The Study of Genetics of Flower Color in Faba Bean Reveals Generous Diversity to Be Used in the Horticulture Industry

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Abstract. The horticulturally valuable traits of faba bean are poorly explored, including the available information on the genetics of flower color and pattern. This lack of understanding has reduced the inclusion of unique flower color into the horticultural-type faba bean market. The modes of inheritance of two flower colors (red petals and yellow spot on wing petals) were examined through the development of multiple F₂ segregating populations. The inheritance of red flower was confirmed for two recessive genes and yellow wing spot inheritance was confirmed for a single recessive gene. These populations led to the discovery of combinations of red and yellow flower color that have not been previously reported. The solid wing petal color gene was confirmed as a single recessive gene. Understanding the inheritance of flower color in faba bean can lead to improvement of current vegetable types and opens up possibilities for ornamental markets.

Faba bean (*Vicia faba*) is a protein-rich, nutritious legume crop (Khazaei and Vandenberg, 2020; Warsame et al., 2018). It can be consumed as both grain (pulse) and vegetable. In its horticultural form, pods are harvested when the seeds are filling, and the seeds are removed for fresh vegetable consumption (Anthony, 2017). The floriculture industry is another unexplored market for faba bean, as it exhibits large variation for many ornamental traits, including flower color. Its flowers are typical papilionaceous, with five petals consisting of one standard, two wings, and two parts fused into a keel (Duc, 1997). The wild-type flower is white petalled with a pronounced black spot on each wing petal and dark striae on the standard petal. The known flower colors in faba bean are wild type, white (absence of any pigments in the wing petals), yellow

wing spots, solid brown, pink, diffused yellow, and red (Cabrera, 1988; Duc et al., 2015). Flower color has been used as a morphological indicator of high (wild-type) tannin content as one of the important antinutritional factors in seeds. The seeds of colored-flowered faba beans have high tannin content and white-flowered faba beans are low in tannin (Martin et al., 1991; Zanutto et al., 2020a).

In 1930, the first genetic study of faba bean flower color examined the inheritance of white flower, where it was determined to be a single recessive gene (Erith, 1930). Later reports have shown that white flower color, together with tannin-free seedcoat, is determined by two complementary zero tannin genes, *zt1* and *zt2* (Crofts et al., 1980; Picard, 1976; Rowlands and Corner, 1962). These genes have pleiotropic effects causing non-expression of the stipule spot and stem color (Metz et al., 1992). The *zt1* and *zt2* genes have been genetically mapped in faba bean chromosomes 2 and 3, respectively (Gutierrez and Torres, 2019; Zanutto et al., 2020a). Previous work on the genetics of red and yellow-spotted flowers has been limited to the work of Sjödin (1971) and Cabrera (1988). Sjödin (1971) identified four loci that affect flower color and denoted them as *sp-a* (refers to *zt1*), *sp-b* (refers to *zt2*), *dp-a*, and *dp-b*. Red flower color appears when the locus *dp-a* is homozygous recessive and the yellow wing spot appears when *dp-b* is homozygous recessive. The monogenic inheritance of the solid wing color was confirmed by Cabrera (1988) and then determined to be segregating independently of the standard petal color. There has been limited research on the yellow-spotted mutant. However, both

Sjödin (1971) and Cabrera (1988) performed a cross between a yellow-spotted type and a wild-type plant that indicated monofactorial inheritance. In addition, Cabrera and Martin (1989) showed that yellow pigment in flowers had pleiotropic effects resulting in yellow testa in faba bean.

The first step for developing successful horticultural-type faba beans is an understanding of the genetics of the potential aesthetic traits, especially flower color. Faba beans have large clusters of fragrant flowers in an array of colors and patterns. By creating combinations of these unique characteristics together with reduced toxic constituents (vicine and convicine, v-c), faba bean has the potential to enter the ornamental and floriculture markets. Therefore, the primary aims of this study were to investigate the genetic control of red flower color and yellow wing spot along with their interactions in faba bean. The motivation of this research was to facilitate the expansion of faba bean into the ornamental market.

