	0.137	-0.23	0.141	-0.373	0.135	0.163	0.166	0.572	-0.024	0.439	0.362	0.527	1						
DPPH	-0.008	-0.13	0.368	-0.329	0.517	-0.373	0.342	0.257	0.087	0.752*	0.709*	0.241	0.624	1					
FRAP	0.15	-0.23	0.218	-0.186	0.541	-0.225	0.48	0.323	-0.108	0.656*	0.628	0.277	0.777**	0.776**	1				
TRP	0.231	-.717*	-0.38	-0.414	.702*	-0.236	0.155	-0.087	-0.141	0.734*	0.783**	0.239	0.561	0.622	0.681*	1			
HRSA	0.395	-0.13	-0.12	-0.125	-0.55	0.765**	-0.424	0.61	-0.252	-0.59	-0.512	0.178	0.415	-0.243	0.044	-0.189	1		
NORSA	0.148	-0.59	-0.55	-0.432	0.2	0.064	-0.165	-0.579	0.018	0.407	0.557	-0.368	-0.151	0.05	-0.158	0.370	-0.425	1	
SY	-.020	.021	.068	-.246	.132	-.125	-.127	-.420	.136	.126	.188	-0.838**	-.209	.170	.098	-.038	-.275	.241	1

*=Significant at 5%, **=Significant at 1%

association was observed between bioactive compound accumulation and seed coat color (Lee *et al*., 2020). Black colored Arka Garima showed maximum phenol content followed by grey colored BL-2 and UPC-628 genotypes. Cream colored Pusa Sampada recorded lowest level of phenol content. Phenolic acid, flavanoids and procynidins are the dominant phenolic compounds present in legumes (Zhang *et al*., 2015). Dehulling of pulses resulted in removal of large amounts of polyphenols as they are concentrated in seed coat portion (Singh *et al*., 2017). Phenols serve as free radical scavengers, reducing agents and chelators of metal ions so variation of phenolic content with seed coat color can be used by plant breeder as a guide line for breeding new cultivars rich in antioxidant potential (Djordievic *et al*., 2011). A highly positive correlation of phenol was found with DPPH activity (r=0.755*, p<0.05), ferric reducing antioxidant power (FRAP, r=0.676*, p<0.05) and total reducing power (TRP, r=0.730*, p<0.01). On the other hand, high concentration of phenols is antinutritional and known to decrease the digestibility of proteins, minerals, fats and carbohydrates and also lower the activity of digestive enzymes (Othman *et al*., 2007). The tannin content also varied with seed coat color with highest in black colored Arka Garima (1.6 mg/g) and lowest in cream colored Pusa Sampada (0.6 mg/g). In different colored cowpea seeds, tannin content was found in the range of 0.3 to 6.9 mg CE/g (Plahar *et al*., 1997). High tannin content had shown positive correlation with DPPH free radical scavenging activity (r=0.703*, p<0.05), ferric reducing antioxidant power (r=0.635*, p<0.05) and total reducing power (r=0.783**, p<0.01) (Table 7). However, in high concentrations, tannins bind with protein through hydrogen binding and hydrophobic interactions, thereby reducing their nutritional quality (Wang and Saun, 2006). Total flavonoid content (mg RE/g) among different cowpea genotypes ranged from 0.41 to 0.98 with mean value of 0.69. The flavonoid content was also found significantly high in black color Arka Garima genotype and minimum in grey color PFC-39 genotype. Black beans were observed to contain primarily 3-o-glucosides of malvidin, petudin and delphinidin(Liu *et al*., 2008). In our study, flavonoids did not show any correlation with antioxidant activity. Flavonoids accumulation in plants is directly related to nutrient deficiency, reactive oxygen species (ROS) production and metal toxicity (Agati *et al*., 2015). Saponin content (mg saponin/g) in cowpea genotypes was in the range of 2.8 to 9.8 with an average value of 5.4. The saponin range was less (1.70 to 3.50 mg/g) in lentil genotypes (Ahuja *et al*., 2015). Saponin content positively correlated with ferric reducing antioxidant power (r=0.777**, p<0.01) (Table 7). It lowers harmful LDL-cholesterol level and stimulates immune response (Segev *et al*., 2010). However, saponins also show lytic action on erythrocyte membrane when consumed in larger amounts. Nowadays, several strategies are available to overcome the effects of antinutrient factors without changing the nutrition of food items. These include fermentation, debranning, autoclaving, soaking, and microwave, etc. (Samitya *et al*., 2020)

