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ABSTRACT

Cultivated varieties of oilseed Brassica belonging to seven different taxa were scored from 1 to 5 based on seed coat color. *Brassica rapa* var. yellow sarson (1.18) group scored the lowest mean value while *B. napus* (4.84) group scored highest. The average oil content ranged between 37.19 % (*Eruca sativa*) and 43.33 % (yellow sarson). The mean crude fiber content was found highest in Brown sarson group (10.99 %) and lowest in yellow sarson (7.47 %). *B. carinata* (1.73 %) showed highest total phenol content while it was lowest in Toria (1.2 %). We also observed that there is a significant negative correlation between crude fiber content and total phenols (r= -0.23). Flavonoid content was highest in the *Eruca sativa* group (1160 µg/g QE) and lowest in yellow sarson (472.94 µg/g QE). The significant negative correlation between flavonoid content and oil content (r= - 0.76) shows that high flavonoid Brassica lines can be expected to have low oil content. There was a positive correlation between seed coat color and crude fiber content (r= 0.61). Hence it is hypothesized that seed coat color can be considered as a morphological marker or index for identification of low fiber lines of oilseed Brassica.

INTRODUCTION

Oilseed Brassica is the source of the third most consumed edible oil in the world after soybean and oil palm (Chandra *et al.,* 2013). It is one of the major sources of edible oil and seed meal with potential nutritional value. Since India is one of the places of origin of this crop, it has a rich and diverse germplasm of all the cultivated species of oilseeds belonging to the Brassicaceae family including *Brassica juncea*, *Brassica rapa* (yellow sarson, brown sarson and toria), *Brassica napus*, *Brassica carinata* and *Eruca sativa*. Apart from having very high oil content (>42%), its de-oiled meal is a source of high quality protein (>40%) and can be used as animal feed for dairy cows, pigs and poultry (Naczk *et al.*, 1998). The canola meal is reported to contain 21.5 % neutral detergent fiber (NDF) and 17.5 % acid detergent fiber (ADF) (Bell and Keith, 1991). However, high fiber seed meal is also not preferable for feeding non ruminant animals as it causes indigestion. Another major drawback for the use of rapeseed meal as animal feed is phenolic compounds such as phenolic acids and condensed tannins that are present in levels almost five times higher than that found in soybean meals (Nazck *et al.*, 2002). This study was intended to quantify various biochemical properties in different species of oilseed Brassica and see how it correlated with seed coat color. The idea was to link seed color to any of the quality parameters for easier initial screening of Brassica lines for quality.

The seed coat color in oilseed Brassica ranges from yellowish to dark brown or black. And the yellow seeded lines are considered advantageous as that they have thinner seed coat resulting in 5-7 % more oil content and increased value for seed meal due to lower fiber, higher protein and more digestible energy (Bell, 1993). Yellow seeded lines delivered more metabolizable energy to poultry compared to the dark seeded lines. According to an earlier study, yellow seeded *B. juncea*, *B. napus* and *B. rapa* had low fiber content compared to their black seeded counterparts (Slominski *et al.*, 1999). It may be noted that the cause for seed coat pigmentation has been reported to be different for different species of oilseed Brassica and it functions as a quantitative trait. It has been reported that the QTL for seed color have an effect on phenolic and fiber content in oilseed rape (Rezaeizad *et al.*, 2011). The genes Transparent testa glabra 1 (*TTG1*) encoding WD-40 protein (Zhang *et al.,* 2009) and Transparent testa 8 (*TT8*) encoding a transcription factor (Li *et al.,* 2012) has been suggested to be involved in seed pigmentation. Hence, the breeding programmes aimed at developing yellow seeded varieties can target the genes involved in the phenylpropanoid pathway which leads to the production of both the flavonoids and some of the fiber components. Also, SSR markers have been identified that can be used for marker assisted breeding for development of yellow-seeded *B. rapa*, introgression of yellow seed color in *B. napus* as well as cloning of genes under this QTL (Kebede *et al.*, 2012).

