GENETIC LINKAGE ANALYSIS OF INCOMPLETE DOMINANT PAIR IN COMMON BEAN CULTIVARS (*PHASEOLUS VULGARIS*) BY USING DIRECT CALCULATION AND MAXIMUM LIKELIHOOD ESTIMATE CALCULATION METHODOLOGY

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ABSTRACT

In this study, the vine cultivar. “Haibushi” had a normal purple stem and red flower colour crossed with a vine-less cv. “Morocco” had a short green stem and white flower colour. 1) The objective of this study was to explain how to use the maximum likelihood estimate calculation compared with direct calculation methodology on the result of the common bean cross (F_2) for higher school biology education. 2) To analyse the recombinant pair of common bean genes. First, the expected genotypes constructed value of F_2 characteristics linkage was calculated by the direct calculation method. Then the Maximum Likelihood Estimate (MLE) method was used to calculate the reliability of the direct calculation method. Results showed that F_1 had a normal mixed stem and pink flower colour. F_2 populations obtained from all F_1 self-fertilised plants have the ratio 1:2:1 in purple: mixed: green in stem colour and red: pink: white in flower colour with p-value>0.05. The results of the chi-square tests of genetic linkage in stem colour and flower colour characteristics showed no significant differences between observed and expected values. The result indicated that genetic linkage and map distance calculation was 9.7% to 21.6% of incomplete dominance characteristics pair, stem colour, and flower colour. The result of the MLE calculation method showed that four of five of the F_2 populations did not detect significant differences between observed and expected values because r-*values* from the direct calculation and MLE methods resembled. Thus, the genetic linkage calculated by direct calculation used in this study was acceptable. The proportion 9:7 caused by gene epistasis from this research was suited to explainability. Students use Punnett’s table to produce the genotypes proportion then use the Excel software to construct the recombinant genotypes instead of MLE which is complicated for high school students.

Keywords: genetic linkage, Phaseolus vulgaris, direct calculation method, Maximum Likelihood Estimate, high school biology education.

1. Introduction

Genetic factors responded to phenomena expression as the flower colour, and seed coat colour in F_2 offspring generation (Herniter et al., 2024). Non-Mendelian inheritance is proposed as other concepts are not described in Mendel’s law which is that it is not more popular for high school students to practicum their learning. Those non-Mendel’s laws included incomplete dominance, codominance, multiple alleles, sex-linkage traits, and multigene trait events (Strome et al., 2024). Thomas Hunt Morgan found the genetic linkage, using the backcross population of fruit flies. The fruit fly has no di-phenotype male and female, and there was no self-fertilisation. In high school, using fruit flies for the general study was difficult because teachers had to keep parents trained by feeding them. It was easy to use self-fertilisation in plants to produce an F_2 population (Morgan T. H., 1911), but in genetic linkage, calculating the recombination value was very difficult due to the dominance characteristic involved in both homozygous and heterozygous genotypes, and it was also difficult to separate the homozygous and heterozygous on observation phenotypes. The incomplete dominance was described in Cambodian high school textbooks and students learned that the hetero type should be an intermediate characteristic. A direct calculation method was introduced to calculate the genetic linkage mathematically. The direct calculation method was used in any research field in terms of different calculation parameters, such as estimating the ecosystem levels (Vogt et al., 1998), and the expected genetic parameter of the Turkey common bean (Bilir et al., 2019), mating ecology (Gardner and Ross, 2014), and mathematical approach (Pupeikis, 2000). Two allele probabilities of the F_2 diploid population could be the individual shared (Lipatov et al., 2015), they can also be calculated by directly expecting the genotype value to be reconstructed. In the other report, Joseph 1973 assumed the minimum-steps method verified by Maximum Likelihood Estimation (Josep F., 1973). In this case, there were no reports that direct calculation methods were largely explained in high school biology education. Thus, in this study, two incomplete dominance characteristics were found from crossing between two cultivars in the common bean (*Phaseolus vulgaris* L.). By the way, this segregation did not follow Mendelian 3rd law. Therefore, the use of common beans with incomplete dominance characteristics in the genetic linkage for education material tried to develop for biology experimental classes in high school education.

