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Research Paper

Relationship between seed coat colour and seed vigour in cowpea (*Vigna unguiculata* Walp (L.)

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ABSTRACT

In the present study, the 25 genotypes were grouped into two seed color groups including pigmented (n = 19; black, brown, dark brown, grey, dark grey seed color) and unpigmented (n = 6; cream and white seed color) for studying the association of seed coat color with seed vigor. Despite high germination (>84%) of all genotypes in the laboratory, unpigmented genotypes recorded low (34-54%) field emergence than pigmented genotypes (52-78%). Rapid initial (30 min) rate of water uptake (-0.75), water uptake at one hour of imbibition (-0.70**), low proportion of seed coat (0.67**), and greater electrical conductivity of seed leachate (-0.72**) was associated with low field emergence in unpigmented cowpea genotypes. No significant correlation or association was observed between field emergence and laboratory germination in the present investigation suggesting that laboratory germination can not be used for predicting field emergence in cowpea. Instead, the electrical conductivity of seed leachate could be used to predict the field emergence of cowpea.**

Key words: Accelerated aging test, Conductivity, Cowpea, Field emergence, Seed vigor

INTRODUCTION

Cowpea [*Vigna unguiculata* Walp (L.)], also known as Lobia, is a self-pollinated kharif pulse crop native to the semi-arid tropics. It is a good source of plant-derived protein, amino acids, and essential nutrients. The cowpea seeds contain about 250 mg/g of protein, which is comparable to the protein content of soybean (Boukar *et al*. 2011, Kumar *et al*. 2017). In addition, cowpea seed contains many essential nutrients like iron (53.2 mg/kg), zinc (38.1 mg/kg), magnesium (1915 mg/kg), and calcium (826 mg/kg) (Boukar *et al*. 2011). The cowpea succulent leaves and pods are consumed as vegetables, while the seeds after grinding into a fine powder are used for making porridge (da Silva *et al*. 2018). It is grown for its seed (shelled green or dry), pods, and/or leaves, which are eaten fresh as green vegetables, while the dried grain is used to make snacks and main meal recipes (Akhtar *et al.* 2022). Considering the importance of cowpea as all aboveground parts are utilized in some form or other, it is very helpful in meeting the nutritional requirements of vegetarian people. After the pods are picked, the rest of the cowpea plant is utilized as healthy cattle fodder. It is considered a multipurpose crop, supplying food and fodder for humans and cattle, while also providing farmers and grain traders with a valuable revenue-generating product (Singh *et al*. 2006).

Seed is considered the basic input of agriculture and its quality determines the potential of any high-yielding varieties (Singh *et al.* 2013, Tiwari 2022). Genetic purity, physical purity, germination, seed vigor, moisture content, seed health, and physical attributes like size, weight, color, etc. are the seed quality parameters (Lamichaney *et al*. 2017). Seeds possessing high vigor and laboratory germination are expected to produce a good stand of the crop in the field and ultimately better harvest. Therefore, the availability of quality seeds is an important factor in enhancing production and productivity (Singh *et al.* 2018). In legumes, seed coat color has been associated with seed vigor such that pigmented seeds are understood to have better vigor (ability to cope better with different stresses) as compared to unpigmented seeds. A wide range of variability exists for seed coat pigmentation in cowpea. Such positive association of seed coat colour with seed vigour is reported in other legumes such as chickpea (Lamichaney *et al*. 2016, Lamichaney *et al*. 2017, Lamichaney and Katiyar 2017), rajmash (Dickson and Petzoldt 1988, Lamichaney *et al*. 2021), fababean (Kantar *et al*. 1996, Peksen 2007), flax (Saeidi 2008), soybean (Mohammadi *et al*. 2011, Sooganna *et al*. 2016, 2021), and cowpea (Peksen *et al*. 2004). Therefore, the present experiment was conducted to study the relationship between seed coat color and seed vigor in cowpea differing in seed coat pigmentation.