There are certain external factors that may also affect the seed coat color. Specific genotypes do not exhibit same phenotypic characteristics in all environmental conditions. Also, their growth responses vary with the which ultimately decides its selection for a particular trait (Dinda *et al.,* 2015). The role of environmental growing conditions, temperature and UV radiation in regulation of tannin biosynthesis and condensation on seed color has been reported earlier (He *et al*., 2008). It has also been proposed that environmental factors such as temperature and light also play a role in the pigmentation (Qu *et al*., 2013). The reason for such changes may be that at high temperatures the phenylpropanoid biosynthetic genes are down-regulated or the enzymes are deactivated. It has been suggested that there could be alternate metabolic pathways including lignin, melanin as well as enzymes involved in oxidative reactions such as polyphenol oxidases that may play a role in determining seed color (Yu, 2013). Condensed tannins also known as proanthocyanidins (PAs) get deposited in the palisade cells during early stages of development and later gets oxidised to form brown colored PAs (Haughn and Chaudhury, 2005). PAs are considered as antinutritional factors as they form indigestible, astringent or bitter-tasting complexes with proteins. They are responsible for the dark color of the seed coat. Mutation in flavonoid biosynthetic pathways that lead to failure of deposition of PAs in seed produces transparent seed coat that makes the yellow embryo visible resulting in yellow seed color (Badani *et al*., 2006). The paper deals with quantification of the total phenolic content and flavonoid content under the assumption that the relative abundance of PAs would have an influence on these parameters.

MATERIALS AND METHODS

Seeds of 45 different cultivars of rapeseed-mustard including 15 varieties of *Brassica juncea* and 5 varieties each of *Brassica rapa* var. Yellow sarson, *Brassica rapa* var. Toria, *Brassica rapa* var. Brown sarson, *Brassica napus*, *Brassica carinata* and *Eruca sativa* were selected for the study.

Seed color scoring

The seeds were visually examined and scored based on seed coat color following the method of Bagheri *et al.* (2012). Scoring was done from 1 to 5 with maximum score given to the darkest seed color. The *Brassica juncea* varieties Basanti, CS-52, RCC-4, RGN-73 and DRMR-1288 were used as reference standards for yellow, reddish brown, brown, dark brown and black seed coat colors respectively. Seed coat colors that are intermediate to these 5 colors were scored accordingly by assigning fractional values.

Total Phenol and Crude fiber

Total phenols and crude fiber content were measured by non destructive method using Bruker matrix-1 FT-NIR as reported in a previous study (Bala and Singh, 2013).

Oil Content and Total Flavonoids

Oil content was determined gravimetrically after extracting with hexane. Total flavonoid content was estimated in methanolic extracts prepared from the seed meal using a spectro UV-Vis Double beam UVD-3500 spectrophotometer (Zhishen *et al*., 1999). Flavonoid content was expressed as quercetine equivalents(QE).

Statistical analysis

The experiment was conducted in CRD design with three replications for each parameter. Data was analysed using a mixed model of analysis of variance (ANOVA) with species as fixed effect and genotypes nested within species as a random effect using the statistical programming language R. The species effect was tested against the random effect of genotypes nested within species. The mean differences between species were compared by Tukey's test using the R package Tuckey C. $P < 0.05$ was considered as statistically significant.

RESULTS AND DISCUSSION

Variation in seed coat color among different species of rapeseed-mustard

Yellow seed coat color in Brassica oilseeds is actually due to the transparent testa which makes the yellow embryo clearly visible from outside (Sagasser *et al.,* 2002; Bharati and Khurana 2003). Considerable variation in seed coat color was observed among the 45 lines. The seed color score ranged from 1.1 to 4.9 (Table 1). It was Ragini and Jhumka varieties that belonged to *B. rapa* ssp. yellow sarson group that scored the lowest making them the lightest colored seeds among the 45 varieties .*B. napus* varieties Sheetal, Neelam and GSL-1 scored the highest (4.9) and are the darkest. It was observed that *Brassica napus* varieties had the highest mean seed color score (4.84) (fig 1 and 2) followed by Brown sarson (4.54), Toria (4.40) and