Materials and Methods

Plant material. Eight faba bean inbred lines were used for this study. The flower color characteristics and origin of these genotypes are given in Table 1. The flower color of parental lines are presented in Fig. 1A–D. Aurora/2, IG 114476 (accession DOI: 10.18730/8VJQF), Rinrei (Fukuta et al., 2004), and IG 12658 (accession DOI: 10.18730/60V47) were the sources of wild-type flower color. CDC Snowdrop and Disco/2 were used as sources of white flower (low tannin) carrying *zt1* and *zt2* genes, respectively. Red-flowered genotype P47-1 was derived from the vegetable faba bean “1778 (Crimson-Flowered),” which was listed by Fearing Burr in 1863 in a horticultural seed catalogue (Burr, 1863; Watson, 1996). The source of yellow wing spot flower was a spontaneous mutation (Sjödin, 1971).

Hybridization. To study the genetics of red flower, the crosses P47-1 × Aurora/2, P47-1 × IG 114476, P47-1 × Rinrei, P47-1 × IG 12658, P47-1 × CDC Snowdrop, P47-1 × Disco/2, and P47-1 × Gelber were prepared along with their reciprocals. Crosses Aurora/2 × Gelber, P47-1 × Gelber, CDC Snowdrop × Gelber, and Disco/2 × Gelber were made to study the inheritance of yellow wing spot. Crosses were prepared by hand in the insect-proof growth chambers of the College of Agriculture and Bioresources at the University of Saskatchewan, Canada.

Growing conditions. Seeds of parental lines were scarified, inoculated with *Rhizobium leguminosarum* bv. *viciae* and then planted into 4-L pots of soilless growing mixture No. 3 (Sun-Gro Horticulture, Agawam, MA) and placed in a growth chamber. The light conditions in the phytotron chamber were 16-h days at a photon flux of 300 μmol·m⁻²·s⁻¹ followed by 8 h of dark. The temperature was set to 21 °C during the light interval, and to 18 °C for the dark interval.

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Table 1. Overview of the color characteristics of floral tissues, tannin content, origin, and percent homozygosity of faba bean inbred lines used in this study.

Inbred line	Standard color	Wing color	Wing spot	Tannin	Origin/Source	% Homozygosity
Aurora/2	White	White	Black	High	Sweden	92.9
CDC Snowdrop	White	White	Absent	Low (<i>zt1</i>)	CDC, Canada	95.0
Disco/2	White	White	Absent	Low (<i>zt2</i>)	INRA, France	99.0
Gelber	White	White	Yellow	High	Svalöf, Sweden	98.5
P47-1	Red	Red	Trace of brown	High	CDC, Canada	98.9
IG 114476	White	White	Black	High	Bangladesh	99.4
Rinrei	White	White	Black	High	Japan	99.2
IG 12658	White	White	Black	High	Ethiopia	99.5

CDC = Crop Development Center; INRA = Institut National de la Recherche Agronomique.

Fig. 1. Flower color diversity of parental lines used in this study. (A) Wild type. (B) Yellow wing spotted. (C) White. (D) Red.

Plants were watered as necessary and fertilized with a blend of 15–30–15 (N–P–K) biweekly after the 10th node stage. The parental lines, F₁ and F₂ seeds were grown into 10-cm pots under similar growing conditions explained previously to score them all at the same growing conditions. Biological control was used using both beneficial mites (*Amblyseius cucumeris*) and nematodes (*Steinernema feltiae*) to suppress the thrips population.

Phenotyping. The flower color was scored visually for each plant by recording the standard color, wing color, and presence of a wing spot. The flower color of the parent lines in this study were evaluated according to the horticultural color chart (Wilson, 1938). The term “solid” is used to describe a flower phenotype where the wing petal is totally

pigmented and “spot” refers to the phenotype of observable wing spots.

Parental line genotyping. The eight parental lines were genotyped using 875 single nucleotide polymorphism (SNP) markers developed by Webb et al. (2016) to examine their percent homozygosity. DNA was extracted from a seedling leaf of a single plant of each parent line. Ten discs (diameter 5 mm) from healthy, newly expanded leaves of the parental lines were shipped to the LGC Genomics laboratory (LGC Genomics, Beverly, MA) for genomic DNA extraction and SNP genotyping according to the manufacturer’s instructions.

Statistical analysis. Statistical goodness of fit of observed offspring segregation ratios to their expected counterparts for flower color characteristics were determined by the standard χ^2 test using R Statistical Computing (R Development Core Team, 2019). The expected number of offspring in each cross was obtained from the hypothesis of either one or two unlinked recessive genes inherited in a simple Mendelian fashion. Cross P47-1 × Gelber was evaluated as a trihybrid gene model.

