The Relationship Between Seed Coat Colour, Seed Quality and Yield Related Traits in Yardlong Bean (*Vigna Unguiculata* (L.) Walp.ssp. *Unguiculata* cv.-gr. *Sesquipedalis*)

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Short Report

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Abstract

Yardlong bean genotypes have wide variability in seed coat colours. This research sought to investigate association of seed coat colour with germination, yield and disease resistance of yardlong bean genotypes. Total nine yardlong beans genotypes three from each with different seed coat colours *i.e.* brown, black and bicolor were selected for this study. Eight important seed and horticultural traits like seed length, seed width, seed thickness, days of 50% flower, days of mature pods, average pod length, average pod width and pod yield per plant were recorded in all set of genotypes. Bicolor seed coat genotype exhibited highest germination percentage (85-100%) followed by brown (71-84%) and black (43-59). Water uptake capacity was found highest in bicolor genotype *i.e.* RCPY-2 (240%) followed by YB-7 (131.87%) and RCPY-1 (141.17%). Average pod length and pod yield per plant was also found highest in bicolor seed coat coloured genotype RCPY-2 (37.64 ± 11.03 g) and (1036.66 ± 115.9 g/plant) respectively. We also studied the disease resistance to yellow mosaic disease caused by mungbean yellow mosaic India virus (MYMIV) in field condition. Results from preliminary screening for MYMIV showed that bicolor seeds were resistant to MYMIV. The RCPY-2, a bicolored genotype was recorded lowest disease incidence, which can use as donor to MYMIV resistance breeding of Yardlong bean.

Introduction

Yardlong bean alternatively known as Chinese long bean, string bean, snake bean, snake pea, snap pea, *bodi*, pea-bean and asparagus bean (*Vigna unguiculata* (L.) Walp.ssp. *unguiculata* cv.-gr. *sesquipedalis*) is a dicotyledonous crop in the order Fabaceae, subfamily Faboideae (Syn. Papilionoideae), tribe Phaseoleae, subtribe Phaseolinae, genus *Vigna* and section *Catiang* (Timko and Singh, 2008). *Vigna* which contains several important cultivated legume species including cowpea (*V. unguiculata* (L.) Walp.ssp. *unguiculata*), blackgram (*V. mungo* (L.) Hepper), mungbean (*V. radiata* (L.) Wilczek), bambara groundnut (*V. subterranea*), moth bean (*V. aconitifolia*), adzuki bean (*V. angularis*) and rice bean (*V. umbellata*). Yardlong bean and cowpea are in the subgenus *Vigna*, whereas the mungbean and blackgram are in the subgenus *Ceratotropis* (Pasquet, 2001). Unlike other *Vigna* crops, yardlong bean is grown primarily for its crisp and tender pods, which can be eaten raw or cooked. Yardlong bean characterized by its very long (30–90 cm in length) succulent pods with seeds usually 8–12 mm long whereas cowpea has shorter pod (20–30 cm in length) (Verdcourt 1970). It is rich source of protein (25%), carbohydrates (64%), vitamins, fiber and folic acid (Hall, 2012; Timko and Singh, 2008). The world production of yardlong bean exceeds 17 million tons and productivity is 13,450 kg/ha (Huque et al., 2012) but productivity of India is very low and therefore high-yielding varieties need to be developed through utilization of valuable local and overseas germplasm collections. It is generally considered to be drought resistant compared to other leguminous crops (Belko et al. 2013; Fatokun et al. 2016). However, the ability to cope with low water potential vary significantly by the particular genotype (Watanabe et al. 1997 ; Muchero et al. 2008).

The seed coat acts as a barrier between the internal structures of the seed and the external environment and this further contributes to the protection of the embryo from mechanical injuries and attacks of pest

diseases (Tiryaki et al. 2016; Weber et al. 1996). Additionally, seed coat colour due to pigmentation is an important quality trait, domestication trait and morphological marker, and is closely associated with water intake and drought (Ayeh et al. 2021). In addition, the seed coat ensures that seeds survival of seed in harsh environmental conditions (de Souza and Marcos-Filho, 2001). Research findings have proven that seed size and coat colour are important characteristics for distinguishing different varieties of *Vicia sativa* (Buyukkartal et al. 2013).

Pod yield of yardlong bean falls far below the potential of the crop due to many problems (Kongjaimun et al. 2010). High among production constraints of yardlong beans are insect pests attack, diseases infestation and weather failure (Dugje et al. 2009). Yellow Mosaic Disease (YMD) is the most important viral disease in yardlong bean reported from entire India. The key symptoms are bright yellow patches on the leaves interspersed with green areas, and slight puckering; seed production is affected (Naraini 1960).