The Maximum Likelihood Estimation (MLE) is a method used to estimate the parameters of a mathematical model (Allard R.W, 1956). Allard 1956 reported this MLE method to estimate recombination value heredity in the F_2 population. He proposed the expected genotype value from an incomplete dominance gene pair by using the probability of recombination rate (r) as the parameter. However, this formula made it difficult for high school students to practice their calculations. Using MLE here aimed to verify whether the direct calculation method used in this study is reliable or not.

2. Methods and Materials

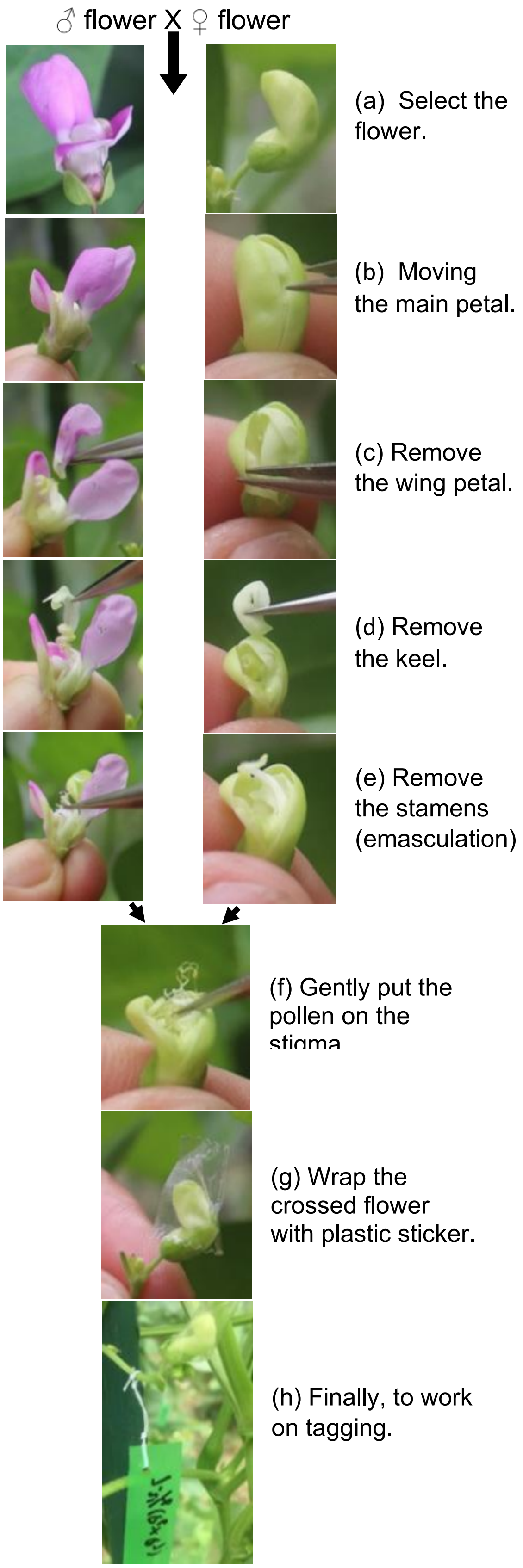
2.1 Plant Materials Selection

The crossing chose the commercial common bean vine cultivar Haibushi as a male parent and a vine-less cultivar Morocco as the female parent. The cultivar Haibushi has normal purple stems, red flowers, and black seed phenomena. The cultivar Morocco had a short green stem colour, white flowers, and white seeds phenomena. These two different parental phenomena are simple to recognise and perform cross techniques.

2.2 Crossing Method

The crossing method used in this study was adapted from techniques of bean crossing performed by Steve Temple et al., 1987. They established two ways of hand pollination: (1) pollination without the emasculation of a female flower and (2) pollination with the emasculation of a female flower. The crossing technique in this study used pollination with the emasculation of the female flower to ensure that self-fertilising is blocked. First, a flower bud (before the flower opened stage) was selected from the Morocco cultivar as a female flower, and an opened flower was selected from the Haibushi cultivar as a male flower (Figure 1a). The techniques of female flower emasculation and collection of pollen from male flowers were done in the same steps (Figure 1b-e). The emasculation of female flowers was done very carefully. The female flower was emasculated by gently holding the flower between the thumb and index finger, then using the forceps' tip to open the petal and wing petal and fold it out direction until the keel is found (Figure 1bc). Gently removed the keel which covered the free stigma, then used forceps to cut all stamens from the part around the stigma (Figure 1d). Then use a pressure plastic bottle with cleaning water to spray on the stigma to ensure that all pollen is removed. For the male parent flower, a forceps' tip collects the stamen and then attaches to the female emasculated stigma (Figure 1f). Finally, fold the female's petal to close the crossed pistil, then cover the pistil with a plastic sticker to keep the moisture inside the flower for fertilisation support and to protect the crossing flower from insect attaching (Figure1g). Finally, the crossed flower was labelled (Figure 1h).