Results

All inbred lines used as parents were highly homozygous except Aurora/2 (Table 1). The genotyping call on parental lines is presented in Supplemental Table 1. Counts for red and non-red flowers for each cross are shown in Table 2. F₁ plants from all “red × non-red” and their reciprocal crosses produced wild-type flowers. The F₂ results suggested that the red flower phenotype was controlled by two recessive genes (15 non-red:1 red). However, when the red-flowered genotype was crossed with the wild-type genotypes or white-flowered carrying *zt1* gene, a better fit to the expected segregation ratio was observed compared with crosses with the yellow-spotted and *zt2* genotypes (Table 2). The F₂ progenies of cross IG 114476 × P47-1 segregated 85 wild type, 18 solid brown, 17 pink, and 7 solid red flowers. This ratio fit to 9:3:3:1 Mendelian ratio of the double recessive gene model ($\chi^2 = 6.053$, $P = 0.109$); however, no similar ratio was found for other “red × non-red” cross combinations or their reciprocal crosses.

In “wild-type × yellow spotted” crosses, two distinct wing spot color phenotypes were observed: black and yellow. The F₂ segregation ratio confirmed a monogenic Mendelian

inheritance (three black spotted:one yellow spotted), with black spotted being dominant to yellow-spotted wing petals. The F₂ populations involving *zt1* (CDC Snowdrop) and *zt2* (Disco/2) fit a 9:3:4 recessive epistasis where white flower was epistatic over the yellow spot (Table 3). When both white and yellow genes were present as homozygous recessive alleles, the solid white wing was expressed.

The χ^2 test for the solid and spotted wing phenotype is presented in Table 4. All 11 populations fit the segregation ratio of 3:1 for solid wing:spotted wing. This confirms that the solid wing phenotype is controlled by a single recessive gene. This gene controls the spot pattern on the wing petal, rather than petal color, like the other genes discussed previously.

The genotype and phenotype of cross P47-1 × Gelber are given in Table 5. This cross was evaluated as a trihybrid. Eight distinct phenotypic classes were observed. The F₁ plants produced wild-type flowers. In F₂ phenotypes, solid brown, solid yellow, pink/brown (standard/wing), pink/yellow, and red/yellow were observed.

Discussion

Here we present results of multiple F₁ crosses and F₂ populations derived from contrasting parental lines from different origins and flower colors. Our results confirmed the previous results on the genetics of red and yellow-spotted wing flowers (Cabrera, 1988; Sjödin, 1971). Our crosses also led to the discovery of combinations of red and yellow flower colors that have not been previously reported. As most faba bean pollen and flowers contain the pyrimidine glycosides v-c (Khazaei et al., 2019), the incorporation of low v-c into new flower phenotypes is critical.

Gregor Mendel’s laws for inheritance studies are still relevant today. There are several examples of plants that follow Mendelian inheritance patterns for flower color. Aside from pea (*Pisum sativum*), Mendel’s model plant (Ellis et al., 2011), the flower colors of many other ornamental plants are controlled by qualitative genes that follow Mendelian genetics (Anderson, 2005). The inheritance of anthocyanin in petunia (*Petunia hybrida*) flowers is controlled by two independent genes (Griesbach, 1996, 2005). In Stokes aster (*Stokesia laevis*), flower color is controlled by at least three loci and

Table 2. Observed offspring segregation ratios, value of χ^2 test and corresponding P value for non-red and red-flowered (standard, wings, and keel) for F_1 and F_2 generations of faba bean crosses.