MATERIALS AND METHODS

Experimental details

The laboratory and field experiments were conducted at the ICAR-Indian Institute of Pulses Research (ICAR-IIPR), Kanpur. The experimental material for the present study consisted of 25 genotypes of cowpea (PL1, GP7, GP17, GP18, GP30, PL2, DC15, DC16, CO4, TPTC29, Pusa Phalguni, PCP306-01, RC101, GC3, CH46, C152, PL3, RC19, CO7, Goa Cowpea 3, EC458425, IC201095, Kashi Kanchan, EC97749 and EC724047). These genotypes were obtained from the Division of Crop Improvement, ICAR-IIPR, Kanpur. The details of these genotypes are given in Table 1. For association studies among seed traits with seed coat color, the genotypes possessing black, brown, dark brown, grey, and dark grey are grouped as pigmented (n = 19) and genotypes with cream and white seed coat color are grouped as un-pigmented (n = 6).

Seed moisture content estimation

For seed moisture estimation, the weight of an empty moisture can was recorded (W1). The seeds were coarsely ground and placed in a moisture can weighed (W2) and were kept with the lid open in a hot air oven maintained at 130 ºC for 1h. After which it was allowed to cool for 5 minutes in a desiccator and weighed again (W3). The moisture content was estimated as

$$\textit{Moisture content (\%)} = \frac{\text{W2} - \text{W3}}{\text{W2} - \text{W1}} \times 100$$

Seed physical characters and water uptake

A sample of hundred seeds was counted manually and weighed for the 100–seed weight determination. Seed coat from seeds were removed carefully after soaking in distilled water for 4-5 h. The seed coat and cotyledons were then dried at 80 ºC for 24 hrs and weighed separately. The proportion of the seed coat was then calculated using the following formula.

$$\text{Proportion of seed coat (\%)} = \frac{\text{Weight of seed coat}}{\text{Weight of seed coat and cotyledon}} \times 100$$

For estimation of water uptake ratio, fifty seeds were weighed and soaked in 250 ml deionized water and were kept at 20 ºC for varying duration. The seeds were removed, blotted dry, and re-weighed after 30 min, 1, 2, 3, 4, 5, 6, and 24 h. The gain in seed weight was calculated and expressed as a percentage.

Germination and field emergence test

The germination test was carried out by using the between-paper method. One hundred pure seeds in four replications were placed inside moist germination paper and then incubated at 25 ºC in the dark for 8 days. After 8 days, the seeds and seedlings were categorized as normal seedlings, abnormal seedlings, dead seeds, hard seeds, and freshly ungerminated seeds. The value of normal seedlings was computed as germination percent. Field emergence was tested by hand-sowing seeds in 10 m rows (100 seeds per row) in three randomized rows during the summer and *Kharif* season of 2021. Spacing of 45 × 10 cm was maintained between rows and plants respectively. The number of seedlings that emerged were counted daily for 21 days and computed as field emergence percent.

Electrical conductivity

Fifty seeds in four replications were weighed and soaked in 250 ml of deionized water at 20 ºC for 24 h. Two beakers containing only 250 ml deionized water were also taken which serve as blank. The electrical conductivity of the seed leachate was measured according to ISTA (2015) using an electrical conductivity meter.

$$\text{Electrical Conductivity } (\mu\text{S/cm/g}) = \frac{\text{Conductivity reading } \ \mu\frac{\text{S}}{\text{cm}} - \text{Background reading}}{\text{Weight of replicate}}$$

Accelerated aging test

For accelerated aging of cowpea seeds, the seeds were placed inside a muslin cloth and were suspended above water in a sealed desiccator maintained at 41.3 ± 1 ºC for 72 h. Thereafter, germination and related parameters were determined following the protocol as mentioned earlier.

RESULTS AND DISCUSSION

Laboratory germination of all genotypes exceeded 84.80%, which was higher than the minimum requirement of 75% as per the seed standards of the Indian Minimum Seed Certification Standards (IMSCS). Overall, the maximum germination percent was noted in C-152 (94.00 %) while the minimum germination percent was noted in GC-3, CO-4 (79.00 %). The mean laboratory germination among pigmented and un-pigmented genotypes was 85.00 and 84.17, respectively, suggesting no differences in laboratory germination among these two color groups. The maximum field emergence was recorded in Goa cowpea-3 (78.00 %) while minimum emergence was recorded in PCP-306-01 (34.00 %) with an average of 57.84% in the summer season. In *Kharif*, the maximum emergence was recorded in RC-19 (72.50 %) and the minimum emergence was recorded in Pusa Phalguni (31.00 %) with an average of 57.84%. The mean emergence in pigmented and unpigmented seed color groups during summer and *Kharif* was 62.87 and 62.95; 41.92 and 41.67, respectively (Figure 1A). The maximum seedling vigor index I and seedling vigor index II were Pusa Phalguni (3545.53) and Kashi Kanchan (19.41), while the minimum was observed in GP-18 (1281.73 and 6.98), with an average of 2377.85 and 12.70, respectively. The mean seedling vigor index I among pigmented and un-pigmented genotypes was 2252.33 and 2779.34, respectively, showing un-pigmented genotypes to have better seedling vigor index I. The mean seedling vigor index II among pigmented and un-pigmented genotypes was 13.46 and 10.28, respectively, showing pigmented genotypes to have better seedling vigor index II (Figure 1B).