Figure 1: The crossing



(a) Select the flower.

(b) Moving the main petal.

(c) Remove the wing petal.

(d) Remove the keel.

(e) Remove the stamens (emasculation)

(f) Gently put the pollen on the stigma

(g) Wrap the crossed flower with plastic sticker.

(h) Finally, to work on tagging.

2.3 The F_2 Genotype Construction Methods

In the meiosis phase, the F_1 genotype was designed as $\frac{st^{pig}-fl^{pig}}{g-w}$ Where st^{pig} and fl^{pig} represented the purple stem colour and red flower colour alleles, while the g and w represented the green stem colour and white flower colour alleles, respectively. Then, four chromosome phenomena were produced, in which two (st^{pig}-fl^{pig}) and (*g*-*w*) were parental genotypes, and two other (st^{pig}-*w*) and (*g*-fl^{pig}) were linked genotypes. The construction of phenotypes and genotypes was produced by using the Punnet square (Table 1).

Table 1: The F$_2$ phenotypes and genotypes construction.

F$_1$ **gamete**		♀ pistil			
		st^{pig}-fl^{pig}	**st^{pig}-w**	**g-fl^{pig}**	**g-w**
♂ **pollen**	**st^{pig}-fl^{pig}**	$\frac{st^{pig}-fl^{pig}}{st^{pig}-fl^{pig}}$ purple-red	$\frac{st^{pig}-w}{st^{pig}-fl^{pig}}$ purple-pink	$\frac{g-fl^{pig}}{st^{pig}-fl^{pig}}$ mixed-red	$\frac{g-w}{st^{pig}-fl^{pig}}$ mixed-pink
	st^{pig}-w	$\frac{st^{pig}-fl^{pig}}{st^{pig}-w}$ purple-pink	$\frac{st^{pig}-w}{st^{pig}-w}$ purple-white	$\frac{g-fl^{pig}}{st^{pig}-w}$ mixed-pink	$\frac{g-w}{st^{pig}-w}$ mixed-white
	g-fl^{pig}	$\frac{st^{pig}-fl^{pig}}{g-fl^{pig}}$ mixed-red	$\frac{st^{pig}-w}{g-fl^{pig}}$ mixed-pink	$\frac{g-fl^{pig}}{g-fl^{pig}}$ green-red	$\frac{g-w}{g-fl^{pig}}$ green-pink
	g-w	$\frac{st^{pig}-fl^{pig}}{g-w}$ mixed-pink	$\frac{st^{pig}-w}{g-w}$ mixed-white	$\frac{g-fl^{pig}}{g-w}$ green-pink	$\frac{g-w}{g-w}$ green-white

From Table 1, the sixteen F$_2$ offspring have been produced by self-fertilisation from four F$_1$'s gametes (pistil and pollen gametes). Thus, those offspring were designed as: one purple-red, two purple-pink, two mixed-red, one purple-white, four mixed-pinks, two mixed-white, one green-red, two green-pink, and one green-white. The "*mixed"* here represented the new stem colour of F$_2$ offspring phenomena which stands in the middle of parental stem colour (purple and green) and "*pink*" represented the middle flower colour between red and white phenomena (Incomplete dominance characteristic).