Development of new varieties addressing the various problems can help reduce the yield deficit. However, breeding against constraints without consumer acceptability, considerations may result in the rejection of the improved varieties. Seed coat colour is an important criterion for acceptability of any improved varieties. Relationship between seed coat colour with yield traits and disease resistance is increased the acceptability of any varieties. This study was therefore, designed to evaluate the genotypes of different seed coat colour and their possible correlation with pod yield and disease resistance.

Material And Methods

Plant material and seed multiplication

In yardlong bean three seed coat colour is visible mostly visible in eastern India. Based on seed coat colour total nine genotypes, three genotypes of each seed coat was selected (Table 1). Genotype evaluation and seed multiplication was done at the experimental field of ICAR-Research Center for Eastern Region, Patna, Bihar, India. All genotype and their seeds were grown and purified more than two years before included in this study. Agronomical interventions like plot preparation, fertilizers, fungicides and insecticides application were conducted based on the recommended practices for the crop. Proper care was taken to segregate each other to assure individuality of genotype. Diversifying qualitative characters like flower color, pod color, seed color and some selected yield parameters like days of 50% flower, days of mature pods, average pod length, average pod width and pod yield per plant were measured as per minimum descriptors of NBPGR.

Table 1
Source of genotypes used in this study

S.N	Genotype	Source	Seed coat colour	Plant growth habit	Pod pigmentation
1	YB-7	Bangalore, India	Bicolour	Pole	Light green
2	RCPY-2	Patna, India	Bicolour	Pole	Light green
3	RCPY-1	Motihari, India	Bicolour	Pole	Dark green
4	IC-626154	Kerala, India	Brown	Pole	Light green
5	IC-622602	Kerala, India	Brown	Pole	Light green
6	IC-626152	Kerala, India	Brown	Pole	Light green
7	IC-622601	Kerala, India	Black	Pole	Purple spot on whole pod surface
8	IC-626147	Kerala, India	Black	Pole	Light green pod with purple tip
9	IC-626153	Kerala, India	Black	Pole	Light green pod with purple tip

Seed quality and imbibition pattern

To determine the rate of water absorption, the initial weight of dry seeds was recorded on a digital balance in gram, and the seeds were then placed in a 100 ml beaker with 50 ml of water, covered with an aluminium foil, and held at 25°C. To determine water uptake in three different seed coat colours, the seeds of each genotype were weighed at one-hour interval for the first ten hours, then at 12-hour intervals until a plateau was reached. The surface of the seeds were dried using absorbent paper before and after each weighing, and the seeds were then placed back in the beaker. The percentage of water uptake (%) was calculated as the increase in seed weight relative to the initial weight of the seeds.

Seven seeds of each genotype whose initial weights were measured were left to uptake water in moist paper in four replications at 25°C. The seeds were weighed in 1h intervals. The study was ended when germination started. Amounts (%) of water uptake was calculated according to Demir et al. (2008) as follows:

$$W_2 = (100 - A/100 - B) \times W_1$$

Where: W_2 – indicates final seed weight; W_1 – indicates initial seed weight; A – indicates initial seed moisture content; B – indicates the final seed moisture content that seedlots reached

Standard germination test was conducted by keeping 10 seeds in germination paper (200 × 200 mm) for 14 days at 25°C. Seeds producing minimum 2 mm radical were counted as germinated seed.

Mosaic severity recording

Disease reaction was scored when 95% of the infector rows (PS 16) showed BCMV incidence under field conditions. The resistant plants did not show any mosaic symptoms on leaves or pods during the entire growth period, while the susceptible plants showed various grades of yellowing depending on the stages at which infection occurred. The disease rating was done using modified rating scale of Singh et al. 1988 (Table 2). Genotypes having different seed coat were classified into four reactions that is, susceptible (S), moderately susceptible (MS), moderately resistant (MR) and highly resistant (R) on the basis of disease scores and type of symptoms (Table 2).

Table 2
Reaction rating for Mung bean yellow mosaic India virus (MYMIV)

Types of symptoms		Reaction
1	No visible symptoms or yellow specks on leaves or pods	R (Resistant)
2	Minute yellow specks on leaves without any symptoms on pods	MR (Moderately Resistant)
3	No visible symptoms on leaves till flowering and showing yellowing and mottling of pods at maturity	MS (Moderately Susceptible)
4	Pronounced yellow mottling of foliage and discoloration of leaves and yellowing of pods and seeds	S (Susceptible)

Results And Discussion

Agro-morphological characterization

The clear variation was observed, as the colour of pod varied from light green to dark green with and without purple tip. In spite of different seed coat colour all plants are pole type and need support to climb up (Table 1). Black seed coloured genotype (IC-630383, IC-626147) yielded green pod with purple tip (Fig. 1a) while patches of purple colour were observed in whole pod in the genotypes IC-622601 (Fig. 1b). Most of the economical important traits found in IC-622601 were similar to wild type *Vigna* sp. having an indeterminate growth habit, bearing small seeds, differs in the extent of hard seededness and pod dehiscence from the cultivated species as reported by several researcher (Hammer 1984; Andargie et al.,2014). These results indicated that, there is a strong relationship between black seed coat colour and evolution of yardlong bean. Additionally pigmentation in immature pod and can be considered as important morphological marker in breeding programs. Correlation between pod tip colours with black

seed coat colour was also reported by Herniter et al., 2018. But different type of gene involved in seed coat colour, pod tip colour and pod pigmentation and their interaction is need to be studied.