Table 1. Detail information of the genotypes used in the study

S.No	Genotype	Testa Colour	Initial seed moisture content (%)
1	PL-1	Black	9.27
2	GP-7	Black	8.88
3	GP-17	Black	9.20
4	GP-18	Black	8.88
5	GP-30	Black	9.05
6	PL-2	Brown	9.48
7	DC-15	Brown	8.63
8	DC-16	Brown	9.60
9	CO-4	Brown	9.51
10	TPTC-29	Brown	9.63
11	Pusa Phalguni	White	8.86
12	PCP-306-01	White	7.18
13	RC-101	White	8.75
14	GC-3	Cream	10.58
15	CH-46	White	9.28
16	C-152	Dark brown	8.92
17	PL-3	Dark brown	9.85
18	RC-19	Dark brown	9.76
19	CO-7	Dark brown	7.95
20	Goa Cowpea	Dark brown	9.42
21	EC-458425	Cream	9.05
22	IC-201095	Grey	9.14
23	Kashi Kanchan	Dark grey	9.78
24	EC-97749	Dark grey	9.97
25	EC-724047	Dark grey	9.58

The maximum 100 seed weight was recorded in GP-30 (14.24 g) whereas the minimum 100 seed weight was recorded in EC-724047 (5.54 g), with an average of 10.99 g for *Kharif* season, and in the summer season, the maximum 100 weight was recorded in CO-4 (15.46 g) whereas minimum 100 seed weight was recorded in EC-724047 (5.77 g), with an average of 11.36 g. The mean 100 seed weight in both the season among pigmented and un-pigmented genotypes was 11.4-11.8 g and 9.5-9.75g, respectively. The maximum proportion of seed coat was recorded in EC-724047 (14.99 %) whereas the minimum was recorded in Pusa Phalguni (7.54 %), with an average of 11.39 %. The mean proportion of seed coats among pigmented and un-pigmented genotypes was 11.9 and 8.4%, respectively, showing pigmented genotypes to have a higher proportion of seed coat (Figure 1C).

Significant differences were observed between non-pigmented and pigmented cowpea genotypes in terms of water uptake. Percent increase in water uptake of colored genotypes ranged from 37.47 % (EC 714047) to 133.61% (IC 201095), while in the case of un-pigmented cowpea genotypes it varied between 105.26% (PCP 306-01) and 121.54% (GC 3) at 24 h of imbibition. Moreover, pigmented and unpigmented genotypes differed in the rate of water absorption depending on the imbibition time. For the initial first hour of imbibition, the water uptake by most of the colored genotypes was less than 30% with some genotypes like GP 30 and PL 2 imbibing less than 5% water. On the contrary, all non-pigmented genotypes imbibed more than 40% water, and notably, the genotypes Pusa phalguni, RC 101, and CH 46 had more than 50% water uptake. It is important to note that the un-pigmented cowpea genotypes reached their maximum water imbibing capacity within 2 hours of imbibition, whereas pigmented cowpea genotypes continued to imbibe water even up to 6 h of imbibition (Figure 2).

Further, the seed moisture content was comparable among these genotypes. The electrical conductivity of seed leachate showed a significant variation among these genotypes, recorded maximum in Kashi Kanchan (51.3 µS/cm/gm),

Table 2. Electrical conductivity (EC) and germination after accelerated ageing test (AAT) in pigmented and unpigmented seed colour groups.

