

Research Article

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New insight into inheritance pattern of seed coat colour in adzuki bean

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Abstract

Seed coat colour in adzuki bean is an important quality trait and closely associated with anthocyanin metabolism pathways. To further understand the inheritance of seed coat colour pattern, the inheritance between multiple seed coat colours and ivory seed were analysed using $F_{1,2}$, $F_{2,3}$ and $F_{3,4}$ populations derived from five bi-parental crosses. The differences between ivory and red mottle on ivory are controlled by a single recessive R locus and RI locus, respectively. Green, light brown and golden are all dominant to red and governed by two loci. The B (brown) locus shows dominant epistasis over T locus. The R (red) locus was recessive epistasis to B (black), T (light brown), G (golden), GR (green) and RI (red mottle on ivory) loci. The new insight into the strong recessive epistasis of the R locus will be important for gene mapping and cloning, candidate gene functional validation and quality improvement in adzuki bean.

Introduction

Adzuki bean (*Vigna angularis* L.), with folk name of ‘red small bean’ in China, was grown in more than 30 countries but mainly in Asia (Gohara *et al.*, 2016; Belfry and Sikkema, 2018). Adzuki bean is considered to have high medicinal value because of its antioxidant activity from phenolic compounds (Gohara *et al.*, 2016). Seed coat colour has been correlated with the content of phenolic compounds (Kim *et al.*, 2011). Seed coat colour is an important domestication trait and quality trait. Seed coat colour has varied from black mottle on grey to red during the domestication of adzuki bean.

The seed coat is developed from the integument so is controlled by the maternal genotype, which can delay its phenotype expression for a half generation. Takahashi and Fukuyama (1917) first reported the genetic analyses of four loci including red (R), green (G), brown (F) and red inhibition (H). The genetic relationship of ivory, red and light grey was further analysed (Naruwa, 1976). The seed coat colour loci $OLB1$, $OLB2$, sdc (light brown/red) and $sdc3.1.1$ (ivory/red) were mapped in linkage group LG1 (Isemura, *et al.*, 2007; Kaga *et al.*, 2008). The IVY (ivory yellow) and POB (pale olive buff) genes were mapped in LG8 and LG10, respectively (Horiuchi *et al.*, 2015). We analysed the genetic relationship between red and other seed coat colours and showed that black, light brown, brown, black mottle on red, black mottle on grey, and golden were dominant to red seed coat colour, but ivory seed coat was the only trait recessive to red seed coat (Chu *et al.*, 2021a). We mapped $VaSDC1$ (Li *et al.*, 2017) and demonstrated that the function of $VaSDC1$ (B Locus) was involved in the formation of black seed coat colour (Chu *et al.*, 2021b).

The research on seed coat colour inheritance in adzuki bean is mainly focused on the difference between red and other colours. Ivory seed coat is particularly important in explaining the genetic mechanism of seed coat colour in adzuki bean but has rarely been investigated. In this study, we analysed the genetics of ivory over black, green, golden, light brown, and red mottle on ivory using six $F_{2,3}$ and one $F_{3,4}$ segregating populations derived from the bi-parental crosses between ivory and these seed coat colours. The study provides new information about the inheritance pattern net of seed coat colour in adzuki bean.

Materials and methods

Plant materials

Six different bi-parental crosses between ivory and black, green, golden, light brown and red mottle on ivory were constructed (Fig. 1, Table 1). Norin3 (NL3) is a commercial variety with an ivory seed coat. AG118, GM170, GM537, AG89 and AG49 have black, golden and red mottles on ivory, light brown and green seed coats, respectively. The seed coat colour of individuals in $F_{1,2}$, $F_{2,3}$ and $F_{3,4}$ segregating populations was observed.



