Mechanism for the Formation of Distinctive Seedcoat Colour in Yellow-Seeded Rape (*Brassica napus* L.)

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ABSTRACT

Three pairs of different genetic near-isogenic lines (NILs) of black- and yellow-seeded *Brassica napus* as experimental materials were used in the study. The results are as follows: The activities of phenylalanine ammonia lyase (PAL), polyphenol oxidase (PPO) in the black-seeded lines were much higher than those in the yellow ones under the same genetic background. The isozyme patterns of PPO in the seedcoat were investigated. There were more PPO isozyme bands in the black-seeded lines than in the yellow ones under the same genetic background and there was 1 special band in the black-seeded lines. The plants of yellow- and black-seeded NIL were treated with red light or blue light during seed development. The result showed that the activity of PAL, the content of polyphenols, anthocyanidins and melanin pigments in the seedcoat increased in the red light treatment. The plants of yellow- and black-seeded *B.napus* were treated with PAL-inhibiter or promoter of protein synthesis or PPO inhibiter or reductant during seeds development. The results indicated that content of polyphenols, anthocyanidins and melanin pigments in the seedcoat were decreased.

Key words: *Brassica napus* L. – Yellow-Seed – Seedcoat Color – Mechanism

INTRODUCTION

Yellow-seeded (*B. napus* L.) breeding has in recent years attracted the attention of rapeseed experts for the fact that they are characterized by higher oil content, higher protein content and good quality of the oil. However, the unstability of the shade of its yellowness and the percentage of the yellow seed hinders its extension in production. There have been some reports of the studies on seedcoat colour of yellow-seeded rapeseed[1,2], and these studies focused on the substances related to yellow seed or black seed. In the present study, we adopted the approach to adjust enzyme activity and metabolism path to investigate the major factors controlling the synthesis of pigments so as to understand the mechanism(s) of the variation in seedcoat colour.

MATERIALS AND METHODS

Test materials were 3 pairs of NILs: L1 (black seed) vs. L2 (yellow seed); L3 (black seed) vs. L4 (yellow seed); L5 (black seed) vs. L6 (yellow seed). Three experiments were carried out: Light treatment: at the initial flowering stage of the plants, shelters of red and blue plastic film were put up over plants, seed samples were taken after maturation. Reagent treatments: phenylalanine ammonia lyase(PAL)inhibitor: *p*-hydroxybenzoic acid; polyphenol oxidase inhibitor: polyvinyl pyrrolidone(PVP); reductant: ascorbic acid Vc ; protein synthesis promoter: urea. Enzyme activity determination: the samples were taken for determination enzyme activity starting from the 20th day after flowering till seed maturation at 5-day intervals.

RESULTS AND DISCUSSION

Dynamic variations of PAL, PPO and POD in the seedcoat of yellow- and black-seeded NIL (*B. napus*): The activity of PAL, PPO and POD in the seedcoat of both yellow- and black-seeded rapeseed rose with seed development to their peaks followed by a decline. The difference in activity in various development stages was highly significant statistically. The peaks of PAL activity occurred around 34 days after flowering in the black-seeded NILs and 41day in the yellow-seeded NILs. The peaks of PPO and POD activity occurred around 41days after flowering in all of the NILs. The activity of the above enzymes was highly significantly higher in the black-seeded NILs than in the yellow-seeded NILs, indicating that the three enzymes have great influence in the pigments formation of the seedcoat.

Isozymes analysis of the enzymes related to PPO and POD: The spectrum-model of PPO isozymes shows that all the genotypes shared 4 common bands and that the black-seeded genotypes had 1-3 more bands than their yellow-seeded counterparts, one band among them is specific to black- seeded NILs. It can be seen from the spectrum-model of POD isozymes that all the NILs shared 2 common bands and that the black-seeded NILs had 1 more band than their yellow-seeded counterparts, one band among them may be taken as the characteristic band for black-seeded genotypes.

Effects of reagents on the pigments synthesis in seedcoat: The results of the treatments with *p*-hydroxybenzoic acid, urea, PVP and Vc in the process of seed development are following.