Along with these qualitative traits some of selected seed parameters like seed length, seed width, seed thickness and yield related traits like days of 50% flower, days of mature pods, average pod length, average pod width and pod yield per plant were measured to study the relationship between seed coat colour, seed quality and yield related traits. The general statistical parameters for various characters are presented in Table 3.

Table 3
Estimates of variance for the traits studied in yardlong bean genotypes

Parameters	Seed length (cm)	Seed width (cm)	Seed Thickness (cm)	Days of 50% flower	Days of mature pods	Average pod length (cm)	Average pod width (cm)	Pod yield per plant (g)
Mean	1.1011	0.5078	0.4078	54.222	58.667	31.058	1.1500	878.22
SD	0.2707	0.0756	0.0524	8.8003	11.192	10.810	0.6230	148.47
SE Mean	0.0902	0.0252	0.0175	2.9334	3.7305	3.6033	0.2077	49.490
C.V.	24.581	14.894	12.847	16.230	19.076	34.805	54.174	16.906
Minimum	0.8000	0.4300	0.3300	43.000	41.000	17.600	0.6000	650.00
Maximum	1.5300	0.6700	0.5000	70.000	77.000	44.250	2.6000	1170.0

Correlation study (Table 4) among these quantitative traits indicated the significant positive correlation between seed length, seed width ($r=0.577$) and seed thickness ($r=0.608$). Days to 50% flowering trait was negatively significant with average pod length ($r=-0.7974$) and pod yield per plant ($r=-0.4767$). Days of 50% flower and days to mature pods showed significant positive ($r=0.7421$) relationship with each other. Seed length ($r=0.6063$) and average pod length ($r=0.6401$) recorded highly significant positive correlation between pod yield per plant. However, days of 50% flower and days to mature pods showed significant negative correlation with pod yield per plant. Interestingly, days to 50% flowering indicated negative correlated with all three seed dimension parameters, seed length ($r=-0.3208$), seed width ($r=-0.3485$) and seed thickness ($r=-0.1452$). Correlation study specified that there is a strong relationship between seed dimension and earliness of genotype as days to 50% flowering is indicator of earliness. Flowering time and seed size are the important traits for adaptation in Legumes. Early phenology (time of flowering, pod filling and maturity) enhance its adaptation to short season environments. Similar relationship was reported by Sundaram et al. (2019) found in *kabuli* chickpea.

Table 4
Correlation analysis of morphological traits in yardlong bean

Parameters	Seed length (cm)	Seed width (cm)	Seed Thickness (cm)	Days of 50% flower	Days of mature pods	Average pod length (cm)	Average pod width (cm)	Pod yield per plant (g)
Seed length (cm)	1							
Seed width (cm)	0.5778	1						
Seed Thickness (cm)	0.6085	0.8063	1					
Days of 50% flower	-0.3208	-0.3485	-0.1452	1				
Days of mature pods	-0.0193	0.0448	0.0007	0.7421	1			
Average pod length	0.5702	0.5296	0.3300	-0.7974	-0.5183	1		
Average pod width	0.5022	0.3383	0.6798	0.3842	0.4706	-0.1308	1	
Pod yield per plant (g)	0.6063	0.0415	0.1945	-0.4767	-0.5326	0.6401	-0.0623	1

Imbibition pattern

Significant differences were observed between genotype of different seed coat with respect to increase in seed mass after 1hrs of soaking. The trend showed a general increase on average until after 24 hrs (Fig. 2). All the genotypes selected for this study were of the same moisture content (*10.3%). Imbibition curves indicated that water absorption and weight gain of seed were highest in bicolor seed coat coloured genotypes compared to brown and black seed coat coloured genotypes used in the study (Fig. 2).

