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Full Length Article

Seed Coat Color Determines Seed Germination, Seedling Growth and Seed Composition of Canola (*Brassica napus*)

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Abstract

Canola (*Brassica napus* L.) seeds were sorted based on seed coat color into black (BL), dark brown (DBR) and light brown (LBR). Germination, seedling growth and seed composition (oil, protein and soluble sugar contents) were evaluated among seeds of different categories. From dark to light seed coat colors, germination percentage and germination index (GI) declined and mean germination time (MGT) increased; emergence percentage, seedling vigor index (SVI), shoot and root length decreased; seed oil content decreased but protein and soluble sugar content increased. Correlation matrix indicated that oil content was positively, but the protein and soluble sugar content were negatively correlated to seed germination and seedling growth. Almost all seed germination characteristics were either negatively or positively correlated to those of seedling growth ($r = -0.94$ to 0.99, $P=0.00$ to 0.03) with the exception between germination percentage and shoot length ($r = 0.52$, $P = 0.08$). It was concluded that seeds in the BL category showed fast germination, high emergence percentage and high seedling establishment, and the science behind this is that seed coat color determines seed composition, which further affects seed germination and seedling growth.

Keywords: Canola; Seed coat color; Germination; Seedling growth; Seed composition

Introduction

Canola (*Brassica napus* L.) is grown in many parts of the world as an important source of edible oil. Seed yield and oil content determine the oil yield, thus increasing seed yield remains an important objective in canola cultivation. Seed germination and successful seedling establishment are two independent events determining crop yield (Jain and Staden, 2007). Therefore, in order to obtain high yield, seeds with high germination potential and seed vigor should be selected to ensure optimum seedling establishment until they can photosynthesize independently (Bewley and Black, 1994).

However, stage of seed development at harvest influences canola seed quality, which may influence seed vigor and seedling growth (Elias and Copeland, 2001). Furthermore, seed coat color is an important indicator of maturity and differences in seed color within a cultivar are associated with harvesting seeds at different developmental stage (Elias and Copeland, 2001; Atis *et al.*, 2011). Previous studies on a number of crops indicated that seed coat color affected seed germination percentage, emergence percentage, seedling dry weight and water uptake ratio. For example, in guar (*Cyamopsis tetragonoloba* L. Taub) seeds, black colored seeds had a greater water uptake rate and higher germination than dull-white-colored seeds (Liu *et al.*, 2007). In *Atriplex cordobensis* seeds, light-brown and reddish colored seeds had greater germination percentages and seedling dry weight than dark-brown colored seeds (Aiazzi *et al.*, 2006). In red clover, yellow colored seed lots showed the highest germination percentage and emergence percentage and shortest mean germination time (Atis *et al.*, 2011).

Studies on seed coat color of B. *napus* were mostly focused on different genotypes or species for plant breeding purposes. Downey *et al.* (1975) reported that seed in naturally occurring yellow seeded genotypes in *B. rapa*, *B. juncea* and *B. carinata* contains greater oil, higher protein and lower fiber contents than the black/brown seeded genotypes. Plant breeders around the world have been trying to develop yellow seeded B. *napus* genotypes using crosses which involve naturally occurring yellow seeded *Brassica* species (Rahman and McVetty, 2011). Consequently, most of research efforts focus on characteristics of different genotypes with seed coat colors in *B. napus* and molecular markers associated with seed color traits. However, little work has been done on effects of seed coat color within one accession/cultivar on germination and seedling growth of canola.

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The objective of this study was to determine how seed coat color affects seed germination and seedling growth within a genotype. In addition, the relationships among seed germination, seedling growth and oil, protein and soluble sugar contents from seeds in different categories were also analyzed.