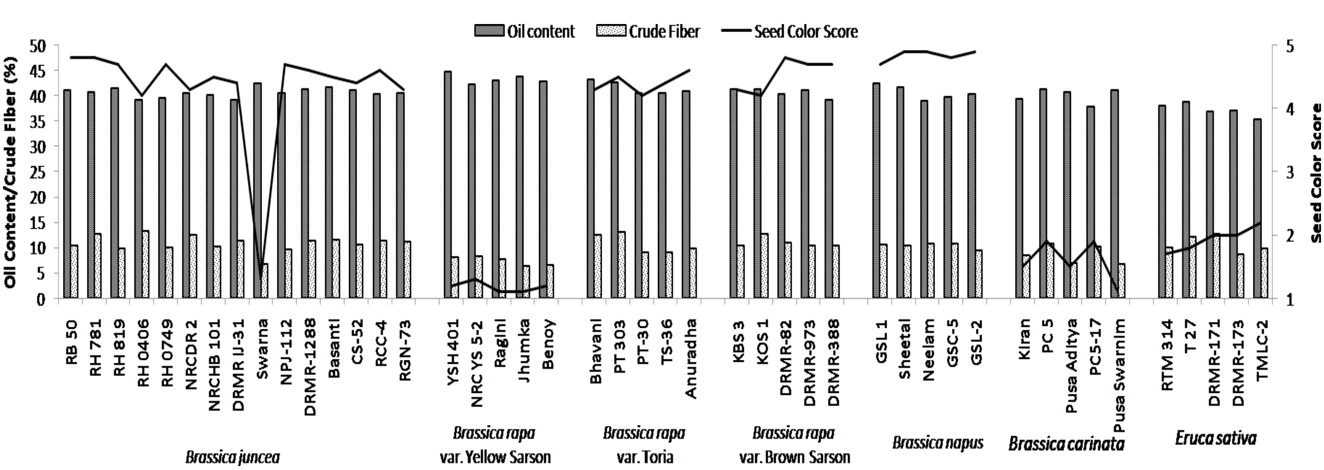

Figure 1: Graphical representation of variation in seed color, fiber and oil content among different species of oilseed Brassica

Table 1: Seed coat color, oil, crude fiber, total phenols and total flavonoids among different species and genotypes of oilseed Brassica

Taxa	Variety	Seed colour score	Oil content (%)	Crude fiber (%)	Total phenols (%)	Total flavonoids (μg/g QE)
Brassica juncea	RB 50	4.8	41	10.34	1.69	733.33
	RH 781	4.8	40.77	12.67	1.52	639.22
	RH 819	4.7	41.5	9.91	1.87	568.63
	RH 0406	4.2	39.22	13.39	1.41	662.75
	RH 0749	4.7	39.46	10.11	1.52	815.69
	NRCDR 2	4.3	40.45	12.54	1.54	611.76
	NRCHB 101	4.5	40.14	10.14	1.81	674.51
	DRMR IJ-31	4.4	39.17	11.31	1.5	576.47
	Swarna	1.3	42.52	6.85	1.78	631.37
	NPJ-112	4.7	40.5	9.73	1.91	600.00
	DRMR-1288	4.6	41.22	11.44	1.72	745.10
	Basanti	4.5	41.58	11.57	1.76	686.27
	CS-52	4.4	41.05	10.69	2.09	737.25
	RCC-4	4.6	40.41	11.41	1.54	741.18
	RGN-73	4.3	40.52	11.25	1.54	776.47
Brassica rapa var. yellow sarson	YSH 401	1.2	44.8	8.15	1.33	443.14
	NRC YS 5-2	1.3	42.19	8.34	1.33	517.65
	Ragini	1.1	43.08	7.79	1.41	443.14
	Jhumka	1.1	43.74	6.43	1.32	486.27
	Benoy	1.2	42.85	6.66	1.29	474.51
Brassica rapa var. Toria	Bhavani	4.3	43.23	12.44	1.2	541.18
	PT 303	4.5	42.53	13.1	1.12	705.88
	PT-30	4.2	40.47	9.07	1.29	658.82
	TS-36	4.4	40.54	9.03	1.22	576.47
	Anuradha	4.6	40.86	9.91	1.17	705.88
Brassica rapa var. Brown Sarson	KBS 3	4.3	41.25	10.44	1.05	607.84
	KOS 1	4.2	41.19	12.63	1.13	807.84
	DRMR-82	4.8	40.26	10.94	1.75	705.88
	DRMR-973	4.7	41.18	10.42	1.15	623.53
	DRMR-388	4.7	39.08	10.51	0.75	537.25
Brassica napus	GSL 1	4.7	42.38	10.65	1.14	725.49
	Sheetal	4.9	41.66	10.49	1.36	725.49
	Neelam	4.9	39.07	10.82	1.41	564.71
	GSC-5	4.8	39.66	10.72	1.28	745.10
	GSL-2	4.9	40.41	9.54	1.33	670.59
Brassica carinata	Kiran	1.5	39.42	8.58	1.52	678.43
	PC 5	1.9	41.19	10.85	1.5	839.22
	Pusa Aditya	1.5	40.78	7.01	2.09	749.02
	PC5-17	1.9	37.88	10.25	1.63	839.22
	Pusa Swarnim	1.1	41.12	6.69	1.89	713.73
Eruca sativa	RTM 314	1.7	38.06	10.01	1.48	945.10
	T 27	1.8	38.69	12.17	1.21	1066.67
	DRMR-171	2	36.79	12.63	1.08	1121.57
	DRMR-173	2	37.04	8.78	1.44	1243.14
	TMLC-2	2.2	35.37	9.89	1.19	1423.53