Table 1. Effects of reagents on the contents of protein, polyphenols, anthocyanidins and melanin

Treatment	Seedcoat color	protein content	polyphenols content	anthocyanidins content	melanin content
p-hydroxybenzoic acid	Yellow	4.07	-10.3	-21.4	-4.90
	Black	1.83	-7.92	-11.2	-5.31
urea	Yellow	1.02	-1.68	-2.21	-1.24
	Black	1.34	-2.74	-3.27	-1.49
PVP	Yellow	0.94	-0.47	-1.34	-7.22
	Black	2.44	-1.50	-2.20	-3.6
Vc	Yellow	2.17	-2.61	-1.54	-7.1
	Black	2.91	-4.52	-3.66	-12.0

Note: The figure in the table represents the percentage of increase as compared with the control.

The data indicated that the formation of seedcoat pigments may be influenced by the PAL-inhibiter or promotion of protein synthesis *p*-hydroxybenzoic acid and urea, and be influenced by PPO inhibiter PVP or reductant Vc.

Effects of red light and blue light on PAL activity and colour of the seedcoat: PAL activity in the seedcoat in the red light treatment was not significantly different from that of the natural light treatment but was significantly higher than that in the blue light treatment. PAL activity in the yellow-seeded NILs was lower than in the black-seeded NILs, which suggesting that PAL is activated by red light, the effect is greater in the yellow-seeded NILs than in the black-seeded NILs. No significant differences were observed between the red light treatment and the natural light treatment in protein, polyphnols, anthocyanidins and melanin pigments in the seedcoat of both black- and yellow-seeded NILs. In contrast, the contents of polyphnols, anthocyanidins and melanin pigments were significantly lower and protein content was significantly higher in the blue light treatment than in the red one, which suggesting that blue light is favorable for the enhancement of protein synthesis in the seedcoat and has an inhibitive effect on the formation of polyphnols, anthocyanidins and melanin pigments by inhibiting PAL activity, the influence being greater in the yellow-seeded NILs than in the black-seeded NILs.

DISCUSSION

The pigments in plant are synthesized main through the shikimic-phenylalanine pathway, in which the enzymes PAL, PPO and POD play a key role. PAL catalyzes deamination of phenylalanine to form phenylacrylic acid and ends in the formation of polyphenols. Polyphenols are oxidized by PPO to form quinones, which are brown in tint. When quinines are polymerized, melanin pigments are produced. POD catalyzes the oxidation of various substances in living things. High PAL activity promotes the deamination of phenylalanine and thus decrease the amount of the raw material for protein synthesis, a mechanism favorable for the formation of pigments. In the present experiment PAL activity in the seedcoat of black-seeded NILs was found to be significantly higher than in that of yellow-seeded NILs, indicating that PAL activity plays a crucial role in the formation of seedcoat pigments. The effect of different lights on the seedcoat colour of yellow-seeded plants could be observed with naked eye, but such an effect could hardly appreciated with naked eye in black-seeded genotypes. Such results suggest that

PAL is the key factor for the pigments synthesis in yellow-seeded lines, however, there may be many pathways existing in the formation of pigments in the black-seeded rapeseed.

In the process of seed development, PPO activity in the seedcoat in black-seeded NILs was significantly higher than that in yellow-seeded NILs, indicating the important role of PPO activity in the formation of seedcoat pigments. In the treatment of the plants with the PPO inhibitor PVP, PVP was found to reduce the contents of melanin pigments with a greater effect. Since melanin pigments are the major substances responsible for the coloration of seedcoat, PPO is thought to be an important factor for the coloration of seedcoat. POD activity in the seedcoat of black- seeded NILs was significantly higher than that in yellow-seeded NILs, indicating that POD activity had some influence on the coloration of seedcoat. In the treatment of the plants with the reductant Vc, the colour of the seedcoat of black-seeded rape became lighter, suggesting an association between the formation of seedcoat pigments and oxidation within the plant.