Materials and Methods

Seed Material

This study was carried out at the Ministry of Agriculture (MOA) key laboratory of Huazhong Crop Physiology, Ecology and Production, College of Plant Science and Technology, Huazhong Agricultural University, China. Canola conventional cultivar - Hua-Shuang No. 5 from Huazhong Agriculture University is a winter type oilseed cultivar widely grown in Hubei province of China. Seeds were collected, dried and stored in the refrigerator at 4°C until experimentation. About 10 g cleaned seeds were grouped into black (BL), dark brown (DBR) and light brown (LBR) seed coat colors (Fig. 1) by naked eyes, the proportion of each seed coat color category was calculated and replicated four times (Table 1).

Germination Trials

Seeds were surface sterilized with 1% sodium hypochlorite solution for 15 min and then rinsed with distilled water six times. Thereafter, seeds were blotted dry to remove excess water and allowed to dry at room temperature until their initial weight. Filter papers was sterilized by autoclave and germination test was carried out in polyethylene boxes（12 cm length × 12 cm breadth × 6 cm height）containing three sheets of sterile filter paper moistened with 10 ml distilled water in the growth chamber. The germination boxes were also sterilized with 1% NaClO solution for 15 min and rinsed before use. The temperature of the growth chamber was maintained at 25±1°C/20±1°C based on a 12 h photoperiod (4800 lx).

A seed was considered to be germinated when the 1-2 mm radicle appeared. Germination percentage was recorded each day until the 7^{th} day when the seedling emergence percentage was recorded and the seedlings were harvested. Ten seedlings were measured for root and shoot length.

Mean Germination Time (MGT) =∑(Dn)/∑n, (Ellis and Roberts, 1981), Germination Index (GI) = ∑(D/n) (ISTA, 1999), where n is the number of seeds germinated on day D (D representing the number of days since sowing) and Seedling Vigor Index (SVI) = percentage germination × seedling length (Shoot length + Root length) were calculated (Baki and Anderson, 1973).

Chemical Analysis of Seed Composition

Soluble sugar content: Total soluble sugars were determined by the phenol-sulphuric acid method (Pei *et al.*, 2010). The fine powder of seeds was boiled in distilled water for 30 min twice. The extract was filtered through two layers of cheesecloth into a flask. The filtrate (0.5 mL) was mixed with 1.5 mL distilled water and 1 mL of 9% phenol, and then 5 mL H_2SO_4. Mixtures were kept at room temperature for 30 min. Color change was estimated using a UV spectrophotometer at 485 nm. The soluble sugar concentration was determined using a standard curve.

Oil content: Seed oil content was determined using the Soxhlet extraction according to the official method (Harwood, 1984). From each category, 1 g seed was ground and extracted with petroleum ether in a Soxhlet apparatus for 6 h. Petroleum ether was evaporated under reduced pressure using a Rota vapor. Oil content was expressed as % of seed dry weight.

Protein content: Seeds were ground and digested according to Rutherford *et al*. (2008). The digested solution was measured using a Segmented Flow Analyzer, an automated colorimetric analyzer (Futura, Alliance Instruments, Frépillon, France), then the total nitrogen content was calculated by the nitrogen concentration. Crude protein concentration was estimated by applying the factor N × 6.25 to the total nitrogen content. Protein contents were expressed in percentage (%) of dried seeds.

Experimental Design and Statistical Analysis

The experiment was arranged in a completely randomized design with four replications and 100 seeds per replicate. Statistical analyses were conducted with SAS system V8.0. Data for germination and emergence percentage were subjected to arcsine transformation before analysis of variance (ANOVA). The differences between the means were compared by Duncan's new multiple range test ($P<0.05$).

Results

Seed Germination

The canola germination was significantly affected by seed coat color. From darker to lighter seed coat colors, the germination declined rapidly. The germination percentage in BL seed coat color was 89.83% on the 2^{nd} day, while, 67.58% and 56.83% in the DBR and the LBR seed coat color categories, respectively. Later on, from 3^{rd} to 7^{th} day, the difference of germination percentage became smaller and the final germination percentage at 7^{th} was significantly different ($P < 0.05$), showing 99.25%, 95.33% and 89.50% in BL, DBR, LBR seed coat color categories, respectively (Fig. 1). Similar trend was observed for GI and MGT with decreased GI and increased MGT from darker to lighter seed coat color categories (Fig. 2a and b).