Cross	F_1	F_2			P
		Non-red	Red	χ^2 (15:1), df=1	
Red $\times$ wild type					
P47-1 $\times$ Aurora/2	Wild type	76	14	13.300	0.000
Aurora/2 $\times$ P47-1	Wild type	109	7	0.769	0.381
P47-1 $\times$ IG 114476	Wild type	116	7	0.066	0.798
IG 114476 $\times$ P47-1	Wild type	289	22	0.360	0.550
P47-1 $\times$ Rinrei	Wild type	143	5	2.080	0.149
Rinrei $\times$ P47-1	Wild type	38	3	0.080	0.778
P47-1 $\times$ IG 12658	Wild type	376	23	0.161	0.689
Pooled		1147	81	0.251	0.616
Red $\times$ White (<i>zt1</i>)					
P47-1 $\times$ CDC Snowdrop	Wild type	187	10	0.463	0.496
CDC Snowdrop $\times$ P47-1	Wild type	89	7	0.178	0.673
Pooled		276	17	0.100	0.751
Red $\times$ White (<i>zt2</i>)					
P47-1 $\times$ Disco/2	Wild type	136	0	9.067	0.003
Disco/2 $\times$ P47-1	Wild type	87	2	2.434	0.119
Pooled		223	2	11.625	0.001
Red $\times$ Yellow spotted					
P47-1 $\times$ Gelber	Wild type	310	8	7.568	0.006
Gelber $\times$ P47-1	Wild type	60	1	2.213	0.137
Pooled		370	9	9.714	0.002
Pooled data (all)		2016	109	4.554	0.033

Table 3. Observed offspring segregation ratios, value of χ^2 test and corresponding P value for flower yellow wing spot for F_1 and F_2 generations involving yellow wing spotted faba bean (Gelber).

Cross	F_1	F_2			P
		Black spot	Yellow spot	White	
Wild type $\times$ Yellow spotted					
Aurora/2 $\times$ Gelber	Wild type	61	19	—	χ^2 (3:1), df=1
P47-1 $\times$ Gelber	Wild type	54	15	—	0.067
White $\times$ Yellow spotted					P
CDC Snowdrop (<i>zt1</i>) $\times$ Gelber	Wild type	128	41	49	χ^2 (9:3:4), df=2
Disco/2 (<i>zt2</i>) $\times$ Gelber	Wild type	75	21	32	0.791
					0.673
					0.779

Table 4. Observed offspring segregation ratios, value of χ^2 test and corresponding P value for spotted and solid wing petals for F_1 and F_2 generations of faba bean crosses.

Cross	F_1	F_2			P
		Spotted wing	Solid wing	χ^2 (3:1), df=1	
Aurora/2 $\times$ P47-1	Spotted	93	27	0.400	0.527
P47-1 $\times$ Aurora/2	Spotted	66	24	0.133	0.715
IG 114476 $\times$ P47-1	Spotted	241	69	1.243	0.265
P47-1 $\times$ IG 114476	Spotted	88	40	2.667	0.102
Rinrei $\times$ P47-1	Spotted	36	5	3.585	0.053
P47-1 $\times$ Rinrei	Spotted	118	33	0.797	0.372
P47-1 $\times$ IG 12658	Spotted	304	93	0.525	0.469
Gelber $\times$ P47-1	Spotted	47	14	0.137	0.712
P47-1 $\times$ Gelber	Spotted	244	64	2.926	0.087
CDC Snowdrop $\times$ Gelber	Spotted	96	32	0.000	1.000
Disco/2 $\times$ Gelber	Spotted	169	48	0.960	0.327
Pooled		1502	449	4.105	0.043

is consistent with simple Mendelian genetics (Barb et al., 2008). A three-gene model was also proposed for the inheritance of flower color in *Anagallis monelli* (Freyre and Griesbach, 2004). Due to these consistencies over many species, the hypothesis for flower color in faba bean was developed based on simple Mendelian genetic models. They have also been used to describe many other traits in faba bean such as hilum color, seedcoat color, flower color, pigmentation and

dwarfism (Khazaei et al., 2014; O'Sullivan and Angra, 2016; Zannotto et al., 2020a).

The genetics of red flower color had limited published work in this species. A publicly available red-flowered faba bean called "1778" was the initial source of the red flower used to develop P47-1, the red-flowered line used in this study. In pea, two genes influenced the regulation of purple flower color (Moreau et al., 2012), which is in agreement with our results on faba bean. Our finding that the yellow

wing spot is controlled by a single recessive gene, is consistent with the findings of Cabrera (1988); however, the relationship between yellow wing spot and white wing has not been previously reported. Our results showed an epistatic relationship for wing spot color with both *zt1* and *zt2*. The yellow pigment appears to be a mutation of the black pigment.