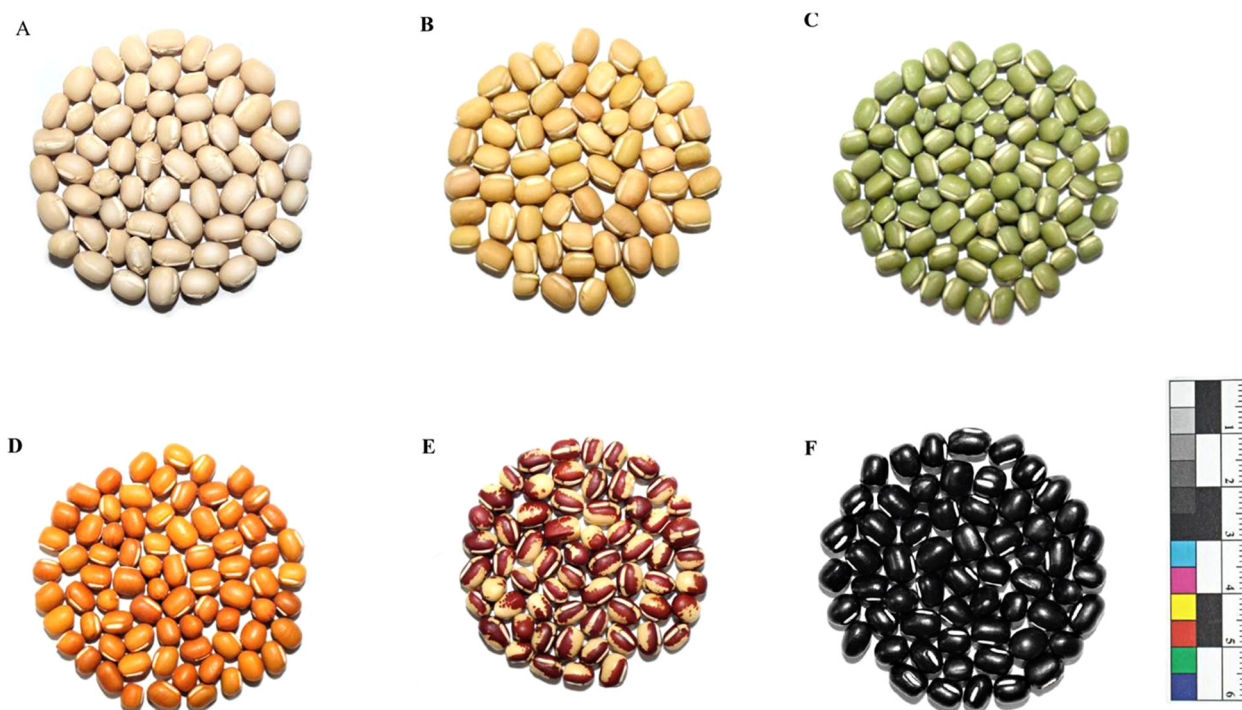


Figure 1. Parent seed coat colour of the crosses in adzuki bean a: NL3 (Ivory); b: AG89 (Light brown); c: AG49 (Green); d: GM170 (Golden); e: GM537 (Red mottle on ivory); f: AG118 (Black).

F_1 , F_2 and F_3 generations were grown at the Experimental Farm of Beijing University of Agriculture (BUA) in 2020, 2021 and 2022. Each line of F_3 families was derived from an F_2 individual. Eighty seeds were planted for each line. The parents were planted on both edges of each population. Each parent was planted into two rows. Rows were 3 m long and 45 cm apart; 40 seeds were planted evenly in each row.

Data analysis

The seed coat colour phenotypes and segregation ratios of $F_{2,3}$ individuals were investigated. χ^2 test was applied to the goodness

of fit to various genetic ratio colours. The gene numbers regulating different seed coat colours were predicted based on the segregation data of these crosses and Mendel's law. The χ^2 test was conducted using SPSS20.0 software.

Results

Analysis of $F_{1:2}$ phenotypes and genetic relationships

The genotype of the seed coat was the same as its female parent so the coat colour of $F_{0:1}$ hybrid seed was dependent on the genotype of its female parent. The seed coat colours of NL3 \times AG118,

Table 1. The information of parents and the crosses in adzuki bean

Cross combination	Female parent seed coat colour	Male parent seed coat colour	Generation	$F_{2,3}$ Population	Grown
NL3 \times AG89	NL3 (ivory)	AG89 (light brown)	F_1	201	2020
			F_2		2021
NL3 \times AG49	NL3 (ivory)	AG49 (green)	F_1	398	2020
			F_2		2021
GM170 \times NL3	GM170 (golden)	NL3 (ivory)	F_1	219	2020
			F_2		2021
NL3 \times GM537	NL3 (ivory)	GM537 (red mottle on ivory)	F_1	136	2020
			F_2		2021
GM537 \times NL3	GM537 (red mottle on ivory)	NL3 (ivory)	F_1	156	2020
			F_2		2021
NL3 \times AG118	NL3 (ivory)	AG118 (black)	F_1	638	2020
			F_2		2021