Seedling growth: The seedling growth, in terms of the emergence percentage, SVI, shoot and root length, was

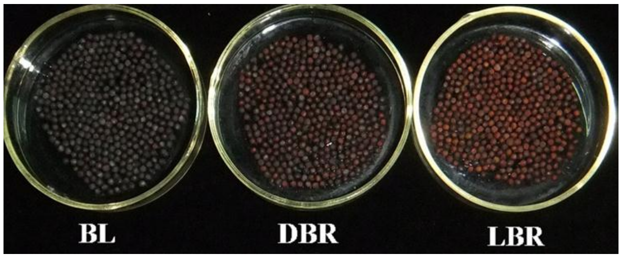

Fig. 1: Seed coat color differences in the three seed categories black (BL), dark brown (DBR) and light brown (LBR)

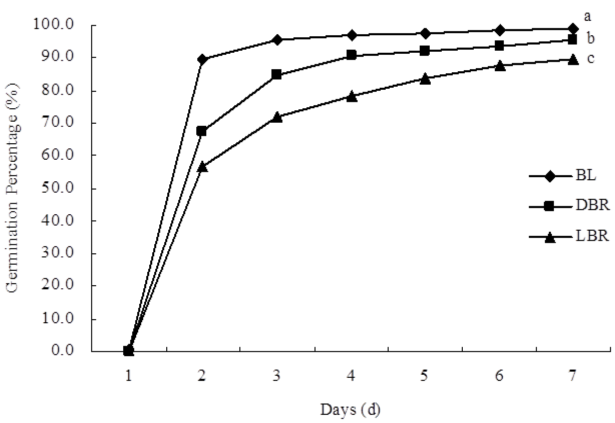

Fig. 2: Germination progress of canola with different seed coat colors. The different lower case letters at the end of each line indicate significant difference of the final germination percentage (%) ($LSD_{0.05} = 1.20$)

also affected by seed coat colors. From darker to lighter seed coat color categories, all seedling growth characteristics decreased. The emergence percentages and SVI of BL, DBR, LBR seed coat color categories were significantly different with 89.50, 76.92, 60.08% and 894.34, 815.43, 745.51, respectively (Fig. 3a and b). The shoot and root lengths from BL, DBR to LBR seed coat color categories showed a general decrease, but only the differences of shoot and root lengths between BL and LBR seed coat color categories were statistically significant (P<0.05; Fig. 3c and d).

Seed composition: Seed coat color was significantly associated with seed oil, protein and soluble sugar contents. The oil content of seeds in the BL seed coat color category was 41.13% and significantly (P < 0.05) higher than DBR (40.58%) and LBR (40.60%) categories (Fig. 4a). The protein content increased significantly (P < 0.05) from darker to lighter seed coat color, showing 27.51%, 29.90% and 30.70% in BL, DBR and LBR seed coat color categories, respectively (Fig. 4b). For soluble sugar content, LBR seed coat color category showed the highest values of 7.26%, significantly (P < 0.05) higher than DBR (5.96%) and BL (5.80%) categories with the later two showing no significant difference (P > 0.05) (Fig. 4c).

Table 1: The proportion of each seed coat color category in the seed lot

Seed coat color	Proportion (%)
BL	74.73±1.01a
DBR	14.39±0.82b
LBR	10.88±0.47c

All data was represented as mean of four replications ±SE (n=4). Within a column, different lowercase letter indicated significantly different at P < 0.05 by Duncan's multiple range test.