Antioxidant potential

Antioxidant activity of pulses is related to the seed coat color as higher antioxidant activity was observed in dark colored seed compared to light colored (Chaver Mendoza *et al*., 2019). In our study also significant variation between seed coat color and antioxidant activity was observed (Fig. 1). Black colored Arka Garima (65.2%, 30.0%, 18.6%) showed the highest DPPH, FRAP and TRP activities. The light colored Pusa Sampada (35.8%, 10.8% and 3.4%) showed the lowest DPPH, FRAP and TRP antioxidant activities, respectively (Fig.1a-c). Our findings showed consistency with previous study showing high antioxidant activity in pigmented cowpea inbred lines over non-pigmented inbred lines (Nzaramba *et al*.,

Table 7. Correlation between antinutritional factors, antioxidant potential and color values of cowpea seeds

	L	a*	b*	hº	Chroma
Phenol	-0.816**	0.724*	-0.512	-0.689*	-0.242
Tannin	-0.758*	0.564	-0.567	-0.633*	-0.353
Flavonoid	-0.254	0.108	-0.023	-0.403	0.075
Saponin	-0.705*	0.344	-0.243	-0.529	-0.077
DPPH activity	-0.742*	0.425	-0.384	-0.514	-0.211
FRAP activity	-0.837**	0.534	-0.406	-0.577	-0.243
TRP	-0.843**	0.203	-0.786**	-0.785**	-0.651*
HRSA	0.145	-0.414	0.218	0.149	0.105
NORSA	-0.076	0.166	-0.189	-0.103	-0.155