2.4 Calculation Recombination Rate (*r*) from Observed Population

In Table 1, the expected genotypes and phenotypes were designed using the Punnet square model. The recombination rate was calculated by observing the F$_2$ characteristic segregation population by using Excel software as the total number of chromosome linkage/total number of chromosomes by the formula: $r = \frac{Total\ Number\ of\ Recombination\ Chromosome}{Total\ Number\ of\ Observed\ Chromosome}$. Where *r* is the recombination rate. Then, $r = \frac{(st^{pig}-w)+(g-fl^{pig})}{(st^{pig}-fl^{pig})+(g-w)+(st^{pig}-w)+(g-fl^{pig})}$. By using the theoretical proportion, the Total proportion of all four-chromosome genotype $st^{pig}-fl^{pig}, g-w, st^{pig}-w$, and $g-fl^{pig}$ equal to 1. Since there are two (st^{pig}-fl^{pig}) and (*g*-*w*) parental genotypes, and two $st^{pig}-w =$ and $g-fl^{pig}$ were linked genotypes. Therefore, the genes of $st^{pig}-w =$ gene of $g-fl^{pig} = \frac{r}{2}$, and genotype of $st^{pig}-fl^{pig} = gene\ of\ \ g-w = \frac{1-r}{2}$.

2.5 Direct Calculation Method for Reconstruction of the Expected Genotypes from Phenotypes Value by using Recombination Rate (*r*)

The expected genotype value = ♀genotype × ♂genotype × number of F$_2$ population (n). All the expected F$_2$ genotype value formulas are shown in *Table 2*, sixteen genotypes and nine phenotypes of the F$_2$ population were produced. To solving genetic linkage, the study directly calculated the expected genotypes from the observed phenotypes using recombination rate (*r*).

Table 2: The formula used to calculate the expected value of genotype characteristics linkage pair, stem colour and flower colour by using the Punnett square.

F₁ gametes		*purple-red*	*purple-white*	*green-red*	*green-white*
		$\frac{1-r}{2}$	$\frac{r}{2}$	$\frac{r}{2}$	$\frac{1-r}{2}$
purple-red	$\frac{1-r}{2}$	$(\frac{1-r}{2})^2 n$	$\frac{r(1-r)}{4}n$	$\frac{r(1-r)}{4}n$	$(\frac{1-r}{2})^2 n$
purple-white	$\frac{r}{2}$	$\frac{r(1-r)}{4}n$	$(\frac{r}{2})^2 n$	$(\frac{r}{2})^2 n$	$\frac{r(1-r)}{4}n$
green-red	$\frac{r}{2}$	$\frac{r(1-r)}{4}n$	$(\frac{r}{2})^2$	$(\frac{r}{2})^2 n$	$\frac{r(1-r)}{4}n$
green-white	$\frac{1-r}{2}$	$(\frac{1-r}{2})^2 n$	$\frac{r(1-r)}{4}n$	$\frac{r(1-r)}{4}n$	$(\frac{1-r}{2})^2 n$

Using the Punnett square model in Table 2, the reconstructed genotypes from nine phenotypes frequency given as formula below:

$$Purple-d\ =\frac{st^{pig}-fl^{pig}}{st^{pig}-fl^{pig}}=\frac{(1-r)^2}{4}n,$$

$$Purple-Pink=\frac{st^{pig}-fl^{pig}}{st^{pig}-w}+\frac{st^{pig}-w}{st^{pig}-fl^{pig}}=\frac{r(1-r)}{2}n,$$

$$Purple-White=\frac{st^{pig}-w}{st^{pig}-w}=\frac{r^2}{4}n,$$

$$Mixed-d\ =\frac{st^{pig}-fl^{pig}}{g-fl^{pig}}+\frac{g-fl^{pig}}{st^{pig}-fl^{pig}}=\frac{r(1-r)}{2}n,$$

$$Mixed-Pink=\frac{st^{pig}-fl^{pig}}{g-w}+\frac{g-w}{st^{pig}-fl^{pig}}+\frac{st^{pig}-w}{g-fl^{pig}}+\frac{g-fl^{pig}}{st^{pig}-w}=\frac{r^2+(1-r)^2}{2}n,$$

$$Green-d\ =\frac{g-fl^{pig}}{g-fl^{pig}}=\frac{r^2}{4}n,$$

$$Green-Pink=\frac{g-fl^{pig}}{g-w}+\frac{g-w}{g-fl^{pig}}=\frac{r(1-r)}{2}n$$

$$Green-White=\frac{g-w}{g-w}=\frac{(1-r)^2}{4}n.$$

Then use the expected genotypes and observed phenotypes value to calculate in chi-square test by Chi-square formula:

$$Chi-square=\sum\ \frac{(Observed\ Value-Expected\ Value)^2}{Expected\ Value}.$$