Pigmented		
Genotype	EC (µS/cm/g)	Germination after AAT
PL-1	20.07	50.00
GP-7	16.06	38.00
GP-17	26.83	60.00
GP-18	27.92	30.00
GP-30	20.24	26.00
PL-2	26.09	58.00
DC-15	19.04	72.00
DC-16	21.89	74.00
CO-4	21.40	8.00
TPTC-29	13.81	30.00
C-152	29.10	82.00
PL-3	21.10	64.00
RC-19	10.53	46.00
CO-7	24.91	40.00
Goa Cowpea-3	10.33	58.00
IC-201095	14.91	70.00
Kashi Kanchan	17.36	32.00
EC-97749	37.20	54.50
EC-724047	24.71	57.00
Average	21.24	49.97
Minimum	10.34	8.00
Maximum	37.20	82.00

Unpigmented		
Genotype	EC (µS/cm/g)	Germination after AAT
EC-458425	40.56	32.00
Pusa Phalguni	37.70	58.00
PCP-306-01	39.34	18.00
RC-101	51.29	20.00
GC-3	23.83	42.00
CH-46	47.15	2.00
Average	39.98	28.67
Minimum	23.84	2.00
Maximum	51.30	58.00

while minimum electrical conductivity was recorded in Goa cowpea-3 (10.33 µS/cm/g), with an average of 25.73 µS/cm/g of seed. The mean electrical conductivity of seed leachate among pigmented and un-pigmented genotypes was 21.24 and 39.98 µS/cm/g, respectively, showing pigmented genotypes to have lower electrical conductivity. The germination after accelerated aging at 41 ±

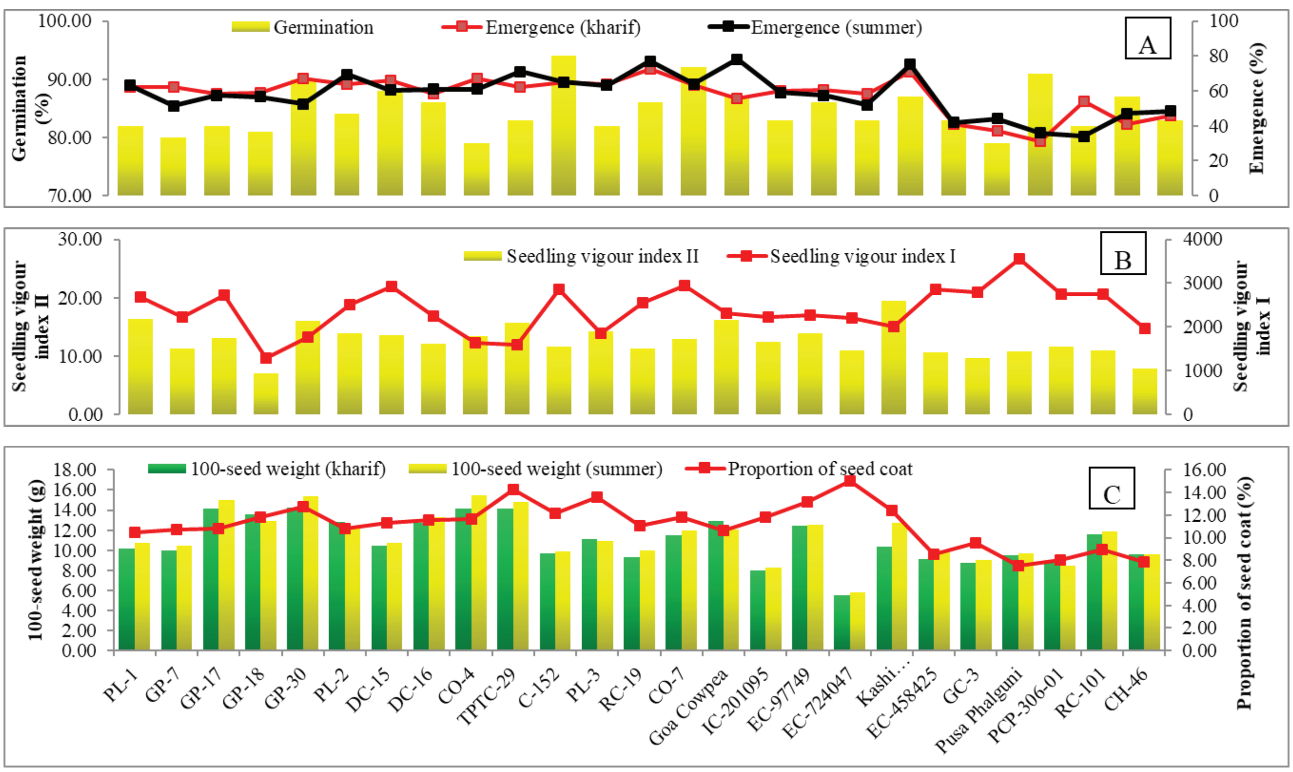


Fig. 1. Laboratory germination, field emergence (A), vigor index I, vigor index II (B), one hundred seed weight, and proportion of seed coat (C) of twenty-five cowpea genotypes. Genotypes (6) represented at the end of the graph are of non-pigmented type.