*=Significant at 5%
**=Significant at 1%

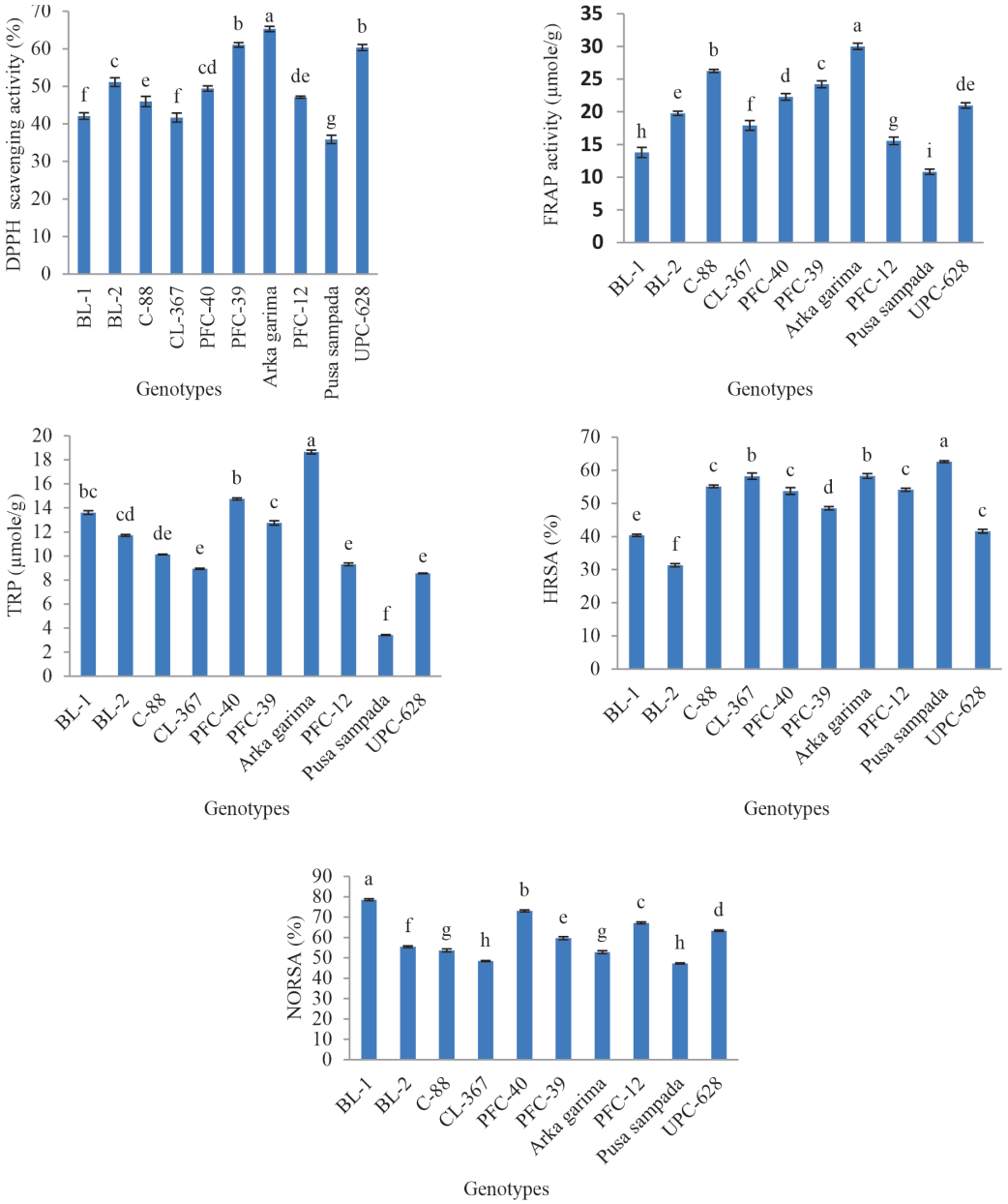

Fig.1. Evaluation of (a) DPPH free radical scavenging activity, (b) total reducing power, (c) FRAP activity, (d) hydroxyl radical scavenging activity and (e) nitric oxide radical scavenging activity in cowpea seeds. Vertical bars show mean ± SD of triplicates. Values with different letters are significantly different (p<0.05).

2005). Similarly, red colored rice grain showed 3.6 fold higher antioxidant activity than white colored rice grain(Fasahat *et al*., 2012) . In other study, FRAP activity was observed maximum in dark color chickpea seeds than light color chickpea seeds (Sreeramulu *et al*., 2009). The positive correlation of FRAP activity with DPPH free radical scavenging activity (r=0.777**, p<0.01) and TRP (r=0.691*, p<0.05) was observed (Table 6). The DPPH, FRAP and TRP activities varied with polyphenol content of seed coat as these activities showed strong positive correlation with total phenol (r=0.755*, p<0.05, r=0.676*, p<0.05and r=0.783**, p<0.01) and tannins (r=0.703*, p<0.05, r=0.635*, p<0.05, r=0.783**, p<0.01), respectively. In contrast, hydroxyl radical scavenging activity (HRSA) was maximum in cream colored Pusa Sampada cultivar and minimum in dark brown BL-2 cultivar. So HRSA does not seem to respond with seed coat color in our study. Hydroxyl radical is the most reactive free radical and reacts with biological molecules such as DNAs, proteins, and lipids thus causing chemical modifications of these molecules. There are several research reports on the oxidative damage of DNA due to the ˙OH radical (Sharma *et al*., 2012). The nitric oxide radical scavenging rate (NORSA) was in the range

of 47.2% to 78.5% with an average value of 59.9%. The highest NORSA was found in greyish brown BL-1 genotype and lowest in Pusa Sampada. In a previous study, nitric oxide radical scavenging activity was highest in black color soybean genotypes and ranged from 47.86-68.31% (Miglani and Sharma, 2016). Nitric oxide radical is used as widespread signaling molecule and participate in some cellular functions of the body. It also acts as a neurotransmitter and an important mediator of the immune response (Fang *et al*., 2002).

Seed yield

Seed yield (q/ha) of different cowpea cultivars revealed variation from 6.9 to 15.8 and 8.0 to 17.3 during the years 2015-16 and 2016-17, respectively (Table 5). The maximum seed yield was found in PFC-39 cultivar during both the years. The minimum seed yield was observed in genotype *Arka Garima* during 2015-16 and PFC-12 during 2016-17. An average seed yield was observed maximum during current year (11.3 q/ha) as compared to the previous one (10.9 q/ha). In previous studies, the seed yield of different cowpea cultivars varied from 10.10 to 14. 20 q/ha (Basaran *et al.,* 2011) and 11.7 to 16.1 q/ha (Kumar and Salaich, 2015). The seed yield negatively correlated with the flavonoids at 1% significance

To conclude, seed coat color plays a major role in determining phytochemical content and antioxidant potential of cowpea genotypes. Black colored Arka Garima possessed maximum DPPH, FRAP and TRP along with highest tannin and phenol content. Only HRSA was observed maximum in light colored genotype Pusa Sampada. Therefore, it can be assumed that colored cowpea genotypes have high biological potential for the prevention of various pathological diseases. On the other hand, PFC-39 has highest yield potential, high crude protein, starch, phenol and tannin content and medium range of antioxidant activity and antinutritional factors. Antioxidant rich Arka Garima can be involved in breeding programme with high yielding PFC-39 genotype for the production of healthy and functional foods that can be further used as nutraceuticals.

Authors' contribution

Conceptualization of research work and designing of experiments(MG); Execution of field/lab experiments and data collection(HK, PDPS); Data analysis and interpretation of results(HK); Preparation of manuscript (MG,HK).

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