2.6 Maximum Likelihood Estimate Calculation Method

Maximum Likelihood Estimate methods were used to estimate the recombination rate from the F₂ population (Allard R.W., 1956). This calculation showed probability. Using the *r* parameter, *the r*-value producing the highest probability was the most reliable, and the chi-square value using this *r*-value could show the minimum value and *the p*-value as highest. The probability function of observed genotypes can be expressed from a multinomial distribution. The probability of frequencies of appearance of observed phenotype number of F₂ is as: $P(r)=\frac{n!\prod_{i=1}^{m}\ (f_{k_i})^{k_i}}{\prod_{i=1}^{m}\ k_i!}$. Where *m* is the number of observed phenotypes, *n* is the population values $(n=k_1+k_2+\ldots+k_m)$, k_i is the observed value and f_{k_i}: the frequency phenotype with recombination rate value.

Note: (*i*) $n!=n(n-1)\ldots.2.1$,

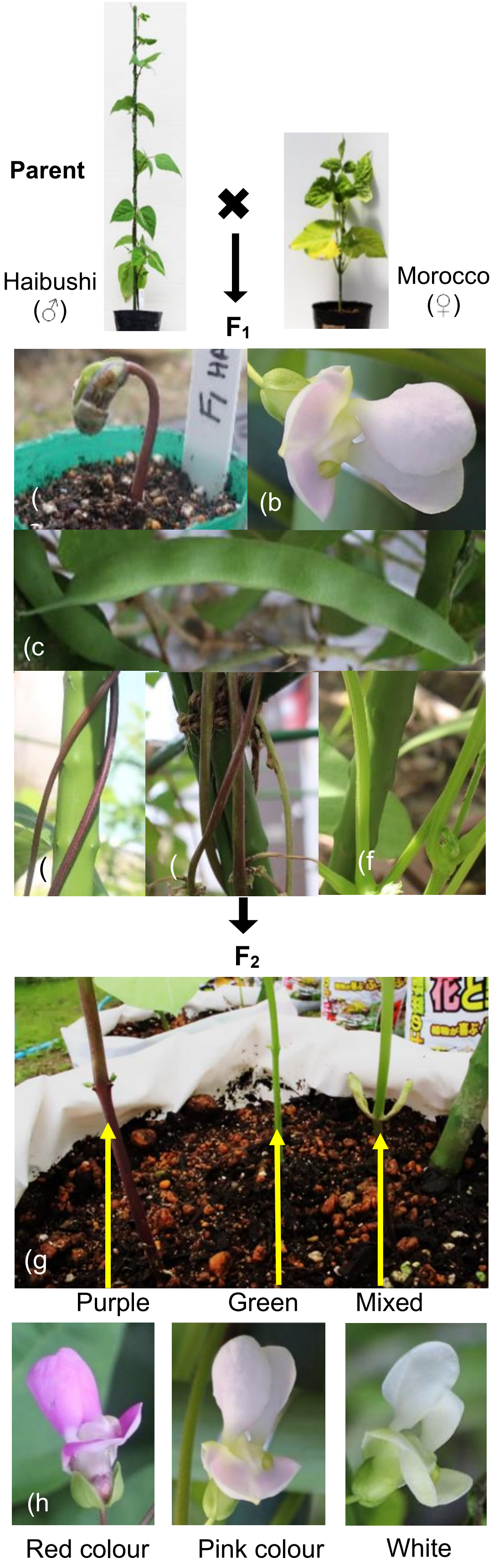
(*ii*) $\prod_{i=1}^{m}\ \ k_i!=k_1!\,k_2!\ldots!\,k_m!$

(*iii*) $\prod_{i=1}^{m}\ \ (f_{k_i})^{k_i}=(f_{k_1})^{k_1}(f_{k_2})^{k_2}\ldots.(f_{k_m})^{k_m}$.