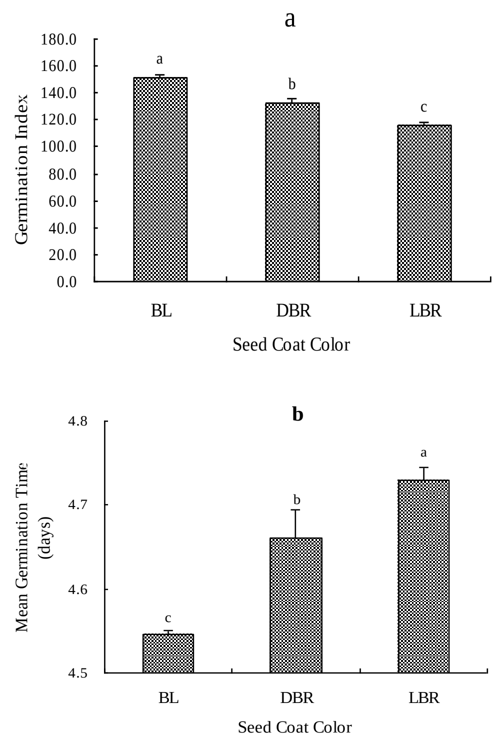

Fig. 3: Germination characteristics of canola with different seed coat colors. (a) Germination index ($LSD_{0.05} = 3.46$).

Correlation of Characters among Seed Coat Color Categories

The oil content was negatively correlated to the protein content (r = -0.69, P = 0.01), but correlation was insignificant negative with the soluble sugar content (r=-0.36, P = 0.24). Protein content was significantly correlated to the soluble sugar content (r = 0.72, P = 0.01). The oil content was positively correlated with seed germination and seedling growth. Seeds with higher oil content showed higher germination percentage (r=0.61, P=0.04) and faster germination speed as reflected by higher GI (r = 0.68 , P = 0.01) and lower MGT (r = -0.73, P = 0.01), also higher emergence percentage (r = 0.62, P = 0.03), SVI (r = 0.66, P = 0.02) and longer root length (r = 0.63, P=0.03), but the correlation between oil content and shoot length was not significant (r = 0.23, P = 0.48). The protein and soluble sugar content were negatively correlated with seed