Table 3. Correlation of various seed physical and physiological parameters.

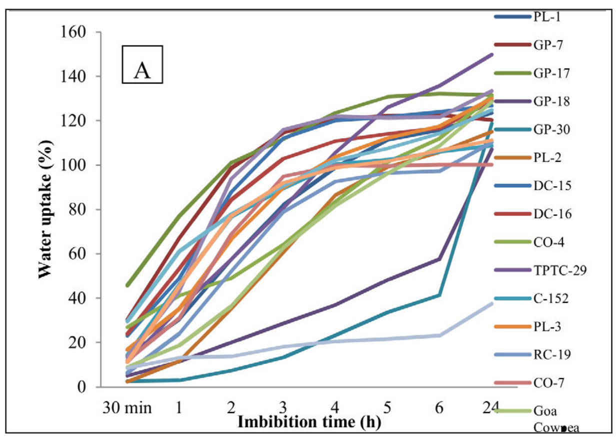
Traits		Germ	SVI-I	SVI-II	Emerge	EC	AAT	SW	PSC	Water uptake (hour)							
										30 min	1h	2h	3h	4h	5h	6h	24h
Germ		1.00															
SVI-I		0.43	1.00														
SVI-II		0.22	-0.1	1.00													
Emerge		0.14	-0.42	0.58	1.00												
EC		0.08	0.31	-0.5	**-0.72**	1.00											
AAT		0.35	0.35	0.16	0.26	-0.36	1.00										
SW		0.04	-0.41	0.38	0.37	-0.16	-0.18	1.00									
PSC		0.04	-0.54	0.41	**0.67**	-0.56	0.31	0.2	1.00								
Water uptake (h)	30 min	-0.17	0.41	-0.37	**-0.75**	0.63	-0.35	-0.21	-0.75	1.00							
	1h	-0.09	0.49	-0.34	**-0.7**	0.57	-0.2	-0.24	-0.69	0.96	1.00						
	2h	-0.04	0.54	-0.24	**-0.5**	0.39	0.04	-0.26	-0.57	0.79	0.91	1.00					
	3h	-0.02	0.54	-0.106	-0.29	0.19	0.17	-0.2	-0.48	0.62	0.77	0.96	1.00				
	4h	-0.05	0.47	0.04	-0.12	0.06	0.21	-0.06	-0.39	0.51	0.66	0.88	0.97	1.00			
	5h	-0.1	0.36	0.14	-0.02	-0.03	0.18	0.08	-0.33	0.43	0.58	0.78	0.90	0.98	1.00		
	6h	-0.12	0.28	0.21	0.03	-0.07	0.15	0.17	-0.29	0.38	0.52	0.72	0.84	0.94	0.99	1.00	
	24h	-0.11	-0.16	0.38	0.23	-0.3	0.01	0.59	-0.09	0.08	0.16	0.31	0.43	0.57	0.69	0.76	1.00

Where, Germ - laboratory germination; SVI-I – Seedling vigour index –I; SVI-II – Seedling vigour index-II, Emerge – field emergence; EC – electrical conductivity of seed leachate; AAT – germination after accelerated ageing test; SW – 100-seed weight; PSC – proportion of seed coat, rate of water uptake after 30 min, 1, 2, 3, 4, 5, 6 and 24 h of imbibition.

1°C for 3 days showed marked differences in the cowpea genotypes, being recorded maximum in C 152 (82.00%), while CH 46 recorded minimum (2.00%) germination after accelerated aging, with an average of 44.3%. The mean germination after accelerated aging among pigmented and un-pigmented genotypes was 49.97 and 28.67%, respectively, showing pigmented genotypes to have higher germination after accelerated aging (Table 2).