Table 2: ANOVA showing significant variation in Seed coat color, oil, crude fiber, total phenols and total flavonoids among different species and genotypes

Source	df	Seed colour score	Oil content	Crude fiber	Total phenols	Total flavonoids
Residuals	90	0.01	0.0001	0.0001	0.0001	0.0001
Taxa	6	43.02 **	50.56 **	30.92 **	1.1 **	682212.82 **
Variety	38	0.89 **	3.77 **	6.38 **	0.12 **	25725.49 **

B. juncea (4.32) having scored on par with each other. Available reports shows that naturally occurring *B. napus* has black seed coat color and interspecific crosses with *B. carinata* (BBCC) and *B. rapa* var. Yellow sarson (AA) had to be done to develop yellow seeded type *B. napus* (AACC) (Rahman, 2001). Also, the black seeded type was found to be dominant over yellow and seed color was controlled by three independent duplicated genes in *B. napus* (Deynze and Pauls 1993). *B. juncea* seeds were mostly dark brown seeded and had scores just below *B. napus* varieties. The inheritance of seed color in *B. juncea* has been found to be under control of maternal genotype and brown seeded trait was dominant over yellow-seeded trait (Xu *et al.*, 2010). This is probably why most *B. juncea* varieties are dark seeded. *B. rapa* var. Yellow sarson

Table 3 : Correlation between seed color, oil content, crude fiber, total phenols and flavonoids

	Seed color score	Oil content	Crude fiber	Total phenols	Total flavonoids
Seed Colour Score	1				
Oil content	-0.03	1			
Crude Fiber	0.61**	-0.28**	1		
Total phenols	-0.06	0.07	-0.23**	1	
Total flavonoids	-0.16	-0.76**	0.23**	-0.05	1

had the lowest score (1.18) as they had light yellow seed coat color, followed by *B. carinata* and *Eruca sativa* (1.94). The scores suggest that *B. napus*, *B. juncea*, Brown sarson and toria are dark brown to black in color. Previous studies in *B. rapa* have shown that seed color is under the control of maternal genotype and is governed by a single gene and yellow seed color was partially dominant over black seed color (Zhi-Wen *et al.*, 2005). This may be the reason why yellow seeded type of *B. rapa* is occurring naturally. *B. carinata* and *Eruca sativa* were reddish brown to brown in color. It has been found that PAs were deposited in entire seed coat of black seeded *B. napus* and in patches in yellow seeded lines (Marles and Gruber, 2004).