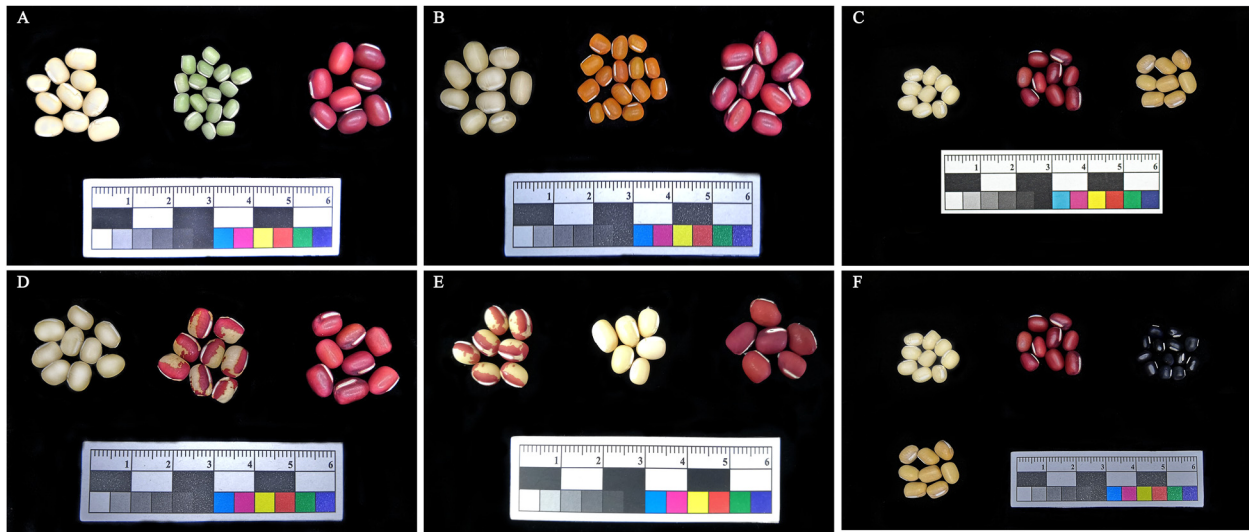


Figure 2. Segregation of seed coat colour in $F_{2,3}$ populations. a: Phenotypic segregation of NL3 × AG49 F_2 population. b: Phenotypic segregation of GM170 × NL3 F_2 population. c: Phenotypic segregation of NL3 × AG89 F_2 population. d: Phenotypic segregation of NL3 × GM537 e: Phenotypic segregation of GM537 × NL3 F_2 populations. f: Phenotypic segregation of NL3 × AG118 f_2 population.

NL3 × AG49, GM170 × NL3, NL3 × GM537, GM537 × NL3 and NL3 × AG89 in $F_{1,2}$ populations were black, green, golden, red, red and light brown, respectively. The results indicated that the five seed coat colours were dominant to ivory. The seed coat colour of $F_{1,2}$ from the reciprocal crosses between ivory and red mottle on ivory was red and revealed that red was dominant to both red mottles on the ivory and ivory seed coat.

Segregation of seed coat colour and Chi-square test in $F_{2,3}$ populations

The red seed coat individuals which did not exist in bi-parents appeared in the crosses between ivory and green, golden and light brown. The segregation ratio of green (golden or light brown): red: ivory was 9:3:4 in $F_{2,3}$. Two genetic loci controlled the difference between green (golden, light brown) and ivory. Green (*GR*), golden (*G*) and light brown (*T*) were all dominant to red. Red was dominant to ivory (*R*). The χ^2 test values of $F_{2,3}$ population derived from NL3 × AG49 and NL3 × AG89 were 5.85 and 1.62 and that of 1.36 from GM170 × NL3, lower than $\chi^2_{(df=2)} P=0.05 = 5.99$ (Fig. 2a–c; Table 2). Two genetic loci governed the difference between green (golden or light brown) and ivory. The interaction of the *R* locus over *GR*, *G* and *T* loci was recessive epistasis.