3. Result and Discussion

In the cross between the "Haibushi cultivar" with long and purple stems and the "Morocco cultivar" with short and green stems, all F_1 offspring were long and colour stem plants (Figure 2a-e*)*. The long stem characteristic of F_1 plants looked completely dominant on short stem characteristics because all F_1 progenies showed long stems. The mixed stem colour characteristics of F_1 plants showed the incomplete dominance of purple stem and green stem. The cross between the red flower Haibushi cultivar and the white flower Morocco cultivar, F_1 offspring were all pink flower plants (Figure 2b). Pink flower characteristics are incompletely dominant between red and white flower characteristics. The F_2 population was segregated into nine different phenotypes, including purple-red, purple-pink, purple-white, mixed-red, mixed-pink, mixed-white, green-red, green-pink, and green-white plants in the characteristics pair of stem colour *(*Figure 2g) and flower colour *(*Figure 2h*)*. The term Mixed colour here refers to the colour of the offspring stem in the middle between the green and purple parents' colour. The phenotype frequency from observed data of F_2 rejected Mendel's proportions 9:3:3:1 and 1:2:1: 2:4:2: 1:2:1, thus, the genetic linkage was hypothesised.

Figure 2: F_1 and F_2 characteristics. a) Young F_1, b) F_1 flower, c) fruit, d) Haibushi stem colour, e) F_1 stem colour, f) Moroco stem colour, g) F_2 stem colour and h) F_2 flower colour.



3.1 Incomplete Dominant of F_2 Characteristics Segregation Pair, Stem Colour and Flower Colour

F_2 characteristics stem colour and flower colour were statistics analysed by chi-square test among 263 F_2 plants obtained from six F_1 populations (Table 3). The stem colour characteristics, the F_2 plants were segregated into three different phenotypes purple, mixed (immediate colour), the green stem colour (*Figure:2g*), and flower colour (Figure:2h). The segregation ratios were 1:2:1 in purple, mixed, and green in stem colour with p-value > 0.05, 2 degrees of freedom (Table 4). The flower characteristics, F_2 populations were also segregated into three different phenotypes included red, pink (immediate colour), and white flower colour, and the segregation ratio was 1:2:1 of red: pink: white colour flowers with p-value > 0.05, 2 degrees of freedom (Table 5). This ratio of 1:2:1 followed Mendel's incomplete dominance inheritance characteristics.

Table 3: F_2 phenotypes segregation and frequency.

F_1 code	*F_2 phenotypes*								
	purple-red	purple-pink	purple-white	mixed-red	mixed - pink	mixed-white	green-red	green-pink	green-white
14-HAI	3	4	0	5	13	0	2	1	12
11-HAI	1	8	0	5	16	1	0	3	7
12-HAI	2	3	0	2	17	1	0	1	8
15-HAI	7	1	0	6	24	0	0	4	15
8-HAI	9	1	0	7	15	2	0	3	17
10-HAI	7	5	0	3	8	1	1	5	7
independent frequency	*1/16*	*2/16*	*1/16*	*2/16*	*4/16*	*2/16*	*1/16*	*2/16*	*1/16*

Table 4: The incomplete dominance of F_2 characteristics in stem colour.

F_1 code	*n*	*x^2*	*deg. of freedom*	*p-value*	*proportion*
14-HAI	40	3.60	2	0.165	1:2:1
11-HAI	41	0.26	2	0.874	1:2:1
12-HAI	34	2.0	2	0.367	1:2:1
15-HAI	57	4.40	2	0.110	1:2:1
8-HAI	54	4.37	2	0.112	1:2:1
10-HAI	37	4.62	2	0.099	1:2:1

Table 5: The incomplete dominance of F_2 characteristic in flower colour.

F_1 code	n	x^2	deg. of freedom	p-value	proportion
14-HAI	40	0.55	2	0.757	1:2:1
11-HAI	41	4.31	2	0.115	1:2:1
12-HAI	34	3.35	2	0.187	1:2:1
15-HAI	57	0.15	2	0.924	1:2:1
8-HAI	54	5.07	2	0.079	1:2:1
10-HAI	37	0.51	2	0.773	1:2:1