Crude fiber content correlates significantly with seed coat color

Yellow seeded genotypes are said to be superior in meal quality in *B. napus* as they have thinner seed coat due to which they can be expected to have higher protein content and less fiber components (Rahman and McVetty, 2011; Liu *et al.,* 2012). In our study, *B. juncea* variety RH-406 showed the highest crude fiber content (13.39 %) while yellow sarson variety jhumka showed the lowest fiber content (6.43 %) (Table 1). The mean crude fiber content was highest in Brown sarson (10.99 %) followed by *B. juncea* (10.89 %), toria (10.71 %) and *Eruca sativa* (10.70 %) which had mean values on par with each other, while the lowest fiber content was in the yellow sarson group (7.47 %). A significant positive correlation was observed between seed coat color and crude fiber content (r=0.61) (Table 3). Previous reports shows that the palisade layer of seed coat is about two-thirds thinner in yellow seed coat lines than in black/brown seed coat due to lower fiber content (Stringam *et al.*, 1974). A major QTL with effects on seed color, condensed tannin content and seed acid detergent fiber (ADF) was mapped in black-seeded × yellow-seeded doubled haploid (Snowdon *et al.*, 2010).

Earlier reports also have confirmed that seed meal from yellow-seeded varieties contains higher protein and lower fiber which enhance the quality of poultry and livestock feed based on rapeseed meal (Shirzadegan and Robbelen, 1985). A study comparing the meal quality of *B. rapa*, *B. juncea* and *B. napus* reported that the yellow seeded *B. napus* had the lowest fiber content (271 g/kg) while the black seeded *B. napus* had the maximum fiber content (352 g/kg). The meal from yellow seeded *B. napus* was found to be the best feed for broilers in terms of metabolizable energy (Slominski *et al.*, 1999). This gives the idea that seeds with dark seed coat color can be expected to have higher fiber content. As yellow sarson, *B. carinata*, *Eruca sativa* as well as yellow seeded *B. juncea* varieties like swarna are having low fiber content, they can be used as sources of good quality animal feeds.

Highest oil content of 44.8 % was recorded by YSH-401 (Table 1) which is a yellow sarson variety while the lowest was recorded by TMLC-2 (35.37 %) an *Eruca sativa* variety. Yellow sarson varieties showed the highest mean oil content (43.33 %) followed by toria (41.53 %), *B. napus* (40.64 %) and *B. juncea* (40.63 %). Our observations regarding the negative correlation between crude fiber and oil content (r= -0.28) were also in agreement with earlier reports (Naczk *et al.*, 2002). But we did not see much significant correlation (Table 3) between seed color and oil content (r= 0.03) which is contrary to the popular notion that light colored seeds are high in oil content compared to dark seeded ones.

Phenolic compounds and their role in imparting seed coat color

It has been reported that deposition of PAs is strongly reduced in yellow seeded *B. carinata* than those with black seeds suggesting the role of PAs in imparting dark seeded trait (Li *et al.*, 2010). Also in *Arabidopsis thaliana* which is a related species, deposition of PAs has been described and is said to happen exclusively in the inner integument and chalaza zone (Dean *et al.,* 2011). The biosynthesis of PAs and phenolic lignin has common precursors and are expected to both contribute to seed color.

In this study, the highest phenol content was observed in the *B. juncea* variety CS-52 and the lowest in DRMR-388 a brown sarson variety. The mean value was highest in *B. carinata* (1.73 %) followed by *B. juncea* (1.68 %) and *B. napus* (1.30 %) (Table 1). At the same time, highest total flavonoid content was recorded by TMLC-2 an *Eruca sativa* variety. In fact all *Eruca sativa* varieties showed very high levels of total flavonoids compared to other species. They had an average value of 1160 μg/g QE. The lowest flavonoid content was observed in yellow sarson varieties YSH-401 and ragini (443.14 μg/g QE). The mean value for yellow sarson varieties was 472.94 μg/g QE. Low levels of PAs may be the reason why yellow seeded lines are having low total flavonoid content.

We also observed that there is a significant negative correlation between crude fiber content and total phenols (r= -0.23) (Table 3). This suggests that in high fiber lines the biosynthetic pathway is more biased towards fibers given the fact that both have common precursors. Surprisingly, the correlation of seed coat color with total phenols (r= -0.06) and total flavonoids were not significant enough to support the earlier reports suggesting that flavonoid compounds such as proanthocyanidins (PAs) are primarily responsible for the seed coat color. Our study is in agreement with previous findings (Lipsa *et al.*, 2007) which states that seed coat color do not correlate significantly with total flavonoid content.

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