In the reciprocal crosses of red mottle on ivory and ivory, the red seed individuals which did not exist in bi-parents were segregated out. The segregating ratio of red: red mottle on ivory: ivory was 9:3:4 in $F_{2,3}$. Red was dominant to both red mottle on ivory (*RI*) and ivory (*R*). The χ^2 goodness of fit test for NL3 × GM537 and GM537 × NL3 was 3.96 and 3.01, lower than $\chi^2_{(df=2)} P=0.05 = 5.99$ (Fig. 2d and e; Table 2). Two genetic loci controlled the difference between red mottle on ivory and ivory. The interaction between *R* and *RI* loci was recessive epistasis.

In the cross generation between ivory and black seed coat appeared light brown and red which did not exist in the parents. The segregation in $F_{2,3}$ fitted the Mendel's law of three genes with a ratio of black: ivory: light brown: red = 36:16:9:3. Black was dominant to light brown (*B*), red (*R*) and ivory (*r*). The χ^2 values

was 6.86 lower than $\chi^2_{(df=3)} P=0.05 = 7.82$ (Fig. 2f; Table 2). *B* locus over *R* locus was dominant epistasis, and the interaction of *R* locus over the other two loci was recessive epistasis. The recessive epistasis of *R* locus was stronger than the dominant epistasis of *B* locus. We predicted the genetic backgrounds of the seed coat colours (online Supplementary Table S1).

Segregation verification of GM537 × NL3 and NL3 × GM537 by F_3 families

To further verify the genotypes of $F_{2,3}$ seeds of GM537 × NL3 and NL3 × GM537, the segregation of colours from F_3 families was analysed and assessed using χ^2 tests (Table 3). The segregating ratio of these two populations were red: red mottle on ivory: ivory = *R_RI*: *R_riri*: *rr* = 9:3:4. Based on Mendel's law, there were four different genotypes in the red progeny of F_2 individuals and the theoretical segregation ratio is *RIri Rr:RIri RR:RIRI Rr:RIRI RR* = 4:2:2:1. Individuals of F_3 families from F_2 parents with heterozygous genotypes will have different phenotypes. Phenotypes of F_3 families derived from F_2 red parents are expected to be red, red mottle on ivory and ivory individuals: families with red and ivory individuals: families red and red mottle on ivory: pure red families = 4:2:2:1. The χ^2 value for these families was 2.056 and less than $\chi^2_{(df=2)} P=0.05 = 5.99$.

There are two different genotypes in red mottle on ivory progeny of F_2 individuals, and the theoretical segregation ratio is *riri Rr:riri RR* = 2:1. The phenotype of F_3 families corresponding to red mottle on ivory progeny of F_2 individuals are predicted to be red mottle on ivory and ivory individuals: pure red mottle on ivory families = 2:1. The χ^2 value for these families was 0.675 and less than $\chi^2_{(df=1)} P=0.05 = 3.84$. There are three different genotypes in the ivory progeny of F_2 individuals, and the theoretical segregation ratio is *RIRI rr:RIri rr:riri rr* = 1:2:1 with the expected recessive epistasis of *R* locus. The phenotype of these F_3 families is predicted to be all ivory. The χ^2 test results verify the seed coat colour difference prediction between red mottle on ivory and ivory and the recessive epistasis of the *R* locus (Table 3).

Table 2. Segregation ratio of seed coat colour derived from six crosses in adzuki bean

Cross combination	Seed coat colour of $F_{1,2}$	Number of $F_{2,3}$	Phenotype1	Phenotype2	Phenotype3	Phenotype4	Theoretical segregation ratio	χ^2
NL3 × AG89	Light brown	201	Theoretical value	Red	Ivory		9:3:4	1.62
			Observed value	112	44	45		
NL3 × AG49	Green	398	Theoretical value	Red	Ivory		9:3:4	5.85
			Observed value	232	86	80		
GM170 × NL3	Golden	219	Theoretical value	Red	Ivory		9:3:4	1.36
			Observed value	223.88	74.63	99.5		
NL3 × AG118	Black	638	Theoretical value	Black	Light brown	Red	36:16:9:3	6.86
			Observed value	331	172	95		
NL3 × GM537	Red	136	Theoretical value	Red	Red mottle on ivory		9:3:4	3.96
			Observed value	358.88	159.5	89.72		
GM537 × NL3	Red	156	Theoretical value	Red	Ivory		9:3:4	3.01
			Observed value	86	37	33		
			Observed value	87.75	29.25	39		

Note: When degrees of freedom are 1, 2 and 3 numbers of $\chi^2_{P=0.05}$ are 3.84, 5.99 and 7.82, respectively.