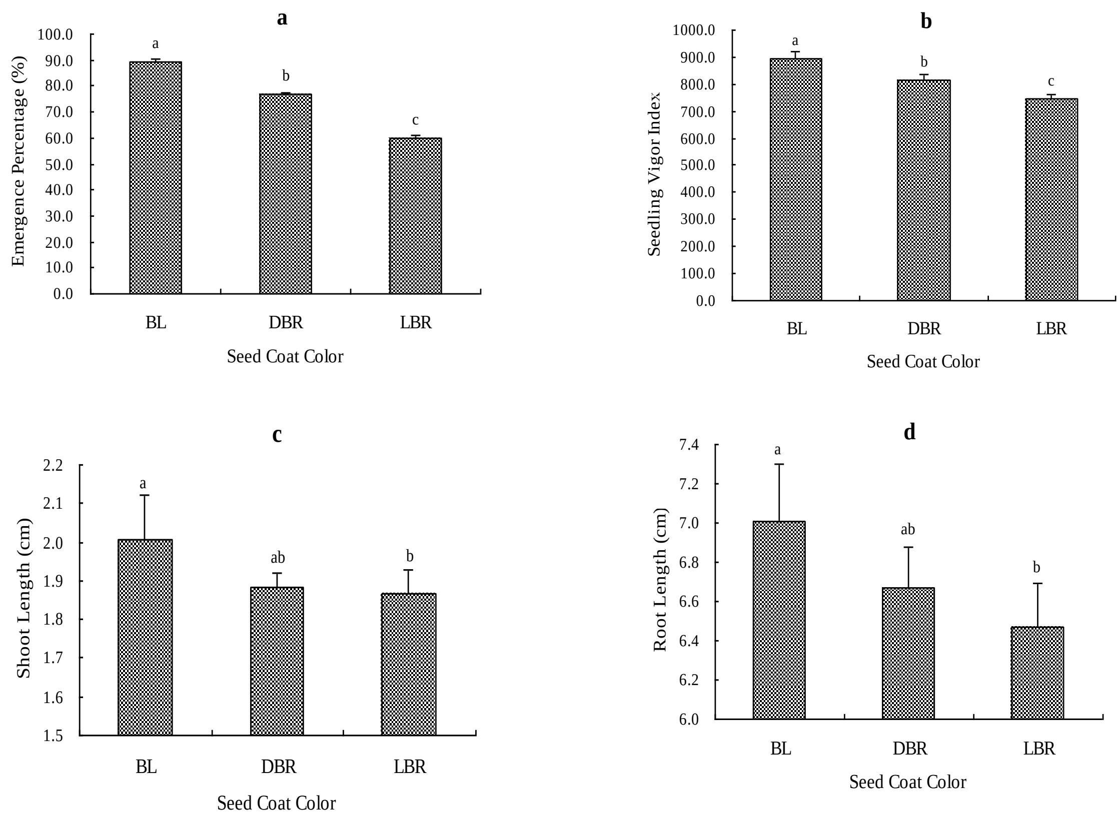

Fig. 4: Seedling growth of canola with different seed coat colors. (a) Emergence percentage ($LSD_{0.05}$ = 1.31). (b) Seedling vigor index ($LSD_{0.05}$ = 33.73). (c) Shoot length ($LSD_{0.05}$=0.13). (d) Root length ($LSD_{0.05}$ = 0.39)

germination and seedling growth. Seeds with higher protein and soluble sugar content showed lower germination percentage (r = -0.89, P = 0.00 and r = -0.91, P = 0.00), slower germination speed as reflected by lower GI (r=-0.93, p =0.00 and r = -0.86, p = 0.00) and longer MGT (r = 0.92, P = 0.00 and r = 0.78, P = 0.00), also lower emergence percentage (r = -0.91, P = 0.00 and r = -0.91, p = 0.00), SVI (r = -0.88, p = 0.00 and r = -0.84, P = 0.00) and lower shoot length (r = -0.67, P = 0.02) and root length (r = -0.62, p=0.03 and r = -0.61, P = 0.03). The correlation between soluble sugar content and shoot length was not significant (r= -0.44, P = 0.15). Almost all the examined indicators of seed germination were correlated with seedling growth (r=-0.94 to 0.99, P = 0.00 to 0.03) with the exception of correlation between germination percentage and shoot length (r = 0.52, P = 0.08) (Table 2).

Discussion

The study revealed that better seed germination, seedling growth and higher oil content were observed in seeds with darker seed coat color. On the other hand, correlation matrix showed that seed germination and seedling growth were positively correlated with oil content, but negatively correlated with protein and soluble sugar content. Furthermore, seedling growth was significantly correlated with seed germination.

Seeds in the darker seed coat color showed higher seed vigor with high germination percentage, GI, and lower MGT. Considering the different seed coat colors in one genotype, the variable response may be explained by maturity, the immature seeds were in lighter color compared to mature ones in canola (Elias and Copeland, 2001) as observed for BL seeds. Similarly, Elias and Copeland (2001) reported that canola seeds of all cultivars had greater germination and vigor at harvest maturity than physiological maturity. Samarah and Abu-Yahya (2008) indicated that germination of chickpea increased with rapid development in crop maturity and obtained the maximum germination when harvested at harvest maturity. This may be also explained by the physiological changes i.e., hormonal reduction and/or nutrient increase, which occurs after physiological maturity to promote germination (Khan, 1971). In present study, the oil content was highly correlated to seed germination (Table 2) and the oil content generally increased with seed development (Vera *et al.,* 2007). This probably might explain why the highest oil content, germination percentage and fastest germination speed were observed in the BL seed coat color category. Similarly, Hu *et al.* (2009) indicated that cultivars with high oil content

Table 2: Correlation coefficients (r) among germination, seedling growth and seed composition with seed coat color