Water uptake is reported to be highest in bicolor seed RCPY-1 followed by RCPY-2 and germination of seed is also fastest in bicolor genotypes (YB-7, RCPY-2 and RCPY-1) as shown in Fig. 3 and 4. The highest water uptake during seed germination was observed in bicolor seed coat colour genotypes (RCPY-2>RCPY-1>YB-7). When the reading was taken on the 3rd day, it was observed that germination speed was also fastest in the bicolor genotypes compared to brown and black seed coat colour. The results showed that bicolor coloured seeds imbibed water more rapidly than black and brown coloured seeds. Fig. 4 (a, b) shows the speed of germination of different genotypes at 3rd day. These findings are similar

to those by Chibarabada et al. (2014) who reported that dark coloured Bambara seeds absorbed water slowly than other coloured seeds. This delay in absorption may be caused by substances present on the seed coat or due to the internal part of the seeds, which can block the metabolism preparatory to germination or prevent the free access of oxygen to the embryo or the release of carbonic gas. Various kinds of germination inhibitors, such as tannins (responsible for dark coloured seed coat), phenolic acids, alkaloids and aldehydes are known (Marcos Filho 2005). It was interesting to notice that genotype IC-630383 showed highest root elongation (Fig. 4a) among all genotype and moderate rate of water uptake. This genotype appears to be potential donor for abiotic stress-resilient breeding programs.

Disease screening

Yellow mosaic disease (YMD) is most devastating disease of cultivated legumes in South-East Asia. In Eastern India, YMD is caused by Mungbean yellow mosaic India virus (MYMIV) and belonging to the genus Begomovirus of the family Geminiviridae (Haq et al. 2010). MYMIV is widely distributed, causing 10–100% yield losses once infected in standing crop of yardlong bean (Nene 1969, Nene et al. 1972, Chenulu et al. 1979, Marimuthu et al. 1981). In the fields, the leaves of symptomatic plants showed severe mosaic with bright yellow and vein-clearing symptoms and as the season advanced, the leaves showed bright yellow mosaic symptoms followed by necrosis and the death of infected plants.

The per cent disease intensity among the genotypes of different seed coat colour ranged from 6.25 to 78.25%. All three bicolour seed coat colour genotypes (YB-7, RCPY-2 and RCPY-1) were resistant to MYMIV. However, brown seed coat coloured lines showed moderate resistance (IC-622602, IC-626152) to resistant (IC-626154) disease reaction (Table 5). Genotype IC-626154, showed minimum mosaic incidence (6.25%). Black coloured seeds showed varied response to MYMIV. Among black coloured seeded genotype, IC-630383 was moderately resistant and IC-622601 was resistant. However, genotype IC-626147 was found to be moderately susceptible against MYMIV with highest mean mosaic incidence 78.25%. Our findings concurred with previous findings by Islam et al. (2003) indicating the resistance of dark-coloured common bean cultivars involved in resistance to diseases and pests, and reducing tannin concentration may have a negative effect on plant resistance.

Table 5
Reaction of yardlong bean genotypes against *Yellow bean mosaic virus* in field condition

Genotypes	Seed colour	Mean(^x) mosaic incidence (%)	Disease Reaction
YB-7	Bicolour	12.00 (20.24) ^b	Resistant
RCPY-2	Bicolour	8.00 (16.39) ^a	Resistant
RCPY-1	Bicolour	8.50 (16.94) ^a	Resistant
IC-626154	Brown	6.25 (14.47) ^a	Resistant
IC-622602	Brown	38.50(38.32) ^c	Moderately Resistant
IC-626152	Brown	35.75(36.70) ^c	Moderately Resistant
IC-630383	Black	34.35 (35.88) ^{c,d}	Moderately Resistant
IC-626147	Black	78.25(62.21) ^e	Moderately Susceptible
IC-622601	Black	15 (22.71) ^b	Resistant
* Figures in parentheses are angular transformed values			
'x' Means followed by the same letter in a column within each year are not significantly different (P≤0.05) according to Fisher's protected least significant difference test using values after angular transformation of the proportion of wilt.			

Conclusion

This study reported relationship between seed coat colour and water uptake relation and yield related traits in yardlong bean genotypes. Bicolour genotype RCPY-2 was identified as high yielding and disease resistant genotype and advanced form of cultivated type while black seed coat were wild type. Seed coat may be used as an important parameter to prescreen high yield genotypes of yardlong bean. Additionally, the knowledge of seed coat colour and its linkage with identified potential germplasm appears to be possible donor for yellow mosaic disease resistant and water stress-resilient yardlong bean breeding programs.

Declarations

Conflict of interest

The authors declare that they have no conflict of interest on the manuscript's content and study undertaken.

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Figures

Figure 1

1a. Black seed coat coloured genotype (IC-630383, IC-626147) yielded green pod with purple tip; **1b.** Black seed coat coloured genotype patches of purple colour were observed in whole pod in the genotypes IC-622601

Figure 2

Imbibition curves of yardlong bean genotypes of different seed coat colour

Figure 3

Water uptake and germination percentage of yardlong bean genotypes of different seed coat colour (bicolored, brown, black)

Figure 4 (a)

Figure 4 (b)

Figure 4

(a) showed the speed of germination of black seed coat yardlong bean genotype

(b) showed the speed of germination of bicolored seed coat yardlong bean genotype