Table 3. Segregation analyses of adzuki bean GM537 × NL3 and NL3 × GM537 F3 families

Hybridized combination		Red families (120 lines)	Theoretical segregation ratio	χ^2	Red mottle on ivory families (60 lines)	Theoretical segregation ratio	χ^2	Ivory families (60 lines)
GM537 × NL3	Theoretical value	53.3:26.6:26.6:13.3	4:2:2:1	2.056	40:20	2:1	0.675	60
NL3 × GM537	Observed value	46:30:28:16			37:23			60

Note: When degrees of freedom are 1, 2 and 3 numbers of $\chi^2_{P=0.05}$ are 3.84, 5.99 and 7.82, respectively. There are four different genotypes in red families. The theoretical segregation ratio is $RlriRr:RlriRR:RlRiRr:RlRiRR = 4:2:2:1$. There are three different genotypes in ivory families. The theoretical segregation ratio is $RlriRr:RlriRr:ririRr = 1:2:1$. There are two different genotypes in red mottle on ivory families. The theoretical segregation ratio is $ririRr:ririRR = 2:1$.

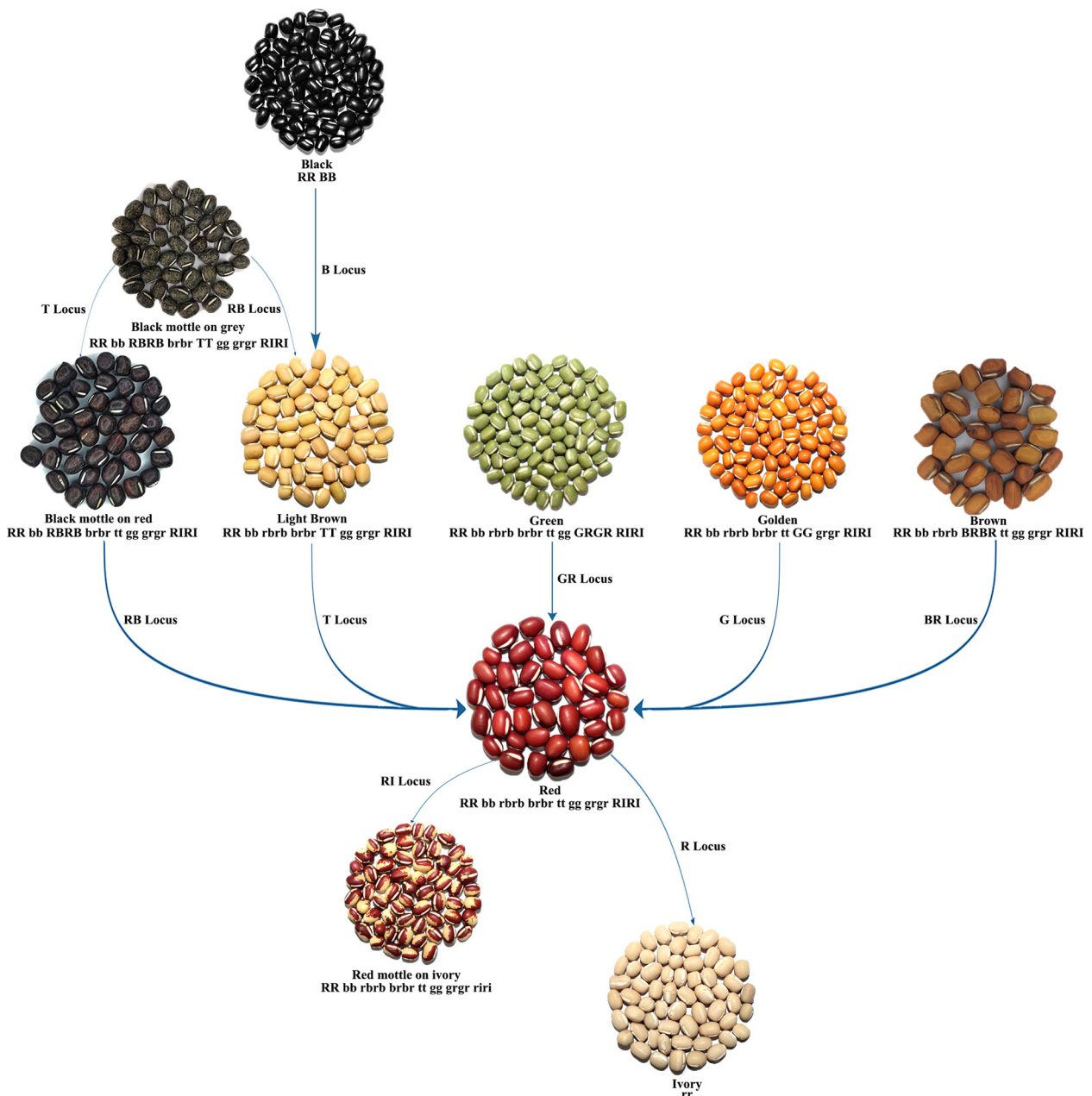


Figure 3. Dominant and recessive inheritance of seed coat colour loci in adzuki bean.

Discussion

During the domestication of adzuki bean, a red seed coat was selected and retained for cultivation. The red seed coat colour was preserved during the domestication of adzuki beans. However, the red seed coat colour is recessive to most other seed coat colours, except for ivory. Therefore, the study of the genetic mechanism of ivory seed coat colour is very important for adzuki bean domestication. Our result demonstrated that red is dominant to ivory and controlled by the *R*. This conclusion is consistent with the previous study (Horiuchi *et al.*, 2015; Chu *et al.*, 2021a).

The segregating ratios of the 9:3:4 in the crosses between golden, green, light brown and ivory showed that two loci controlled the differences between golden/ivory, green/ivory and light brown/ivory, respectively. Among them, the interaction between *R* locus and *GR* locus, *G* locus and *T* locus is recessive epistasis, respectively. When the *R* locus is recessive *rr*, the seed coat colour is all ivory whatever the genotype of another locus is. The *GR* locus, *G* locus and *T* locus can regulate the difference between green to red, golden to red and light brown to red, respectively, when *R* locus is *R*. The recessive epistasis of *rr* in *R* locus to other loci in adzuki bean seed coat colour was first identified in this study. The genetic relationship between red mottle on ivory, green and red has also been discovered for the first time, respectively.