Characters	Germination Index	Mean Germination Time (days)	Establishment Percentage (%)	Seedling Vigor Index	Shoot Length (cm)	Root Length (cm)	Oil Content (%)	Protein Content (%)	Soluble Sugar Content (%)
Germiantion Percentage (%)	0.96**	-0.91**	0.99**	0.93**	0.52	0.67*	0.61*	-0.89**	-0.91**
Germiantion Index		-0.98**	0.98**	0.94**	0.62*	0.70*	0.68**	-0.93**	-0.86**
Mean Germination Time (days)			-0.94**	-0.90**	0.61*	0.67*	-0.73**	0.92**	0.78**
Emergence Percentage (%)				0.94**	0.61*	0.67*	0.62*	-0.91**	-0.91**
Seedling Vigor Index					0.57	0.87**	0.66*	-0.88**	-0.84**
Shoot Length (cm)						0.29	0.23	-0.67*	-0.44
Root Length (cm)							0.63*	-0.62*	-0.61*
Oil Content (%)								-0.69*	-0.37
Protein Content (%)									0.72**

Numbers followed by * indicate significance at $P < 0.05$ and by ** indicate significance at $P < 0.01$ (n=12)

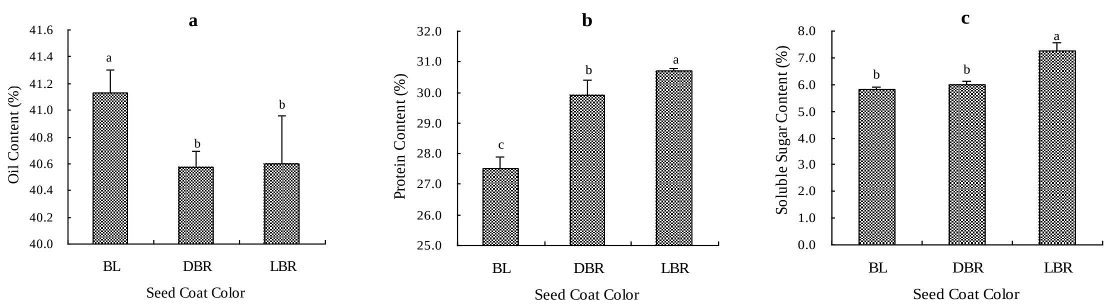

Fig. 5: Seed composition of canola with different seed coat colors. (a) Oil content ($LSD_{0.05}$ = 0.38). (b) Protein content ($LSD_{0.05}$ = 0.58). (c) Soluble sugar content ($LSD_{0.05}$=0.32)

had nearly 20% higher germination rate than cultivars with low oil content. The reason may be the lipids supplied energy requirements for germination (Hu *et al.*, 2009).

The indicators used in present study can be related to seed germination – germination percentage, GI, MGT; seedling growth - shoot length, root length and SVI; and related to seed quality - oil, protein and soluble sugar content. All seedling growth related traits were affected by seed coat color and significantly associated with seed germination (r = -0.94 to 0.99, P = 0.00 to 0.03) with the exception of shoot length and germination percentage (r=0.52, P = 0.08). Meanwhile, oil content was positively correlated to all the examined indicators of seedling growth with the exception of shoot length (r = 0.23, P = 0.48). The protein content and shoot length was negatively correlated (r= -0.67, P = 0.02) (Table 2) and explain why higher emergence percentage, SVI, root and shoot length was observed in darker seed coat color category.

Seed coat acquires its color with maturity. Differences in seed color within species are associated with seed harvesting from different developmental stage of fruit and some genetic differences (Atis *et al.*, 2011). In order to avoid the genetic differences of seed color, the conventional variety of canola - Hua-shuang 5 was chosen to conduct the study. In spite of harvesting at the same time, seeds with different coat colors may be attributed to fruit and seed set at different stages, hence difference in maturity and seed coat color. For example, seeds from the main stem became mature earlier than those on branches; seeds from the bottom of the stem became mature earlier than on the top.

Conclusion

We can conclude that seeds with BL seed coat color showed fast germination, high emergence percentage and better seedling stand. Seed coat color determines seed composition as indicated by high soluble sugars, which further affects seed germination and seedling growth. Nonetheless, how seed composition determines seed germination and seedling growth and maturity time affects seed coat color need to be investigated.

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