Flower color in the P47-1 $\times$ Gelber population and its reciprocal segregated for three genes, making them trihybrids. Trihybrids are much more complex to classify due to the large population size required. In our study, the population sizes were not large enough to accurately test trihybrid segregation ratios of 27:9:9:3:3:3:1. These crosses had similar phenotypes to dihybrid crosses with red flower (red $\times$ non-red), but there was another set of phenotypes in which the brown pigmentation was substituted for yellow. We performed test crosses to determine the genotypes of the rare phenotypes. A cross between a solid yellow flower phenotype and a red flower phenotype resulted in an F_1 plant with dark brown flowers. Another cross between a pink/yellow flower and red flower resulted in a 1:1 ratio of red: pink/brown flowered F_1 hybrids. These test cross phenotypes were consistent with the proposed genotypes in Table 5, and indicate the logical assumption that the phenotype expressing both yellow and red is the most recessive genotype, expressing both the double recessive red genes and the single recessive yellow gene (Table 5) for the trihybrid cross of Gelber $\times$ P47-1 and its reciprocal.

Throughout the crosses, many colors and patterns were recorded, although additional rare phenotypes were discovered (e.g., pink standard/yellow wing and red standard/yellow wing, Table 5) by combining the yellow spot mutation with the double recessive red flower. Previous reports indicate the existence of a solid yellow flower, and the pink and yellow flower (Cabrera, 1988), but there are no reports of the existence of a red and yellow flower. This phenotype arose from a trihybrid cross between red-flowered and yellow spot flowered (P47-1 $\times$ Gelber). The red and yellow flower phenotype occurs only in the complete homozygous recessive genotype from the trihybrid, which is expected at a frequency of 63:1 genotype in a trihybrid.

Faba bean flowers exhibit color variation within the same color classes, and varying color patterns. The standardized color of the parent source of crimson red-flowered in this study was Rhodamine purple 29/1 according to the horticultural color chart (Wilson, 1938). Very few of the red flowers in the F_2 populations fit this same color standard. There were gradients of colors within the same color classes, with clear variation in intensity and hue. Within the red phenotype, for example, the pigmentation exhibited hues from burgundy to purple. For example, the red flower phenotype derived from crosses with IG 114476 and Rinrei was a cooler tone and appeared purple, whereas red flower types derived from an Aurora/2 background had warmer red tones. Environmental

Table 5. Genotypes proposed for cross P47-1 × Gelber along with the F₁ and segregating trihybrid F₂ faba bean plants.

Parental line / Cross	Genotype	Phenotype (Standard/Wing)	Photo
Parental line			
P47-1	<i>r₁r₁r₂r₂YY</i>	Red
Gelber	<i>R₁R₁R₂R₂yy</i>	Yellow wing spot
F ₁	<i>R₁-R₂-Y-</i>	Wild type
F ₂	<i>r₁r₁R₂-Y-</i>	Solid brown
F ₂	<i>R₁-r₂r₂Y-</i>	Pink/Brown
F ₂	<i>r₁r₁R₂-yy</i>	Solid yellow
F ₂	<i>R₁-r₂r₂yy</i>	Pink/Yellow
F ₂	<i>r₁r₁r₂r₂yy</i>	Red/Yellow

conditions such as temperature, light intensity, pH, or even insect pressure may influence the flower color (Oh et al., 2014). Another unique aspect of this work was the presence of reciprocal crosses to study the genetics of red flower color. When P47-1 was used as a male parent, a better fit to 15:1 ratio was observed than a female parent (Table 2). Faba bean flower color varies in intensity, hue, and pattern, properties that are influenced by the environmental stresses that can cause anthocyanin upregulation, the maternal effects, and genotype-specific gene interactions with color.

Our preliminary results of biochemical analysis revealed that red flower color in faba bean was produced by anthocyanins. For brown, pink, and yellow flowers, no anthocyanins were found in flower tissue (manuscript in preparation). Anthocyanins are red-purple pigments that can be found in many plant species (reviewed in Ng and Smith, 2016). A recent study also showed that flavonols were abundant in flowers of white-flowered faba beans (Zanotto et al., 2020b).

Conclusions

In faba bean, red flower and yellow wing spot are controlled by double recessive and single recessive genes, respectively. Although this explanation seems straightforward, faba bean flower color has many other environmental and genetic influences. Faba bean flower color also had epistatic genetic relationships between colors and within specific populations. This study was not only able to determine the inheritance of two unique faba bean flower colors, but a new flower color phenotype was reported for the first time.

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