3.2 Expected Genotypes from Observed Phenotypes by Direct Calculation Method

F_2 populations obtained from five F_1 plants, 11-HAI (n=41), 12-HAI (n=34), 15-HAI (n=57), 8-HAI (n=54) and 10-HAI (n=37) were used in this study. All plant phenotype was inputted into an Excel database, and each plant's data observed in both colour characteristics were changed to estimated genotype combinations on the chromosome by using recombination (*r*) value. From these estimated genotype combinations on the chromosome, the r-value is calculated by the total recombinant chromosome number/total observed chromosome number. The results of the gene linkage study of stem colour and flower colour characteristics significantly differ between the expected number and observed number of F_2 plants because all p-values>0.05 from the chi-square test (Table 4, and Table 5*).* Each r-value calculated for each population was 0.2125, 0.1029, 0.0965, 0.1204, and 0.2162, respectively. The chi-square test between the observed value and reconstructed expected value of genotypes using r-value indicated that only observed population 11-HAI deviated from the expected value and other populations did not have significant differences, so these recombinant values were acceptable. Two weak points of these calculations were imaged; (1) to change from phenotype to genotype, a couple of heterozygosis genotypes, mixed-pink, could not divide between parent pair of chromosomes, st^{pig}-fl^{pig} and *g*-*w*, and recombinant pair of chromosomes, st^{pig}-*w* and *g*-fl^{pig}. In this study, the number of plants in each population was not so many, the expected value of F_2 plants with both different recombinant chromosome pairs, calculated by $\frac{r^2}{2}n$ was about 1 or 2 when r=0.1. So, students could be ignoring this value. If the *r*-value is too big or the number of F_2 plants is also big, then consideration is needed. (2) this method, the *r*-value was different by each population, thus teachers have to explain to the students this reason. However, the differences depended on the observed value, so when F_2 populations obtained from the same F_1 plants cultivated repeat, it looked like the same *r*-value may be present. The results of linked incompletely dominant characters will be good materials used in high school genetic education because students could image genotypes on a chromosome from phenotype. Gene-linkage inheritance, which confirmed two loci involved during gene expression event, (Herniter et al., 2024). Nevertheless, mostly backcross populations were used in the linkage study, students understood linkage calculation as several individuals with recombinant phenotypes per total number of individuals and no chromosome images. Common bean stem colour and flower colour results are based on F_2 population results; thus, it is important to have chromosome images for students to calculate genotypes to phenotypes procedure. Frank 2010 demonstrated the calculation methods for biology and biotechnology (Frank H. S., 2010). In Chapter 10, he suggested a formula to calculate the expected value, but those formulas seemed so difficult for the students at the high school level.

3.3 Confirmation of recombination value by MLE calculation in segregation between incomplete dominant pair, stem, and flower colour

A couple of incomplete dominant characters were then detected r-value by the MLE calculation method to verify the direct calculation method. This MLE calculation aimed to find out the differences between the parameter r-value in the direct calculation method and the r-value in MLE (Table 6 and Table 7). Results showed that in a population of 10-HAI, 8% of differences were presented between direct calculation methods and MLE, while other populations were less than 4.5% (Table 6). These results suggested that detecting a recombination value of nearly 0.5 as the nearly equal independent value was unstable for a small number of individuals in populations. Thus, it could not decide whether the recombination value obtained from the direct calculation method could be used. However, understanding MLE methods to calculate recombinant value might be too tricky for high school students (Allard, 1956). So, the comparison of detection recombinant value between the direct calculation method and MLE should be calculated by the smaller recombinant value as 30%-40% of other coupled characters.

Table 6: chi-square test of expected genotypes by direct calculation method.

F_1 code	n	r	x^2	degree of freedom	p-value
11-HAI	41	0.21	14.50	7	0.01<p<0.05
12-HAI	34	0.10	5.63	7	0.5<p<07
15-HAI	57	0.10	12.36	7	0.05<p<0.1
8-HAI	54	0.12	14.03	7	0.05<p<0.1
10-HAI	37	0.22	6.10	7	0.3<p<0.5

Table 7: Comparison between r-value of the direct calculation method and the r-value of MLE method.

F_1 code	n	$r(x^2)$	r(MLE)	X^2	degree of freedom	p-value
11-HAI	41	0.21	0.24	14.49	7	0.07
12-HAI	34	0.10	0.11	5.63	7	0.69
15-HAI	57	0.09	0.1	12.36	7	0.136
8-HAI	54	0.12	0.13	14.03	7	0.13
10-HAI	37	0.21	0.13	6.93	7	0.54