The segregating ratios of the reciprocal crosses between red mottle on ivory and ivory are both 9:3:4. Different from other crosses, red was dominant to both red mottle on ivory and ivory seed coat. The interaction between *R* locus and *RI* locus is also recessive epistasis. The segregation of $F_{3,4}$ was analysed to verify this result. The genetic pattern of the red mottle in this study is different from that of the black mottle seed coat in a previous study (Kaga *et al.*, 2008; Chu *et al.*, 2021a). The genetic analyses between red and red mottle on ivory have not been reported, and further research will be needed.

The segregation model in $F_{2,3}$ populations derived from the cross between black and ivory was controlled by 3 loci. The ratio of 36:16:9:3 supported the recessive epistasis of *rr* genotype and the dominant epistasis of *B*₋. The recessive epistasis of the *rr* genotype was greater than the dominant epistasis *B*₋. We show that *VaSDC1*, located in the *B* locus, increased the expression level of flavonoid metabolism pathway genes (Chu *et al.*, 2021b). We deduce that the ivory seed coat *Norin3* might be a loss of function mutant of upstream key genes of the flavonoid metabolism pathway. However, further identification of molecular markers and candidate gene functional validation should be performed.

Overall, our study provides new insight into the inheritance of adzuki bean seed coat colours. Summarizing the results of genetic analysis between ivory seed coat and different seed coat colours in adzuki bean, we propose that there is strong recessive epistasis of *R* locus over other loci. A new model is proposed to integrate the genetic analyses in this study with our previous model (Chu *et al.*, 2021a) (Fig. 3). This research will lay the foundation for gene

mapping and cloning, function validation, insight into the genetic mechanism of seed coat colour and quality improvement in adzuki bean.

Supplementary material. The supplementary material for this article can be found at <https://doi.org/10.1017/S1479262123000746>

Author contributions. WP designed and managed the project and revised the manuscript. YK and CLW coordinated the project and experiments. CLW and WKL wrote the primary manuscript. WP and XGH proofread the manuscript and figure. ZP and HJ prepared the Materials. WKL analysed the data. ZB and HJ cultivated and managed the experimental accessions, segregated the population and identified the phenotypes. All authors contributed to the article and approved the submitted version.

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Competing interests. The authors declare that they have no conflict of interest.

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