The result indicated that the germination percent was comparable among pigmented and unpigmented genotypes, but the field emergence was low in unpigmented genotypes as compared to pigmented genotypes suggesting the vigor differences among these two color groups. The unpigmented group even had better shoot length, root length, seedling length, and seedling vigor index I than pigmented genotypes. The differences in vigor between pigmented and unpigmented genotypes were further confirmed when tested for vigor parameters like germination after accelerated aging and electrical conductivity test, where the unpigmented genotypes recorded poor germination after accelerated aging and higher electrolyte leakage as evident from higher electrical conductivity. Our results corroborated with the findings of Lamichaney *et al*. (2016, 2017) in chickpea, Kantar *et al*. (1996) in fababean, and Peksen *et al*. (2004) in cowpea. Differences in field emergence of cowpea genotypes differing in seed coat colour may be attributed to the occurrence of pre-emergence damping off (Aveling *et al*. 2005).

In cowpea seeds, the seed coat acts as a modulator between the external environment and inner structures of the seed and is a vital determinant of having high vigor and emergence in the field (Souza and Filho 2001), by protecting the living embryo, regulating the imbibition process such that the embryo does not get affected by imbibitional damage, by facilitating gas exchange between environment and embryo. Therefore, in the present study, the vigor differences between pigmented and un-pigmented cowpea genotypes in terms of field



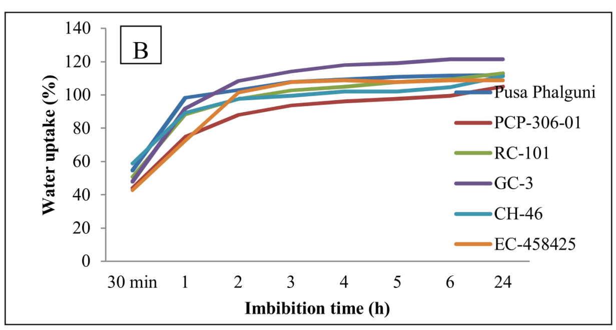


Fig. 2. Cumulative water absorption of pigmented (A) and unpigmented (B) cowpea genotypes.

emergence were due to differences in the physical seed coat structure between these two color groups. Significant differences existed for hundred-seed weight and proportion of seed coat among the cowpea genotypes studied. The mean hundred-seed weight of non-pigmented cowpea genotypes was 9.6 g, while pigmented cowpea had a 100-seed weight of 11.5g. The colored cowpea genotypes had a higher seed coat ratio than white colored cowpea genotypes. Seed coat, its content, thickness, and composition are plant defensive traits (Zhang *et al*. 2016), and non-pigmented cowpea appears to possess less of them.

Further, the electrical conductivity values also varied significantly within and between the cowpea seed group. The majority of the un-pigmented cowpea genotypes had leachate conductivities of greater than 35 μS/cm/ g seed, while all other colored cowpea genotypes had low leachate conductivity (<30 μS/cm/g seed). The correlation studies (Table 3) revealed that the field emergence had a significant and positive association (0.67**) with the proportion of seed coat, suggesting the unpigmented cowpea genotypes with a low proportion of seed coat had lower field emergence. Also, the field emergence had a significant and negative association with electrical conductivity (-0.72**), water uptake at 30 minutes of imbibition (-0.75**), and water uptake at one hour of imbibition (-0.7**), which suggests that the unpigmented genotypes, owing to low seed coat content imbibe water rapidly resulting into imbibitional damage as shown by higher electrical conductivity, thus recorded low field emergence. Generally, it is expected that the better the laboratory germination better the field emergence. However, no significant correlation or association was observed between field emergence and laboratory germination in the present investigation suggesting that laboratory germination could not be used for predicting field emergence in cowpea. Instead, the electrical conductivity of seed leachate could predict the field emergence of cowpea better. Similar observations on the electrical conductivity of seed leachate to be a better indicator of field emergence than laboratory germination has been reported in chickpea (Lamichaney *et al*. 2016), sunflower (Szemruch *et al*. 2019), and common bean (Kolasinska *et al*. 2000, da Silva *et al*. 2013).

There is a higher demand of white seed coloured (unpigmented) cowpea varieties due to absence of anti-nutritional components such as tannins, phenolics etc. However, the result of present investigation concludes that there exist a direct association between seed coat colour and seed vigour i.e. cowpea genotype with pigmented seed had higher vigour as compared to cowpea genotypes with unpigmented seed. Hence, there is a need to consider seed coat pigmentation as an important seed vigour trait in cowpea redding programme.

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