4. The Applicability for High School Biology Education

The estimating genotype was considered essential to recognise the recombinant genotype for the student by meiosis. In this estimation, students could consider that recombination occurred on a chromosome. In F_2 segregation, students learned Punnett square methods in independence. An independent case could be considered a recombination value at 0.5. The four kinds of coupled genotype frequency were the same as 1/4 in each gamete-coupled genotype. Thus, to introduce recombinant value, four types of coupled genotypes were described using the r-value. The formula of expected value was described by r-value. All recombinant chromosomes could be estimated in incomplete dominance character pairs, and the recombinant value was calculated by directly calculating methods as r=total recombinant chromosomes/total estimated chromosomes. In the confirmation of the reliability of r-value calculating of direct calculated methods by Maximum Likelihood Estimation methods, two r-values were similar to each other. To produce an experimental genetic class that included both Mendel's laws and genetic linkage, the independent colour of the stem and flower was considered a better material. Here, it was proposed that the new experimental class, which was docking between plant hormone content and whole genetic study, could be produced by using common beans. Although these contents are divided into other grades, a curriculum reconstructed could be needed as likely contents accumulate.

5. Conclusion

For these common bean cultivars could be concluded the recombination rate was ranked 9% to 21% representing genetic linkage in incomplete dominance pair, stem colour, and flower colour. The method to calculate the expected number of F_2 genotypes and phenotypes by direct calculation introduced in this study is useful for high school biology education in common bean genetic linkage. The result of this genetic linkage in incomplete dominance pairs, stem colour, and flower colour of common beans are suitable reference materials for further genetic linkage research. In the class, teachers can use the data of F_2 offspring from this research to teach students. Teachers can lead students to design the experiment as project-based learning. Students can do ground-based practicals on the common bean crossing technique. Through this ground-based practical, they investigated the handling work and recognised the phenomena of pollen and pistils possible to cross-fertilise. The MLE method should be difficult for high school students to use to calculate the genetic recombinant of common beans. Thus, the direct calculation method using Excel software is introduced as a crucial simple calculation for students. Using Punnett's square to produce the genotype's proportion and then using the Excel software to construct the recombinant genotypes could be used for simple handling calculations by high school students.

References

Allard, R. W. (1956*).* Formulas and tables to facilitate the calculation of recombination values in heredity. *Journal of Agricultural Science* Published by the California Agricultural Experiment Station (Vol. 24).

Bilir, Ö., Özmen, C. Y., Özcan, S., & Kibar, U. (2019). Genetic analysis of Turkey common bean (Phaseolus vulgaris L.) genotypes by simple sequence repeats markers. *Russian Journal of Genetics*, *55*(1), 61-70.

Frank H. S. (2010). *Calculations for Molecular Biology and Biotechnology*. *The Real-Time Polymerase Chain Reaction (RT-PCR)*, 2nd Edition, 212–214.

Gardner, A., & Ross, L. (2014). Mating ecology explains patterns of genome elimination. *Ecology Letters*, *17*(12), 1602-1612. https://doi.org/10.1111/ele.12383

Herniter, I. A., Muñoz-Amatriaín, M., Lo, S., Guo, Y. N., Lonardi, S., & Close, T. J. (2024). Identification of candidate genes controlling red seed coat color in cowpea (Vigna unguiculata [L.] Walp). *Horticulturae*, *10*(2), 161. https://doi.org/10.3390/horticulturae10020161

Felsenstein, J. (1973). Maximum likelihood and minimum-steps methods for estimating evolutionary trees from data on discrete characters. *Systematic Biology*, *22*(3), 240-249. http://www.jstor.org/stable/2412304

Lipatov, M., Sanjeev, K., Patro, R., & Veeramah, K. R. (2015). Maximum likelihood estimation of biological relatedness from low coverage sequencing data. *BioRxiv*, 023374. https://doi.org/10.1101/023374

Morgan, T. H. (1911). Random segregation versus coupling in Mendelian inheritance. *Science*, *34*(873), 384-384.

Pupeikis, R. (2000). Closed-loop robust identification using the indirect approach. *Informatica*, *11*(3), 297-310.

Strome, S., Bhalla, N., Kamakaka, R., Sharma, U., & Sullivan, W. (2024, July 1). Clarifying Mendelian vs non-Mendelian inheritance. *Genetics*, Vol. 227. Oxford University Press. https://doi.org/10.1093/genetics/iyae078

Vogt, K. A., Vogt, D. J., & Bloomfield, J. (1998). Analysis of some direct and indirect methods for estimating root biomass and production of forests at an ecosystem level. *Plant and Soil*, 200(1), 71-89.